EFFECTS OF PRENATAL EXPOSURE OF DECABROMINATED DIPHENYL ETHER (PBDE 209) ON REPRODUCTIVE SYSTEM IN MALE MICE

Hsu P-C[1], Tseng L-H[1,2], Lee C-W[1]

[1]Department of Safety Health and Environmental Engineering, National Kaohsiung First University of Science and Technology, Kaohsiung, TAIWAN; [2]Department of Occupational Safety and Hygiene, Tajen University, Pingtung, TAIWAN

Introduction

Decabrominated diphenyl ether (PBDE 209) accounts for approximately 82% of total PBDE production[1]. It main application is in high impact polystyrene used for electronic enclosures and flame retard upholstery fabric. It is additive flame retardants so that they are not bound in polymer products and can leak into the environment. They were recently detected worldwide in human milk, blood, indoor environment and the food at the highest levels. Exposure to PBDEs, however, mainly comes through the consumption of foodstuffs such as in freshwater fish, marine species, chickens, eggs and mixed meat products[2,3]. Some of the brominated flame retardants (BFRs) might elicit thyroid hormones and affect reproductive in vivo. Recent investigation found that female mice fed a single low dose of pentabrominated diphenyl ether (PBDE 99), produced offspring with decreased sperm counts and neuro-behavior deficiencies[4]. It is, however, not known if PBDE 209 has a similar effect on male reproductive system. For this purpose, the objective of this study is to determine whether treatment of prenatal BFRs, PBDE 209, affects the sperm and testicular functions in mice.

Materials and Methods

Pregnant CD-1 mice (five dams per each group) were treated once daily by gavage with PBDE 209 at doses of 0, 10, 500, and 1500 mg/kg BW from gestation day 0 (GD 0) to GD17. Selection of given dose was based on our previous study[5]. PBDE 209 was dissolved in corn oil. Control mice received vehicle only. On postnatal day (PND) 21, three male pups in each litter were selective according to similar mean body weight. At PND 71, the animals were anesthetized by CO_2 and the reproductive organ were removed and weighed. Relative organ weight was calculated by calculating the ratio between organ weight and body weight. The testis samples were used for evaluating spermatid number. Epididymal perm count, motility, sperm chromatin structure analysis (SCSA), mitochondrial membrane potential (MMP), and reactive oxygen species (ROS) generation were measured. Testis Index was expressed as (testis length × testis width) / body mass. Computer-assisted sperm analysis (CASA) was obtained for velocity indices with a Hamilton Thorn Research motility analyzer (version HTMIVOS Specification, Beverly, MA, USA) at a temperature of 37 ^{o}C. CASA was gained for sperm velocity parameters: curvilinear velocity (VSL, μm/s), average path velocity (VAP, μm/s), straight-line velocity (VSL, μm/s),

amplitude of lateral head displacement (ALH, mm), and beat cross frequency (BCF, Hz). SCSA, MMP, and ROS generation were analyzed by flow cytometry (FCM).

Results and Discussion

For body weights of offspring, repeated-measures analysis showed neither a treatment-by-age interaction during lactation (Table 1 and Fig.1). There were significant differences in right testis index, relative right testis and total testis weights of mice prenatally exposed to 1500mg/kg of PBDE 209 than in controls (Table 1). There were no significant differences in sperm count, motility, VCL, VAP, VSL, and ALH between mice prenatally exposed to PBDE 209 and the controls (Table 2). Cauda epididymal sperm was analyzed by FCM SCSA to investigate whether there was any changes in sperm chromatin DNA integrity. There were significant differences in XαT and COMPαT in the 10 mg/kg and 1500 mg/kg groups compare with the control or 500mg/kg groups (Table 3). The group exposed to 1500 mg/kg was found to have a significantly fewer high MMP sperm than control groups. To evaluate sperm ROS generation, we measured H_2O_2 and $O_2^{-\bullet}$ in the groups treated with PBDE 209. While we found a significant increase in the generation of sperm $O_2^{-\bullet}$ in the 500 mg/kg/day group compared to that of the control group. (Table 3). It the first report to demonstrate that *in utero* exposure to PBDE 209 might affect male reproductive system. A recent study found that individual fetal cord blood concentrations did not differ from maternal concentrations in serum in Indiana[6]. Because fetal cord blood levels are similar to levels found in breast milk, that study indicated that PBDEs might enter the fetus through the placenta[6]. If PBDEs are transferred as efficiently through breast milk as they are through the placenta, both fetuses and infants are being exposed to potentially high levels of harmful chemicals at critical developmental stages. Our study investigate that PBDE 209 might through the placenta to impair male reproductive system in mice. Future investigations should be carried out on the effects of different types of PBDE congeners on causing sperm dysfunctions, and which pathways are responsible for this effect.

