Introduction

Organotin compounds are chemicals widely used in agriculture and industry (Piver 1973, World Health Organization 1980). Tetrasubstituted organotin compounds are mainly used as intermediates in the preparation of other organotin compounds. Trisubstituted organotin compounds have biocidal properties and are used in agriculture as fungicides and acaricides, as rodent repellents, and mulluscicides, and are widely used as antifoulants in ship paints and underwater coatings. Especially, triphenyltins (TPTs) and tributyltins (TBTs) have been used extensively in antifouling products such as algaecides and mulluscicides. Disubstituted organotin compounds are commercially the most important derivatives, and are mainly used in the plastics industry, particularly as heat and light stabilizers for polyvinyl chloride (PVC) plastics to prevent degradation of the polymer during melting and forming of the resin into its final products, as catalysts in the production of polyurethane foams, and as vulcanizing agents for silicone rubbers. Monosubstituted organotin compounds are used as stabilizers in PVC films. Widespread use of organotin compounds has caused increasing amounts to be released into the environment. The most important nonpesticidal route of entry for organotin compounds into the environment is through leaching of organotin-stabilized PVC in water (Quevauviller et al. 1991), and the use in antifouling agents, resulting in the introduction of organotin into the aquatic environment (Maguire 1991). Data are available regarding the detection of butyltin and phenyltin compounds in aquatic marine organisms (Sasaki et al. 1988, Fent and Hunn 1991, Lau 1991) and marine products (Suzuki et al. 1992, Belfroid et al. 2000, Tsuda et al. 1995, Ueno et al. 1999, Toyoda et al. 2000). Food chain bioamplification of butyltin in oysters (Waldock and Thain 1983), mud crabs (Evans and Laughlin 1984), marine mussels (Laughlin et al. 1986), Chinook salmon (Short and Thrower 1986), and dolphin, tuna, and shark (Kannan et al. 1996), and of phenyltin in carp (Tsuda et al. 1987) and horseshoe crab (Kannan et al. 1995) has been reported. These indicate that organotin compounds accumulate in the food chain and are bioconcentrated, and that humans can be exposed to organotin compounds via seafood. The World Health Organization (WHO) reported in 1980 that the estimated mean total daily intake of tin by humans ranged from 200 μg to 17 mg. Recently, Tsuda et al. (1995) reported that the daily intakes in Shiga prefecture in Japan were 0.7 to 5.4 μg in 1991 and 0.7 to 1.3 μg in 1992 for TPT and 4.7 to 6.9 μg in 1991 and 2.2 to 6.7 μg in 1992 for TBT. Toyoda et al. (2000) also showed that the daily intakes in Japanese consumers, based on analysis with the 1998 total diet samples, were 0.09 μg for TPT, 0 μg for diphenyltin (DPT), 1.7 μg for TBT, and 0.45 μg for dibutyltin (DBT). These values are lower than the acceptable daily intake for TPT according to the JMPR (Joint Meetings of the FAO [Food and Agriculture Organization] and World Health Organization

Panel of Experts on Pesticides Residues), 25 μg (World Health Organization 1992), and the guidance value for oral exposure to tributyltin oxide (TBTO), 18 μg (International Programme on Chemical Safety 1999a). Thus, the levels of organotin compounds in seafood are not considered to be sufficiently high to affect human health (Tsuda et al. 1995, Ueno et al. 1999). However, Belfroid et al. (2000) noted that more research on residual TBT levels in seafood is needed before a definitive conclusion on possible health risks can be drawn.

In recent years, adverse effects of environmental chemicals on the reproductive success of wildlife populations have been reported (Colborn et al. 1993). These phenomena may result from interference with the endocrine system. Disturbances of hormonal regulation during pre- and postnatal development may produce deleterious effects on reproduction and development. TPT and TBT are suspected to be endocrine disruptors (Japan Environment Agency 1998). TBT and TPT are known to have strong effects on the development of imposex (imposition of male sex characteristics on females) in the rock shell (Horiguchi et al. 1996, 1997a), and this condition may bring about reproductive failure and a consequent population decline.

Although the toxicity of organotins has been extensively reviewed (World Health Organization 1980, Snoeij et al. 1987, Winship 1988, Boyer 1989, International Programme on Chemical Safety 1999a, b), the reproductive and developmental toxicity of these compounds is not well understood. In this chapter, we summarize the findings of the studies on reproductive and developmental effects of organotin compounds.

Effects on Aquatic Organisms

Imposex on Gastropods

TBT causes reproductive toxic effects in marine gastropods, which were represented by some masculinizing effects including *imposex* or *pseudohermaphodi(ti)sm*. The imposition of male sex organs (a penis and vas deferens) on female mud snails (*Nassarius obsoletus*) was found in near harbors, and the degree of penis development and frequency of imposex were positively correlated to the seawater TBT concentration (Smith 1981a, b). Imposex has been induced experimentally by treatment with 4.5 to 5.5 μg/L of TBT compounds for 60 days. In field studies in southeastern England, imposex has been reported in declining populations of the common dogwhelk (Bryan et al. 1986, 1987, 1989, Gibbs and Bryan 1986, Davies et al. 1987, Gibbs et al. 1987).

