LOW DOSE EFFECTS ON THE RAT FEMALE REPRODUCTIVE SYSTEM FOLLOWING EXPOSURE TO A SINGLE ADMINISTRATION OF PBDE-47

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Introduction

Polybrominated diphenyl ethers (PBDEs) are persistent and bioaccumulative compounds used as flame retardants added to textiles, polyurethane foams, plastics, and electronics. A survey of the literature by Hites[1] regarding concentrations of PBDEs in human samples from Europe, Asia and North America taken between 1970 and 2002 revealed that there has been an exponential increase in total PBDE body burdens with a doubling time of approximately 5 years. A study in weanling rats revealed that some commercial mixtures of PBDEs reduced serum total thyroxine which was associated with upregulation of uridinediphosphate-glucuronosyltransferase activity[2]. Thyroid hormones play a critical role in neurological development and exposure to PBDEs has been shown to cause neurobehavioral alterations in mice [3,4]. Other studies in rats have shown effects on behavior as well as alterations to the male and female reproductive systems [5,6,7] following exposure to the PBDE-99 congener. The present study evaluates the effects of exposure to low doses of PBDE-47, which is the predominant congener found in environmental and human samples, on the developing reproductive system of the female rat. In order to avoid having to extrapolate data obtained with high doses to those relevant for human exposure, we chose to evaluate doses much lower than those commonly used in toxicological studies. The highest level of PBDE-47 found in human female serum in a study from the San Francisco area[8] was 511 ng PBDE-47/g lipid. Another study in Indiana[9] sampling pregnant women reported the highest level as 310 ng PBDE-47/g lipid in maternal serum and the highest level reported in breast milk in a study from Texas[10] was 272 ng PBDE-47/g lipid. Based on a fat content of 14 % of bodyweight, our doses of 700 and 140 µg/kg BW result in levels approximately 10 and 2 times higher, respectively, than that reported from the San Francisco area.

Materials and Methods

Gravid Wistar rats were exposed to a single administration of either pharmacological grade peanut oil as vehicle or 140 or 700 µg PBDE-47/kg BW (2,2', 4, 4'- tetrabromodiphenyl ether) on gestational day 6. The goitrogen, 6-n-propyl-2-thiouracil (PTU), served as a reference control and was administered in the drinking water (5mg/L) to another group of rats from gestational day 7 to the end of lactation on postnatal day (PND) 21. The F1 offspring were sacrificed on PND 38 (16-20/group) when selected organ weights were recorded and trunk blood was collected to determine serum estradiol concentrations (N=12-17/group) using a competitive radioimmunoassay kit according to the manufacturer's instructions (Diagnostic Products Corporation Biermann GmbH, Bad Nauheim, Germany). Ovaries were fixed in Bouin and serially sectioned for ovarian follicle counts (N=10/group) or frozen in liquid nitrogen and stored at -80°C for analysis of aromatase activity (N=11-12/group) by measuring the stereospecific release of tritiated water from [1β-^{3}H]androstenedione. The statistical analyses were performed with Graph Pad Prism Version 3 software. Means from the PTU group were compared to control using the unpaired student's t-test. The means from the PBDE-47 groups were compared to control by ANOVA followed by the Dunnett's test. Medians from the PTU group were compared to control using Mann Whitney and those from the PBDE-47 groups were compared to control using Kruskal Wallis. The influence of body weight on organ weights was evaluated with linear regression analysis. Based on these analyses, liver weights were analyzed by ANCOVA using bodyweight and dose as independent variables (SAS Version 9.1).

Results and Discussion

Compatible to previous reports that PBDEs can reduce serum total thyroxine concentrations, we observed a decrease in serum thyroxine (free) on PND 14 in the female offspring exposed to PBDE-47. This decrease was transient and not observed on the other days measured (PNDs 1 and 22). Serum thyroxine (free) was reduced in the PTU-exposed offspring on PNDs 14 and 21 and not on PND 1 (Kuriyama SN, unpublished).

Body and Organ Weights of the F1 Offspring on PND 38 There was a reduction in body and paired ovarian weights in the group exposed to PTU which is in accordance with another report in rats following oral PTU treatment from PND 21-40[11] and one following exposure from PND 1-40[12]. Ovarian weight was also reduced in the 140 µg PBDE-47/kg BW group. The adjusted liver weight means were significantly lower in both groups exposed to PBDE-47. *Ovarian Follicle Numbers, Ovarian Aromatase Activity and Serum Estradiol Concentrations of the F1Offspring on PND 38* In the PTU group there was a significant reduction in the number of primordial and tertiary follicles and in the 700 µg PBDE-47/kg BW group, the number of secondary and tertiary follicles was lower compared to control (Table 1). The serum estradiol concentrations were reduced in both of these treatment groups as well (Figure 1). In cultured porcine granulosa cells, findings from Maruo *et al.*[13] suggest that thyroid hormone and FSH synergize to stimulate differentiation of granulosa cells and to activate steroidogenic enzymes such as aromatase and, therefore, we evaluated whether the lowered circulating estradiol concentrations were associated with alterations in whole ovary aromatase activity. We found no differences in aromatase activity (Figure 2) among the groups to explain the decreased circulating estradiol levels. Statistically significant alterations in folliculogenesis were observed in the PTU offspring and those exposed to the higher dose of PBDE-47. Both substances caused similar effects on the antral follicle numbers. The decrease in tertiary follicles seen in these groups is compatible with the observed reduction in circulating estradiol levels as antral follicles are a major source of estrogen. In both cases, the drop in the number of antral follicles was not due to an increased rate in atresia. PTU exposure also resulted in a 50% reduction in primordial follicles, an effect not seen with PBDE-47. This dramatic decrease in primordial follicles poses the possibility that these animals may experience early sexual senescence.

