adrenal glan 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 TBTCl 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 TBTCl affected humoral and cell-mediated immunity, and the number and function of cells involved in the hostís immunosurveillance mechanisms against tumors and vital infections in rat offspring.

Postnatal behavioral changes in pups of SD rats that received TBTCl 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.1

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 DBTCl were also studied in Wistar rats. Female rats were given DBTCl 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 DBTCl 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 TBTCl 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 μmol/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, TBTCl, DBTCl, and MBTCl 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 TBTCl or during the susceptible period to teratogenesis of DBTCl. Pregnant rats were given TeBT, TBTCl, or DBTCl during the period of susceptibility to the teratogenesis of TBTCl, on days 13 to 15 of pregnancy (Ema et al. 1996a). TeBT caused an increased incidence of cleft palate at 1832 mg (5280μmol)/kg. TBTCl 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 DBTCl 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)mg/kg. These results indicate that there are differences in the manifestation and degree of developmental toxicity among TeBT, TBT, and DBT. Pregnant rats received TBTCl, DBTCl, or MBTCl during the period of susceptibility to teratogenesis of DBTC, on days 7 to 8 of pregnancy (Ema et al. 1995b). TBTCl at 40 and 80 mg/kg caused an increase in postimplantation embryolethality, but no increase in fetal malformations. DBTCl 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 MBTCl even at 1500 mg/kg. These results indicate that the developmental toxicity of DBTCl is different from that of TBTCl and MBTCl in the level of susceptibility and spectrum of toxicity. A lack of developmental toxicity of MBTCl was also reported by Noda et al. (1992a). MBTCl 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 MBTCl does not participate in the induction of the developmental toxicity of butyltins.

In Vitro *Dysmorphogenic 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 μg/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 dysmorphogenic action. Yonemoto et al. (1993) determined the relative teratogenic potencies of TBTO, TBTCl, (3-OH) hydroxybutyl dibutyltin chloride (3-OHHDBTCl), DBTCl, and MBTCl by comparing developmental hazard estimates using rat embryo limb bud cell cultures. The organotin compounds tested, except for MBTCl, 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 TBTCl, 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 TBTCl 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 DBTCl 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 DBTCl. 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 monosubstituted organotins. DBTCl exerts dysmorphogenic effects on postimplantation embryos *in vitro*. The dysmorphogenic concentrations of DBTCl 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 DBTCl may be attributable to a decline in the susceptibility of embryos to the dysmorphogenesis of DBTCl 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)
TMTCl	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)
MMTCl	SD rat	243 mg/L	As above	As above	As above	
TMTCl	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)
TMTCl	THA rat	5–7 mg/kg	Day 12 of pregnancy	ip	Disruption of learning acquisition	Miyake et al. (1989)
THTCl	SD rat	5 mg/kg	Day 6–20 of pregnancy	Gavage	Increased spontaneous activity, increased d-amphetamine-stimulate rearing	Gårdlund et al. (1991)
DMTCl	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% DOTTG and 20% MOTTG)						Faqi et al. (2001)
	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	

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% monooctyltin 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, clavicula 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 TMTCl possesses developmental neurotoxic effects in postnatal rat offspring, even at doses that induced no maternal toxicity. The learning deficiency induced by prenatal TMTCl may be due to hippocampal lesions. Prenatal treatment of maternal toxic doses of TMTCl adversely affected survival and growth of offspring. Prenatal treatment of THTCl is also reported to induce behavioral changes in postnatal offspring. An increased number of cleft palates were observed in fetuses of rats given DMTCl during organogenesis at a severely maternal toxic dose. A mixture of DOTTG 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. TPTCl during early pregnancy caused implantation failure. Implantation failure due to TPTCl 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, TBTCl 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. TBTCl and DBTCl during early pregnancy caused implantation failure in rats. Implantation failure due to TBTCl and DBTCl, at lower doses than TBTCl, may be mediated via the suppression of uterine decidualization and correlated with the reduction in serum progesterone levels. Administration of MBTCl 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 TBTCl 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. DBTCl exerts dysmorphogenic effects on postimplantation embryos *in vitro*. The phase specificity for the in vivo teratogenic effects of DBTCl may be attributable to a decline in the susceptibility of embryos to the dysmorphogenesis of DBTCl 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 THTCl 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.

References

Adeeko, A., Li, D., Forsyth, D.S., Casey, V., Cooke, G.M., Barthelemy, J., Cyr, D.G., Trasler, J.M., Robaire, B., and Hales, B.F. (2003) Effects of in utero tributyltin chloride exposure in the rat on pregnancy outcome, *Toxicological Sciences*, 74, 407–15.

Baroncelli, S., Karrer, D., and Turillazzi, P.G. (1990) Embryotoxic evaluation of bis(tri-*n*-butyltin)oxide (TBTO) in mice, *Toxicology Letters*, 50, 257–62.

Baroncelli, S., Karrer, D., and Turillazzi, P.G. (1995) Oral bis(tri-*n*-butyltin) oxide in pregnant mice. I. Potential influence of maternal behavior on postnatal mortality, *Journal of Toxicology and Environmental Health*, 46, 355–67.

Belfoid, A.C., Purperhart, M., and Ariese, F. (2000) Organotin levels in seafood, *Marine Pollution Bulletin*, 40, 226–32.

Bettin, C., Oehlmann, J., and Stroben, E. (1996) YBY-induced imposex in marine neogastropods is mediated by an increasing androgen level, *Helgoländer Meeresunters*, 50, 299–317.

Boyer, I.J. (1989) Toxicity of dibutyltin, tributyltin and other organotin compounds to humans and to experimental animals, *Toxicology*, 55, 253–98.

Bryan, G.W., Gibbs, P.E., Hummerstone, L.G., and Burt, G.R. (1986) The decline of the gastropod *Nucella lapillus* around South-West England: evidence for the effect of tributyltin from antifouling paints, *Journal of the Marine Biological Association of the United Kingdom*, 66, 611–40.

Bryan, G.W., Gibbs, P.E., Burt, G.R., and Hummerstone, L.G. (1987) The effect of tributyltin (TBT) accumulation on adult dog-whelks, *Nucella lapillus*: long-term field and laboratory experiments, *Journal of the Marine Biological Association of the United Kingdom*, 67, 525–44.