Acknowledgements

This study was supported by the National Science Council under grant NSC 93-2621-Z-327-001.

References

1. BSEF. 2003. http://www.bsef-site.com/docs/ BFR_vols_2001.doc
2. Sjödin A, Hagmar L, Klasson-Wehler E, Björk J, Bergman Å. *Environ Health Perspect* 2000;108:1035.
3. Domingo JL. *J Chromatogr A* 2004;1054:321.
4. Kuriyama SN, Talsness CE, Grote K, Chahoud I. *Environ Health Perspect* 2005;113:149
5. Tseng LH, Lee CW, Pan MH, Tsai SS, Li MH, Chen JR, Lay JJ, Hsu PC. *Toxicology* (In Press).
6. Mazdai A, Dodder NG, Abernathy MP, Hites RA, Bigsby RM. *Environ Health Perspect* 2003;111:1249.

Table 1. Reproductive organ weights of male mice prenatally exposed to PBDE 209.

	PBDE 209 (mg/kg BW/day)			
	0	10	500	1500
Body weight (g)	41.28 ± 2.00	44.52 ± 1.79	43.64 ± 1.32	41.18 ± 1.67
Absolute testis parameters				
Right testis weight (g)	0.115±0.003	0.108±0.003	0.108±0.003	0.105±0.003
Left testis weight (g)	0.103±0.002	0.102±0.002	0.099±0.002	0.096±0.002
Total testis weight (g)	0.22±0.01	0.21±0.01	0.21±0.01	0.20±0.01
Right testis lenth (mm)	7.73±0.09	7.77±0.09	7.78±0.09	7.64±0.09
Right testis width (mm)	5.21±0.09	5.11±0.09	5.03±0.09	5.08±0.09
Left testis lenth (mm)	7.39±0.09	7.53±0.09	7.44±0.09	7.40±0.09
Left testis width (mm)	4.96±0.07	5.05±0.07	4.96±0.07	4.99±0.07
Right testis index	1.24±0.02	1.19±0.02	1.17±0.02	1.12±0.03*
Left testis index	1.10±0.03	1.12±0.03	1.09±0.02	1.07±0.03
Epididymis weight (mg)	0.072±0.002	0.074±0.002	0.073±0.002	0.071±0.002
Cauda epididymis weight (mg)	24.0±0.9	25.4±0.9	24.5±0.9	23.4±0.9
Seminal vesicles weight (mg)	0.18±0.009	0.19±0.009	0.18±0.009	0.16±0.009
Relative testis ratio parameters				
Right testis weight (mg/g BW)	0.34±0.008	0.32±0.008	0.33±0.008	0.31±0.008*
Left testis weight (mg/g BW)	0.31±0.007	0.30±0.007	0.30±0.007	0.28±0.007
Total testis weight (mg/g BW)	0.65±0.014	0.62±0.014	0.62±0.014	0.59±0.014*
Epididymis (mg/g BW)	0.215±0.006	0.215±0.006	0.22±0.006	0.208±0.006
cauda Epididymis (mg/g BW)	0.072±0.003	0.075±0.003	0.074±0.003	0.069±0.003
Seminal vesicles (mg/g BW)	0.53±0.03	0.56±0.03	0.55±0.03	0.48±0.03

Data represents mean ± S.E.M. * $P<0.05$ as compare with control group.

