In Utero and Lactational Exposures to Low Doses of Polybrominated Diphenyl Ether-47 Alter the Reproductive System and Thyroid Gland of Female Rat Offspring

Chris E. Talsness,1 Sergio N. Kuriyama,1 Anja Sterner-Kock,2 Petra Schnitker,2 Simone Wichert Grande,1 Mehdi Shakibaei,3 Anderson Andrade,1 Konstanze Grote,1 and Ibrahim Chahoud1

1Department of Toxicology, Institute of Clinical Pharmacology and Toxicology, Campus Benjamin Franklin, Charité University Medical School Berlin, Berlin, Germany; 2Department of Veterinary Pathology, Freie Universität Berlin, Berlin, Germany; 3Department of Anatomy, Ludwig-Maximilians-University Munich, Munich, Germany

BACKGROUND: Polybrominated diphenyl ethers (PBDEs) are capable of disrupting thyroid hormone homeostasis. PBDE-47 (2,2′,4,4′-tetrabromodiphenyl ether) is one of the most abundant congeners found in human breast adipose tissue and maternal milk samples.

OBJECTIVES: We evaluated the effects of developmental exposure to low doses of PBDE-47 on the female reproductive system.

METHODS: Pregnant Wistar rats were administered vehicle (peanut oil) or PBDE-47 [140 or 700 µg/kg body weight (bw)] on gestation day (GD) 6, or 5 mg 6-n-propyl-2-thiouracil (PTU)/L in the drinking water from GD7 through postnatal day (PND) 21.

RESULTS: In female offspring sacrificed on PND38, there was a significant decrease in ovarian weight after exposure to PTU or 140 µg/kg PBDE-47. Alterations in folliculogenesis were apparent: we observed a decrease in tertiary follicles and serum estradiol concentrations in the offspring exposed to either PTU or 700 µg/kg PBDE-47. PTU exposure also resulted in a decrease in primordial follicles. On PND100, persistent effects on the thyroid glands included histologic and morphometric changes after exposure to either PTU or PBDE-47. No relevant changes in reproductive indices were observed after mating the exposed F1 females with nontreated males.

CONCLUSIONS: Administration of PBDE-47 at doses relevant to human exposure led to changes in the rat female reproductive system and thyroid gland.

KEY WORDS: development, endocrine disruption, in vivo, PBDE-47, reproductive system, thyroid.

Environ Health Perspect 116:308–314 (2008). doi:10.1289/ehp.10536 available via http://dx.doi.org/
[Online 3 December 2007]
modulate estrogen action in various species, including timing of seasonal reproduction and lordosis behavior in rodents (Vasudevan et al. 2002). In addition, late uterine responses to estradiol administration have been shown to be diminished in hypothyroid rats (Gardner et al. 1978), and thyroidectomy of sexually immature rats has been shown to delay vaginal opening and to result in smaller ovaries, as well as uteri and vaginas that are not well developed (reviewed by Doufas and Mastorakos 2000). In porcine granulosa cell culture, follicle-stimulating hormone (FSH) and thyroid hormone act synergistically to stimulate granulosa cell differentiation and function (Maruo et al. 1987). Because of the interplay between the hypothalamic–pituitary–thyroid and the hypothalamic–pituitary–ovarian axes, we designed this study to evaluate the effects of low doses of 2,2′,4,4′-tetrabromodiethyl ether (PBDE-47) on the developing reproductive system of the female rat. The results from the male studies will be presented elsewhere. PBDE-47 is one of the predominant congeners found in humans; Johnson-Restrepo et al. (2005) reported PBDE-47 concentrations of 1.3–2,700 µg/kg lipid in human adipose tissues samples collected in New York City. We treated pregnant rats to a single dose of PBDE-47 at 140 or 700 µg/kg bw (2,2′,4,4′-tetrabromodiethyl ether, 98% purity; LG Chem Promochem GmbH, Wesel, Germany) by gavage (10 mL/kg bw) on GD6. An additional group, serving as reference control, was administered PTU (Sigma-Aldrich Chemicals GmbH, Steinheim, Germany). The gravid dams were given 5 mg/L PTU in the drinking water GD7 through postnatal day (PND) 21.