Imposex has not just occurred at a regional level, but worldwide on a global scale. Imposex in dogwhelk was not only reported in England, but in Scotland, the Netherlands, and the coastline of the North Sea. Imposex in other whelk species occurred in Canada, West Africa, New Zealand, Australia, Malaysia, Singapole, Indonesia, and Japan (Fent 1996, Horiguchi et al. 1996). Imposex among prosobranchs is known to occur in around 70 species of 50 genera, although some species are less susceptible to TBT compounds (Fioroni et al. 1991, Fent 1996).

TPT also induced imposex in *Thais clavigera* at the same potency as TBT (Horiguchi et al. 1997a). Although, in *Nucwlla lapillus*, TPT did not induce imposex, tripropyltin (TPrT) had a small effect on the development of imposex (Bryan et al. 1988). DBT and monobutyltin (MBT) did not induce imposex in the gastropod species examined. Three trisubstitution compounds (TBT, TPT, TPrT) and monophenyltin (MPT) easily induced imposex in some species, among the eight organotins, i.e., MBT, DBT, TBT, tetrabutyltin (TeBT), MPT, DPT, TPT, and TPrT. (Bryan et al. 1988, Hawkins and Hutchinson 1990, Horiguchi et al. 1997b).

The early studies in the 1980s reached some common conclusions, which are described below (Eisler 2000). Imposex correlated with the body burden of tributyl- and dibutyltin, but not with the tissue concentration of arsenic, cadmium, copper, lead, silver, or zinc. Forty-one percent of females had male characteristics, when the body burden reached to 1.65 mg Sn/kg of dry soft parts, by exposing with 0.02 μg Sn/L for 120 days. Imposex in immature females is caused above the concentration of around 1 ng/L (Sn) in seawater. At higher concentrations of TBT, the oviduct had been blocked, resulting in sterilization. Declining dogwhelk populations could be caused by aborting capsules, sterility, and premature death, which were characterized by a moderate to high degree of imposex, fewer female functions, fewer juveniles, and scarcity of laid egg capsules.

There is also a great variety of gradations of imposex in different species. The intensity is characterized by a classification system, which distinguishes six stages with a few different types, mainly based on a Vas Deferens Sequence (VDS) index (Oehlmann et al. 1991). Imposex development occurred in three variations: (1) a small penis without penis duct, (2) a short distal vas deferens section, or (3) a short proximal vas deferens section (stage 1). At stages 2 and 3 the male sex characteristics of each type are developed continuously. Stage 4 is characterized by a penis with penis duct and a complete vas deferens, and represents the last stage of fertility. The reproductive failure or sterility is induced in later stages. At stage 5 the vagina is replaced with a small prostate gland, the vagina opening is blocked by vas deferens tissue, or the incompletion of the pallial oviduct closure occurs. Abortive egg capsules fill the lumen and vestibulum of the capsule gland and evoke an intense swelling of the gland at stage 6 (Bettin et al. 1996). High TBT exposure in the early stages of life induced gametogenesis or sex changes characterized by a suppression of oogenesis and commencement of spermatogenesis in females (Gibbs et al. 1988, Fioroni et al. 1991, Oehlmann et al. 1991, 1996, Horiguchi et al. 2002). It was thought that the initial phases of imposex corresponding to VDS stages 1 and 2 may be reversible; however, advanced phases of imposex and sterilization with gross morphological changes corresponding to VDS stage 5 and 6 would be irreversible (Fent 1996).

Although many morphological aspects of pseudohermaphodi(ti)sm caused by TBT have been investigated, the biochemical mechanism has been indistinct. It is known that a neurotropic hormone called the penis morphogenic factor (PMF) develops male normal differentiation in mollusks (Féral

and LeGall, 1983). Co-localization of TBT with PMF in ganglia suggested that PMF release through TBT's neurotropic action induced masculinization in females (Bryan et al. 1989). Other studies indicated increased testosterone levels detected in female dogwhelk exposed to TBT, and that testosterone injection without TBT induced penis development in females (Spooner et al. 1991, Stroben et al. 1991). The later studies suggested that TBT disturbed the P-450–dependent aromatization of androgens to estrogen, and a nonsteroidal specific aromatase inhibitor–induced imposex similar to TBT (Bettin et al. 1996). However, the PMF has not been well characterized, and the role of vertebrate sex steroids is not known in gastropods to date. A recent study proposed that the combination of changes in the neuropeptide (APGWamide), which is considered to be a PMF in mud snails, and steroid hormones would lead to imposex induction at extremely low doses of TBT (Oberdörster and McClellan-Green 2002).