Bryan, G.W., Gibbs, P.E., Hummerstone, L.G., and Burt. G.R. (1988) Comparison of the effectiveness of tri-*n*-butyltin chloride and five other organotin compounds in promoting the development of imposex in the dogwhelk *Nucella lapillus*, *Journal of the Marine Biological Association of the United Kingdom*, 68, 733–44.

Bryan, G.W., Gibbs, P.E., Hummerstone, L.G., and Burt, G.R. (1989) Uptake and transformation of 14-C labelled tributyltin chloride by the dog-whelk, *Nucella lapillus*: Importance of absorption from the diet, *Marine Environmental Research*, 28, 241–5.

Bui, Q.Q., Tran, M.B., and West, W.L. (1986) A comparative study of the reproductive effects of methadone and benzo[*a*]pyrene in the pregnant and pseudopregnant rat, *Toxicology*, 42, 195–204.

Chernoff, N., Setzer, R.W., Miller, D.B., Rosen, M.B., and Rogers, J.M. (1990) Effects of chemically induced maternal toxicity on prenatal development in the rat, *Teratology*, 42, 651–8.

Clark, R.L., Anderson, C.A., Prahalada, S., Robertson, R.T., Lochry, E.A., Leonard, Y.M., Stevens, J.L., and Hoberman, A.M. (1993) Critical developmental periods for effects on male rat genitalia induced by finasteride, a 5 alpha-reductase inhibitor, *Toxicology and Applied Pharmacology*, 119, 34–40.

Colborn, T., vom Saal, F., and Soto, A. (1993) Developmental effects of endocrine-disrupting chemicals in wildlife and humans, *Environmental Health Perspectives*, 101, 378–84.

Cooke, G.M., Tryphonas, H., Pulido, O., Caldwell, D., Bondy, G.S., and Forsyth, D. (2004) Oral (gavage), in utero and postnatal exposure of Sprague-Dawley rats to low doses of tributyltin chloride. Part I: toxicology, histopathology and clinical chemistry, *Food and Chemical Toxicology*, 42, 211–20.

Crofton, K.M., Dean, K.F., Boncek, V.M., Rosen, M.B., Sheets, L.P., Chernoff, N., and Reiter, L.W. (1989) Prenatal or postnatal exposure to bis(tri-*n*-butyltin)oxide in the rat: postnatal evaluation of teratology and behavior, *Toxicology and Applied Pharmacology*, 97, 113–23.

Cummings, A.M. (1990) Toxicological mechanisms of implantation failure, *Fundamental and Applied Toxicology*, 15, 571–9.

Davies, J.M., Bailey, S.K., and Moore, D.C. (1987) Tributyltin in Scottish sea lochs, as indicated by degree of imposex in the dogwhelk, *Nucella lapillus* (L.), *Marine Pollution Bulletin*, 18, 400–4.

Davis, A., Barale, R., Brun, G., Forster, R., Güunther, T., Hautefeuille, H., van der Heijden, C.A., Knaap, A.G.A.C., Krowke, R., Kuroki, T., Loprieno, N., Malaveille, C., Merker, H.J., Monaco, M., Mosesso, P., Nuebert, D., Norppa, H., Sorsa, M., Vogel, E., Voogd, C.E., Umeda, M., and Bartsch, H. (1987) Evaluation of the genetic and embryotoxic effects of bis(tri-*n*-butyltin)oxide (TBTO), a broad-spectrum pesticide, in multiple in vivo and in vitro short-term tests, *Mutation Research*, 188, 65–95.

De Feo, V.J. (1963) Temporal aspect of uterine sensitivity in the pseudopregnant or pregnant rat, *Endocrinology*, 72, 305–16.

Doering, D.D., Stechelbroeck, S., Doering, T., and Klingm, D. (2002) Effects of butyltins on human 5-reductase type 1 and type 2 activity, *Steroids*, 67, 859–67.

Eisler, R. (2000) Tin, in *Handbook of Chemical Risk Assessment: Health Hazards to Humans, Plants, and Animals, Volume 1 (Metals)*, Boca Raton, FL, Lewis Publishers, 551–603.

Ema, M. and Harazono, A. (2000) Adverse effects of dibutyltin dichloride on initiation and maintenance of rat pregnancy, *Reproductive Toxicology*, 14, 451–6.

Ema, M. and Harazono, A. (2001) Toxic effects of butyltin trichloride during early pregnancy in rats, *Toxicology Letters*, 125, 99–106.

Ema, M. and Miyawaki, E. (2001) Role of progesterone on suppression of uterine decidualization and implantation failure induced by triphenyltin chloride in rats, *Congenital Anomalies*, 41, 106–11.

Ema, M. and Miyawaki, E. (2002) Suppression of uterine decidualization correlated with reduction in serum progesterone levels as a cause of preimplantation embryonic loss induced by diphenyltin in rats, *Reproductive Toxicology*, 16, 309–17.

Ema, M., Itami, T., and Kawasaki, H. (1991) Teratogenicity of di-*n*-butyltin dichloride in rats, *Toxicology Letters*, 58, 347–56.

Ema, M., Itami, T., and Kawasaki, H. (1992) Susceptible period for the teratogenicity of di-*n*-butyltin dichloride in rats, *Toxicology*, 73, 81–92.

Ema, M., Kurosaka, R., Amano, H., and Ogawa, Y. (1995a) Further evaluation of the developmental toxicity of tributyltin chloride in rats, *Toxicology*, 96, 195–201.

Ema, M., Kurosaka, R., Amano, H., and Ogawa, Y. (1995b) Comparative developmental toxicity of butyltin trichloride, dibutyltin dichloride and tributyltin chloride in rats, *Journal of Applied Toxicology*, 15, 297–302.

Ema, M., Iwase, T., Iwase, Y., and Ogawa, Y. (1995c) Dysmorphogenic effects of di-*n*-butyltin dichloride in cultured rat embryos, *Toxicology in Vitro*, 9, 703–9.

Ema, M., Kurosaka, R., Amano, H., and Ogawa, Y. (1996a) Comparative developmental toxicity of di-, tri- and tetrabutyltin compounds after administration during late organogenesis in rats, *Journal of Applied Toxicology*, 16, 71–6.

Ema, M., Iwase, T., Iwase, Y., Ohyama, N., and Ogawa, Y. (1996b) Change of embryotoxic susceptibility to di-*n*-butyltin dichloride in cultured rat embryos, *Archives of Toxicology*, 70, 742–8.

