THYROID HORMONE LEVELS AND HEPATIC ENZYME ACTIVITY IN LACTATING DAMS AFTER GESTATIONAL EXPOSURE TO LOW DOSE PBDE 47

Sergio Noboru Kuriyama[1], Antonio A. Fidalgo-Neto[2], Simone W. Grande[1], Zeynep Akkoc[1], Cristina A.M. de Souza[1], Ibrahim Chahoud[1]

[1]Institute of Clinical Pharmacology and Toxicology, Dept. Toxicology, Campus Benjamin Franklin, Charité University Medical School Berlin.
[2]Laboratory of Environmental Toxicology, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil.

Introduction

Polybrominated diphenyl ethers (PBDEs), a class of widely used flame retardants, are found extensively in the environment (shown by several studies on sentinel animal species), as well as in humans. In rodents, technical commercial PBDE mixtures and individual congeners have shown to interfere with thyroid hormone homeostasis, produce a mix-type induction of hepatic microsomal enzymes, disrupt spontaneous behaviour, impair learning and memory and alter the cholinergic transmitter system [(1-11)]. In rat and mice, some technical PBDE commercial mixtures such as DE-71 and Bromkal 70 and the congener PBDE 47 have shown to decrease circulating thyroid hormone levels [(1-3;12;13).] PBDEs are also able to induce both hepatic phase I and phase II detoxification enzymes, demonstrated by several investigations in laboratory animals. For example, induction of ethoxyresorufin-O-deethylase (EROD), pentoxyresorufin-O-despenthylase (PROD) and uridinediphospho-glucuronosyltransferase (UDPGT) has been shown in rodents and cell lines after exposure to technical mixtures or individual congeners [(1;12-14)]. However, these studies deal with doses much higher than that found in human tissues, highlighting the importance of assessing the adverse effects of doses close to human exposure levels. PBDE 47 is the most predominant congener found in environmental and human samples (including human milk) [(15)] and, therefore, hazard identification is extremely important for human risk assessment.

We administered a single dose to gravid dams on gestation day 6 of either 140 µg/kg BW or 700 µg/kg BW of the congener, 2,2'4,4'-tetrabromo diphenyl ether (PBDE 47). These doses are pertinent to human exposure levels because a study by She *et al.* found a mean level of 33.3 µg PBDE 47 /kg fat in human breast adipose tissue with a range from 7.01 to 196 µg PBDE 47 /kg fat [15]. In this study, thyroid hormone levels and hepatic enzyme activity were evaluated in lactating dams after *in utero* administration of low dose PBDE 47.

Materials and Methods

Animals and treatment: Wistar dams (N control=16; PTU=17; PBDE 140=19 and PBDE 700=18) were treated by gavage on gestation day 6 with a single dose of 140 or 700 µg PBDE 47/kg body weight or peanut oil (control). An additional group was administered the goitrogen, PTU (6-n-propyl-2-thiouracil), which served as a reference control. PTU was given to the gravid dams by placing 5mg/L PTU in the drinking water on gestation days 7 through postnatal day 21. On PND 1, about half of the treated females (control=9, PTU=9; PBDE 140=10 and PBDE 700=9) were killed by decapitation and liver was weighed and frozen at –80°C. Serum was collected from trunk blood, divided in five aliquots and stored at –20°C. At the end of lactation (PND 22), the procedure performed on PND 1 was repeated with the remaining dams. Total serum thyroxine (T4), free serum thyroxine (FT4) and TSH were measured using the enzyme immunoassay (ELISA) kit purchased from DRG diagnostics – GmbH, Germany. **Enzyme assay:** EROD, PROD and UDPGT activity was measured in dams (n=6 per group) on PND 1 and 22. Ethoxyresorufin-*O*-deethylase (EROD) and pentoxyresurufin-*O*-despenthylase (PROD) activities were measured in a 96-multiwell plate microassay adapted essentially from Burke et al. (1985) [16] and Pohl & Fouts (1980) [17]. The UDP-glucuronosyltransferase (UDPGT) activity was determined essentially as reported by Bock *et al.* (1983) [18], modified by Martin & Black (1994) [19]. UDPGT activity was measured with a spectrophotometric assay using UDP-glucuronic acid (UDPGA) and p-nitrophenol as substrates. **Statistical analysis:** Data are expressed as mean ± SD (standard deviation) and groups were tested by analysis of variance (ANOVA) followed by the Dunnett t-test. Differences were considered statistically significant when $P \leq 0.05$.