Effects on Fish

TBT or TPT exposure in early life stages induces altered embryonic development, and delayed or inhibited hatching in fish. Exposure of TBT or TBT to minnow eggs and larvae at concentrations of 0.2 to 18 μg/L in the water in which the fish lived induced dose-dependent morphological effects on larvae. Marked body axis deformations were observed at more than about 4 μg/L exposure, and incomplete hatching occurred at similar concentrations in 10 to 30% of larvae. At 15.9 μg/L of TPT exposure, hatching was delayed and the hatching rate was reduced significantly (Fent and Meier 1992, 1994). Developmental defects, such as skeletal abnormality and retarded yolk sac resorption, occurred in zebrafish larvae at more than 25 μg/L of triphenyltin acetate (TPTA) exposure, and hatching delay was found at more than 0.5 μg/L (Strmac and Braunbeck 1999). These developmental effects in fish were caused not only by organotin compounds, but also by a variety of contaminants (i.e., heavy metals, chlorinated hydrocarbons, altered pH), suggesting that such alteration would be classified as a nonspecific reaction to organic toxicants (Fent 1996, Strmac and Braunbeck 1999).

Some reproductive effects (i.e., reduced fecundity and sperm counts) in fish were reported. Reproductive success of three-spine stickleback with TBT exposure were examined over a 7-month period; no effects were detected in relation to fecundity, number of hatched fry, or frequency of malformed fry. However, no changes were found in the gonad somatic index (GSI; ovary weight ratio to total body weight); by the 7-month TBT treatment (2 μg/L) despite increasing GSI in controls, which suggested a lack of maturation of egg tissue and consequently a potential reduced fecundity (Holm et al. 1991). In sheepshead minnows, reduction in both total and percent viable eggs was found at more than 1.3 μg/L of TBT exposure, although the reductions were not statistically significant (Manning et al. 1999). TBT exposure to Japanese medaka at 1 mg/kg body weight caused a reduction of the spawning frequency (Nirmala et al. 1999). Additionally, environmentally relevant concentrations of

TBT induced significantly decreased sperm counts in guppies (11.2 to 22.3 ng/L for 21 days), and decreased sperm motility at concentrations less than 1 μg/L (Haubruge et al. 2000, Kime et al. 2001).

Effects on Other Organisms

Despite a great number of studies on imposex in snails and a comparable number of toxicity reports on fish, there is little information on development and reproductive effects on other species by organotin compounds. It was reported that imposex has not only been found in gastropods, but also been induced in Japanese freshwater crabs by TBT (Takahashi et al. 2000). In crabs, imposex has also occurred in males, which is characterized by dual-gender imposex (either a female genital opening or a single ovary occurred in males). Malformations during limb regeneration occurred in fiddler crabs (Weis and Kim 1988) and in axolotl, induced by TBT (Scadding 1990).

Summary of Effects on Aquatic Organisms

TBT or TPT causes the imposition of male sex organs (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, and advanced phases of imposex and sterilization with gross morphological changes are irreversible. The biochemical mechanism studies suggested that the induction of either neurotropic hormone or androgen titer would lead to imposex at an extremely low dose of TBT. Also, TBT or TPT exposure in the early life stages of fish causes altered embryonic development, impaired morphological development, and delayed or inhibited hatching, and reduces fecundity and sperm counts. 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 an important issue in aquatic ecosystems.

Effects on Experimental Animals

Reproductive Toxicity of Phenyltin Compounds

Reproductive Toxicity of Triphenyltins

TPTs have been reported to be insect chemosterilants (Kenaga 1965). Reproductive studies on TPTs are presented in Table 3.1. Several reports on male reproductive toxicity have been published. Male Sharman rats were given a diet containing triphenyltin hydroxide (TPTH) at 50, 100, or 200 ppm and then mated with untreated females repeatedly five times (Gaines and Kimbrough 1968). Reduced fertility, such as decreases in the total number of matings, total number of litters born alive, and ratio of number of litters to number of matings, accompanied by a marked reduction in food consumption

Table 3.1 Reproductive Toxicity of Phenyltin Compounds

Compounds	Animals	Dose	Days of Administration	Route	Reproductive and Developmental Effects	Author(s)
TPTH	Sharman rat	100–200 ppm	64–238 days	Diet	Decreased no. of matings Decreased no. of litters born alive Decreased ratio of no. of litters to no. of matings	Gains and Kimbrough (1968)
TPTA or TPTCl	Holtzman rat	20 mg/kg	19 days	Diet	Decreased tesicular size Change in testicular morphology	Pate and Hays (1968)
TPTA or TPTCl	Holtzman rat	20 mg/kg	20 days	Diet	Impairment of spermatogenic process	Snow and Hays (1983)
TPTA	ICR/Ha Swiss mouse	2.4–12 mg/kg	1 day	ip	No dominant lethal effect	Epstein et al. (1972)
		6 mg/kg	5 days	Gavage	No dominant lethal effect	
TPTH		1.3–8.5 mg/kg	1 day	ip	No dominant lethal effect	
		11 mg/kg	5 days	Gavage	No dominant lethal effect	
TPTA or TPTCl	Holtzman rat	20 mg/kg	4–24 days	Diet	Decreased no. of mature follicles Increased incidence of atresia in early follicle growth Decreased no.of corpora lutea	Newton and Hays (1968)
TPTCl	Wistar rat	4.7–6.3 mg/kg	Days 0–3 of pregnancy	Gavage	Decreased pegnancy rate, decreased fetal wt.	Ema et al. (1997a)
		12.5–25 mg/kg	Days 4–6 of pregnancy	Gavage	Decreased pregnancy rate	
TPTCl	Wistar rat	4.7–6.3 mg/kg	Days 0–3 of pseudopregnancy	Gavage	Suppression of uterine decidualization	Ema et al. (1999a)
DPTCl	Wistar rat	16.5–24.8 mg/kg	Days 0–3 of pregnancy	Gavage	Decreased pregnancy rate, preimplatation loss, decreased fetal wt.	Ema et al. (1999b)
		33.3 mg/kg	Days 4–7 of pregnancy	Gavage	Effects as above, postimplantation loss	
DPTCl	Wistar rat	4.1–24.8 mg/kg	Days 0–3 of	Gavage	Suppression of uterine decidualization	Ema and Miyawaki (2002)