Ema, M., Miyawaki, E., Harazono, A., and Ogawa, Y. (1997a) Effects of triphenyltin chloride on implantation and pregnancy in rats, *Reproductive Toxicology*, 11, 201–6.

Ema, M., Harazono, A., Miyawaki, E., and Ogawa, Y. (1997b) Effect of the day of administration on the developmental toxicity of tributyltin chloride in rats, *Archives of Environmental Contamination and Toxicology*, 33, 90–6.

Ema, M., Miyawaki, E., Harazono, A., and Ogawa, Y. (1998) Reproductive effects of butyl benzyl phthalate in pregnant and pseudopregnant rats, *Reproductive Toxicology*, 12, 127–32.

Ema, M., Miyawaki, E., and Kawashima, K. (1999a) Suppression of uterine decidualization as a cause of implantation failure induced by triphenyltin chloride in rats, *Archives of Toxicology*, 73, 175–9.

Ema, M., Miyawaki, E., and Kawashima, K. (1999b) Adverse effects of diphenyltin dichloride on initiation and maintenance of pregnancy in rats, *Toxicology Letters*, 108, 17–25.

Ema, M., Miyawaki, E., and Kawashima, K. (1999c) Developmental toxicity of triphenyltin chloride after administration on three consecutive days during organogenesis in rats, *Bulletin of Environmental Contamination and Toxicology*, 62, 363–70.

Ema, M., Miyawaki, E., and Kawashima, K. (2000) Critical period for adverse effects on development of reproductive system in male offspring of rats given di-*n*-butyl phthalate during late pregnancy, *Toxicology Letters*, 111, 271–8.

Ema, M., Harazono, A., Hirose, A., and Kamata, E. (2003) Protective effects of progesterone on implantation failure induced by dibutyltin dichloride in rats, *Toxicology Letters*, 143, 233–8.

Epstein, S.S., Arnold, E., Andrea, J., Bass, W., and Bishop, Y. (1972) Detection of chemical mutagens by the dominant lethal assay in the mouse, *Toxicology and Applied Pharmacology*, 23, 288–325.

Evans, D.W. and Laughlin, R.B., Jr. (1984) Accumulation of bis(tributyltin) oxide by the mud crab, *Rhithropanopeus harrisii*, *Chemosphere*, 13, 213–9.

Faqi, A.S., Schweinfurth, H., and Chahoud, I. (1997) Determination of the no-effect dose of bis(tri-*n*-butyltin)oxide (TBTO) for maternal toxicity and teratogenicity in mice, *Congenital Anomalies*, 37, 251–8.

Faqi, A.S., Schweinfurth, H., and Chahoud, I. (2001) Developmental toxicity of an octyltin stabilizer in NMRI mice, *Reproductive Toxicology*, 15, 117–22.

Farr, C.H., Reinisch, K., Holson, J.F., and Neubert, D. (2001) Potential teratogenicity of di-*n*-butyltin dichloride and other dibutyltin compounds, *Teratogenesis, Carcinogenesis, and Mutagenesis*, 21, 405–15.

Fent, K. (1996) Ecotoxicology of organotin compounds, *Critical Reviews in Toxicology*, 26, 1–117.

Fent, K. and Hunn, J. (1991) Phenyltins in water, sediment, and biota of freshwater marinas, *Environmental Science and Technology*, 25, 956–63.

Fent, K. and Meier, W. (1992) Tributyltin-induced effects on early life stages of minnows *Phoxinus phoxinus*, *Archives of Environmental Contamination and Toxicology*, 22, 428–31.

Fent, K. and Meier, W. (1994) Effects of triphenyltin on fish early life stages, *Archives of Environmental Contamination and Toxicology*, 27, 224–31.

Féral, C. and LeGall, S. (1983) The influence of a pollutant factor (tributyltin) on the neuroendocrine mechanism responsible for the occurrence of a penis in the female of *Ocenebra erinacea*, in *Molluscan neuro-endocrinology*, Lever, J. and Boer, H., Eds., Amsterdam: North Holland Publishing Co., 173–5.

Fioroni, P., Oehlmann, J., and Stroben, E. (1991) The pseudohermaphroditism of prosobranchs; morphological aspects, *Zoologischer Anzeiger*, 226, 1–26.

Fish, R.H., Kimmel, E.C., and Casida, J.E. (1976) Bioorganotin chemistry: reactions of tributyltin derivatives with a cytochrome P-450 dependent monooxygenese enzyme system, *Journal of Organometallic Chemistry*, 118, 41–54.

Gaines, T.B. and Kimbrough, R.D. (1968) Toxicity of fentin hydroxide to rats, *Toxicology and Applied Pharmacology*, 12, 397–403.

Gårdlund, A.T., Archer, T., Danielsson, B., Fredriksson, A., Lindqvist, N.G., Lindstrom, H., and Luthman, J. (1991) Effects of prenatal exposure to tributyltin and trihexyltin on behaviour in rats, *Neurotoxicology and Teratology*, 13, 99–105.

Giavini, E., Prati, M., and Vismara, C. (1980) Effects of triphenyltin acetate on pregnancy in the rat, *Bulletin of Environmental Contamination and Toxicology*, 24, 936–9.

Gibbs, P.E. and Bryan, G.W. (1986) Reproductive failure in populations of the dog-welk, *Nucella lapillus*, caused by imposex induced by tributyltin from antifouling paints, *Journal of the Marine Biological Association of the United Kingdom*, 66, 767–77.

Gibbs, P.E., Bryan, G.W., Pascoe, P.L., and Burt, G.R. (1987) The use of the dog-whelk, *Nucella lapillus*, as an indicator of tributyltin (TBT) contamination, *Journal of the Marine Biological Association of the United Kingdom*, 67, 507–23.

Gibbs, P.E., Pascoe, P.L., and Burt, G.R. (1988) Sex change in the female dog-whelk, *Nucella lapillus*, induced by tributyltin from antifouling paints, *Journal of the Marine Biological Association of the United Kingdom*, 68, 715–31.

Harazono, A. and Ema, M. (2000) Suppression of decidual cell response induced by tributyltin chloride in pseudopregnant rats: as a cause of early embryonic loss, *Archives of Toxicology*, 74, 632–7.

Harazono, A. and Ema, M. (2003) Suppression of decidual cell response induced by dibutyltin dichloride in pseudopregnant rats: As a cause of early embryonic loss, *Reproductive Toxicology*, 17, 393–9

Harazono, A., Ema, M., and Kawashima, K. (1998a) Evaluation of malnutrition as a cause of tributyltin-induced pregnancy failure in rats, *Bulletin of Environmental Contamination and Toxicology*, 61, 224–30.

