# Effects of developmental low dose PBDE 47 exposure on thyroid hormone status and serum concentrations of FSH and inhibin B in male rats

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## Introduction

Several persistent halogenated organic compounds such as PCBs, dioxins and more recently, polybrominated diphenyl ethers (PBDEs) have been shown to disrupt thyroid hormone homeostasis in experimental animals[1]. Particular concern exists regarding exposure to these compounds during critical periods of development when thyroid hormones orchestrate the growth and differentiation of many organs including the brain and the testis[1,2].

**While the contamination levels of PCBs and other persistent organic pollutants have declined in the past years, increasing levels of PBDEs have been detected in environmental and human samples including human breast milk[3,4,5]. PBDEs are produced in large quantities and used worldwide as flame retardants in electrical appliances, carpets and furniture upholstery. Similar to other halogenated environmental organic pollutants, PBDEs seem to present a wide range of toxic effects including reproductive, endocrine, neurobehavioral and hepatic toxicity[6].**

**Recently, it has been demonstrated that *in utero* and pubertal exposures to DE-71 (a commercial mixture containing mostly tetra- and penta-bromodiphenyl ethers) significantly reduce thyroxine levels (T4) in rats[7,8]. The present study has focused on the possible effects of the tetra-BDE congener 2,2'4,4'-tetrabromo diphenyl ether (PBDE 47) on thyroid hormone status and associated changes on FSH and inhibin B levels in the developing male rat. We administered a single dose to gravid dams on gestation day 6 of either 140 µg/kg BW or 700 µg/kg BW PBDE 47. These doses are pertinent to human exposure situation because a study by She et al.[3] 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.**

## Materials and methods

Animals and treatment: **Pregnant Wistar rats (N= 16-19/group) 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 (0.0005%) from gestation day 7 through postnatal day 21. On postnatal day 1 (PND1), 7-9 litters from each treatment group were selected and the pups**

**were killed by decapitation to collect blood for hormonal analysis. On PND 14 and PND 22 (weaning) 6-9 litters from each treatment group were selected and 2 male pups per litter were killed by decapitation to collect blood and organs. Brain, testis, liver, spleen and kidney weights from male offspring on PND 22 were registered.**

Hormonal analysis**: trunk blood was collected and allowed to clot on an ice bath (4°C) for 2 h. Serum was removed after centrifugation and stored at –20°C until analysis. Serum was pooled on a litter basis (N= 6-9) due to the small volume available at the ages evaluated (PND 1, 14 and 22). Total serum thyroxine (T4), free thyroxine (FT4), total triiodothyronine (T3), free triiodothyronine (FT3), thyroid stimulating hormone (TSH) and inhibin B levels were determined using immunoassay (ELISA) kits from DRG (Germany) according to the manufacturer's instructions. Serum follicle stimulating hormone (FSH) levels were measured by an immunoassay (ELISA) kit purchased from Amersham Biosciences.**

**Statistics:** Data were analyzed by analysis of variance (ANOVA). Differences between groups were tested by the Dunnet multiple comparisons test. Differences were considered to be statistically significant at a probability level of 5% ($p < 0.05$).

**Table 1. Thyroid hormone concentrations in male offspring rats exposed *in utero* to low doses of PBDE 47**

| Hormones | Treatment | | | |
|---|---|---|---|---|
| | Control | PTU | PBDE 47 140 µg/kg | PBDE 47 700 µg/kg |
| PND 1 | **N = 7** | **N = 9** | **N = 10** | **N = 9** |
| **T4 (ng/mL)** | **40.03 ± 4.72** | **31.56 ± 4.94 *** | **39.22 ± 5.25** | **37.57 ± 4.98** |
| **FT4 (pg/mL)** | **4.04 ± 2.48** | **2.57 ± 1.63** | **2.27 ± 1.15** | **2.67 ± 1.40** |
| **T3 (ng/mL)** | **2.39 ± 1.14** | **1.94 ± 0.70** | **1.95 ± 0.62** | **1.28 ± 0.43 *** |
| **FT3 (pg/mL)** | **0.45 ± 0.21** | **0.52 ± 0.14** | **0.62 ± 0.19** | **0.65 ± 0.40** |
| **TSH (ng/mL)** | **8.36 ± 6.44** [$] | **8.40 ± 1.05** [$$] | **9.10 ± 4.75** [$$] | **5.40 ± 2.63** [$$] |
| PND 14 | **N = 6** | **N = 6** | **N = 8** | **N = 8** |
| **T4 (ng/mL)** | **110.09 ± 13.56** | **28.78 ± 8.97 *** | **98.32 ± 8.14** | **103.85 ±13.87** |
| **FT4 (pg/mL)** | **8.33 ± 1.05** | **2.99 ± 1.20 *** | **6.91 ± 1.38** | **6.63 ± 1.85** |
| **T3 (ng/mL)** | **3.65 ± 0.77** | **3.94 ± 0.78** | **2.25 ± 0.46 *** | **2.83 ±0.54 *** |
| **FT3 (pg/mL)** | **1.00 ± 0.39** | **1.75 ± 0.45 *** | **1.18 ± 0.08** | **1.17 ± 0.23** |
| **TSH (ng/mL)** | **9.37 ± 0.78** | **8.50 ± 1.05** | **8.02 ± 1.06 *** | **8.63 ± 0.93** |
| PND 22 | **N = 7** | **N = 8** | **N = 9** | **N = 9** |
| **T4 (ng/mL)** | **89.23 ± 8.31** | **41.77 ± 4.43 *** | **96.18 ± 13.88** | **100.73 ± 8.23 *** |
| **FT4 (pg/mL)** | **15.11 ± 3.62** | **7.84 ± 2.37 *** | **17.07 ± 3.06** | **18.54 ± 3.80** |
| **T3 (ng/mL)** | **4.55 ± 0.40** | **4.32 ± 0.66** | **4.46 ± 0.43** | **4.44 ± 0.96** |
| **FT3 (pg/mL)** | **2.16 ± 0.57** | **2.04 ± 0.39** | **2.46 ± 0.42** | **2.54 ± 0.33** |
| **TSH (ng/mL)** | **31.43 ± 11.44** | **23.50 ± 6.74** | **20.16 ± 4.18 *** | **23.66 ± 7.76** |