and weight gain, were observed at 100 or 200 ppm for 64 days. At these doses, food consumption later improved, and with it, fertility. Dietary exposure to triphenyltin acetate (TPTA) or triphenyltin chloride (TPTCl) at 20 mg/kg for 19 days produced marked effects on body weight, testicle size, and testicular structure in male Holtzman rats (Pate and Hays 1968). Microscopic examinations revealed degenerative changes, such as a decrease in the number of layers per tubule, a depletion of the more advanced cell forms from the tubules, and a closing of the tubule lumina. Effects were more pronounced in rats treated with TPTA. TPTA or TPTCl at 20 mg/kg in feed for 20 days was reported to cause an impairment of the spermatogenic process in male Holtzman rats; complete recovery of the spermatogenesis was observed after feeding a normal diet for 70 days (Snow and Hays 1983). No mutagenicity was detected in dominant lethal assay in which male ICR/Ha Swiss mice were given a single intraperitoneal injection of TPTA at 2.4 or 12 mg/kg or TPTH at 1.3 or 8.5 mg/kg, or given TPTA at 6mg/kg or TPTH at 11 mg/kg by gavage on 5 successive days and then mated with untreated females, and pregnancy outcome was determined on day 13 of pregnancy (Epstein et al. 1972).

Adverse effects on female reproductive toxicity were also reported. Dietary TPTA and TPTCl at 20 mg/kg for 4 days produced significant changes in the ovarian tissue, including a decreased number of mature follicles, an increased incidence of atresia in early follicle growth, and a pronounced decrease in the number of corpora lutea in female Holtzman rats (Newton and Hays 1968). These effects were regarded as a decrease in ovulation, and thus decreased fertility. The adverse effects of TPTCl on the initiation and maintenance of pregnancy were determined after administration to the mother during early pregnancy (Ema et al. 1997a). Following successful mating, female Wistar rats were given TPTCl by gavage on days 0 to 3 of pregnancy at 3.1, 4.7, or 6.3 mg/kg or on days 4 to 6 of pregnancy at 6.3, 12.5, or 25.0 mg/kg, and pregnancy outcome was determined on day 20 of pregnancy. TPTCl totally prevented implantation in a dose-dependent manner. The pregnancy rate was decreased after administration of TPTCl on days 0 to 3 at 4.7 and 6.3 mg/kg and on days 4 to 6 at 12.5 and 25.0 mg/kg. Preimplantation loss was increased after administration of TPTCl on days 0 to 3 at 4.7 mg/kg and higher. In females having implantations, the numbers of implantations and live fetuses, and the incidences of pre- and postimplantation embryonic loss in the TPTCl-treated groups were comparable to the controls. These results indicate that TPTCl during early pregnancy causes failure in implantation and has greater antiimplantation effects when administered during the preimplantation period than the periimplantation period.

The function of the uterine endometrium is one of the principle factors in embryonic survival. Uterine decidualization is required for normal implantation, placentation, and therefore normal gestation in rats. The uterine growth induced by endometrial trauma in pseudopregnant animals mimics the decidual response of the pregnant uterus that occurs after embryo implantation (Cummings 1990, Kamrin et al. 1994). The decidual cell

response (DCR) is a model for maternal physiological events that are associated with implantation (Cummings 1990). This technique can distinguish between the adverse effects of chemical compounds in the maternal and fetal compartments, and has been used to evaluate the reproductive toxicity of chemical compounds (Spencer and Sing 1982, Bui et al. 1986, Cummings 1990, Kamrin et al. 1994, Ema et al. 1998). The effects of TPTCl on the reproductive capability of the uterus, as a cause of implantation failure, were evaluated using pseudopregnant rats (Ema et al. 1999a). Female Wistar rats were given TPTCl by gastric intubation at 3.1, 4.7, or 6.3 mg/kg on days 0 to 3 of pseudopregnancy. Between 11:00 and 13:00 on day 4 of pseudopregnancy, induction of DCR was performed via midventral laparotomy under ether anesthesia, and experimental decidualization was initiated by scratching the antimesometrial surface of the endometrium with a bent needle. The uterine weight on day 9 of pseudopregnancy served as an index of the uterine decidualization (De Feo 1963). A decrease in the uterine weight, which indicates suppression of the uterine decidualization, was detected at 4.7 and 6.3 mg/kg. TPTCl at 4.7 and 6.3 mg/kg also produced a decrease in the serum progesterone levels in female rats on day 4 and on day 9 of pseudopregnancy. These doses caused an increase in implantation failure (preimplantation embryonic loss) in female rats given TPTCl on days 0 to 3 of pregnancy (Ema et al. 1997a). These results suggest that TPTCl causes the suppression of uterine decidualization correlated with the reduction in serum progesterone levels, and these participate in the induction of implantation failure due to TPTCl. Protective effects of progesterone against suppression of uterine decidualization and implantation failure induced by TPTCl were examined (Ema and Miyawaki 2001). The hormonal regimen, consisting of progesterone and estorone supported decidual development in ovariectomized rats given TPTCl. The pregnancy rate and number of implantations in groups given TPTCl at 4.7 or 6.3 mg/kg in combination with progesterone were higher than those in the groups given TPTCl alone. These results indicate that the TPTCl-induced suppression of uterine decidualization is mediated, at least partially, by ovarian hormones, and that progesterone protects against TPTCl-induced implantation failure.