Harazono, A., Ema, M., and Kawashima, K. (1998b) Evaluation of early embryonic loss induced by tributyltin chloride in rats: Phase- and dose-dependent antifertility effects, *Archives of Environmental Contamination and Toxicology*, 34, 94–9.

Harazono, A., Ema, M., and Ogawa, Y. (1996) Pre-implantation embryonic loss induced by tributyltin chloride in rats, *Toxicology Letters*, 89, 185–90.

Haubruge, E., Petit, F., and Gage, M.J. (2000) Reduced sperm counts in guppies (*Poecilia reticulata*) following exposure to low levels of tributyltin and bisphenol A, *Proceedings of the Royal Society of London. Series B. Biological Sciences*, 267, 2333–7.

Hawkins, L.E. and Hutchinson, S. (1990) Physiological and morphogenetic effects of monophenyltin trichloride on *Ocenebra erinacea* (L.), *Functional Ecology*, 4, 449–54.

Holm, G., Norrgren, L., and Linden, O. (1991) Reprodictive and histophathological effects of long-term experiemental exposure to bis(tributyltin) oxide (TBTO) on the three-spined stickeback, *Gasterosteus aculeatus* Linnaeus, *Journal of Fish Biology*, 38, 373–86.

Horiguchi, T., Shiraishi, H., Shibata, Y., Morita, M., and Shimizu, M. (1996) Imposex and organotin contamination in gastropods after the regulation of organotin use in Japan, *Abstract Book of SETAC 17th Annual Meeting*, 177.

Horiguchi, T., Shiraishi, H., Shimizu, M., and Morita, M. (1997a) Imposex in sea snails, caused by organotin (tributyltin and triphenyltin) pollution in Japan: a survey, *Applied Organometallic Chemistry*, 11, 451–5.

Horiguchi, T., Shiraishi, H., Shimizu, M., and Morita, M. (1997b) Effects of triphenyltin chloride and five other organotin compounds on the development of imposex in the rock shell thais clavigera, *Environmental Pollution*, 95, 85–91.

Horiguchi, T., Kojima, M., Kaya, M., Matsuo, T., Shiraishi, H., Morita, M., and Adachi, Y. (2002) Tributyltin and triphenyltin induce spermatogenesis in ovary of female abalone, Haliotis gigantea, *Marine Environmental Research*, 54, 679–84.

International Programme on Chemical Safety (IPCS) (1999a) *Concise International Chemical Assessment Document, No. 14 Tributyltin Oxide*, IPCS, Geneva, World Health Organization.

International Programme on Chemical Safety (1999b) *Concise International Chemical Assessment Document, No. 13 Triphenyltin Compounds*, Geneva, World Health Organization.

Ishizaka, T., Suzuki, T., and Saito, Y. (1989) Metabolism of dibutyltin dichloride in male rats, *Journal of Agricultural and Food Chemistry*, 37, 1096–101.

Itami, T., Ema, M., Amano, H., Murai, T., and Kawasaki, H. (1990) Teratogenic evaluation of tributyltin chloride in rats following oral exposure, *Drug and Chemical Toxicology*, 13, 283–95.

Iwai, H., Wada, O., and Arakawa, Y. (1981) Determination of tri-, di-, and monobutyltin and inorganic tin in biological materials and some aspects of their metabolism in rats, *Journal of Analytical Toxicology*, 5, 300–6.

Japan Environment Agency (1998) *Strategic Programs on Environmental Endocrine Disruptors '98*, Tokyo, Environmental Health Department.

Kamrin, M.A., Carney, E.W., Chou, K., Cummings, A., Dostal, L.A., Harris, C., Henck, J.W., Loch-Caruso, R., and Miller, R.K. (1994) Female reproductive and developmental toxicology: overview and current approaches, *Toxicology Letters*, 74, 99–119.

Kannan, K., Tanabe, S., and Tatsukawa, R. (1995) Phenyltin residues in horseshoe crabs, *Tachypleus tridentatus* from Japanese coastal waters, *Chemosphere*, 30, 925–32.

Kannan, K., Corsolini, S., Focardi, S., Tanabe, S., and Tatsukawa, R. (1996) Accumulation pattern of butyltin compounds in dolphin, tuna, and shark collected from Italian coastal waters, *Archives of Environmental Contamination and Toxicology*, 31, 19–23.

Karrer, D., Baroncelli, S., and Turillazzi, P.G. (1995) Oral bis(tri-*n*-butyltin) oxide in pregnant mice. II. Alterations in hematological parameters, *Journal of Toxicology and Environmental Health*, 46, 369–77.

Kenaga, E.E. (1965) Triphenyl tin compounds as insect reproduction inhibitors, *Journal of Economic Entomology*, 58, 4–8,

Kime, D.E., Huyskens, G., McAllister, B.G., Ruranguwa, E., Skorkowski, G., and Ollevia, F. (2001) Tributyltin disrupts the vertebrate reproductive system at muliple sites, *Abstracts 11th Annual Meeting of SETAC Europe*, Madrid, May 6–10, 195.

Kimmel, E.C., Fish, R.H., and Casida, J.E. (1977) Bioorganotin chemistry. Metabolism of organotin compounds in microsomal monooxygenase system and in mammals, *Journal of Agricultural and Food Chemistry*, 25, 1–9.

Krowke, R., Bluth, U., and Neubert, D. (1986) In vitro studies on the embryotoxic potential of (bis[tri-*n*-butyltin])oxide in a limb bud organ culture system, *Archives of Toxicology*, 58, 125–9.

Kumasaka, K., Miyazawa, M., Fujimaki, T., Tao, H., Ramaswamy, B.R., Nakazawa, H., Makino, T., and Satoh, S. (2002) Toxicity of the tributyltin compound on the testis in premature mice, *Journal of Reproduction and Development*, 48, 591–7.

Lau, M.M. (1991) Tributyltin antifoulings: a threat to the Hong Kong marine environment, *Archives of Environmental Contamination and Toxicology*, 20, 299–304.

Laughlin, R.B., Jr., French, W., and Guard, H.E. (1986) Accumulation of bis(tributyltin) oxide by the marine mussel *Mytilus edulis*, *Environmental Science and Technology*, 20, 884–90.