Results and Discussion

Administration of low dose PBDE 47 on gestation day 6 caused hypothyroxinemia in dams at the beginning of lactation. As expected, the reference group exposed during gestation/lactation to the goitrogen PTU showed a severe hypothyroxinemia, displaying T4 levels about 50% of that observed in control animals (Table 1). Dams exposed to 700µg PBDE 47 show a significant decrease in T4 and TSH levels only in the beginning of lactation, returning to normal levels on PND 22 (Table 1). The PBDE-induced hypothyroxinemia is consistent with other short-term and long-term studies on technical mixture or pure PBDEs exposure in rodents [2;3;12]. Two studies from the same laboratory show that 14 days exposure to the congener PBDE 47 caused a significant decrease in T4 levels when animals were exposed to 18 mg PBDE 47 / kg [2;3]. It is interesting to note that when PBDE 47 is combined either to PCBs (Aroclor mixture) or chlorinated

Table 1: Thyroid hormone levels in dam's serum during lactation. Animals were exposed to a single dose of PBDE 47 on gestation day 6 or gestational and lactational exposure to the goitrogen PTU via drinking water.
Values are mean ± standard deviation. Statistical analysis was performed per day using ANOVA followed by Dunnett t-test and significance (*) was confirmed when $p<0.05$.

Parameters		Control	PTU	PBDE 140	PBDE 700
PND 1 $	T4 (ng/mL)	108.58 ± 17.28	57.90 ± 15.39 *	103.65 ± 24.59	82.62 ± 16.98 *
	FT4 (pg/mL)	24.72 ± 3.29	17.27 ± 4.04 *	25.24 ± 4.12	23.77 ± 3.28
	TSH (ng/mL)	32.58 ± 8.74	39.89 ± 11.48	35.96 ± 9.85	12.60 ± 10.96 *
PND 22 §	T4 (ng/mL)	71.84 ± 11.15	41.87 ± 5.61*	70.54 ± 5.94	74.41 ± 12.20
	FT4 (pg/mL)	22.91 ± 4.74	13.98 ± 3.72 *	27.80 ± 3.76	23.37 ± 4.43
	TSH (ng/mL)	28.36 ± 19.40	19.52 ± 12.66	27.61 ± 14.76	21.92 ± 3.40

$ (N of animals): control = 8, PTU = 9, PBDE 140 = 10 and PBDE 700 = 9
§ (N of animals): control = 7, PTU = 8, PBDE 140 = 9 and PBDE 700 = 9

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synergistic effect was observed showing a drastic reduction in total T4 and free T4 levels [2]. It seems that increased elimination of thyroid hormones, especially T4, via UDPGT induction is not the main mechanism involved in PBDE induce hypothyroxinemia as we did not observe significant induction in UDPGT levels in the same period in dams treated with 700µg PBDE 47. This evidence is supported by previous observation that the relationship between serum T4 depletion and induction of the T4-UDPGT activity is not clear in PBDE exposure and does not seem to be the main mechanism involved in the hypothyroidism-induced by PBDEs [1-3]. However, further investigations should be done in order to identify the mechanism underlying PBDE 47-induced hypothyroxinemia at the low dose level.