Reproductive Toxicity of Diphenyltin Compounds

Oral TPT is metabolized to DPT, MPT, and further to inorganic tin in rats (Kimmel et al. 1977, Ohhira and Matsui 1993 a, b). Reproductive toxicity studies on DPTs are also published (Table 3.1). The adverse effects of diphenyltin dichloride (DPTCl) on the initiation and maintenance of pregnancy, and the role of DPT in the implantation failure of TPT were evaluated. Following successful mating, DPTCl was given to Wistar rats by gavage on days 0 to 3 of pregnancy at 4.1, 8.3, 16.5, or 24.8 mg/kg or on days 4 to 7 of pregnancy at 8.3, 16.5, 24.8, or 33.0 mg/kg (Ema et al. 1999b). The pregnancy rate was decreased after administration of DPTCl on days 0 to 3 at 24.8 mg/kg and on days 4 to 7 at 33.0 mg/kg. The incidence of preimplantation loss was increased at 16.5 mg (equivalent to 48 μmol)/kg on days 0 to 3. In

females having implantations, the incidences of pre- and postimplantation embryonic loss in the groups given DPTCl on days 0-3 were comparable to the controls. The incidence of postimplantation embryonic loss was increased after administration of DPTCl on days 4 to 7 at 33.0 mg/kg. These results indicate that DPTCl during early pregnancy causes implantation failure, and that DPTCl has greater effects on reproduction when administered during the preimplantation period rather than the periimplantation period. Following administration on days 0 to 3 of pregnancy, the increased incidence of preimplantation embryonic loss was induced by TPTCl, a parent compound of DPTCl, at 4.7 mg (equivalent to12 μmol)/kg and higher (Ema et al. 1997a), or DPTCl at 16.5 mg (equivalent to 48 μmol)/kg. If, on a mole-equivalent basis, a metabolite is as, or more, effective than the parent compound, this is consistent with the view that the metabolite is the proximate toxicant or at least an intermediate to the proximate toxicant. Thus, it seems unlikely that only DPTCl and/or its further metabolites can be considered the agents responsible for the antiimplantation effects of TPTCl. As for the metabolism of phenyltin, however, Ohhira and Matsui (1993b) showed that TPT compound was formed in the liver of the DPTCl-treated rat by metabolism of DPTCl, and suggested that part of the administered DPT compound has some harmful effect as the TPT compound in rats, and this must be taken into consideration in toxicological research on DPT. Further studies are needed to clarify the difference in the reproductive toxicity induced by TPT and DPT, and to identify the proximate or ultimate toxicant of phenyltins. The effects of DPTCl on the reproductive capability of the uterus were evaluated in pseudopregnant rats according to the procedure described above. Female Wistar rats were given DPTCl by gastric intubation on days 0 to 3 of pseudopregnancy at 4.1, 8.3, 16.5, or 24.8 mg/kg (Ema and Miyawaki 2002). Suppression of uterine decidualization was observed at 16.5 mg/kg and higher. A decrease in the serum progesterone levels in pseudopregnant rats was also found on day 4 and on day 9 of pseudopregnancy at 16.5 mg/kg and higher. These doses induced an increase in preimplantation embryonic loss in female rats given DPTCl on days 0 to 3 of pregnancy (Ema et al. 1999b). No changes in serum estradiol levels in pseudopregnant rats were noted. These results suggest that DPTCl causes the suppression of uterine decidualization correlated with the reduction in serum progesterone levels. These are responsible for the DPTCl-induced implantation failures. The hormonal regimen consisting of progesterone and estrone supported decidual development in ovariectomized rats given DPTCl (Ema and Miyawaki 2002). The pregnancy rate and number of implantations in groups given DPTCl at 16.5 or 24.3 mg/kg in combination with progesterone were higher than those in the groups given DPTCl alone. These results show that the DPTCl-induced suppression of uterine decidualization is mediated, at least partially, by ovarian hormones, and that progesterone protects against the DPTCl-induced implantation failure.