Lehotzky, K., Szeberenyi, J.M., Gonda, Z., Horkay, F., and Kiss, A. (1982) Effects of prenatal triphenyl-tin exposure on the development of behavior and conditioned learning in rat pups, *Neurobehavioral Toxicology and Teratology*, 4, 247–50.

Maguire, R.J. (1991) Aquatic environmental aspects of non-pesticidal organotin compounds, *Water Pollution Research Journal of Canada*, 26, 243–360.

Manning, C.S., Lytle, T.F., Walker, W.W., and Lytle, J.S. (1999) Life-cycle toxicity of bis(tributyltin) oxide to the sheepshead minnow (*Cyprinodon variegatus*), *Archives of Environmental Contamination and Toxicology*, 37, 258–66.

Miyake, K., Misawa, T., Aikawa, H., Yoshida, T., and Shigeta, S. (1989) The effects of prenatal trimethyltin exposure on development and learning in the rat, *Japanese Journal of Industrial Health*, 31, 363–71 (in Japanese).

Miyake, K., Misawa, T., and Shigeta, S. (1990) Toxicity of bis (tri-*n*-butyltin) oxide on learning and development in the rat, *Nippon Eiseigaku Zasshi*, 45, 926–34 (in Japanese).

Miyake, K., Misawa, T., and Shigeta, S. (1991) The effects of prenatal triphenyltin exposure on learning and development in the rat, *Nippon Eiseigaku Zasshi*, 46, 769–76 (in Japanese).

Newton, D.W. and Hays, R.L. (1968) Histological studies of ovaries in rats treated with hydroxyurea, triphenyltin acetate, and triphenyltin chloride, *Journal of Economic Entomology*, 61, 1668–9.

Nirmala, K., Oshima, Y., Lee, R., Imada, N., Honjo, T., and Kobayashi, K (1999) Transgenerational toxicity of tributyltin and its combined effects with polychlorinated biphenyls on reproductive processes in Japanese medaka (*Oryzias latipes*), *Environmental Toxicology and Chemistry*, 18, 717–21.

Noda, T. (2001) Maternal and fetal toxicity of dimetyltin in rats, *Journal of Health Science*, 47, 544–51.

Noda, T., Morita, S., and Baba, A. (1993) Teratogenic effects of various di-*n*-butyltins with different anions and butyl(3-hydroxybutyl)tin dilaurate in rats, *Toxicology*, 85, 149–60.

Noda, T., Morita, S., and Baba, A. (1994) Enhanced teratogenic activity of di-*n*-butyltin diacetate by carbon tetrachloride pretreatment in rats, *Food and Chemical Toxicology*, 32, 321–7.

Noda, T., Morita, S., Shimizu, M., Yamamoto, T., and Yamada, A. (1988) Safety evaluation of chemicals for use in house-hold products (VIII)—teratological studies on dibutyltin diacetate in rats, *Annual Report of Osaka City Institute of Public Health and Environ Sciences*, 50, 66–75 (in Japanese).

Noda, T., Morita, S., Yamano, T., Shimizu, M., and Yamada, A. (1991a) Effects of triphenyltin acetate on pregnancy in rats by oral administration, *Toxicology Letters*, 56, 207–12.

Noda, T., Morita, S., Yamano, T., Shimizu, M., Nakamura, T., Saitoh, M., and Yamada, A. (1991b) Teratogenicity study of tri-*n*-butyltin acetate in rats by oral administration, *Toxicology Letters*, 55, 109–15.

Noda, T., Yamano, T., Shimizu, M., Saitoh, M., Nakamura, T., Yamada, A., and Morita, S. (1992a) Comparative teratogenicity of di-*n*-butyltin diacetate with *n*-butyltin trichloride in rats, *Archives of Environmental Contamination and Toxicology*, 23, 216–22.

Noda, T., Nakamura, T., Shimizu, M., Yamano, T., and Morita, S. (1992b) Critical gestational day of teratogenesis by di-*n*-butyltin diacetate in rats, *Bulletin of Environmental Contamination and Toxicology*, 49, 715–22.

Noda, T., Yamano, T., and Shimizu, M. (2001) Effects of maternal age on teratogenicity of di-*n*-butyltin diacetate in rats, *Toxicology*, 167, 181–9.

Noland, E.A., Taylor, D.H., and Bull, R.J. (1982) Monomethyl-and trimethyltin compounds induce learning deficiencies in young rats, *Neurobehavioral Toxicology and Teratology*, 4, 539–44.

Oberdörster, E. and McClellan-Green, P. (2002) Mechanisms of imposex induction in the mud snail, *Ilyanassa obsoleta*: TBT as a neurotoxin and aromatase inhibitor, *Marine Environmental Research*, 54, 715–8.

Oehlmann, J., Stroben, E., and Fioroni, P. (1991) The morphlogical expression of imposex in *Nucella lapillus* (Linnaeus) (Gastropoda: Muricidae), *Journal of Molluscan Studies*, 57, 375–90.

Oehlmann, J., Stroben, E., Fioroni, P., and Markert, B. (1996) Tributyltin (TBT) effects on *Ocinebrina aciculata* (Gastropoda: Muricidae): imposex development, sterilization, sex change and population decline, *The Science of the Total Environment*, 188, 205–23.

Ogata, R., Omura, M., Shimasaki, Y., Kubo, K., Oshima, Y., Aou, S., and Inoue, N. (2001) Two-generation reproductive toxicity study of tributyltin chloride in female rats, *Journal of Toxicology and Environmental Health A*, 63, 127–44.

Ohhira, S. and Matsui, H. (1993a) Gas chromatographic determination of inorganic tin in rat urine after a single oral administration of stannous chloride and mono-, di-, and triphenyltin chloride, *Journal of Chromatography*, 622, 173–8.

Ohhira, S. and Matsui, H. (1993b) Metabolism of diphenyltin compound in rat liver after a single oral administration of diphenyltin dichloride, *Journal of Agricultural and Food Chemistry*, 41, 607–9.

Omura, M., Ogata, R., Kubo, K., Shimasaki, Y., Aou, S., Oshima, Y., Tanaka, A., Hirata, M., Makita, Y., and Inoue, N. (2001) Two-generation reproductive toxicity study of tributyltin chloride in male rats, *Toxicological Sciences*, 64, 224–32.

Pate, B.D. and Hays, R.L. (1968) Histological studies of testes in rats treated with certain insect chemosterilants, *Journal of Economic Entomology*, 61, 32–4.