**Materials and Methods**

**Animals and housing.** Virgin female Wistar rats (HsdCpb:WU; Fa. Harlan-Winkelmann, Borchern, Germany) weighing 200 ± 15 g were allowed to acclimate in our facility for 2 weeks. The rats were housed at a temperature of 21 ± 1 ºC and 50 ± 5% relative humidity with constant light/dark periods of 12 hr each. Tap water and rodent chow (Altromin 1324; Altromin GmbH, Lage, Germany) were given *ad libitum*. Two females were placed with one male for 3 hr on 8 consecutive days. Daily vaginal smears were examined for the presence of sperm. The day of sperm detection was considered day 0 of gestation. The pregnant females were housed in Type III macronol cages with stainless steel covers and wood shavings (Altromin GmbH). The animals were treated humanely, and care was taken to ease suffering. The experimental protocol was approved by the Berlin Agency for Health and Social Welfare in accordance with the German National Animal Protection Law (Tierschutzgesetz 1998).

**Treatment.** Three groups of females with sperm-positive vaginal smears were administered either pharmacologic grade peanut oil (Henry Lanotte GmbH, Bremen, Germany) as vehicle or PBDE-47 at 140 or 700 µg/kg bw (2,2′,4,4′-tetrabromodiethyl ether, 98% purity; LGC Promochem GmbH, Wesel, Germany) by gavage (10 mL/kg bw) on GD6. A second set of female offspring was necropsied during estrus (based on vaginal cytology) on approximately PND100. We counted only follicles in the drinking water GD7 through postnatal day (PND) 21.

**End points.** The number of litters was recorded for each end point. Eight dams from each group were sacrificed 27 days postpartum, and the ovaries were weighed and evaluated using light microscopy. The F1 offspring were weaned on PND22 and sacrificed on PND38; organ weights were recorded, and samples were either frozen at −80 ºC for measurement of aromatase activity or placed in Bouin fixative for histology. Trunk blood was collected, and the obtained serum samples were frozen at −20 ºC until analysis. We measured the estradiol concentration in serum samples using a competitive radioimmunoassay kit according to the manufacturer’s instructions (Diagnostic Products Corporation, Bie ern GmbH, Bad Nauheim, Germany). Counts per minute were detected and data were interpolated with a Cobra Auto-Gamma Counting System (Packard Instrument Company, Meriden, CT, USA).

**Light microscopy.** We collected the ovaries (n = 4) from dams 28 days postparturition, and the ovaries (n = 4–6), uteri (n = 10–12), vaginas (n = 6–7), and thyroids (n = 10–12) from F1 female offspring (approximately 100 days of age) during estrus. All tissues were fixed in Bouins solution, dehydrated in ethanol, and embedded in paraffin. Sections (5 µm thick for the thyroid and 3 µm for all other tissues) were stained with H&E.

**Thyroid morphometry.** We analyzed H&E-stained sections (5-µm) of the thyroid gland by standard point counting (Cruz-Orive and Weibel 1990; Serakides et al. 1999) to determine the proportions of colloid, follicular epithelium, and stroma. Photomicrographs of 10 fields per thyroid were taken at 200× magnification using a Zeiss Axioshot light microscope (Zeiss, Oberkochen, Germany) fitted with a Sony 3CCD camera (AVT Hor, Aalen, Germany). A grid with 300 intersections (points) was superimposed on each field; one of the three structural components under each intersection was identified and counted, giving a total of 3,000 points per animal.

**Electron microscopy.** Tangential sections were made in the ovary and thyroid gland (n = 3) using a razor blade. Subsequently, the ovaries were cut crosswise for preparation of ultrathin sections. All samples were fixed in 1%...
Table 1. Body weight and paired ovarian weight of dams (F0) 27 days postparturition.

| Treatment | Body weight (g) | Paired ovaries (mg) |
|-----------|----------------|---------------------|
| Control   | 232 ± 6        | 92 ± 4              |
| PTU       | 248 ± 18       | 101 ± 4             |
| 140 µg PBDE-47/kg | 237 ± 16 | 108 ± 4**           |
| 700 µg PBDE-47/kg | 232 ± 7   | 91 ± 4              |

n = 8 per group. Body weights are presented as mean ± SD, and paired ovary weights are mean ± SE adjusted for body weight.

*p < 0.05, and **p < 0.01 by ANOVA.

