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MATERNAL EXPOSURE TO LOW DOSE 2,2´,4,4´,5 PENTABROMO DIPHENYL ETHER (PBDE 99) IMPAIRS MALE REPRODUCTIVE PERFORMANCE IN ADULT RAT OFFSPRING

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Introduction

Among the large variety of EDCs occurring in the environment, special concern over the polybrominated diphenyl ethers (PBDE) have broadened in the last 5 years. Although levels of DDT, dioxins and PCBs are declining in developed countries, an exponential increase on PBDE levels in human tissue and wild life has been detected[1]. Exposure to PBDE mixtures or single congeners has resulted in a mixed induction of CYP450-dependent enzymes, showing increased activity of EROD and PROD in livers of mice and rats. In addition, genotoxicity has been observed in recombination assays[2], and neurotoxicity has been reported in mice exposed during development [3;4]. Acute and sub-chronic exposures of adult mice to a PBDE mixture cause dose-dependent reductions in serum concentrations of total thyroxin (TT4), and stress-induced elevations in plasma corticosterone [5]. Further, some hydroxylated metabolites of PBDE congeners exhibit a higher potency *in vivo* than T4 in competitive binding to human transthyretin (TTR), the transport protein mediating transfer of thyroid hormones across the placenta and into the brain.

Despite the broad spectrum of PBDE-related effects, no data on sexual development and reproductive performance have been reported. There is concern regarding male reproductive health and environmental pollutants. Decreases in sperm concentration/volume and increases in testicular cancer over the past 50 year provides a clue that such a decline in human male reproductive health might be associated with environmental factors [6;7]. Chemicals that are able to alter the hormonal homeostasis during critical periods of development (pre- or early postnatal) may induce several reproductive adverse effects. Although in animal studies, alterations of sexual development (sperm quality, fertility index and sexual behavior) have been demonstrated to be a sensitive end points for EDCs [8-12], effects of PBDEs on male reproductive performance are unknown.

Since EDCs possess variable dose-related effects, assessment of low dose exposures are important to obtain a complete picture for human risk assessment. For that purpose, we evaluated the effects of *in utero* exposure to a low dose of PBDE 99 on the male reproductive system in the adult rat. The low dose was calculated to be approximately 100- and 500-fold higher than human breast milk concentration reported for women in industrialized countries.

Methods and Materials

Animals and treatment: Using a dose equation previously described by our group [13], Wistar dams (n=10) were treated by gavage on gestation day 6 with a single dose of 60 or 300 µg PBDE 99/kg body weight or peanut oil (control). A reference control group composed of dams treated with

0.5% of the goitrogen, 6n-propyl-2- thiouracil (PTU), in drinking water (about 940 µg PTU / kg b.w./d) from GD 7 to 21 was included. Dams were allowed to deliver and after weaning, the offspring were housed by gender (n=5 per cage) until adulthood. Twenty males, representative from all litters, were killed by decapitation on PND 140 and thymus, liver, spleen, testis, epididymis, ventral prostate and seminal vesicle were removed and weighed. **Spermatid number and sperm count:** Testis (without tunica albuginea) and epididymis were minced and homogenized in 10 mL 0.9% NaCl containing 0.5% triton X-100 at medium speed in an IKA-RW 15 tissuemizer (Janke and Kunkel, Staufen in Breisgau, Germany) for one minute. The number of homogenization-resistant spermatids and sperm were counted in a hemocytometer (Buerker). Daily sperm production was calculated dividing the number of homogenization-resistant spermatids by 6.1 to convert to daily sperm production [14]. **Testosterone and LH levels:** After decapitation, trunk blood was collected and allowed to clot on an ice bath (4°C) for 2 hours. Serum was collected via centrifugation (2500 rpm for 15 min) of clotted samples and stored at –20°C for later analyses. Serum testosterone and LH were measured using the enzyme immunoassay (ELISA) kit purchased from DRG diagnostics – GmbH, Germany.

