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Sex differences in effects on sexual development in rat offspring after pre- and postnatal exposure to triphenyltin chloride

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ABSTRACT

Consumers are exposed to organotin compounds (OTCs) via contaminated fish and seafood due to the accumulation of these compounds in marine organisms. Certain OTCs are immunotoxic and may also have endocrine disrupting properties resulting in adverse effects on the reproductive tract in mollusks and mammals. Since effects of *in utero* exposure to endocrine disrupting chemicals on the reproductive system are dependent on the critical window of exposure during its development, we conducted a comprehensive study with the aim to identify the most sensitive window of exposure to TPTCl and to investigate the effects of pre- and postnatal treatment on sexual development in rats. Male and female offspring rats were exposed to 2 or 6 mg TPTCl/kg b.w. and day either *in utero* and during lactation (gestation day 6 until weaning on PND 21) or from gestation day 6 until termination. As previously reported, offspring in the 6 mg TPTCl dose group exhibited high perinatal mortality and therefore no further evaluation was carried out at this dose level (Grote, K., Hobler, C, Andrade, A.J.M., Wichert Grande, S., Gericke, C., Talsness, C.E., Appel, K.E., Chahoud, I., 2007. Effects of in utero and lactational exposure to triphenyltin chloride on pregnancy outcome and postnatal development in rat offspring. Toxicology 238, 177–185). In the present paper, results on postnatal development obtained from surviving offspring of dams exposed to 2 mg TPTCl/kg b.w. are reported. Male offspring were sacrificed on PND 64 or 65 and female offspring at first estrus after PND 58. A clear sex difference in response to treatment was observed. Male postnatal development was severely affected with decreases in body weight gain, reproductive organ weights and testosterone concentration as well as a significant delay in the age at preputial separation. In contrast, females exhibited a precocious completion of vaginal opening while all other endpoints were unaffected. Most of these effects were already present in animals that were only exposed until weaning indicating that these effects may be irreversible and continued treatment until termination had contributed less than expected to the severity of the observed effects. The results of the present study suggest that the sensitive window for the evaluated endpoints seems to be the period of prenatal development and that male offspring rats were more susceptible to treatment.

1. Introduction

Recently, there has been a lot of public concern about the toxicological and ecotoxicological properties of organotin compounds (OTCs). Some tri-substituted OTCs in particular exhibit biocidal properties and are therefore used as fungicides and pesticides in many agricultural applications. Furthermore, they are applied for antifoulant protection on deep-sea ships and smaller vessels and from there they are leaching into the sea. Due to the accumulation of these compounds in marine organisms, consumers are exposed via contaminated fish and seafood (Ebdon et al., 1989; Kannan and Falandysz, 1997; Kannan et al., 1999; Harino et al., 2000; Appel et al., 2000; Appel, 2004). For the assessment of health risks to consumers a group tolerable daily intake (TDI) for tributyltin (TBT), dibutyltin (DBT), triphenyltin (TPT) and dioctyltin (DOT) was established (EFSA, 2004). Experimental studies imply that certain OTCs are immunotoxic and may also have endocrine disrupting properties resulting in adverse effects on the reproductive tract in mollusks and mammals (Penninks et al., 1990; Vos et al., 1990; Raffray and Cohen, 1993; Cooke et al., 2004; Grote et al., 2004; Makita et al., 2005). These endocrine disrupting effects include the development of imposex in gastropods, changes in reproductive organ weights as well as alterations in sexual hormone levels in rats (Horiguchi et al., 1995; Oehlmann et al., 1996;

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Morcillo and Porte, 1999; Omura et al., 2001; Ogata et al., 2001; Hirose et al., 2004; Reddy et al., 2006; Barthelemy et al., 2007). Furthermore, administration of certain OTCs to female rodents during early pregnancy resulted in reduced fertility, implantation failure, post-implantation loss and teratogenicity (Faqi et al., 1997; Ema et al., 1999; Ema and Miyawaki, 2002; Adeeko et al., 2003; Harazono and Ema, 2003).

It is well known that there are fundamental differences between the developing and the adult organism with respect to the exposure and the actions of endocrine-disrupting compounds (EDCs). Effects of *in utero* exposure to EDCs on the reproductive system are dependent on the critical window of exposure during its development. As reproductive functions and processes that develop later in life are largely determined during fetal and postnatal life, the knowledge of developmental stage sensitivity is of particular relevance for understanding the mechanisms involved in the characteristic of the different responses. We conducted a comprehensive study to investigate the effects of triphenyltin chloride (TPTCl) exposure during pre-, post- and peripubertal development on sexual development and reproduction. As previously reported, peripubertal treatment to TPTCl resulted in alterations in sexual development, sexual hormone concentrations and reproductive organ weights in male and female rats (Grote et al., 2004, 2006). The aim of the comprehensive study was to further identify the most sensitive window of exposure to TPTCl and to investigate the effects of pre- and postnatal treatment with TPTCl on sexual development in the male and female rat. Furthermore, we wanted to clarify whether possible effects set during pre- and postnatal development are reversible or would persist and can be detected later in life. For this purpose, rat offspring were exposed to TPTCL *in utero*, during lactation and during the postweaning period and sexual development. To be able to compare results from previous studies, dose selection was based on results from our investigations in male and female pubertal rats (Grote et al., 2004, 2006). As previously reported, treatment with 6 mg TPTCl/kg b.w. resulted in a high mortality rate in dams and a considerable number of offspring of surviving dams died up until PND 4 (Grote et al., 2007). In the present paper, we report on results obtained from surviving offspring of dams that were administered 2 mg TPTCl/kg b.w. Hormone levels, preputial separation and vaginal opening as well as body and organ weights of offspring exposed to 2 mg TPTCl/kg b.w. until termination were evaluated. The results were compared to offspring that were only exposed prenatally and during lactation.

