(1) 村部麻由、王淑一、美留町潤一、山内淳司、三部篤、<u>田上昭人</u> Embryonic Stem Cell test (EST 法)による薬物毒性評価 第 32 回日本小児臨床薬理学会、10 月 21 日〜22 日、2005、東京

(2) 村部麻由、美留町潤一、山内淳司、三 部 篤 、<u>田 上 昭 人</u> In Vitro Embryotoxicity Assays by Using Mouse Embryonic Stem Cells 第 79 回日本薬理学会年会、3月 8 日〜10 日、2006、横浜

G. 知的所有権の取得状況

1. 特許取得

なし

2. 実用新案登録

なし

3. その他

なし

研究成果の刊行に関する一覧表

書籍

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雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Ema M, Kimura E, Matsumoto M, Hirose A, Kamata E	Reproductive and developmenta toxicity screening test of basic rubber accelerator, 1,3-di-o-tolylguanidine, in rats.	Reproductive Toxicology	Epub ahead of print	–	2005
Hasegawa R, Hirata-Koizumi M, Takahashi M, Kamata E, Ema M.	Comparative susceptibility of newborn and young rats to six industrial chemicals.	Congenit Anom (Kyoto).	45	137-145	2005
Hirata-Koizumi M, Hamamura M, Furukawa H, Fukuda N, Ito Y, Wako Y, Yamashita K, Takahashi M, Kamata E, Ema M, Hasegawa R.	Elevated susceptibility of newborn as compared with young rats to 2-tert-butylphenol and 2,4-di-tert-butyl phenol toxicity.	Congenit Anom (Kyoto).	45	146-153	2005
Kuwagata M, Muneoka KT, Ogawa T, Takigawa M, Nagao T.	Locomotor hyperactivity following prenatal exposure to 5-bromo-2'-deoxyuridine: neurochemical and behavioral evidence of dopaminergic and serotonergic alterations.	Toxicol Lett.	152	63-71	2004
Ogawa T, Kuwagata M, Muneoka KT, Shioda S.	Neuropathological examination of fetal rat brain in the 5-bromo-2'-deoxyuridine-induced neurodevelopmental disorder model.	Congenit Anom (Kyoto).	45	14-20	2005
Muotri AR, Nakashima K, Toni N, Sandler VM, Gage FH.	Development of functional human embryonic stem cell-derived neurons in mouse brain.	Proc Natl Acad Sci U S A.	102	18644-8	2005

Hsieh J, Nakashima K, Kuwabara T, Mejia E, Gage FH.	Histone deacetylase inhibition-mediated neuronal differentiation of multipotent adult neural progenitor cells.	Proc Natl Acad Sci U S A.	101	16659-64	2004
Shimozaki K, Namihira M, Nakashima K, Taga T.	Stage- and site-specific DNA demethylation during neural cell development from embryonic stem cells.	J Neurochem.	93	432-9	2005
Kuwata H, Matsumoto M, Atarashi K, Morishita H, Hirotani T, Koga R, Takeda K.	IkappaBNS inhibits induction of a subset of Toll-like receptor-dependent genes and limits inflammation.	Immunity.	24	41-51	2006
Hirotani T, Lee PY, Kuwata H, Yamamoto M, Matsumoto M, Kawase I, Akira S, Takeda K.	The nuclear IkappaB protein IkappaBNS selectively inhibits lipopolysaccharide-induced IL-6 production in macrophages of the colonic lamina propria.	J Immunol.	174	365-3657	2005
Yamamoto M, Yamazaki S, Uematsu S, Sato S, Hemmi H, Hoshino K, Kaisho T, Kuwata H, Takeuchi O, Takeshige K, Saitoh T, Yamaoka S, Yamamoto N, Yamamoto S, Muta T, Takeda K, Akira S.	Regulation of Toll/IL-1-receptor-mediated gene expression by the inducible nuclear protein IkappaBzeta.	Nature.	430	218-222	2004
Miyagawa S, Katsu Y, Watanabe H, Iguchi T.	Estrogen-independent activation of erbBs signaling and estrogen receptor alpha in the mouse vagina exposed neonatally to diethylstilbestrol.	Oncogene.	23	340-349	2004
Watanabe H, Takahashi E, Kobayashi M, Goto M, Krust A, Chambon P, Iguchi T.	The estrogen-responsive adrenomedullin and receptor-modifying protein 3 gene identified by DNA microarray analysis are directly regulated by estrogen receptor.	J Mol Endocrinol.	36	81-89	2006
Takahashi Y, Kitajima S, Inoue T, Kanno J, Saga Y.	Differential contributions of Mesp1 and Mesp2 to the epithelialization and rostro-caudal patterning of somites.	Development.	132	787-96	2005

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chapter 3

Reproductive and Developmental Toxicity of Organotin Compounds

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Contents

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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 tribu-tyltins (TBTs) have been used extensively in antifouling products such as algaecides and mulluscicides. Disubstituted organotin compounds are com-mercially 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. Mono-substituted organotin compounds are used as stabilizers in PVC films. Wide-spread 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 *pseudohermaphrodi(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 (APGWa-mide), 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	2.4–12 mg/kg	1 day	ip	No dominant lethal effect	Epstein et al. (1972)
	Swiss mouse	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 12.5–25 mg/kg	Days 0–3 of pregnancy Days 4–6 of pregnancy	Gavage Gavage	Decreased pegnancy rate, decreased fetal wt. Decreased pregnancy rate	Ema et al. (1997a)
TPTCl	Wistar rat	4.7–6.3 mg/kg	Days 0–3 of pseudopregnancy	Gavage	Suppression of uterine deciduralization	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)
DPTCl	Wistar rat	33.3 mg/kg	Days 4–7 of pregnancy	Gavage	Effects as above, postimplantation loss	Ema and Miyawaki (2002)
DPTCl	Wistar rat	4.1–24.8 mg/kg	Days 0–3 of	Gavage	Suppression of uterine deciduralization	

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 T: TA 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

Metals, Fertility, and Reproductive Toxicity

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