Paule, M.G., Reuhl, K., Chen, J.J., Ali, S.F., and Slikker, W., Jr. (1986) Developmental toxicology of trimethyltin in the rat, *Toxicology and Applied Pharmacology*, 84, 412–7.

Piver, WT. (1973) Organotin compounds: industrial applications and biological investigation, *Environmental Health Perspectives*, 4, 61–79.

Quevauviller, P.H., Bruchet, A., and Donard, O.F.X. (1991) Leaching of organotin compounds from poly(vinyl chloride) (PVC) material, *Applied Organometallic Chemistry*, 5, 125–9.

Sanderson, J.T., Boerma, J., Lansbergen, G.W.A., and van den Berg, M. (2002) Induction and inhibition of aromatase (CYP19) activity by various classes of pesticides in H295R human adrenocortical carcinoma cells, *Toxicology and Applied Pharmacology*, 182, 44–54.

Sasaki, K., Ishizaka, T., Suzuki, T., and Saito, Y. (1988) Determination of tri-*n*-butyltin and di-*n*-butyltin compounds in fish by gas chromatography with flame photometric detection, *Journal of the Association of Official Analytical Chemists*, 71, 360–3.

Scadding, S.R. (1990) Effects of tributyltin oxide on the skeletal structures of developing and regenerating limbs of the axolotl larvae, *Ambystoma mexicanum*, *Bulletin of Environmental Contamination and Toxicology*, 45, 574–81.

Schardein, J. (2000) Hormones and hormonal antagonists, in *Chemically Induced Birth Defects*, 3rd ed., revised and expanded, Marcel Dekker, New York.

Short, J.W. and Thrower, F.P. (1986) Accumulation of butyltins in muscle tissue of chinook salmon reared in sea pens treated with tri-*n*-butyltin, *Marine Pollution Bulletin*, 17, 542–5.

Smith, B.S. (1981a). Male characteristics on female mud snails caused by antifouling paints, *Journal of Applied Toxicology*, 1, 22–5.

Smith, B.S. (1981b). Tributyltin compounds induce male characteristics on female mud snails *Nassarius oboletus = Ilyanasa oboleta*, *Journal of Applied Toxicology*, 1, 141–4.

Snoeij, N.J., Penninks, A.H., and Seinen, W. (1987) Biological activity of organotin compounds—an overview, *Environmental Research*, 44, 335–53.

Snow, R.L. and Hays, R.L. (1983) Phasic distribution of seminiferous tubules in rats treated with triphenyltin compounds, *Bulletin of Environmental Contamination and Toxicology*, 31, 658–65.

Spencer, F. and Sing, L.T. (1982) Reproductive responses to rotenone during decidualized pseudogestation and gestation in rats, *Bulletin of Environmental Contamination and Toxicology*, 28, 360–8.

Spooner, N., Gibbs, P.E., Bryan, G.W., and Goad, L.J. (1991) The effect of tributyltin upon steroid titers in the female dogwhelk, *Nucella lapillus*, and the development of imposex, *Marine Environmental Research*, 32, 37–49.

Strmac, M. and Braunbeck, T. (1999) Effects of triphenyltin acetate on survival, hatching success, and liver ultrastructure of early life stages of zebrafish (*Danio rerio*), *Ecotoxicology and Environmental Safety*, 44, 25–39.

Stroben, E., Oehlmann, J., and Bettin, C. (1991) TBT-induced imposex and role of steroids in marine snails, in *Proceedings Tenth World Meeting of the Organotin Environmental Programme Association*, Berlin, September, 68–73.

Suzuki, T., Matsuda, R., and Saito, Y. (1992) Molecular species of tri-*n*-butyltin compounds in marine products, *Journal of Agricultural and Food Chemistry*, 40, 1437–43.

Takahashi, T., Araki, A., Nomura, Y., Koga M., and Arizono, K. (2000) The occurrence of dual-gender imposex in Japanese freshwater crab, *Journal of Health Science*, 46, 376–9.

Toyoda, M., Sakai, H., Kobayashi, Y., Komatsu, M., Hoshino Y., Horie, M., Saeki, M., Hasegawa, Y., Tsuji, M., Kojima, M., Toyomura, K., Kumano, M., and Tanimura, A. (2000) Daily dietary intake of tributyltin, dibutyltin, triphenyltin and diphenyltin compounds according to a total diet study in Japanese population, *Shokuhin Eiseigaku Zasshi*, 41, 280–6 (in Japanese).

Tryphonas, H., Cooke, G.M., Caldwell, D., Bondy, G., Parenteau, M., Hayward, S., and Pulido, O. (2004) Oral (gavage), in utero and postnatal exposure of Sprague-Dawley rats to low doses of tributyltin chloride. Part II: Effects on the immune system, *Food and Chemical Toxicology*, 42, 221–235.

Tsuda, T., Inoue, T., Kojima, M., and Aoki, S. (1995) Daily intakes of tributyltin and triphenyltin comounds from meals, *Journal of AOAC International*, 78, 941–3.

Tsuda, T., Nakanishi, H., Aoki, S., and Takebayashi, J. (1987) Bioconcentration and metabolism of phenyltin chlorides in carp, *Water Research*, 21, 949–53.

Ueno, S., Susa, N., Furukawa, Y., Komatsu, Y., Koyama, S., and Suzuki, T. (1999) Butyltin and phenyltin compounds in some marine fishery products on the Japanese market, *Archives of Environmental Health*, 54, 20–5.

Waldock, M.J. and Thain, J.E. (1983) Shell thickening in Crassostrea gigas: organotin antifouling or sediment induced? *Marine Pollution Bulletin*, 14, 411–5.

Weis, J.S. and Kim, K. (1988) Tributyltin is a teratogen in producing deformities in limbs of the fiddler crab, *Uca pugilator*, *Archives of Environmental Contamination and Toxicology*, 17, 583–7.

Winek, C.L., Marks, M.J., Jr., Shanor, S.P., and Davis, E.R. (1978) Acute and subacute toxicology and safety evaluation of triphenyl tin hydroxide (Vancide KS), *Clinical Toxicology*, 13, 281–96.

Winship, K.A. (1988) Toxicity of tin and its compounds, *Adverse Drug Reactions and Acute Poisoning Reviews*, 7, 19–38.

World Health Organization (1980) Tin and Organotin Compounds: A Preliminary Review. *Environmental Health Criteria 15*, World Health Organization, Geneva.

