EFFECTS OF *IN UTERO* EXPOSURE TO DECABROMINATED DIPHENYL ETHER (PBDE 209) ON DEVELOPMENTAL TOXICITY AND LIVER ENZYME ACTIVITIES IN MALE MICE

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Introduction

Decabrominated diphenyl ether (PBDE 209) is a flame retardant used in polyurethane foam, high impact polystyrene, and textiles. PBDE 209 has been detected worldwide in human milk, blood, indoor environment and the food at a fairly high level [1,2]. It is the second most used brominated flame retardants (BFRs) in constructed materials next to tetrabromobisphenol A (TBBPA). The potential development toxicity of PBDE 209 is not fully investigated. Prenatal developmental toxicities of PBDE 209 in rats were not found under the oral exposure level of 1000 mg /kg/day following fetal evaluations [3]. However, this study did not follow to the adult stages of rats for evaluating development toxicities of PBDE 209. Some of the BFRs might elicit of uridinediphosphate-glucuronosyltransferase (UDGPT) and ethoxy-resorufin-O-deethylase (EROD) *in vivo*[4]. However, little information is conclusive regarding the development toxicology and hepatic enzyme levels in vivo following exposure to PBDE 209. The current study was designed to determine whether *in utero* exposure to PBDE 209 affects the development and hepatic enzyme levels in male mouse offspring.

Materials and Methods

Animals and treatment: Dose selection was based on our previous study[5]. CD-1 dams (five dam per each group) were treated once daily by gavage with PBDE 209 at doses of 0, 10, 500, and 1500 mg/kg BW/day from gestation day 0 (GD 0) to gestation day 17 (GD17). PBDE 209 was dissolved in corn oil. Control mice received vehicle only. *Observations:* Pregnant mice were weight daily in gestation and laction. Pregnancy duration, litter size, sex ratio, and pup weight were determined on postnatal day 0 (PND 0). Developmental landmarks were recorded duration lactaion. On PND 21, three male fetuses were kept in each litter selected by mean body weight. At PND 71, anogenital distances (AGD) were measured using calipers. Dams and male offspring were euthanized at weaning PND21 and PND 71, individually. Body and organ weight were measured. Hepatic enzyme Assays: On PND 71, male mice were sacrificed, livers were perfused *in situ* with ice-cold 0.05 M

Tris–0.15 M KCl buffer (pH 7.4) and weighed. The livers were then homogenized in the same Tris-KCl buffer. The crude homogenate was centrifuged at 10,000 × *g* for 15 min at 4°C. The pellet from the final centrifugation was resuspended in buffer (0.05 M Tris-HCl, 20% glycerol [v/v], 1 mM EDTA; pH 7.4 at 4°C) and stored at -80°C until assayed. Analyses of EROD and UDPGT were based on other previous studies[6-8].

Results and Discussion

Effects on dams and litter size: For body weights of dams, repeated-measures analysis showed that there was no treatment-by-age interaction during gestation and lactation (data not shown). There was no evidence of any treatment-related effects of gestation length, little size, and pup weight following exposure to PBDE 209. However, there was significant difference on sex ratio in the group exposed to 500mg/kg PBDE 209 (Table 1).

Table 1. Effects of prenatal were exposed to PBDE 209 on days 0 to 17 of gestation of maternal and litter characteristics

	PBDE 209 (mg/kg BW/day)			
	0 (n=5)	10 (n=5)	500 (n=5)	1500 (n=5)
Body weight (g)	41.28 ± 2.00	44.52 ± 1.79	43.64 ± 1.32	41.18 ± 1.67
Gestational length	18.20 ± 0.20	18.80 ± 0.20	18.60 ± 0.40	18.60 ± 0.24
Live pups per litter (PND 0)	12.60 ± 0.93	15.20 ± 0.86	14.60 ± 0.68	13.00 ± 0.45
Sex ratio of live fetuses (M/F)	1.1	0.8	0.5*	0.9
Live pup weight at birth (g)	1.50 ± 0.05	1.41 ± 0.12	1.43 ± 0.06	1.48 ± 0.03
Live pup weight at weaning (g)	9.06±0.36	8.57±0.36	8.13±0.36	9.34±0.36
Dam liver weight (g)	3.08 ± 0.18	3.48 ± 0.22	3.34 ± 0.16	3.23 ± 0.24
Thymus (g)[a]	0.032±0.002	0.033±0.002	0.038±0.002	0.043±0.002*
Thymus/body weigh (%)[a]	0.098±0.08	0.095±0.08	0.114±0.10	0.125±0.11*,#