Summary of Reproductive Toxicity of Phenyltin Compounds

TPTs caused a decrease in male fertility due to degenerative changes in testicular tissue, which were associated with a marked decrease in food consumption. Complete recovery of fertility and impairment of the spermatogenesis was noted following withdrawal of treatment. Female reproductive failure induced by TPTs is more prominent. The harmful effects of TPTs on the ovaries were present after 5 days of treatment, before any significant effects on body weight gain. TPTCl during early pregnancy caused implantation failure at relatively low doses, and TPTCl had greater antiimplantation effects when administered during the preimplantation period. The implantation failure due to TPTCl might be mediated by suppression of uterine decidualization and correlated with the reduction in serum progesterone levels. Implantation failure and suppression of uterine decidualization accompanied with decreased levels of serum progesterone were also observed in rats given DPT, a major metabolite of TPT.

Developmental Toxicity of Phenyltin Compounds

Table 3.2 presents the developmental toxicity studies on phenyltin compounds given to female animals during pregnancy. Several reports on the adverse effects of phenyltins on development of offspring following maternal exposure have been published. Female SD rats were given TPTA by gavage at 5, 10, or 15 mg/kg on days 6 to 15 of pregnancy (Giavini et al. 1980). TPTA caused a decrease in maternal body weight gain at 10 mg/kg and higher, an increase in postimplantation loss at 15 mg/kg, and a reduction of fetal ossification at 5 mg/kg and higher. Teratogenic effects of TPTA were not found even at doses resulting in clear maternal toxicity. Depression of maternal body weight gain and food intake at 9.0 mg/kg and higher, and increase in postimplantation embryonic loss and decrease in fetal ossification at 9.0 mg/kg and higher, but not teratogenic effects, were observed in Wistar rats after administration of TPTA at 1.5, 3.0, 6.0, 9.0, or 12.0 mg/kg by gavage on days 7 to 17 of pregnancy (Noda et al. 1991a). Behavioral effects of prenatal exposure to TPTA were reported. A transient increase in spontaneous locomotor activity and increased mortality during the lactation period were found in pups of CFY rats given TPTA by gavage at 6 mg/kg on days 6 to 14 of pregnancy (Lehotzky et al. 1982). In this study, maternal rats were free of any overt signs of toxicity. Disruptions of learning acquisition, as evidenced by low avoidance rate in the Sidman avoidance test, and prolonged swimming time to the goal, and an increased number of errors in a reversed test in the water E-maze, were observed in postnatal offspring of Tokai High Avoiders (THA) rats received TPTA by gavage on days 6 to 20 of pregnancy at 4 or 8 mg/kg (Miyake et al. 1991). Maternal deaths and decreased weight gain were found at 8 mg/kg, no maternal toxicity was observed at 4 mg/kg, and no malformed offspring appeared in any group.

Table 3.2 Developmental Toxicity of Phenyltin Compounds

Compounds	Animals	Dose	Days of Administration	Route	Reproductive and Developmental Effects	Author(s)
TPTA	Wistar rat	5–15 mg/kg	Days 6–15 of pregnancy	Gavage	Postimplantation loss, delayed ossification	Giavini et al. (1980)
TPTA	Wistar rat	9–12 mg/kg	Days 7–17 of pregnancy	Gavage	Postimplantation loss, delayed ossification	Noda et al. (1991a)
TPTA	CFY rat	6 mg/kg	Days 6–14 of pregnancy	Gavage	Postnatal death, transient increase in spontaneous locomotor activity	Lehotzky et al. (1982)
TPTA	THA rat	4–8 mg/kg	Days 6–20 of pregnancy	Gavage	Disruption of learning acquisition	Miyake et al. (1991)
TPTH	SD rat	20 mg/kg	Days 1–7 of pregnancy	Gavage	Decreased pregnancy rate	Winek et al. (1978)
		15 mg/kg	Days 8–14 of pregnancy	Gavage	Posimplantation loss, decreased fetal wt.	
		15 mg/kg	Days 14–12 of pregnancy	Gavage	Effects as above	
TPTH	SD rat	13 mg/kg	Days 6–15 of pregnancy	Gavage	Postimplantation loss	Chernoff et al. (1990)
TPTCl	Wistar rat	6.3–12.5 mg/kg	Days 7–9 of pregnancy	Gavage	Postimplantation loss	Ema et al. (1999c)
		9.4–12.5 mg/kg	Days 10–12 or 13–15 of pregnancy	Gavage	Postimplantation loss, decreased fetal wt.	

Winek et al. (1978) noted that (1) SD rats given Vancide KS (TPTH) by gavage at 20 mg/kg on days 1 to 7 of pregnancy did not produce pups nor did they exhibit any resorption sites, (2) that only two of the six rats given TPTH at 15 mg/kg on days 14 to 20 of pregnancy produced viable pups, and (3) that four of the six rats given TPTH at 15 mg/kg on days 14 to 20 of pregnancy produced viable pups. Their study was conducted on only a small number of animals and the design of the study was not described in detail. Chernoff et al. (1990) observed a significant decrease in maternal body weight gain and an increase in postimplantation embryonic loss, but not fetal malformations, after administration of TPTH by gavage on days 6 to 15 of pregnancy at 13 mg/kg in SD rats. They stated that there was a correlation between maternal toxicity and fetal weight and/or lethality.