Table 2. Body weight and selected organ weights of F1 females on PND38.

| Treatment | Body weight (g) | Liver (g) | Thyroid (mg) | Uterus (mg) | Paired ovaries (mg) |
|-----------|----------------|----------|-------------|-------------|---------------------|
| Control   | 167 ± 4        | 6.89 ± 0.52 | 10 ± 2      | 470 ± 53    | 98 ± 9              |
| PTU       | 168 ± 6        | 6.28 ± 0.89* | 12 ± 2**    | 480 ± 56    | 100 ± 12            |
| 140 µg PBDE-47/kg | 174 ± 26 | 7.02 ± 0.74 | 10 ± 1      | 461 ± 58    | 98 ± 12             |
| 700 µg PBDE-47/kg | 162 ± 14   | 6.60 ± 0.61 | 11 ± 2      | 464 ± 123   | 103 ± 13            |

n indicates the number of litters. Body and organ weights are presented as mean ± SD (by ANOVA and unpaired t-test). Organ weights are presented as mean ± SE adjusted for body weight (by ANOVA).

*p < 0.05, and **p < 0.01.

Table 3. Ovarian follicle counts for F1 females on PND38.

| Treatment | Follicle type |
|-----------|---------------|
| Control   | Primordial | Primary | Secondary | Tertiary | Atretic |
| (n = 9)   | 78 (62, 100) | 46 (35, 50) | 7 (7, 8) | 13 (9, 16) | 41 (35, 46) |
| PTU       | 42 (28, 78)* | 32 (18, 43)* | 5 (2, 9) | 9 (4, 12)* | 36 (26, 46) |
| 140 µg PBDE-47/kg | 76 (56, 93) | 35 (22, 46) | 4 (4, 8)** | 11 (5, 14) | 40 (36, 52) |
| 700 µg PBDE-47/kg | 82 (69, 105) | 42 (28, 50) | 4 (2, 7)* | 8 (4, 10)* | 45 (29, 56) |

The median number of follicles and (Q1, Q3) presented are from 5 sections per ovary for the primordial and primary follicles and from 25 sections per ovary for the secondary, tertiary, and atretic follicles. The control group includes 9 litters; the PTU and PBDE groups (140 µg/kg and 700 µg/kg) include 10 litters each.

*p < 0.05, **p = 0.06, and * = 0.08 by Kruskall-Wallis test, followed by Dunn’s Multiple Comparison Test and Mann-Whitney test.

Figure 1. Individual serum estradiol concentrations (bars indicate means) of F1 female offspring on PND38 after treatment with vehicle or PBDE-47 (140 or 700 µg/kg bw) to F0 dams on GD6. PTU was administered on GD7–PND21.

Figure 2. Individual ovarian aromatase activity (bars indicate means) of F1 female offspring on PND38 after treatment with vehicle or PBDE-47 (140 or 700 µg/kg bw) to F0 dams on GD6. PTU was administered on GD7–PND21.

Female reproductive performance. At approximately 22 weeks of age, 22–24 female F1 offspring from each group were mated daily with untreated males for 14 days or until a sperm-positive vaginal smear was obtained.

On day 21 of gestation, the dams were sacrificed and the uterus was excised. We determined fetal weight and sex, as well as the numbers of implantations, resorptions, and fetuses. The fetuses were examined for external anomalies; all were cleared for skeletal staining by fixation in 5% formalin for 1 week and then rinsing in water for 2 days. After evisceration, they were placed in a diethyl ether/ethanol solution (1:4) for 1 week and then washed with water. The skeletons were stained with an alizarin/100% potassium hydroxide solution, rinsed with water, placed in a benzyl alcohol/glycerol/ethanol (1:2:2) solution until clear, and then stored in glycerol until examination.

Statistical analyses. We performed statistical analyses using GraphPad Prism, Version 3, software (GraphPad Software Inc., San Diego, CA, USA). We considered the litter as the experimental unit. We compared means from the PTU group with those of controls using the unpaired Student’s t-test; means from the PBDE-47 groups were compared by analysis of variance (ANOVA) followed by the Dunnett’s test. Medians from the PTU group were compared with those of controls using the Mann-Whitney test, and those from the PBDE-47 group were analyzed with the Kruskal-Wallis test and Dunn’s Multiple Comparison Test. The ovarian weights of the dams and the organ weights of the offspring on PND38 were analyzed by analysis of covariance (ANCOVA) (SAS, version 9.1; SAS Institute Inc., Cary, NC, USA) using body weight and treatment as covariables, because statistically significant differences in body weights were ascertained for the PTU group compared with the control group.

Results

Body and ovarian weights of dams. At 27 days postparturition, the dams in the PTU group were heavier than those in the control group (p < 0.05). In the 140-µg PBDE-47 group, there was an increase in mean paired ovarian weight (p < 0.01) (Table 1).

