DEVELOPMENTAL AND SPERM FUNCTION IN CD-1 MICE FOLLOWING BREAST MILK EXPOSURE TO THE DECABROMODIPHENYL ETHER

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

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

Abstract

Breast milk is the ideal nutrient for the newborn, but unfortunately also a route of excretion for some toxic substances. Decabrominated diphenyl ether (PBDE 209) which has recently been shown to increase in human milk. However, the reproductive effects of PBDE 209 through breast-feeding are still unclear. The aim of this study is to assess whether maternal exposure of PBDE 209 via breast milk affects sperm function in offspring mice. Female mice were treated via oral gavage with 10, 100, 500, and 1500 mg/kg/day of PBDE 209 in lactation. During the pup's lactational period, developmental landmarks were recorded. On postnatal day 71, the mice were evaluated for their sperm functions. The results showed that there were no significant differences in the landmark of development between control and PBDE 209-treated groups. Further, there were no significant differences observed in the sperm count, sperm chromatin structure assay, sperm mitochondrial membrane potential, and generation of hydrogen peroxide (H_2O_2) and superoxide anion ($O2^{-\bullet}$).

Introduction

The brominated flame retardants (BFRs) were widely used in the worldwide in the early 1970s[1]. Unfortunately, BFRs may leach into the environment when the products are manufactured, used or disposed[2]. They have been found not only in the environment[3,4,5,6], but also in human blood[7] and in breast milk[8,9]. Some of the brominated flame retardants (BFRs) might elicit thyroid hormones and affect reproductive in vivo[10,11]. Decabrominated diphenyl ether (PBDE 209) account for approximately 28% of all the BFR usage globally[12]. Many researches showed that PBDE 209 had already become a new developing environmental pollutant that was recently detected in human milk[13,14]. For this purpose, the objective of this study is to determine whether treatment of maternal BFRs, PBDE 209, via breast milk have adverse effects the sperm functions in offspring mice.

Materials and Methods

Animals and treatment. The pregnant CD-1 mice were randomly divided into five groups of seven mice each and housed individually. On delivery, the dams were gavaged with 10, 100, 500, and 1500 mg/kg/day of PBDE 209

during lactation period. PBDE 209 was dissolved in corn oil. Control mice received vehicle only. During the pup's lactation period, developmental landmarks including pinnae detachment, body hair fuzz appearance, incisor eruption, ear opening, and eyes opening day were recorded. 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. Epididymal sperm count, motility, sperm chromatin structure analysis (SCSA), mitochondrial membrane potential (MMP), and reactive oxygen species (ROS) generation were measured. SCSA, MMP, and ROS generation were analyzed by flow cytometry (FCM). *Data analysis.* Data were expressed as means ± standard deviation. All statistical analyses were performed on JMP 5.0. For the analysis of male offspring, the litter was considered the experimental unit. A p value of < 0.05 was considered statistically significant.

Results and Discussion

Development landmarks. We observed no significant developmental delays in any of development landmarks, including days of pinnae detachment, body hair fuzz appearance, incisor eruption, ear opening, and eyes opening day in control and PBDE 209-treated pups (Table 1). *Body and organ weights.* Through breast-feeding, the data showed no significant differences in mean body weight between maternal exposure of PBDE 209 exposed and control groups. There were no significant differences in absolute and relative weights of testis, epididymis, cauda epididymis among the five groups exposed to PBDE 209 exposed and the controls (Table 2). *Sperm function.* There were no significant differences in sperm count. However, the 10 mg/kg/day group was significant differences reduced sperm motility compare with the control (Table 3). Cauda epididymal sperm was analyzed by FCM SCSA to investigate whether there were any changes in sperm chromatin DNA integrity, MMP and ROS generation. To evaluate sperm ROS generation, we measured H_2O_2 and $O2^{-\bullet}$. There were no significant differences in αT (%), MMP and ROS generation than control groups (Fig. 1). The αT (%) is the % of sperm with abnormal chromatin DNA structure assessed by flow cytometric analysis with the acridine orange dye. The MMP values represent percentages of sperm population with high mitochondrial membrane potential as assessed by flow cytometric analysis with the 5,5',6,6'-tetrachloro-1,1',3,3-tetraethyl benzimidazolyl carbocyanine iodide (JC-1) probe. The ROS values represent fluorescence intensity using a fluorescent probe, 2',7'-dichlorofluorescin diacetate (DCFH-DA) and hydroethidine, individually.