Following administration of TPTCl by gavage to pregnant Wistar rats, the maternal body weight gain and food consumption were decreased at 3.1 mg/kg and higher on days 7 to 9 of pregnancy, and at 6.3 mg/kg and higher on days 10 to 12 or on days 13 to 15 of pregnancy (Ema et al. 1999c). An increase in the incidence of postimplantation embryonic loss was found in pregnant rats given TPTCl at 6.3 mg/kg and higher on days 7 to 9, and at 9.4 mg/kg and higher on days 10 to 12 and on days 13 to 15. A decreased fetal weight was observed at 12.5 mg/kg on days 10 to 12 and at 9.4 mg/kg and higher on days 13 to 15. No increase in the incidence of fetuses with malformations was detected after administration of TPTCl regardless of the days of administration. These results indicate that TPTCl is developmentally toxic and that TPTCl has greater embryolethal effects when administered during earlier than later stages of organogenesis.

Summary of Developmental Toxicity of Phenyltin Compounds

Maternal exposure to TPTs caused embryonic/fetal death and suppression of fetal growth at maternal toxic doses. TPTs may cause reduction of fetal ossification at doses that are nontoxic to the mother. TPTs did not induce an increased number of fetal malformations even at doses producing 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.

Reproductive Toxicity of Butyltin Compounds

Table 3.3 shows reproductive toxicity studies on butyltins. A decrease in the sperm head count and vacuolization of Sertoli cells were found in ICR mice gavaged with TBTO at 2 and 10 mg/kg twice a week for 4 weeks (Kumasaka et al. 2002). The male reproductive toxicity of tributyltin chloride (TBTCl) was reported in a two-generation reproductive toxicity study using Wistar rats (Omura et al. 2001). F0 females were fed a diet containing TBTCl at 5, 25, or 125 ppm (estimated to be 0.4, 2.0, or 10.0 mg/kg) from day 0 of pregnancy to the day of weaning of F1 rats. Feeding of TBTCl was continued

Table 3.3 Reproductive Toxicity of Butyltin Compounds

Compounds	Animals	Dose	Days of Administration	Route	Reproductive and Developmental Effects	Author(s)
TBTO	ICR mouse	2–10 mg/kg	4 weeks (twice a week)	Gavage	Decreased sperm head count, vacuolization of Sertoli cells	Kumasaka et al. (2002)
TBTCl	Wistar rat	25–125 ppm	2 generations	Diet	Decreased wt of testis and epididymis, decreased spermatid count, decreased levels of serum estradiol, decreased wt. gain of male offspring	Omura et al. (2001)
TBTCl	Wistar rat	5–125 ppm	2 generations	Diet	Decreased birth index, decreased no. and wt. of pups, delayed vaginal opening, increased female AGD, decreased wt. gain of female offspring	Ogata et al. (2001)
TBTCl	Wistar rat	12.2–16.3 mg/kg	Days 0–7 of pregnancy	Gavage	Decreased pregnancy rate, decreased fetal wt.	Harazono et al. (1996)
TBTCl	Wistar rat	16.3–32.5 mg/kg	Days 0–3 of pregnancy	Gavage	Decreased pregnancy rate, decreased fetal wt.	Harazono et al. (1998b)
		16.3–65.1 mg/kg	Days 4–7 of pregnancy	Gavage	Effects as above, postimplantation loss	
TBTCl	Wistar rat	16.3–32.5 mg/kg	Days 0–3 of pseudopregnancy	Gavage	Suppression of uterine decidualization, decreased levels of serum progesterone, increased levels of serum estradiol	Harazono and Ema (2000)
		16.3–65.1 mg/kg	Days 4–7 of pseudopregnancy	Gavage	Suppression of uterine decidualization, decreased levels of serum progesterone	
DBTCl	Wistar rat	7.6–15.2 mg/kg	Days 0–3 or 4–7 of pregnancy	Gavage	Decreased pregnancy rate, pre- and postimplantation loss, decreased fetal wt.	Ema and Harazono (2000)
DBTCl	Wistar rat	7.6–15.2 mg/kg	Days 0–3 or 4–7 of pseudopregnancy	Gavage	Suppression of uterine decidualization, decreased levels of serum progesterone	Harazono and Ema (2003)
MBTCl	Wistar rat	903 mg/kg	Days 0–3 or 4–7 of pregnancy	Gavage	Decreased fetal wt.	Ema and Harazono (2001)

throughout the premating, mating, gestation, and lactation periods, for two generations. TBTCl affected the male reproductive system. The effects of TBTCl in the F2 generation were greater than those in the F1 generation. Body weight gain was consistently suppressed at 125 ppm in F1 and F2 males. The weights of the testis and epididymis were decreased and homogenization-resistant spermatid and sperm counts were reduced mainly at 125 ppm. Ventral prostate weight and spermatid count were decreased at 125 ppm in F1 males and at 25 and 125 ppm in F2 males. The serum 17-estradiol levels were decreased at 125 ppm in F1 and F2 males, but serum levels of luteinizing hormone and testosterone were not decreased. Omura et al. (2001) note that these changes corresponded with those caused by aromatase inhibitor and suggest that TBTCl might cause a weak aromatase inhibition in male rats.