Ovarian histology of dams. We detected no histologic abnormalities in the ovaries (n = 4/group) from the dams of the control group or those exposed to 140 µg PBDE-47/kg bw. One of four animals in the 700-µg PBDE-47 group exhibited slight follicular dilatation indicative of cysts. In the ovary from one animal in the PTU group, we observed expanded interstitial spaces, which is compatible with slight edema (not shown).

Body and organ weights of F1 female offspring on PND38. The mean body weight was significantly lower in offspring exposed to...
PTU than in controls on PND38 (Table 2). This is in contrast to PND1, when there was no statistically significant difference for any of the groups in average pup weight (whole litter body weight divided by the number of pups in the litter). Liver weight was significantly lower in both PBDE-47 groups. Paired ovarian weights were reduced in the 140-µg PBDE-47 group and those exposed to PTU. The change in ovarian weight was not associated with histopathologic alterations at the light microscopic level. Qualitative assessment revealed a decrease in tertiary follicles in the PTU and 700 µg PBDE-47 groups.

**Ovarian follicle numbers, ovarian aromatase activity, and serum estradiol concentrations.** We found statistically significant differences in follicle numbers in the PTU and 700-µg PBDE-47 groups: primordial and tertiary follicles were reduced in the PTU group, and reductions in secondary and tertiary follicles occurred in the PBDE-47 group (Table 3). The reduction in growing follicles in the 140-µg PBDE-47 group did not reach statistical significance. The serum estradiol concentrations were reduced in the treatment groups, and were statistically significant in the PTU and 700-µg PBDE-47 groups (Figure 1). Whole ovarian aromatase activity was similar to control in all treatment groups (Figure 2).

**Body and organ weights of female offspring in estrus on PND100.** We observed no differences in body weight or reproductive organ weights in the treatment groups compared with controls. The only statistically significant changes were a reduction in liver weight and an increase in thyroid weight in the PTU group (Table 4).

**Histology of F1 female offspring on PND100.** At the light microscopic level, the histologic findings of the ovary, uterus, and vagina were unremarkable compared with controls. Evaluation of the thyroid glands revealed occasional follicular cyst formation in the 140-µg PBDE-47 and PTU groups, and only mild cyst formation in the 700-µg PBDE-47 group. There were multiple areas of degenerated follicular epithelium in the 140-µg PBDE-47 group and slight attenuation of the follicular epithelium in the PTU group. Morphometric analyses resulted in compatible results, as thyroid point counting yielded a statistically significant decrease in the number of points overlying the follicular epithelium in the PTU group, as well as an increased number over the colloid in the PTU and 140-µg PBDE-47 groups (Figure 3). The number of points overlying the epithelium in the 140-µg PBDE-47 group was decreased and, although statistical analysis indicated exposure-related differences, the post hoc test for this end point but did not reach statistical significance.

Electron microscopy also revealed detachment of thyroid follicular epithelial cells, which can be found in the colloid (Figure 4).

Ultrastructural analysis of the control ovaries revealed the presence of intact stromal cells with a small number of vesicular structures and a few vacuoles containing small electron dense granular masses (Figure 5A). The stromal cells of the ovary from the PTU-treated group (Figure 5B) have an accumulation of vesicular structures with homogeneously dense granular material. The ovaries from animals treated with 140 (Figure 5C) and 700 µg PBDE-47/kg (Figure 5D) showed an accumulation of vesicular structures with homogeneously dense granular material in the cytoplasm of the stromal cells, which appear to fuse together to form large vacuoles.

**Reproductive performance and teratology.** We found no differences between the control group and any of the treatment groups in terms of the number of live fetuses, fetal weight, or resorption rate. The mean number of implantation sites per dam was significantly increased in the PTU and 140-µg PBDE-47 groups and slightly decreased in the 700-µg PBDE-47 group. A significant increase in thyroid weight was noted in the PTU group. Evaluation of the thyroid glands revealed multiple areas of degeneration in the PTU and 140-µg PBDE-47 groups, and only occasional follicular cyst formation in the control group. The follicular architecture consists of a single layer of thyrocytes, with adjacent cells in close contact (arrowheads) surrounding a colloid-filled lumen (*). Microvilli are present on the luminal side (arrows) of the polarized thyrocytes. In sections from animals exposed to PTU (B), 140 µg PBDE-47/kg (C), and 700 µg PBDE-47/kg (D), the follicles have an irregular, nontypical shape. Numerous follicular cells are detached (+) from the basal membrane, and the follicle cells are swollen and dilated (#). Magnification = 5,000×; bar = 1 µm.
increased in the PTU group. The sex ratio of the F2 animals in the 700-µg PBDE-47 group was approximately one-half that of the control group (Table 5). However, comparison of the altered sex ratio with controls from two different historical experiments (n = 24 and 43 litters) revealed no differences.