Published information on the physiology of PBDE 209 excretion into breast milk is generally limited to pharmaceuticals, however, these results suggested that lactation exposure to PBDE via the breast milk does not act as a developmental and reproductive toxicant in the mice.

Acknowledgements

This study was supported by the National Science Council under grant NSC 97-2221-E-127-003-MY3

References

1. WHO, 1994; Environmental Health Criteria 162.
2. Sjödin A., Carlsson H., Thuresson K., Sjölin S., Bergman Å. and Östman C. *Environ Sci Technol* 2001; 35: 448.
3. Nylund K., Asplund L., Jansson B., Jonsson P., Litzen K. and Sellström U. *Chemosphere* 1992; 24: 1721.
4. Watanabe I., Kashimoto T. and Tatsukawa R. *Chemosphere* 1987; 16: 2389.
5. Sellström U., Jansson B., Kierkegaard A., de Wit C., Odsid T. and Oisson M. *Chemosphere* 1993; 26: 1703.
6. Fangstrom B., Athanasiadou M., Athanassiadis I., Bignert A., Grandjean P., Weihe P. and Bergman Å. *Chemosphere* 2005; 60: 836.
7. Bi X., Qu W., Sheng G., Zhang W., Mai B., Chen D., Yu L. and Fu J. *Environ Pollut* 2006; 144: 1024.
8. Sjödin A., McGahee E.E., III, Focant J.F., Jones R.S., Lapeza C.R., Zhang Y. and Patterson Jr. D.G. *Anal Chem* 2004; 76: 4508.
9. Meironyté D., Norén K. and Bergman Å. *J Toxicol Environ Health A* 1999; 58: 329.
10. Zhou T., Ross D.G., DeVito M.J. and Crofton K.M. *Toxicol Sci* 2001; 61:76.
11. Kuriyama S.N., Talsness C.E., Grote K. and Chahoud I. *Environ Health Perspect* 2005; 113: 149
12. BSEF. 2003. http://www.bsef-site.com/docs/ BFR_vols_2001.doc
13. Chao H.R., Wang S.L., Lee W.J., Wang Y.F. and Päpke O. *Environ Int* 2007; 33: 239.
14. Schecter A.J., Pavuk M., Papke O., Ryan J., Birnbaum L. and Rosen R. *Environ Health Perspect* 2003; 111: 1723.

Table 1. Via the breast milk exposure of PBDE 209 with 10 mg/kg, 100 mg/kg, 500 mg/kg or 1500 mg/kg of PBDE 209 and corn oil on developmental landmarks in offspring.

Parameters	Treatment of PBDE 209 (mg/kg)				
	Control (n = 7)	10 (n = 7)	100 (n = 7)	500 (n = 7)	1500 (n = 7)
Pinna detachment	4.9	5.5	5.0	4.8	4.7
Body hair fuzz appearance	5.2	5.7	5.4	6	5.3
Incisor eruption	9.7	10.2	9.3	9.4	9.3
Eyes opening	16.5	16.5	16.2	17.2	16
Ear opening	15.6	16.7	15.8	15.6	15.6

Table 2. Body and tissue weight between mice via milk exposed to PBDE 209 (10, 100, 500, and 1500 mg/kg) and unexposed controls

Parameters	Treatment of PBDE 209 (mg/kg)				
	Control (n = 7)	10 (n = 7)	100 (n = 7)	500 (n = 7)	1500 (n = 7)
Absolute					
body weight (g)	36.7±2.4	37.2±1.0	37.7±2.8	36.9±4.5	36.7±1.8
Testis (g)	0.21±0.02	0.22±0.01	0.23±0.04	0.22±0.03	0.23±0.03
Epididymis (g)	0.086±0.005	0.084±0.006	0.084±0.006	0.083 ±0.006	0.084 ±0.006
cauda Epididymis (g)	0.030±0.002	0.029±0.005	0.030±0.003	0.029±0.003	0.028±0.003
Relative					
Testis (%)	0.57±0.07	0.58±0.02	0.60±0.10	0.60±0.07	0.63±0.10
Epididymis (%)	0.23±0.02	0.23±0.02	0.22±0.02	0.23±0.01	0.23±0.02
cauda Epididymis (%)	0.081±0.007	0.079±0.013	0.080±0.007	0.080±0.008	0.077±0.009

Data was expressed as means ± S.D.