Regarding female reproductive toxicity, the results with female rats in the above-mentioned two-generation reproduction study were reported by Ogata et al. (2001). Decreases in body weight gain during pregnancy, total number and average body weight of pups, and live birth index were observed at 125 ppm in F0 and F1 dams. Body weight gain was consistently suppressed at 125 ppm in F1 and F2 females. Delayed vaginal opening and impaired estrous cyclicity were found at 125 ppm in F1 and F2 females. The normalized anogenital distance (AGD) was increased at 5 ppm and higher in F1 females on postnatal day 1, and at 125 ppm in F1 and F2 females on postnatal days (PNDs) 1 and 4. These results show that a whole-life exposure to TBTCl affects the sexual development and reproductive function of female rats. They noted that TBTCl-induced increase in female AGD seems to suggest that it may exert a masculinizing (androgenic) effect on female pups.

Female Wistar rats were administered TBTCl by gavage at 8.1, 12.2, or 16.3 mg/kg on days 0 to 7 of pregnancy, and the adverse effects of TBTCl on implantation and maintenance of pregnancy were determined (Hrazono et al. 1996). Decreases in maternal body weight gain at 12.2 mg/kg and higher, and food consumption at 8.1 mg/kg and higher, were found. Implantation failure was found at doses that also produced maternal toxicity. The pregnancy rate was significantly decreased at 12.2 mg/kg and higher. In females having implantations, the numbers of corpora lutea, implantations, and postimplantation loss, were comparable across all groups. To examine whether pregnancy failure was the result of the effects of TBTCl or maternal malnutrition from reduced food consumption, a pair-feeding study was performed. The results show that the pregnancy failure observed in the TBTCl-treated group is due to the effects of TBTCl, not to the maternal malnutrition from reduced food consumption (Harazono et al. 1998a). The adverse effects of TBTCl on implantation and maintenance of pregnancy after administration during the pre- or periimplantation period were evaluated. Female Wistar rats were given TBTCl by gastric intubation on days 0 to 3 of pregnancy at 4.1, 8.1, 16.3, or 32.5 mg/kg, or on days 4 to 7 of pregnancy at 8.1, 16.3, 32.5, or 65.1 mg/kg, and pregnancy outcome was determined on day 20 of pregnancy (Harazono et al. 1998b). TBTCl on days

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. TBTCl 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 TBTCl varies with gestational stage at the time of maternal exposure, and that TBTCl during the preimplantation period causes implantation failure, while TBTCl during the periimplantation period adversely affects the viability of implanted embryos.

Female Wistar rats were given TBTCl by gavage on days 0 to 3 or on days 4 to 7 of pseudopregnancy, and the effects of TBTCl on the uterus, as a cause of implantation failure, were evaluated according to the same procedures described above. After administration of TBTCl 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 TBTCl 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 TBTCl 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 TBTCl.

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 (DBTCl) 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 DBTCl 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 DBTCl 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 DBTCl 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 DBTCl, not to maternal malnutrition from reduced feed consumption, and the lowest dose of DBTCl inducing early embryonic loss was conservatively estimated at 7.6 mg (25 μmol)/kg. An increase in the incidence of implantation failure was observed after administration of TBTCl, the parent compound of DBTCl, at 16.3 mg (50 μmol)/kg and higher on days 0 to 3 and on days 4 to 7 of pregnancy,

respectively. The doses of DBTCl that caused early embryonic loss were lower than those of TBTCl (Harazono et al. 1998b). Thus, it is likely that DBTCl 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 DBTCl at doses that caused implantation failure (Harazono and Ema 2003), and administration of progesterone protected, at least in part, against the DBTCl-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 DBTCl. Administration of butyltin trichloride (MBTCl) 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 MBTCl and/or metabolites are actively involved in the early embryonic loss due to butyltins. The dose levels of DBTCl 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 DBTCl may suggest that DBTCl 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 DBTCl did not affect serum estradiol levels. Thus, the mechanisms of TBTCl and DBTCl adversely affecting ovarian function might be different. Further studies are needed to determine the effects of TBTCl and DBTCl 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 DBTCl, 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 DBTCl at doses that induced implantation failure. Administration of progesterone protected, at least in part, against the DBTCl-induced implantation failure. Administration of MBTCl 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 at. 1990, 1995). In the prenatal study, a decrease in maternal weight gain and fetal weight were found, along with high embryolethality, 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)
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)
TBTCl	Wistar rat	5–25 mg/kg	Days 7–15 of pregnancy	Gavage	Postimplantation loss, delayed ossification	Itami et al. (1990)
TBTCl	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)
		50–100 mg/kg	Days 10–12 of pregnancy	Gavage	Effects as above, cleft palate	
		25–100 mg/kg	Days 13–15 of pregnancy	Gavage	Decreased fetal wt., cleft palate	
TBTCl	Wistar rat	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	Ema et al. (1997b)
TBTCl	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)
		2.5–10 mg/kg	Days 8–19 of pregnancy		Decreased levels of serum thyroxine	
TBTCl	SD rat	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	Cooke et al. (2004)
TBTCl	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 aquisition 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., manibular 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	
DBTA	Wistar rat	15 mg/kg	Days 7–9 of pregnancy	Gavage	Effects as above	Noda et al. (1992b)
		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)