World Health Organization (1992) Fentin, in *Pesticide Residues in Food 1991: Evaluations Part II Toxicology*, World Health Organization, Geneva. Online: http://www.inchem.org/documents/jmpr/jmpmono/v91pr11.htm (accessed 16 June 2004).

Yamabe, Y., Hoshino, A., Imura, N., Suzuki, T., and Himeno, S. (2000) Enhancement of androgen-dependent transcription and cell proliferation by tributyltin and triphenyltin in human prostate cancer cells, *Toxicology and Applied Pharmacology*, 169, 177–84.

Yonemoto, J., Shiraishi, H., and Soma, Y. (1993) In vitro assessment of teratogenic potential of organotin compounds using rat embryo limb bud cell cultures, *Toxicology Letters*, 66, 183–91.

ORIGINAL ARTICLE

Elevated susceptibility of newborn as compared with young rats to 2-*tert*-butylphenol and 2,4-di-*tert*-butylphenol toxicity

Mutsuko Hirata-Koizumi[1], Masao Hamamura[2], Hiromi Furukawa[2], Naemi Fukuda[3], Yoshihiko Ito[3], Yumi Wako[4], Kotaro Yamashita[4], Mika Takahashi[1], Eiichi Kamata[1], Makoto Ema[1], and Ryuichi Hasegawa[1]

[1]National Institute of Health Sciences, Setagaya-ku, Tokyo; [2]Panapharm Laboratories, Uto-shi, Kumamoto; [3]Research Institute for Animal Science in Biochemistry and Toxicology, Sagamihara-shi, Kanagawa; and [4]Kashima Laboratory, Mitsubishi Chemical Safety Institute Ltd, Ibaraki, Japan

ABSTRACT **In order to determine the susceptibility of newborn rats to 2-*tert*-butylphenol (2TBP) and 2,4-di-*tert*-butylphenol (DTBP) toxicity, studies were conducted with oral administration from postnatal days (PND) 4 to 21 and the findings were compared with results for young rats exposed from 5 or 6 weeks of age for 28 days. In the newborn rats, specific effects on physical and sexual development and reflex ontogeny were not observed. While there were no clear differences in toxicological profiles between newborn and young rats, the no-observed-adverse-effect levels (NOAELs) differed markedly. For 2TBP, clinical signs such as ataxic gait, decrease in locomotor activity and effects on liver, such as increase in organ weight, were observed and the NOAELs were concluded to be 20 and 100 mg/kg/day in newborn and young rats, respectively. Based on hepatic and renal toxicity (histopathological changes and increase in organ weight with blood biochemical changes), the respective NOAELs for DTBP were concluded to be 5 and 20 mg/kg/day. Therefore, the susceptibility of newborn rats to 2TBP and DTBP was found to be 4–5 times higher than that of young rats.**

Key Words: 2, 4-di-*tert*-butylphenol, 2-*tert*-butylphenol, susceptibility of newborn rats

INTRODUCTION

Protection of humans against disease and injury caused by chemicals in the environment is the ultimate goal of risk assessment and risk management (Landrigan *et al.* 2004). However, the focus has long been solely on adult exposure and toxicity and the fetus via maternal transfer, with little consideration given to early childhood. In the past decade, stimulated especially by the 1993 US National Research Council (NRC) report *Pesticides in the Diets of Infants and Children* (NAS 1993), recognition that special consideration is required for children in risk assessment has grown. The NRC report noted that 'children are not little adults', because of their unique patterns of exposures to environmental hazards and their particular vulnerability.

For the susceptibility of children to environmental chemicals, the early postnatal period (the suckling period) is of particular note. During this period, the infant could be exposed to various chemicals not only through mothers' milk, but also directly, by having chemical-contaminated baby food, mouthing toys or household materials, and so on; however, current risk assessment gives no consideration to toxic effects resulting from direct exposure to chemicals. An approach that adequately takes into account the susceptibility of infancy is urgently required. However, because there is no standard testing protocol intended for direct exposure of preweaning animals (newborn animals) to chemicals, and toxicity studies using newborn animals are complicated by practical difficulties regarding grouping, direct dosing, and general and functional observation, there is only limited information on susceptibility of the newborn at the present.

We therefore have established a new protocol for repeated dose toxicity studies using newborn rats (newborn rat studies) (Koizumi *et al.* 2001) for systematic application. Results have been compared with those of 28-day repeated dose toxicity studies using young rats (young rat studies) to provide a basis of analyzing susceptibility. Since young rat studies are routinely conducted as one of a battery of minimum toxicity tests and data are stored for many chemicals, comparative analyzes should provide important information for considering effects of direct exposure to chemicals during the suckling period.

We have already reported analytical results for eight chemicals (4-nitrophenol, 2,4-dinitrophenol, 3-aminophenol, 3-methylphenol, 1,3-dibromopropane, 1,1,2,2-tetrabromoethane, 2,4,6-trinitrophenol, and tetrabromobisphenol A) (Koizumi *et al.* 2001, 2002, 2003; Fukuda *et al.* 2004; Takahashi *et al.* 2004; Hirata-Koizumi *et al.* 2005). The susceptibility of newborn rats to the toxicity of the first four agents was four times higher than that of their young counterparts at a maximum. For 1,3-dibromopropane and 1,1,2,2-tetrabromoethane, while the doses causing clear toxicity were lower in newborn rats, doses at which toxic signs began to appear were paradoxically higher in the newborn case. These six chemicals had no impact on development in the newborn period and showed similar toxicity profiles in both age groups. For the other two chemicals, there were marked differences in toxicity profile between the newborn and young rats. Especially, in the case of tetrabromobisphenol A, a specific rather than enhanced renal toxicity was observed in newborn case.

In the present investigation, two *tert*-butylphenols, 2-*tert*-butylphenol (2TBP), and 2,4-di-*tert*-butylphenol (DTBP), were chosen for comparative toxicity analysis. 2TBP has been used in the production of agricultural chemicals, aroma chemicals, and resins (New Chemical Index 2001), and DTBP in the production of antioxidants and ultraviolet absorbers (Chemical Products' Handbook 2004). For either chemical, there is no available toxicity information on human. Regarding toxicity to experimental animals, results from young rat studies of both chemicals are available in

Correspondence: Mutsuko Hirata-Koizumi, BSc, Division of Medicinal Safety Science, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan. Email: mkoizumi@nihs.go.jp

Received June 17, 2005; revised and accepted September 2, 2005.