**Values represent mean ± SE. PND = postnatal day. [$] N = 5; [$$] N = 4**

*** Significantly different from control (p<0.05 – ANOVA – Dunnet).**

**Table 2. Body and organ weights of male offspring rats (PND 22) exposed *in utero* to low doses of PBDE 47**

| Weight | Treatment | | | |
|---|---|---|---|---|
| | Control (N = 14) | PTU (N = 16) | PBDE 47 - 140 µg/kg (N = 18) | PBDE 47 - 700 µg/kg (N = 18) |
| Body weight | **42.6 ± 3.68** | **35.1 ± 5.16 *** | **39.7 ± 5.83** | **37.2 ± 4.60 *** |
| Absolute | | | | |
| **Brain (g)** | **1.42 ± 0.06** | **1.34 ± 0.06 *** | **1.38 ± 0.09** | **1.37 ± 0.05** |
| **Liver (g)** | **1.47 ± 0.19** | **1.25 ± 0.21 *** | **1.41 ± 0.22** | **1.30 ± 0.20** |
| **Spleen (mg)** | **137 ± 26.1** | **110 ± 33.0 *** | **126 ± 26.5** | **123 ± 29.7** |
| **Kidney (mg)** | **226 ± 21.1** | **202 ± 33.0** | **213 ± 34.2** | **211 ± 34.9** |
| **Testis (mg)** | **120 ± 7.80** | **97.6 ± 18.2 *** | **116 ± 16.5** | **106 ± 18.0 §** |
| Relative % | | | | |
| **Brain** | **3.36 ± 0.32** | **3.87 ± 0.51 *** | **3.51 ± 0.36** | **3.74 ± 0.37 *** |
| **Liver** | **3.44 ± 0.26** | **3.55 ± 0.26** | **3.56 ± 0.25** | **3.48 ± 0.20** |
| **Spleen** | **0.31 ± 0.04** | **0.31 ± 0.06** | **0.32 ± 0.04** | **0.33 ± 0.05** |
| **Kidney** | **0.53 ± 0.03** | **0.57 ± 0.05 *** | **0.54 ± 0.03** | **0.57 ± 0.03 *** |
| **Testis** | **0.28 ± 0.01** | **0.28 ± 0.03** | **0.29 ± 0.02** | **0.28 ± 0.02** |

**Values represent mean ± SD. * Significantly different from control (p<0.05 – ANOVA – Dunnet). § p = 0.052**

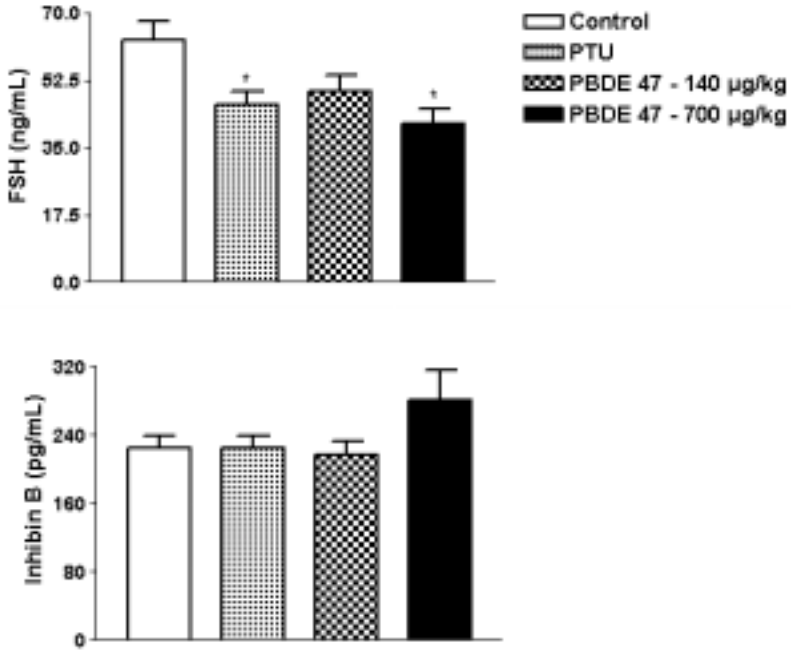


**Figure 1. Effects of *in utero* exposure to PBDE 47 on serum concentrations of follicle stimulating hormone (FSH) and inhibin B in male offspring rats at postnatal day 22. * Significantly different from control (p<0.05 – ANOVA – Dunnet).**

