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# Effects of *in utero* and lactational exposure to triphenyltin chloride on pregnancy outcome and postnatal development in rat offspring

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**Abstract**

The organotin compound (OTC) triphenyltin (TPT) is used extensively as a herbicide, pesticide and fungicide in agriculture as well as, together with tributyltin (TBT), in marine antifouling paints. We studied the effects of *in utero* exposure to 2 or 6 mg triphenyltinchloride (TPTCl)/kg b.w. on pregnancy outcome and postnatal development in rat offspring. Gravid Wistar rats were treated per gavage from gestational day 6 until the end of lactation. In the 6 mg TPTCl dose group gestational mortality in dams as well as an increased incidence of anticipated and delayed parturition was observed. Furthermore, treatment resulted in a significant increase in perinatal mortality, a decrease in lactational body weight gain as well as in delayed physical maturation of offspring. Similarily, exposure to 2 mg TPTCl/kg b.w. resulted in a significant increase in perinatal mortality and in delayed eye opening. Lactational body weight gain and other landmarks of physical maturation were unaffected in the low dose group. We conclude, that *in utero* exposure to TPTCl at the described dose levels severely affected pregnancy outcome and perinatal survival of offspring. These results were unexpected, as in two earlier studies with pubertal rats TPTCl at the same dose levels no signs of general toxicity were observed.


*Keywords:* Triphenyltin chloride; Developmental toxicity; Perinatal mortality; Endocrine disruption

## 1. Introduction

The organotin compound (OTC) triphenyltin (TPT) is used extensively as a herbicide, pesticide and fungicide in agriculture as well as, together with tributyltin (TBT), in marine antifouling paints. The main source of organotin intake for humans is contaminated fish and seafood. This is a result when OTCs leach from ship paint into the aquatic environment (Kannan and Falandysz, 1997; Takahashi et al., 1999; Kannan et al., 1999). Certain OTCs are known hormone disrupters in molluscs (Oehlmann et al., 1996) and have shown to inhibit a variety of enzymes responsible for the production

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of sex steroid hormones in higher male and female organisms (Doering et al., 2002; Lo et al., 2003). In higher species they accumulate in a variety of organs like liver, kidney and brain (Fait et al., 1994) where they are metabolized by specific mechanisms depending on their chemical structure and on species-specific differences. TPT is metabolized by dearylation to the dialkyltins diphenyltin and monophenyltin (for review see Appel, 2004) and it has been shown that it is accumulated to a lesser extent and metabolized faster in rats than in hamsters (Ohhira and Matsui, 1996). OTCs easily cross the placental as well as the blood–brain barrier (Hasan et al., 1984; Adeeko et al., 2003; Cooke et al., 2004) and have shown various effects on the immune (Snoeij et al., 1985, 1986; Vos et al.,1990; Tryphonas et al., 2004) and the reproductive system (Ema et al., 1999; Ogata et al., 2001; Omura et al., 2001; Grote et al., 2004, 2006) in experimental studies. Endocrine disrupters (Eds) are capable of activating or blocking hormone receptors, in particular estrogen or androgen receptors, and may interfere with synthesis and metabolism of steroid hormones. The toxicological mechanisms of OTCs have not yet been fully elucidated. An increase in androgen levels through inhibition of aromatase activity has been made responsible for the induction of imposex in molluscs and has also been observed *in vitro* (Heidrich et al., 2001; Cooke, 2002; Lo et al., 2003). Furthermore, OTCs have been reported to inhibit other key enzymes catalyzing steroid hormone synthesis, like 5α-reductase isoenzymes or 17β-hydroxysteroid dehydrogenase *in vitro* (Doering et al., 2002; Lo et al., 2003).

In a study by Ohno et al. (2005) it has been demonstrated that TPT inhibits 17β-hydroxysteroid dehydrogenase activity in testicular microsomes and testosterone production in Leydig cells of pigs. On the other hand, enhanced enzyme activity and expression of 17β-hydroxysteroid dehydrogenase in human choriocarcinoma cells possibly resulting in an increased biosynthesis of 17β-estradiol is reported by Nakanishi et al. (2006).

Due to these properties OTCs may have the potential to affect hormone-regulated developmental processes during pre- and postnatal development resulting in effects that may not be manifested until the offspring reaches maturity. The developing organism is particularly susceptible to the effects of endocrine disrupters (Gray et al., 1999) and the developmental vulnerability begins at conception and extends through gestation, parturition and the postnatal period up to adolescence. During development the organism undergoes many complex integrated processes and interference with those processes through, e.g. altered cell division, hormone activity or enzyme function can result in significant alterations in physiological developmental processes. The Endocrine Disrupters Screening and Testing Advisory Committee (EDSTAC) devised a rational process for assessing the hazards of environmental endocrine disrupters and recommends a two-tier system, which encompasses a battery of *in vitro* and *in vivo* biological assays. In an earlier study we used a modification of the Rodent 20-day pubertal female assay and the Rodent 20-day pubertal male assay to investigate the effects of TPTCl on pubertal rats (Grote et al., 2004, 2006). The data reported in the present publication are part of a comprehensive study, in which we aimed to further evaluate TPT and its effects on pregnancy outcome and postnatal development of rats and to investigate possible differences of chemical susceptibility/sensitivity of fetuses and neonates in comparison to pubertal rats which we studied earlier at the same dose levels.

## 2. Materials and methods

### 2.1. Animals and animal husbandry

Female Wistar rats (HsdCpb:WU) weighing 200 ± 15 g, were purchased from Harlan Winkelmann, Borchen, FRG. Animals were kept at the Institute of Clinical Pharmacology and Toxicology, Department of Toxicology, Benjamin Franklin Medical Center under specific-pathogen-free (SPF) conditions in climate-controlled rooms and were allowed to adapt to their environment for 2 weeks. The rats were mated daily for 3 h at a ratio of 2 females:1 male. The day of sperm detection in the vaginal smear was determined as day 0 of gestation. Gravid dams were kept individually in Type IV Macrolon® cages. Softwood granulate 8–15® (econ. Altromin, Lage, FRG) was used as bedding for the animals. The rats were housed at a constant light cycle (12 h of light, 12 h of darkness). Relative humidity was 50 ± 5% at a room temperature of 21 ± 1 °C. Animals received autoclaved commercial diet (Altromin®1324, Fa. Altromin Lage, FRG) and tap water ad libitum.

### 2.2. Treatment, period of treatment and endpoints investigated

Gravid dams were randomly assigned to the control group and the two dose groups and were treated daily from gestational day 6 through the end of lactation at dose levels of 2 or 6 mg TPT/kg b.w. Triphenyltin chloride (TPTCl) with a purity of ≥97.0% was purchased from Sigma–Aldrich Chemie GmbH, Steinheim, FRG, and dissolved in pharmaceutical peanut oil. The substance was administered per gavage at a volume of 5 ml/kg. The control group received pharmaceutical peanut oil only. Dose selection was based on results from our previous studies in male and female pubertal rats (Grote et al., 2004, 2006). The day of parturition as well as litter size, litter weight and number of live and stillborn pups on PND 1 were recorded.