2. Materials and methods

2.1. Animals and animal husbandry

Female Wistar rats (HsdCpb:WU), purchased from Harlan Winkelmann, Borchen, FRG, 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 on softwood granulate 8–15® (econ. Altromin, Lage, FRG) at a constant light cycle (12 h of light, 12 h of darkness). The room temperature was 21 ± 1 °C with a relative humidity of 50 ± 5%. Animals received autoclaved commercial diet (Altromin ®1324, Fa. Altromin Lage, FRG) and tap water ad libitum.

2.2. Treatment of dams and offspring

Triphenyltin chloride (TPTCl) with a purity of ≥97.0% was purchased from Sigma–Aldrich Chemie GmbH, Steinheim, FRG, and dissolved in pharmaceutical peanut oil. Gravid dams were randomly assigned to the control and the two treatment groups and treated daily per gavage from gestational days 6 through the end of lactation with the vehicle ($n = 14$), 2 ($n = 17$) or 6 ($n = 16$) mg TPTCl/kg b.w. at a volume of 5 ml/kg b.w. As previously reported (Grote et al., 2007) dams in the 6 mg TPTCl/kg b.w. dose group exhibited a high rate of mortality. Due to the high number of either stillborn or dead offspring until PND 4 and the resulting insufficient number of offspring no further evaluation was carried out at this dose level. All available offspring were carried through the investigation and thus all litters were represented. After weaning on postnatal day (PND) 21 offspring in the treatment group were divided into two groups. In one group treatment was continued with 2 mg TPTCl/kg b.w. until termination, the other group received no further treatment. The same procedure was applied to offspring of the vehicle control, resulting in a separate control group for each 2 mg TPTCl treatment group.

2.3. Mortality and other clinical signs

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

2.4. Preputial separation, vaginal opening and termination

Every day at the same time male offspring of all groups were examined starting on postnatal day (PND) 35 for completion of preputial separation (PS). On the day that PS was achieved, the age of the animal was recorded. On PND 64 or 65 males in all groups were killed by decapitation and trunk blood was collected. Female offspring of all groups were examined daily at the same time of day starting on postnatal day (PND) 30 for completion of vaginal opening (VO). On the day that VO was achieved, the age of the animal was recorded. From PND 58 determination of the estrous cycle was performed through vaginal smear. On the day of estrus females were killed by decapitation and trunk blood was collected. Weights of liver, thymus, and reproductive organs were determined in all offspring (±0.0001 g) with a calibrated electronic scale (econ. Sartorius) and relative organ weights were calculated. Values are presented as mean ± standard deviation.

2.5. Blood collection

Trunk blood was collected into NH_4Heparin tubes (Sarstedt) using a funnel. The tubes were capped instantly and gently shaken to ensure mixing of blood and NH_4Heparin. Directly after collection, the blood was centrifuged for 15 min at 4 °C and 3500×*g*. Aliquots of the plasma were stored in microcentrifuge tubes and stored at −20 °C.

2.6. Determination of hormone concentrations

For the determination of serum testosterone concentrations a double-antibody radioimmunoassay kit (Diagnostic Products Corporation, Los Angeles, USA) was used according to the manufacturer's instructions. Standards and plasma samples were measured in duplicate. The coefficients of intra- and inter-assay variation were less than 8%. Estradiol concentrations in plasma samples were measured with a human steroid assay (Estradiol ELISA, DRG, Marburg, FRG) according to the manufacturers instructions with a sensitivity of 4.6 pg/ml and a coefficient of intraassay variation less than 6.5%. Progesterone concentrations in plasma samples were measured with a human steroid assay (Progesterone ELISA, DRG, Marburg, FRG) according to the manufacturer's instructions with a sensitivity of 0.05 ng/ml and a coefficient of intraassay variation less than 8.3%.

2.7. Ovarian follicle count

The processing and counting of ovarian follicles was performed as described previously by Talsness et al. (2005). Briefly, whole ovaries were fixed in Bouin, dehydrated in ethanol and embedded in paraffin. Subsequently, serial sections were cut every 6 μm and stained with hemotoxylin and eosin. For counting of primordial and primary follicles five sections were taken from the middle of the ovary 240 μm apart. Secondary, tertiary and atretic follicles were counted in every 10th section of the entire ovary. Classification of different ovarian follicle types was carried out according to a modification by Plowchalk et al. (1993) of a scheme by Pedersen and Peters (1968).

2.8. Statistics

Statistical comparisons were conducted using SPSS 11.0. For the evaluation of body weights, organ weights and hormone concentrations the Students *t*-test was applied. Completion of PS and VO was analyzed using the Log Rank-Test, followed by Breslow- and Tarone-Ware-Test.

For statistical confirmation a probability error of 5% ($p < 0.05$) was defined.

3. Results

3.1. Reproductive outcome and general toxicity

Reproductive outcome and postnatal mortality were reported earlier (Grote et al., 2007). Offspring in the 2 mg TPTCl/kg b.w. dose group exhibited early postnatal mortality until PND 4. After this timepoint, no more deaths occurred. No clinical signs of general toxicity were observed in male or female offspring of all treatment groups.