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Effects of peripubertal exposure to triphenyltin on female sexual development of the rat

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Abstract

Triphenyltin (TPT) belongs to the group of organotin compounds which have been shown to affect reproduction in mammals. It is used as a fungicide and antifouling agent and the main source of human exposure is via food. We studied the effects of 2 or 6 mg TPT/kg bw on female sexual development using a modification of the Rodent 20-Day Thyroid/Pubertal Female Assay. Moreover, the effect of TPT before the onset of puberty was investigated. Beginning at postnatal day (PND) 23 female Wistar rats were treated per gavage until either PND 33 or the first estrus after PND 53. A delay in the completion of vaginal opening (VO) was observed in the 6 mg TPT group, while the 2 mg TPT group showed advanced VO. Significantly increased ovarian weights were observed in both treatment groups. Steroid hormone levels and ovarian aromatase activity were affected after exposure to 6 mg TPT/kg bw, while treatment with 2 mg TPT/kg bw resulted in minor changes of these endpoints. We conclude that peripubertal exposure to 6 mg TPT/kg bw, and to a lesser extent to 2 mg TPT/kg bw, affects female sexual development.

Keywords: Triphenyltin chloride; Puberty; Female; Reproduction; Aromatase; Endocrine disruption

1. Introduction

The xeno-androgen, triphenyltin (TPT), belongs to the broad group of organotin compounds (OTC) applied in industry and agriculture. They have been used extensively in antifouling paints for ships and as non-systemic fungicides for crop protection. They leach into aquatic systems resulting in bioconcentration and subsequent exposure of humans via fish and seafood (Tsuda et al., 1995; Kannan and Falandysz, 1997; Kannan et al., 1999; Takahashi et al., 1999). OTCs are endocrine disrupters and have been shown to induce imposex, which is characterized by the development of male sex characteristics in female snails (Gibbs et al., 1991a, 1991b; Oehlmann et al., 1996; Matthiessen and Gibbs, 1998). The suggested underlying mechanism of action is the inhibition of the conversion of androgens to estrogens mediated by the aromatase cytochrome P450 enzyme. Although the toxicity of OTCs has been reviewed extensively (IPCS, 1990; Golub and Doherty, 2004; Hirose et al., 2004), their reproductive and developmental toxicity is

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not well understood. There is evidence that exposure to organotins can also affect mammalian reproduction. In female rat offspring a delay of vaginal opening and a decrease in ovarian weights were reported by Ogata et al. (2001) after in utero exposure to 125 ppm (approximately 10 mg/kg) tributyltinchloride (TBTCl). Wester et al. (1990) observed a decrease in ovarian weights at a dietary dose of 50 mg bis(tri-*n*-butyltin)oxide (TBTO) in a chronic toxicity study in rats. Furthermore, treatment of pseudopregnant rats with dibutyltin and TPT resulted in a decrease in uterine weights and serum progesterone levels (Ema et al., 1999; Harazono and Ema, 2003). In a developmental toxicity study in rats, exposure to 20 mg TBTCl/kg bw during gestation days 0–19 led to increased postimplantation loss, decreased fetal weights and delayed ossification of the fetal skeleton (Adeeko et al., 2003). Reproductive toxicity of TPT has been studied extensively during its registration process as a pesticide and adverse effects on the reproductive tract have been reported at different dose levels (for review see Golub and Doherty, 2004).

Recently, we assessed the effects of two organotin compounds, tributyltin and triphenyltin, on pubertal male rats and found effects on male sexual development (Grote et al., 2004). The present study was carried out to evaluate the effects of peripubertal exposure to TPT on female sexual development using a modified protocol of the Rodent 20-Day Thyroid/Pubertal Female Assay (EDSTAC, 1998). This assay was used to examine possible hormone-like effects of triphenyltin on the neuroendocrine axis during the sensitive period of puberty which could possibly influence the female reproductive tract. The assay is recommended by the US EPA for the evaluation of so called endocrine disruptors (Goldman et al., 2000). Additionally, the effect of TPT exposure on animals before the onset of puberty was investigated.

2. Materials and methods

2.1. Animals and animal husbandry

Female weanling Wistar rats (HsdCpb:WU) were purchased from Harlan Winkelmann, Borchen, FRG. Animals were kept at the Institute of Clinical Pharmacology and Toxicology, Dept. of Toxicology, Benjamin Franklin Medical Center under specific-pathogen-free (SPF) conditions in climate-controlled rooms. Twenty animals for each dosage group were kept together 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 $50 \pm 5\%$ at a room temperature of $21 \pm 1\,^{\circ}C$. 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

Animals were weighed 2 days after weaning (23 days of age) and randomized into treatment groups with 20 animals per group with approximately equal mean body weights (control: 43.9 ± 0.65 g; TPT 2 mg: 44.9 ± 0.7 g; TPT 6 mg: 44.6 ± 0.7 g; mean ± S.E.M.). Animals were treated daily from 23 days of age at dose levels of 2 or 6 mg TPT/kg bw/day. In order to provide comparability between sexes and investigation of specific effects on sexual development, doses were selected based on our previous study in male pubertal rats (Grote et al., 2004), in which mortality and general toxicity were observed after treatment with 12 mg TPT/kg bw. Triphenyltin chloride (purity ≥97.0%) was purchased from Sigma–Aldrich Chemie GmbH, Steinheim, FRG. The substance was dissolved in pharmaceutical peanut oil and administered per gavage at a volume of 5 mL/kg. The control group received pharmaceutical peanut oil only. The following endpoints were investigated:

- body weight gain;
- organ weights (liver, brain and thymus);
- number of animals with completed vaginal opening (VO) on PND 33;
- reproductive organ weights (uterus and ovaries);
- hormone concentrations (estradiol and progesterone);
- aromatase activity in the preoptic area of the brain and in ovaries.

Postnatal day 33 was chosen as the time point for this investigation based on the assumption that 40–60% of the control animals would still exhibit closed vaginas.

2.3. General toxicity

To evaluate for potential general toxicity, body weight (±1 g) was determined daily during the entire period of treatment with a calibrated electronic scale (econ. Sartorius). Animals were daily monitored for mortality and signs of general toxicity. Daily food consumption was roughly estimated.

2.4. Vaginal opening and termination

On PND 33 animals of all treatment groups were investigated between 8:00 and 10:00 for completion of vaginal opening and their number was recorded. For assessment of possible effects of TPT before the onset of puberty 10 animals with closed vaginas from the 0 and 6 mg TPT/kg dose groups were sacrificed on PND 33. In the 2 mg TPT dose group, 14 animals exhibited vaginal opening and, therefore, only 6 animals with closed vaginas were evaluated in this dose group. Ten animals per dose group were kept on treatment until their