Table 1 Ovarian follicle counts and serum estradiol concentrations of the F1 offspring on PND 38

	Primordial	Primary	Secondary	Tertiary	Atretic
Control	**84 (62, 100)**	**44 (35, 55)**	**7 (6, 8)**	**14 (8, 16)**	**40 (35, 46)**
PTU	**42 (28, 76)***	**32 (18, 43)$^{\&}$**	**5 (2, 9)**	**9 (4, 12)***	**36 (26, 46)**
140 µg/kg	**76 (56, 93)**	**35 (32, 46)**	**4 (4, 8)**	**11 (5, 14)**	**40 (36, 52)**
700 µg/kg	**82 (69, 105)**	**42 (28, 50)**	**4 (2, 7)***	**8 (4, 10)***	**45 (29, 56)**

Table 1 The median number of follicles and (Q_1, Q_3) from 5 sections per ovary are presented for the primordial and primary follicles. The median number of follicles and (Q_1, Q_3) from 25 sections per ovary are presented for the secondary, tertiary and atretic follicles. N=10 ovaries and N=12-17 serum samples, * $p<0.05$, $^{\&}$ $p=0.06$, Kruskall Wallis, Mann Whitney, ANOVA and Unpaired t test. PTU=5mg PTU/L tap water on gd 7 - PND21, 140µg/kg=140µg PBDE-47/kgBW on gd 6, 700 µg/kg=700µg PBDE-47/kgBW on gd 6.

Determination of contaminants in human breast milk provides a good indication of the type and extent of exposure of the fetus and neonate to environmental chemicals. It is of concern that the concentrations of PBDEs have been shown to be increasing in human breast milk in countries around the world. Here we show that a single administration of an environmentally relevant dose of PBDE-47 on gestational day 6 caused alterations to the developing rat female reproductive system apparent during puberty; namely changes in ovarian weight, alterations in folliculogenesis and reduction in circulating estradiol concentrations.

Figure 1 Circulating Estradiol Concentration on PND 38

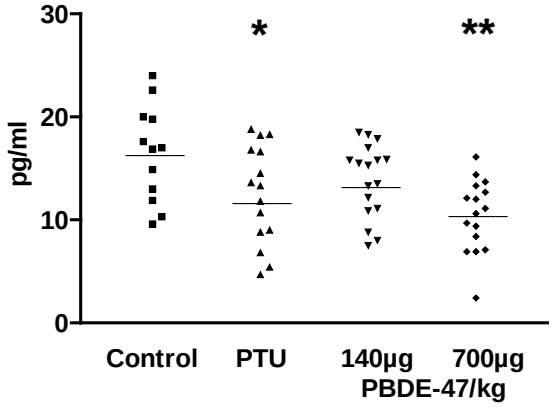

Figure 2 Ovarian Aromatase Activity on PND 38

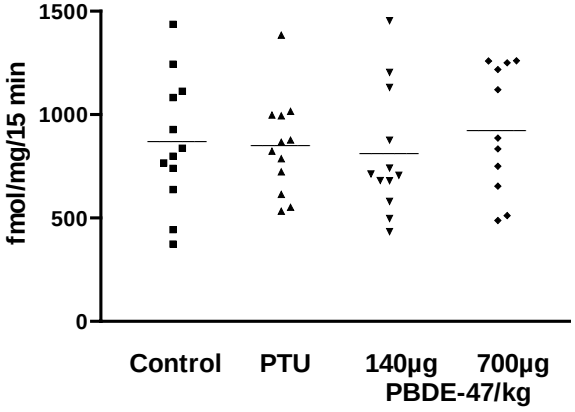

Figure 1 Estradiol concentration is presented as pg/ml serum. Each data point represents one female offspring on PND 38.* $p<0.05$, unpaired t-test, **$p<0.01$, ANOVA followed by Dunnett's test, ─ mean.

Figure 2 Ovarian aromatase activity is presented as fmol ^3H_2O/mg ovarian tissue/15 min. ANOVA and unpaired t-test, N.S., ─ mean.

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