Evaluation of the F2 offspring from the F1 female offspring mated with untreated males revealed two anomalies in one pup (F2) from the 700-µg PBDE-47 group: a shortened mandible accompanied by fused tympanic bone.

Discussion

PBDEs have been shown to alter thyroid hormone homeostasis, and interactions have been reported between thyroid hormones and the reproductive system. We evaluated the influence of early developmental exposure to PBDE-47 on the female reproductive system.

The increase in ovarian weight observed in the dams at the low PBDE dose (140 µg/kg) was not observed in the group exposed to the higher dose of PBDE-47 (700 µg/kg). Characterization of the dose–response relationship was not possible in this study; however, there are reports in the literature describing nonmonotonic dose–response curves after exposures to hormonally active compounds (Almstrup et al. 2002; Muto et al. 2002; Putz et al. 2001; vom Saal et al. 1997), indicating that qualitative differences can exist between low and high doses. Possible mechanisms include differential binding affinities of compounds to steroid receptor isoforms, competition between endogenous and exogenous ligands, and the formation of mixed ligand–receptor complexes versus homodimers and their respective recruitment of activators or repressors of gene transcription. In addition, nongenomic effects of steroids may modify genomic actions yielding nonmonotonic responses (Rochette-Egly 2003).

On PND38, we found a reduction in body weight and paired ovarian weight in the group exposed to PTU, which is in accordance with another study in rats after oral PTU treatment from PND21 to PND40 (Marty et al. 1999) and one after exposure from PND1 to PND40 (Dijkstra et al. 1996). In the present study, we also found reduced ovarian weight in the 140-µg PBDE-47 group. This reduction is in contrast with the increase in ovarian weight in the mothers from the same treatment group and it was also not associated with histologic abnormalities at the light microscopic level.

We observed statistically significant alterations in folliculogenesis in offspring in the PTU group and the 700 µg PBDE-47 group. PTU exposure resulted in a 50% reduction in primordial follicles, posing the possibility that these animals may experience early sexual senescence. (The disadvantages of early menopause in humans include a shorter reproductive life span; also, the onset of menopause can be associated with a variety of health problems such as osteoporosis.) The tertiary follicles were also reduced following exposure to PTU. Modifications to folliculogenesis have been reported in other studies after exposure to PTU (Chan and Ng 1995; Dijkstra et al. 1996), and these data are in agreement with a study performed with ammonium perchlorate (AP), which is used to treat hyperthyroidism (Rochette-Egly 2003). The tertiary follicles were also reduced following exposure to PTU. Modifications to folliculogenesis have been reported in other studies after exposure to PTU (Chan and Ng 1995; Dijkstra et al. 1996), and these data are in agreement with a study performed with ammonium perchlorate (AP), which is used to treat hyperthyroidism (Rochette-Egly 2003). The tertiary follicles were also reduced following exposure to PTU. Modifications to folliculogenesis have been reported in other studies after exposure to PTU (Chan and Ng 1995; Dijkstra et al. 1996), and these data are in agreement with a study performed with ammonium perchlorate (AP), which is used to treat hyperthyroidism (Rochette-Egly 2003).

Table 5. Fertility indices of F1 female offspring after mating with nonexposed males.

| Group          | Total no. of implantation sites | Total no. of live fetuses | Implantation sites per dam (mean ± SD) | Fetuses per dam (mean ± SD) | Mean fetal weight (g) (mean ± SD) | Resorption rate (%) | Sex ratio (median, Q1, Q3) |
|----------------|---------------------------------|---------------------------|----------------------------------------|----------------------------|----------------------------------|---------------------|---------------------------|
| Control (n = 11) | 133                             | 125                       | 12.1 ± 0.8                             | 11.4 ± 0.8                 | 4.7 ± 0.2                        | 6                   | 1.20 (0.84, 2.38)         |
| PTU (n = 17)    | 223                             | 200                       | 13.1 ± 1.2*                            | 11.8 ± 1.8                 | 4.7 ± 0.2                        | 10                  | 1.17 (0.71, 1.45)         |
| 140 µg PBDE-47 (n = 19) | 243                             | 228                       | 12.8 ± 1.6                             | 12.0 ± 2.0                 | 4.7 ± 0.5                        | 6                   | 0.86 (0.53, 1.42)         |
| 700 µg PBDE-47 (n = 17) | 212                             | 202                       | 12.5 ± 1.1                             | 11.9 ± 1.4                 | 4.5 ± 0.3                        | 5                   | 0.65 (0.45, 1.12)*        |