Table 3. Sperm quality of mice via milk exposed to PBDE 209 (10, 100, 500, and 1500 mg/kg) and unexposed controls

Parameters	Treatment of PBDE 209 (mg/kg)				
	Control (n=7)	10 (n=7)	100 (n=7)	500 (n=7)	1500 (n=7)
Sperm count (10^6/ml)	22.3±2.9	22.9±3.4	22.6±4.1	20.4±7.3	22.3±5.5

All data was expressed as means ± S.D.

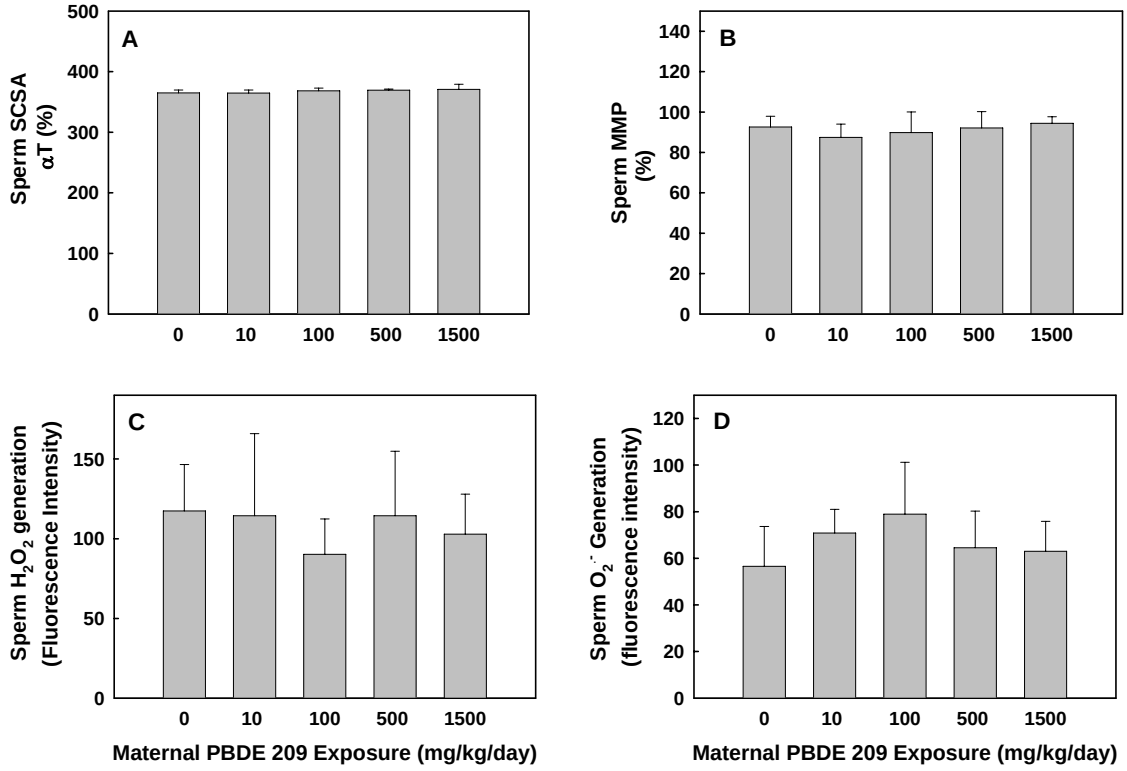

Fig. 1. Lack of effect of via milk exposure to decabrominated diphenyl ether (PBDE 209) and controls on sperm chromatin DNA integrity (A), mitochondrial membrane potential (MMP) (B), generation of reactive oxygen species H_2O_2 (C), and $O2^{-\bullet}$(D). Data are presented as means ± S.D.