The administration of a single low dose of PBDE 47 on gestation day 6 caused mix-type alterations of hepatic biotransformation enzyme revealed as changes in ethoxy and pentoxy resorufin-*O*-dealkylase (EROD and PROD) and uridinediphosphate glucuronosyltransferase (UDPGT) activities in the rat. Dams showed a significant increase in EROD, PROD and UDPGT levels during lactation (figure 1). The congener used in this study (PBDE 47) was reported to be a very weak ligand to the Ah receptor, [14] and was almost inactive in forming the AhR-DRE complex *in vitro* through electrophoretic mobility shift assay (EMSA) [20]. However, in the same study PBDE 47 was shown to inhibit the activation of AhR-DRE complex by TCDD, although this effect was not able to change the induction of CYP1A1 RNAm levels upon co-administration with 0.1 nM TCDD [20]. Hepatic EROD activities, being indicative of specific induction of CYP1A were not induced by PBDE congeners 47 and 99 in liver cell lines from rainbow trout (RTL-W1), rat (H4IIE) and human (HepG2) [14]. Two *in vivo* studies support our findings as they demonstrated that PBDE 47 induces EROD, PROD and to a less extent UDPGT activities in rats and mice exposed for 14 days to higher doses than that used in this study [2;3]. At the lowest PBDE 47 dose tested by Hallgren *et al.* (2002) (1 mg / kg BW), which is much higher than the dose range tested in the present study no effect on EROD and PROD activities were seen,. Differences in the time of exposure (7 weeks old *vs.* adult exposure during gestation) might explain the discrepancies between the two studies, due to the metabolic and physiologic differences associated with the exposure periods [21]. The changes in EROD, PROD and UDPGT activities seen in this study may have a biological relevance as small fluctuations in metabolic clearance / activation can expose the developing organism to high levels of the xenobiotic itself and / or to reactive metabolites.

In the present study we observed changes in maternal levels of thyroid hormones and hepatic enzyme activity during lactation following a single low dose administration of the environmental relevant congener PBDE 47. The developing organism is susceptible to subtle changes occurring during gestation / lactation and, therefore, not only the direct exposure to PBDEs, but also metabolic changes in the maternal organism must be considered when functional changes are observed in the progeny.

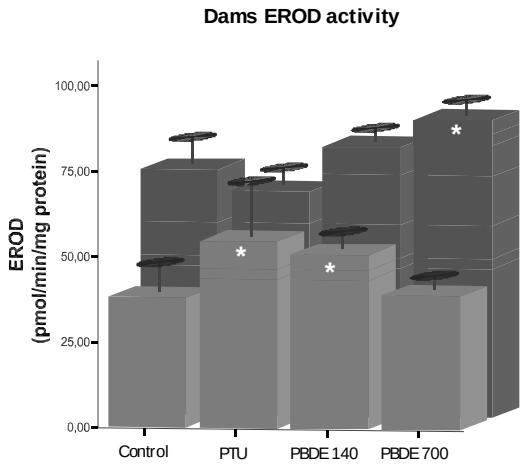
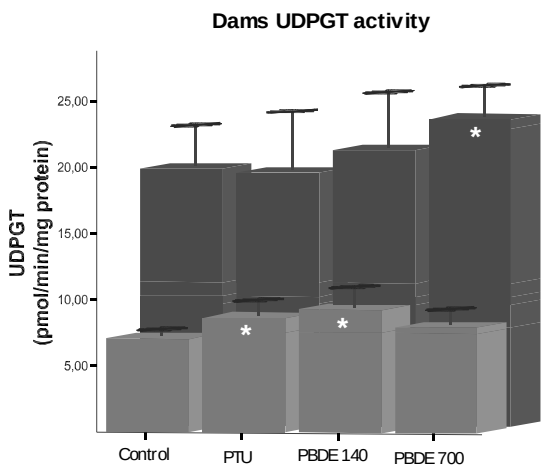
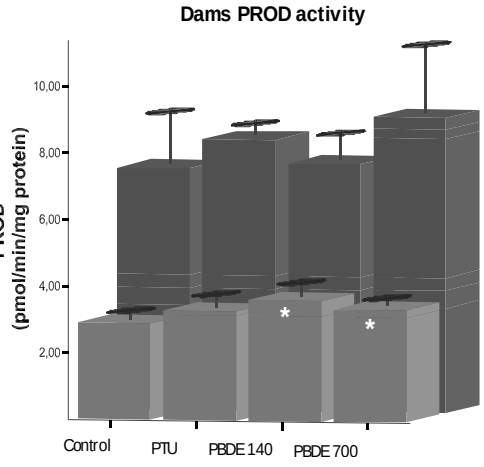


Figure 1: Hepatic EROD activity in lactating mothers after a single low dose of PBDE 47 (140 or 700 µg/kg b.w.) on gestation day 6. Activities are expressed as pmol of resorufin^{-1} minute^{-1} mg of microsomal protein. Bars represent means ± SD and significance was detected by ANOVA followed by Dunnett t test when $p<0.05$.





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