n = number of litters. Sex ratio is calculated as male/female. Analyzed by ANOVA followed by Dunnett’s test; unpaired t-test; Kruskal-Wallis test followed by Dunn’s; and Mann-Whitney test.

*p < 0.05.
preantral follicles, as well as total antral follicles, following in utero and lactational exposures to high doses of AP, whereas lower doses affected only the large antral follicles.

In the present study, exposure to PBDE-47 did not affect primordial follicles as in the PTU group; however, a similar effect on larger follicles was demonstrated, as secondary and tertiary follicles were decreased in the 700-µg PBDE-47 group. The lower number of larger follicles in the PTU and PBDE-47 groups was not due to an increased rate in atresia of this follicle stage. PCB mixtures (Baldridge et al. 2003; Lilienthal et al. 2006) and high doses of PBDE-99 (Lilienthal et al. 2006) have also been shown to alter folliculogenesis. Ovarian folliculogenesis was not altered, however, during adulthood following prenatal and lactational exposure to low doses of PBDE-99 (Talsness et al. 2005).

In the studies by Baldridge et al. (2003, 2004), T4 supplementation was able to ameliorate the effects on the smaller sized follicles, suggesting that thyroid hormone disruption plays a role in the disturbed folliculogenesis of the less mature follicles.

Antral follicles are a major source of estrogen, and we observed a concomitant reduction in circulating estradiol concentrations after exposure to either PTU or PBDE-47. In a study following in utero exposure to 1 or 10 mg PBDE-99/kg, Lilienthal et al. (2006) reported effects on circulating estradiol concentrations. They observed statistically nonsignificant reductions in circulating estradiol concentrations, which were more pronounced in the lower dose group than the higher one, in F1 females on PND21. Estradiol concentrations in males, however, were decreased in a statistically significant fashion on PNDs 21 and 160 (Lilienthal et al. 2006). Evidence suggests that some PBDE congeners and metabolites may affect CYP19 activity. Cantón et al. (2005) reported that aromatase (CYP19) activity evaluated in the H295R human adrenocortical carcinoma cell line showed inhibition of aromatase activity with 6CH3O-PBDE-47. However, in the present study, we found no changes in whole-ovary aromatase activity associated with reduced circulating estradiol concentrations. Some explanations for the decreased estradiol concentrations include the lower number of antral follicles, altered gonadotropins affecting follicular maturation, and the expression of steroidogenic enzymes other than aromatase or an increase in estrogen metabolism.

Tonic levels of FSH play a role in early follicular growth, and rising FSH levels are involved in further follicular maturation when expression of steroidogenic enzymes increases dramatically. The alterations in folliculogenesis and steroidogenesis indicate disruption along the hypothalamic–pituitary–ovarian axis.

At necropsy during estrus on approximately PND100 of the present study, the reduction in body weight the PTU-exposed offspring observed on PND38 was no longer apparent. Persistent adverse effects on the thyroid gland after exposure to PTU was indicated by increased weight of the thyroid gland associated with histologic changes, indicating occasional follicular cyst formation and attenuation of the follicular epithelium. Although no change in thyroid weight was apparent in the animals exposed to 140 µg PBDE-47/kg, we observed similar histologic findings. Thyroid point counting, performed by an observer unaware of the histologist’s findings, supported the histologic observations in the PTU group: the proportion of points over the epithelium were decreased, and the number over the colloid were increased in this group. The same pattern was observed in the morphometric analysis of the 140 µg PBDE-47 group; however, the decrease in the epithelium did not reach statistical significance. Developmental exposure to either PTU or PBDE-47 led to changes in the thyroid tissue, which were apparent in adulthood.

At adulthood, the increased amount of vesicles observed in the ovaries from the offspring exposed to PTU or PBDE-47 exhibited ultrastructural changes similar to those we reported following exposure to PBDE-99 (Talsness et al. 2005). This observation is compatible with nonspecific or uncontrolled synthesis of steroid products.