Toxicity Testing Reports of Environmental Chemicals of the Japanese government (MHLW 2001a, 2001b), but no other data have been reported regarding repeated dose toxicity. Since the young rats were only evaluated for toxicity profiles and no-observed-effect levels, we re-evaluated the results for a more practical evaluation index, the no-observed-adverse-effect level (NOAEL), which could serve as the basis for determining tolerable daily intake (TDI) or acceptable daily intake (ADI) for risk assessment, and conducted comparative analyzes with newborn rats.

MATERIALS

2-*tert*-Butylphenol (2TBP, CAS no. 88-18-6, purity: 99.97%) and 2,4-di-*tert*-butylphenol (DTBP, CAS no. 96-76-4, purity: 99.67%), obtained from Dainippon Ink and Chemicals, Incorporated (Tokyo, Japan), were dissolved in olive oil and corn oil, respectively. The test solutions were prepared once a week as stability for eight days had been confirmed. All other reagents used in this study were specific purity grade.

METHODS

All studies were performed under Good Laboratory Practice conditions and in accordance with 'Guidance for Animal Care and Use' of Panapharm Laboratories Co., Ltd, Research Institute for Animal Science in Biochemistry and Toxicology, or Mitsubishi Chemical Safety Institute Ltd.

Animals

In the newborn rat studies of 2TBP and DTBP, pregnant SPF Sprague-Dawley rats [Crj:CD(SD)IGS] were purchased at gestation days 13–15 from Charles River Japan Inc. (Yokohama, Japan), and allowed to deliver spontaneously. All newborn were separated from dams at postnatal day (PND) 3 (the date of birth was defined as PND 0), and pooled according to sex. At the same time, 12 foster mothers were selected among dams, based on the nursing condition. Each foster mother suckled four male and four female newborn, assigned to each of the four dose groups, including the controls, up to weaning on PND 21 (termination of dosing). After weaning, the animals of the recovery-maintenance group (see Study Design) were individually maintained for nine weeks.

In the young rat studies, 4–5 week-old males and females of the same strain were obtained from the same supplier as for the newborn rat studies, and used at ages of 5–6 weeks after acclimation.

All animals were maintained in an environmentally controlled room at 20–26°C with a relative humidity of 40–70%, a ventilation rate of more than ten times per hour, and a 12:12 h light/dark cycle. They were allowed free access to a basal diet (MF: Oriental Yeast Co. Ltd, Tokyo, Japan, or LABO MR Stock: Nihon Nosan Kogyo Inc., Yokohama, Japan) and water (sterile tap water or well water treated with sodium hypochlorite) throughout.

Study design

1. 18-day repeated dose toxicity study in newborn rats (newborn rat study)

Newborn rats (12/sex/dose) were administered the test substances by gastric intubation on PNDs 4–21. On PND 22, six males and six females in each treated group were sacrificed for autopsy (the scheduled-sacrifice group). The remaining animals in all groups (6 rats/sex/dose) were maintained for nine weeks without chemical treatment and then sacrificed at 12 weeks of age (the recovery-maintenance group).

Based on the results of dose-finding studies conducted prior to the main study, the dose, which would show clear toxicity, was selected as the top dose, that without potentially toxic effects as the lowest dose, and the medium dose was set between them. In the dose-finding study for 2TBP (oral administration from PNDs 4–21), some clinical signs and suppressed body weight gain were observed at 200 mg/kg and an increase in relative liver weight at 60 mg/kg and more. For DBTP (oral administration from PNDs 4–17), all of the four males and four females died at 500 mg/kg, and the death of one of the four males, an increase in serum total cholesterol and phospholipid, and increase in relative liver weight were noted in the 100 mg/kg group. Therefore, the doses were set at 0, 20, 60, or 200 mg/kg/day for 2TBP and at 0, 5, 40, or 300 mg/kg/day for DTBP.

During the study, the rats' general condition was observed at least once a day (details of clinical signs noted in this study are described in 'Glossary of terms for toxicity testing' [NIHS 1994]). Body weight and food consumption (only the recovery-maintenance period) was examined once or more a week. As developmental parameters, fur appearance, incisor eruption, pinna detachment and eye opening were assessed for physical development, and testes descent or preputial separation and vaginal opening for sexual development (OECD 2004). In addition, reflex ontogeny, such as visual placing reflex, and surface and mid-air righting reflexes, were also examined (Adams 1986; Jensh & Brent 1988). Urinalysis (color, occult blood, pH, protein, glucose, ketone bodies, bilirubin, urobilinogen, sediment, specific gravity, and volume of the urine) was conducted in the last week of the recovery-maintenance period.

At PNDs 22 and 85, blood was collected from the abdominal aorta under ether anesthesia (for 2TBP) or from the postcaval vein under pentobarbital sodium anesthesia (for DTBP) after overnight starvation for the scheduled-sacrifice and recovery-maintenance groups, respectively. One portion was treated with EDTA-2K and examined for hematological parameters, such as the red blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, white blood cell count, platelet count, reticulocyte count and differential leukocyte count. In the recovery-maintenance group, part of the blood was treated with 3.8% sodium citrate, and blood clotting parameters such as prothrombin time (PT) and activated partial thromboplastin time (APTT) were examined. Serum from the remaining portions of blood for both the scheduled-sacrifice and recovery-maintenance groups were analyzed for blood biochemistry (total protein, albumin, albumin-globulin ratio [A/G ratio], glucose, total cholesterol, triglycerides, phospholipid, total bilirubin, urea nitrogen [BUN], creatinine, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, alkaline phosphatase, γ-glutamyl transpeptidase [γ-GTP], calcium, inorganic phosphorus, sodium, potassium, and chlorine). Following collection of blood, all animals were sacrificed by exsanguination, and all organs and tissues were macroscopically examined. Then, the brain, pituitary gland, thymus, thyroids, heart, lungs, liver, spleen, kidneys, adrenals, testes, epididymides, and ovaries were removed and weighed. Histopathological examination was conducted for the control and the highest dose groups. The above-listed organs were fixed in 10% buffered formalin-phosphate (following Bouin's fixation for testes and epididymides), and paraffin sections were routinely prepared and stained with Hematoxylin-Eosin for microscopy. For other groups, organs with macroscopically abnormal findings or in which chemical-related effects were evident on microscopic examination for the highest dose group, were similarly investigated.