Effects of Decabrominated Diphenyl Ether on Testis Function in Male Offspring Mice Following *In Utero* Exposure

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Introduction

Polybrominated diphenyl ethers (PBDEs) has raised the focus of interest because of its known neurotoxic and endocrine disrupting (Darnerud et al. 2007; Rice et al. 2007; Viberg et al. 2007). Few studies had shown that PBDEs effect on male fertility (Kuriyama et al., 2005; Tseng et al. 2006). Decabrominated diphenyl ether (PBDE 209) accounts for approximately 82% of total PBDE production (BSEF, 2003). Numerous studies have found that PBDE 209 as a consequence, PBDEs have been found everywhere in the environment, and is universally considered to be an ubiquitous environmental contaminant (Kalantzi et al. 2009; Shaw et al. 2008). In Indiana area, U.S.A. found a high association between maternal and fetal cord blood PBDE concentrations (Mazdai et al. 2003). The china study shows the similar result, maternal samples were correlated with fetal samples which were obtained from the umbilical cord after delivery, the relative distribution between fetal and maternal serum was similar for the PBDEs (Bi et al. 2006). These studies showed that PBDEs might be able to enter the fetus through the placenta. One recent study had found inverse correlations between PBDEs in serum and sperm concentration and testis size in human (Akutsu et al. 2008). If PBDE 209 is transferred as efficiently as those PBDEs through the placenta, it might affect fetal development. Very few studies have been published about reproductive effect of PBDE 209 (Tseng et al. 2006). In this study, we investigate the testis effects of prenatal exposure to PBDE 209 from offspring using laboratory animals.

Materials and Methods

Animals and treatment: Pregnant CD-1 mice were randomly divided into four groups and oral gavage treatment that received vehicle (corn oil) or 10, 500 or 1500 mg/kg mg PBDE 209/kg body weight from gestation day 0 (GD 0) to GD17. On postnatal day (PND) 21, three male pups in each litter were randomly selected and the other pups were sacrificed. On PND 71, male offspring mice were anesthetized by CO_2 and the reproductive organ were removed and weighed. The evaluate testis functions experimental items included (1) daily sperm production (DSP), (2) testes index (TI), and (3)

DNA content analysis of testis cells. *Assessment of TI and DSP:* Testicular length and width were measured with digital form calipers. TI was calculated for each mouse (Glass and Lynch 1981). The right testis was placed in 1 mL Tris buffer (10^{-3} M, pH 7) and homogenized. The testicular sample was diluted ten times with Tris buffer and evaluated with a Buerker hemocytometer (Brand GmbH, Wertheim, Germany) for the sperm heads. DSP and DSP per gram testis (DSP/g) were calculated (Blazak et al. 1985). ***FCM DNA content analysis of testicular cells:*** The procedure proposed by Spanò et al. (1996) was used to prepare testis monocellular suspensions. FCM was used to measure the fluorescence intensity of DNA content of the testicular cells. PI fluorescent emissions were monitored using a 620 nm band-pass filter. Based on the DNA content, main germ cell peaks can be classified into four categories: (1) mature haploid, (2) immature haploid, (3) diploid, and (4) tetraploid. The region between the diploid and tetraploid peaks, called the S-phase, is comprised of cells that actively synthesize DNA (Spanò et al. 1996). The relative proportions of haploid, diploid, S-phase and tetraploid cell types were calculated. *Statistical analysis.* Results were expressed as mean ± standard error of the mean (SEM). All statistical comparisons were performed using one-way analysis of variance (ANOVA), followed by the Tukey-Kramer honestly significant difference (HSD). For the analyses, a p-value of < 0.05 was considered significant.