The mean increased number of implantation sites in the PTU-exposed F1 females was accompanied by a higher resorption rate, resulting in a similar mean number of fetuses compared with the control group. The higher resorption rate in this group is not considered to be biologically significant because resorption rates of ≤10% are within normal limits for our historical controls of this rat strain. We observed a statistically significant alteration in the secondary sex ratio in favor of females after mating of the F1 females from the 700-µg PBDE-47 group. The biological relevance of this finding is low because analyses performed comparing the 700-µg PBDE-47 group with our historical controls indicated no statistically significant differences.

The anomaly observed in one F1 offspring following exposure of the F0 dam to 700 µg PBDE-47/kg on GD6 is one that we have never observed in our rat strain after examining >10,100 fetuses (historical data). In a similar experiment, we also observed skeletal anomalies in offspring from two different mothers exposed in utero and via lactation to 300 µg PBDE-99/kg, which had also never been documented in our laboratory (Talsness et al. 2005). Incomplete bone deposition was observed in the left and right parietal and frontal bones of the skull in one offspring.

Also, in a pup from another litter of the same group, only a portion of the first sacral and caudal vertebrae were absent.

Possible causes for these anomalies may be either spontaneous or substance related. The fact that we have not observed these anomalies in Wistar rats speaks against a spontaneous cause, although it cannot be ruled out. In addition, the F0 generation was treated with a very low dose of PBDE-47, and the anomaly was seen in the F2 generation; this suggests that the anomaly is not directly substance induced, as the congener was probably not present at the time of mating. It is theoretically possible that it is related to an epigenetic modification of the DNA.

### Summary and Conclusions

Data from the present study indicate endocrine disruption following in utero and lactational exposure to environmentally relevant doses of PBDE-47, as the doses used in this study would result in an approximate maternal body burden within or just above the range of concentrations reported in human adipose tissue samples collected in New York City (Johnson-Restrepo et al. 2005). We observed alterations in ovarian folliculogenesis, circulating estradiol concentrations, and persistent changes to both the ovaries and thyroid glands. Legislation banning the marketing and use of the pentaBDE and octaBDE commercial formulations in the European Union and some states of the United States has already occurred, and decaBDE has been banned in Sweden and in Washington and Maine; however, these lipophilic compounds are highly persistent in the environment, and release and exposure will continue for an extended period of time. The European Union is considering a vote to discontinue the planned ban of the decaBDE formulations. The continued use of decaBDE is of concern because of direct exposure to the compound and its debromination to lower brominated congeners. In addition, exposure to the myriad of chemicals in the environment yields the possibility of additive, synergistic, or antagonistic effects. The developing embryo, fetus, and neonate are highly susceptible to exogenous insults, and the magnitude of the current maternal body burden of PBDEs may be of concern for human health.

### References

Almstrup K, Fernandez MF, Petersen JH, Oles N, Skakkebaek NE, Leffers H. 2002. Dual effects of phytoestrogens result in U-shaped dose–response curves. Environ Health Perspect 110:743–748.

Baldridge MG, Stahl RL, Gerstenberger SL, Tripoli V, Hutz RJ. 2003. Modulation of ovarian follicle maturation in Long-Evans rats exposed to polychlorinated biphenyls (PCBs) in utero and lactationally. Reprod Toxicol 17:967–973.

Baldridge MG, Stahl RL, Gerstenberger SL, Tripoli V, Hutz RJ. 2004. In utero and lactational exposure of Long-Evans rats to...
to ammonium perchlorate (AP) disrupts ovarian follicle maturation. Reprod Toxicol 19:155–161.

Boon JP, Lewis WE, Tijmes-A-Choy MR, Altschin CR, Lasz RJ, De Bover J, et al. 2002. Levels of polybrominated diphenyl ether (PBDE) flame retardants in animals representing different trophic levels of the North Sea food web. Environ Sci Technol 36:4025–4032.

Borgeest C, Symonds D, Mayer LP, Hoyer PB, Flaws JA. 2002. Developmental exposure to low dose polybrominated diphenyl ether (PBDE 99) and PCB. Toxicology 220:104–116.

Chen G, Bunce NJ. 2003. Polybrominated diphenyl ethers as Ah receptor agonists and antagonists. Toxicol Sci 76:310–320.

Bruce NJ. 2001. Synthesis of polybrominated diphenyl ether (PBDE) flame retardants in animals representing different trophic levels of the North Sea food web. Environ Sci Technol 36:4025–4032.