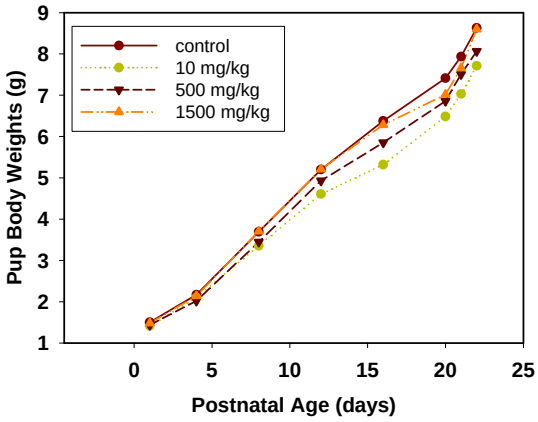

Fig. 1. Lack of prenatal exposure of PBDE 209 on body weights. Data was calculated using repeated-measures analysis of variance.

Table 2. Sperm quality of male mice prenatally exposed to PBDE 209.

	Treatment of PBDE 209 (mg/kg)			
Parameters	Control	10	500	1500
Sperm count (10^6/ml)	15.4±0.39	13.9±0.36	16.0±0.28	16.2±0.36
Sperm motile (%)	72.5±13.5	74.4±10.6	77.0±6.5	70.8±12.9
Velocity of motion				
VCL (μm/s)[a]	149.4±39.2	154.3±23.3	155.5±22.5	149.3±35.1
VAP (μm/s)[b]	80.9±21.2	84.4±13.6	83.3±11.4	82.1±21.2
VSL (μm/s)[c]	59.4±15.4	65.4±10.8	62.7±8.9	63.6±18.3
ALH (μm)[d]	6.5±1.1	7.2±1.1	7.4±1.0	7.0±1.4

All data was expressed in means ± S.D. * $p < 0.05$ as compared with control group. [a] VCL: curvilinear velocity. [b]VAP: Average path velocity. [c]VSL: straight line velocity. [d] ALH: amplitude of lateral head displacement.

Table 3. Epididymal sperm chromatin structure assay (SCSA), mitochondrial membrane potential (MMP) and reactive oxygen species (ROS) generation of male mice prenatally exposed to PBDE 209.

	Treatment of PBDE 209 (mg/kg)			
Parameters	Control	10	500	1500
MMP (%)[a]	77.4 ± 2.9	73.2 ± 2.6	76.2 ± 3.0	65.6 ± 2.7*
Sperm H_2O_2 generation [b]	38.2 ± 2.30	46.72 ± 2.54	43.14 ± 3.43	45.82 ± 2.80
Sperm $O2^{\cdot}$ - generation [b]	230.6 ± 7.29	242.0 ± 9.75	287.04 ± 15.17*	234.34 ±9.45
COMPαT (%)	4.24 ± 0.58	7.37 ± 0.78*[#]	4.87 ± 0.77	6.66 ± 0.78*
XαT	202.0 ± 1.78	214.8 ± 1.64*[#]	204.5 ± 1.67	212.7 ± 1.67*[#]
SDαT	36.5 ± 2.04	39.1 ± 2.43	38.4 ± 3.54	34.5 ± 1.68

All data was expressed in means ± S.E.M. * $p < 0.05$ as compared with control group. [#] $p < 0.05$ as compared with 500 mg/kg group. [a]Values are percentages of sperm population with high mitochondrial membrane potential as assessed by flow cytometric (FCM) analysis with the JC-1 probe. Hight of Mitochondrial membrane potential (MMP) (%). [b] fluorescence intensity.