Results and Discussion

Absolute and relative body weights, spermatogram and hormone levels from F1 offspring exposed *in utero* to PBDE 99 are depicted in Table 1. No sign of maternal toxicity was observed during pregnancy (data not shown). At adulthood, despite similar body weights, changes in both absolute and relatives testes, epididymis and spleen weights (both absolute and relative) were seen, which are indicative of male fertility impairment and immunotoxicity. Moreover, the dose dependent decrease in daily sperm production and decrease in sperm number observed in PBDE- and PTU exposed animals support this notion (Table 1). Although it has been postulated that postnatal exposure to PTU (which induces a transient neonatal hypothyroidism) causes testis enlargement, a increase in sperm production and augmentation of the Sertoli cell population [15], we observed that gestational PTU-exposure permanently impaired male reproductive fertility in F1 offspring (Table 1). Since we observed a severe hypothyroidism in both PTU and PBDE-treated dams at the beginning of lactation (data not shown), gestational hypothyroidism may cause a functional disturbance, probably in Sertoli cell number of the offspring which persists until adulthood. Moreover, the normal levels of testosterone and LH from PTU-and PBDE-treated males support this notion. In order to clarify these questions, a qualitative and quantitative assessment of germ cell population and Sertoli cell number are under way.

The evidence of low dose effects has brought a new challenge for toxicology. The observation of several responses to chemical/physical agent exposure below the no observed adverse effect level (NOAEL) and lowest observed adverse effect level (LOAEL) demonstrate the need to establish new concepts for human risk assessment. Although a linear curve has been assumed for dose-response relationship, experimental data has provided sufficient evidence of non-linearity at dose ranges below to the NOAEL [16]. For example, hormesis, a dose-response relationship phenomenon characterized by low-dose stimulation and high-dose inhibition, has been observed in experimental studies [16] and there are several examples in the literature where low dose effects following exposure to environmental active compounds occur [17]. On this report, we are the first to demonstrate that *in utero* exposure to a low dose of PBDE 99 affects male fertility. Our finding using an animal model corroborates the epidemiological evidence that human exposure to low levels of environmental pollutants is related to the postulated decline in human male reproductive

health. It is noteworthy that the two single doses (60 and 300µg/kg) used in our experiment are well below the NOAEL for penta-BDE (2 mg/kg/d for 90 days)[18]. Although in our study design we can not characterized the dose-response relationships for the parameters investigated, fundamental concepts of linearity and threshold should be carefully revised at dose levels under the NOAEL and LOAEL.

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Table 1: Absolute and relative (% body weight) organ weights, hormone levels, sperm number and daily sperm production in adult offspring (PND 140) exposed pre- and postnatally (via milk) to PBDE 99.

Relative organ weights were compared using Mann Whitney *U* test. The other parameters were analyzed using Student t-test. Values are mean ± standard deviation and significance was confirmed when * $p<0.05$.

Organ weight	Control N=12	PTU N=12	PBDE 60µg/kg N=12	PBDE 300µg/kg N=12
Body Weight (g)	311.7 ± 28.8	335.9 ± 34.5	320.5 ± 20.5	334.9 ± 29.9
Spleen (g)	0.55 ± 0.05	0.63 ± 0.10 *	0.60 ± 0.07 *	0.60 ± 0.07 *
(%)	0.17 ± 0.01	0.19 ± 0.02	0.19 ± 0.02 *	0.18 ± 0.02
Testis (g)	1.57 ± 0.23	1.47 ± 0.34	1.58 ± 0.11	1.53 ± 0.13
(%)	0.51 ± 0.07	0.44 ± 0.11 *	0.49 ± 0.04	0.46 ± 0.05 *
Epididymis (g)	0.58 ± 0.07	0.55 ± 0.08	0.56 ± 0.03	0.58 ± 0.07
(%)	0.19 ± 0.02	0.17 ± 0.02 *	0.18 ± 0.01 *	0.17 ± 0.02 *
Spermatid (10^6) number	266.2 ± 26.0	198.6 ± 34.8 *	182.8 ± 26.4 *	175.0 ± 19.8 *
Daily sperm production ($x10^6$)	43.6 ± 4.3	32.6 ± 5.7 *	30.0 ± 4.3 *	28.7 ± 3.2 *
Sperm number (10^6)	189.6 ± 40.5	143.2 ± 26.6 *	134.7 ± 22.2 *	156.3 ± 28.0 *
LH (ng/mL)	10.8 ± 7.8	12.4 ± 4.6	14.4 ± 7.4	10.3 ± 3.8
Testosterone (ng/mL)	8.7 ± 4.2	10.0 ± 4.9	7.5 ± 3.4	8.4 ± 4.7

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