Chervenick S, Warner D, Motomura H, Kiger J, Flickinger T, et al. 2003. High body burdens of 2,2´,4,4´-tetrabromodiphenyl ether (TBBDE) in California women. Environ Sci Technol 37:2979–2980.

Cone DO, Kohn D, Linn MW, Luhovyy BL, Kettenbach V, et al. 2003. Developmental effects of PBDEs and polychlorinated biphenyls (PCBs) on thyroid hormone and vitamin A metabolism in the mouse. Toxicol Sci 57:473–478.

Cantón RF, Sanderzon JT, Letcher RJ, Bergmann A, van den Berg M. 2005. Inhibition and induction of aromatase (CYP19) activity by brominated flame retardants in H295R human adrenocortical carcinoma cells. Toxicol Sci 80:447–455.

Cestelli R, Faaxs O, Schlimp M, Lichtensteiger W. 2006. Gene expression and estrogen sensitivity in rat uterus after developmentally exposed polybrominated diphenyl ether (PBDE 99) and PCB. Toxicology 220:104–116.

Chen WY, Ng TB. 1995. Effect of hypothyroidism induced by propylthiouracil and thiooeugenol on male and female reproductive systems of neonatal mice. J Exp Zool 273:160–169.

Chen G, Bunce NJ. 2003. Polybrominated diphenyl ethers as Ah receptor agonists and antagonists. Toxicol Sci 76:310–320.

Chen G, Konstantinov AD, Chittagom BN, Moye EM, Botis NC, Bruce NJ. 2001. Synthesis of polybrominated diphenyl ethers and their capacity to induce CYP1A by the Ah receptor. Toxicol Sci 68:359–376.

Cruz-Orive LM, Weibel ER. 1990. Recent stereological methods for cell biology, a brief survey. Am J Physiol 258:1148–1156.

Devine PJ, Payne CM, McCuskey MK, Hoyer PB. 2000. Ultrastructural evaluation of oocytes during atresia in rat ovarian follicles. Biol Reprod 63:1245–1252.

Dijkstra G, de Rooij DG, de Jong FH, van den Hurk R. 1996. The effect of hypothyroidism on ovarian follicular development, granulosa cell proliferation and peripheral hormone levels in the prepubertal rat. Eur J Endocrinol 134:649–654.

Gupta AG, P controvasga A. 2000. The hypothalamic-pituitary-thyroid-axis and the female reproductive system. Ann NY Acad Sci 905:65–76.

Duffy K, Poles G, Driscoll L. 2005. Brief postnatal PBDE exposure alters liver aging and the choline/NMN modulation of attention in rats. Toxicol Sci 89:172–180.

Ellis-Hutchings RG, Cherr GN, Hanna LA, Keen CL. 2006. Regulation of the uterine response to estrogen by thyroid-axis and the female reproductive system. Ann NY Acad Sci 109:399–407.

Ferguson G, Harner T, et al. 2005. Is house dust the missing exposure pathway for PBDEs? An analysis of the urban fate and human exposure to PBDEs. Environ Sci Technol 39:5121–5130.

Fiesel RP, Anderlini P. 1996. Developmental alterations in mice during a critical phase of neonatal brain maturation. Reprod Toxicol 10:771–774.

Fiesel RP, Anderlini P. 1996. Developmental alterations in mice during a critical phase of neonatal brain maturation. Reprod Toxicol 10:771–774.

Fiesel RP, Anderlini P. 1996. Developmental alterations in mice during a critical phase of neonatal brain maturation. Reprod Toxicol 10:771–774.

Fiesel RP, Anderlini P. 1996. Developmental alterations in mice during a critical phase of neonatal brain maturation. Reprod Toxicol 10:771–774.

Fiesel RP, Anderlini P. 1996. Developmental alterations in mice during a critical phase of neonatal brain maturation. Reprod Toxicol 10:771–774.

Fiesel RP, Anderlini P. 1996. Developmental alterations in mice during a critical phase of neonatal brain maturation. Reprod Toxicol 10:771–774.

Fiesel RP, Anderlini P. 1996. Developmental alterations in mice during a critical phase of neonatal brain maturation. Reprod Toxicol 10:771–774.

Fiesel RP, Anderlini P. 1996. Developmental alterations in mice during a critical phase of neonatal brain maturation. Reprod Toxicol 10:771–774.