Results and discussion

Thyroid hormones

In the present study we report changes on thyroid hormone homeostasis in developing male rats exposed to single low doses of PBDE 47 (140 and 700 µg/kg) on gestation day 6. Previous studies reported thyroid hormone changes in developing, pubertal and adult rodents following exposure to relatively high doses of PBDE mixtures or single congeners. Recently, Zhou *et al*[7] reported reductions in total serum thyroxin (T4) concentrations in male fetuses and offspring rats (gestational day 20 and postnatal days 4 and 14) exposed *in utero* and during lactation to 10 and 30 mg DE-71 mixture/kg/day. Hallgren and Darnerud[9] found decreased free serum thyroxin (FT4) but normal TSH levels in female rats exposed during 14 days to PBDE 47 at 18 mg/kg/day.

In the present study no changes were observed on total and free thyroxin (T4 and FT4) and free triiodothyronin (FT3) serum concentrations, with exception of a slight increase in T4 levels in the 700 µg/kg group at PND 22. However, slight but significant reductions were seen on total T3 levels on postnatal days 1 (700 µg/kg) and 14 (140 and 700 µg/kg). Serum TSH concentrations remained unchanged at PND 1 in all groups, but were slightly reduced at PND 14 and 22 in the 140 µg/kg group (table 1). In the group treated with PTU, significant changes in total thyroxin levels were seen at PNDs 1, 14, and 22. Additionally, free thyroxin levels (FT4) were also significantly reduced at PNDs 14 and 22. Despite these changes the levels of TSH remained unchanged at all ages evaluated (table 1). It is important to note that since we expected only slight effects of low doses of PBDE 47 on thyroid hormone homeostasis, the selected dose of PTU (0.0005% in drinking water) was significantly lower than those typically used to induce severe hypothyroidism in experimental animals (0.1% in drinking water). However, we could still detect significant reductions on body and absolute organ weights in PTU treated animals on postnatal day 22 (table 2). In the animals exposed to PBDE 47, reduced body weight and increased brain and kidney relative weights were seen in the highest dose group (table 2). In this same group absolute testis weight was also reduced, but not significantly ($p = 0.052$).

Several mechanisms have been proposed to explain the reductions on thyroid hormone concentrations following exposure to PBDEs including, (a) increased peripheral metabolism; (b) displacement from transport proteins; (c) direct effects on the thyroid gland; and (d) interference with the hypothalamus-pituitary-thyroid axis[7,8,9,10]. However, a clear relationship between thyroid hormones depletion and the above mentioned mechanisms was not yet established[7,9]. It is likely that no single mechanism rather multiple factors are involved on PBDE-induced changes in thyroid hormone status.

**FSH and inhibin B concentrations**

Neonatal hypothyroidism induced by relatively high doses of PTU (0.1 % in drinking water) is associated with several endocrine changes including permanent reductions in serum FSH and increased serum inhibin B levels[11,12,13].

Sharpe and co-workers[13] demonstrated that the increased levels of inhibin B are related to the extended period of Sertoli cell proliferation observed in neonatal hypothyroid rats. On the other hand, reduced levels of FSH in PTU-treated animals were first believed to reflect the increased levels of inhibin B. However, Kirby and colleagues[12] demonstrated that absolute serum concentrations of FSH remained significantly lower in PTU-treated rats despite the removal of the negative gonadal feedback by castration, indicating reduced responsiveness of the pituitary gland

and an overall reduction in gonadotrope synthetic ability. In fact, gonadotropes express T3 receptors and adequate concentrations of thyroid hormones during development seem to be critical for determining gonadotropin production[12].
In the present study serum inhibin B and FSH levels were measured on PND 22 to demonstrate possible effects of PBDE 47 on the testis (Sertoli cell number) and the pituitary gland. Although no statistically significant changes were seen on inhibin B levels, the animals treated with PTU and the highest dose of PBDE 47 showed significantly lower levels of FSH than controls (figure 1). These results indicate that the pituitary gland may be more sensitive to the effects of low doses of PTU and PBDE 47 than the testis. However, we can not rule out the possibility that minor changes in Sertoli cells number may have occurred without significantly altering inhibin B concentrations. Future experiments focusing on inhibin B and FSH levels as well as evaluation of Sertoli cell number and daily sperm production at adulthood will provide a better insight on the low dose effects of PBDE 47 in the hypothalamus-pituitary-testis axis and the possible connections with thyroid hormone disruption.

**Conclusion**

In the present study we report disruption of thyroid hormone homeostasis in the developing male rat after exposure to low doses of PBDE 47 and the reference substance PTU. These alterations were accompanied by reduced FSH levels on PND 22, indicating possible changes in the developing pituitary.

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