Results and Discussion

Body and reproductive organ weights of male offspring mice: The body and organ weights in male offspring of dams administered corn oil (control) or 10, 500, 1500 mg PDBE 209/kg/daily during GD 0 to GD 17 are shown in Table 1. There were no treatment-related effects on body weight or weights of testis, epididymis, and seminal vesicles among the three exposed groups and the controls on PND 71 (Table 1). Although the values did not reach significance, the testis weights in all treatment groups were dose-dependently decreased. ***TI measurement and assessment of DSP:*** The number of homogenization-resistant spermatids are presented in Table 2. No effect of *in utero* PBDE 209 exposure was observed on DSP/testis and DSP/g testis on PND 71. There were also no significant differences in TI among the four groups (Table 2). However, there were decreased DSP and TI in 1500mg/kg-treated. *FCM DNA content analysis of testis cells.* Figure 1 shows the frequency distribution histograms of PI-stained testicular cells as determined by DNA flow cytometry. There were no significant changes in testis DNA content of the four cell types, including mature haploid (elongated spermatids), immature haploid (round spermatids), diploid, and tetraploid as well as that of the S-phase cell. The reason that gestational exposure to PBDE 209 did not affect testis function due to limited sample sizes.

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Table 1. Reproductive organ weights of CD-1 male mice *in utero* exposed to decabrominated diphenyl ether (PBDE 209)

	PBDE 209 (mg/kg BW/day)			
	0 (n=5)	10 (n=5)	500 (n=5)	1500 (n=5)
Initial BW (g)	9.2 ± 0.8	8.6 ± 0.5	8.1 ± 0.7	9.3 ± 0.5
Final BW (g)	33.5 ± 1.0	34.1 ± 0.4	33.24 ± 1.1	34.12 ± 0.7
Testis	217.6 ± 7.3	209.9 ± 9.1	206.9 ± 4.9	200.3 ± 4.1
Epididymis	71.8 ± 2.3	73.5 ± 3.2	73.1 ± 2.2	70.8 ± 3.7
Seminal Vesicles	176.8 ± 8.86	192.4 ± 10.8	184.9 ± 11.8	163.1 ± 6.6

Data represents mean ± SEM

Table 2. Mean daily sperm production (10^6/day), and efficiency of sperm production (10^6/g testis/day) and testis index (TI) in male offspring following prenatal exposure to 10, 500, 1500 mg/kg of PBDE 209 or corn oil on postnatal day (PND) 71. Error bars represent the SEM.

	PBDE 209 (mg/kg BW/day)			
	0 (n=5)	10 (n=5)	500 (n=5)	1500 (n=5)
DSP(10^6/day)	45.6±1.5	44.5±2.6	44.0±1.8	42.2±3.5
DSP/g testis (10^6/g testis/day)	44.8±1.5	43.8±0.9	44.5±0.9	43.1±2.8
TI (R) (cm)	1.10±0.04	1.15±0.01	1.11±0.02	1.08±0.04
TI (L) (cm)	1.19±0.03	1.19±0.02	1.17±0.02	1.15±0.04

Data represents mean ± SEM

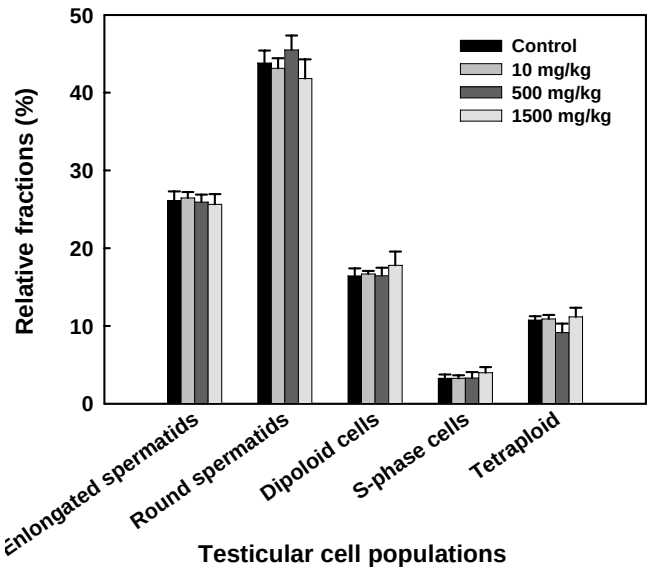

Fig.1. Relative fractions (means ± SEM) of the various testis subpopulations, calculated as % of the histogram area between mice prenatally exposed to PBDE 209 (10, 500, and 1500 mg/kg) and unexposed controls.