Data represents mean ± S.E.M. * $P<0.05$ as compare with controls. [a]: Data from PND 71 of male offspring.

Body and Organ Weights: There were no significant changes in dam weights of brain, adrenal, kidney, spleen and ovary in any of the treatment groups during GD 0 to GD 17 prenatal dosing period (data not shown). There were also no significant changes in male offspring weighs of brain, adrenal, kidney, spleen and liver. However, there were significant differences in absolute and relative thymus weights between control and exposed groups (Table. 1). No evidence of treatment-related effects was found on maternal and offspring body weights.

Effects on the development of F1 male mice: Eye opening had been observed since PND 15 in each group. Repeated measures ANOVA revealed that there are no significant treatment effects on eye opening (Fig.1) and developmental landmarks between groups (Table. 2). Exposure to PBDE 209 resulted in a decrease of AGD in

male offspring on PND 71 at the high doses of 1500 mg/kg. When normalized to body weight, AGD of male offspring declined significantly at the high doses (Fig. 2).

Table 2. Effect of prenatal exposure to PBDE 209 at 10, 500, 1500 mg/kg on developmental landmarks

	PBDE 209 (mg/kg BW/day)			
	0 (n=5)	10 (n=5)	500 (n=5)	1500 (n=5)
Pinna detachment	5.40 ± 0.24	4.80 ± 0.20	5.00 ± 0.00	5.20 ± 0.20
Incisor eruption	10.60 ± 0.40	11.0 ± 0.45	11.6 ± 0.40	10.8 ± 0.37
Hair appearance	6.20 ± 0.2	5.80 ± 0.49	6.40 ± 0.24	6.00 ± 0.32

Data represents mean ± S.E.M.

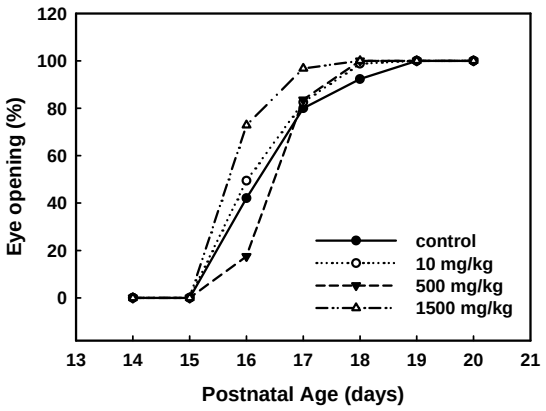

Fig. 1. There was a lack of effect of PBDE 209 on eye opening, determined as the percentage of all pups within a litter with at least one eye open at each age.

Liver Enzymes: This is the first study to investigate developmental toxicity and hepatic enzymes simultaneously following exposure to PBDE209. Liver wet weights of male mice were no different among different treatment groups, whereas liver S9 protein content was decreased of male mice in highest dose group compared to control animals (data not shown). Liver S9 EROD (associated with CYP1A1) was only slightly increased of male mice in highest dose group compared to control animals. Although maternal exposure to the PBDE 209 resulted in significant increases in hepatic EROD and UDPGT activity in male offspring at the exposure level of 1500mg/kg (Figs. 3), our findings suggest that liver S9 4-nitorphenol UDPGT activity was slightly higher in male mice *in utero* exposed to in lowest and highest dose groups. Overall, there are not strongly significant EROD or UDPGT activities induction in 10 week old male mice exposed to PBDE 209 *in utero* maternal oral exposure to PBDE 209 induces developmental toxicity and hepatic enzymes in mice.

Acknowledgements

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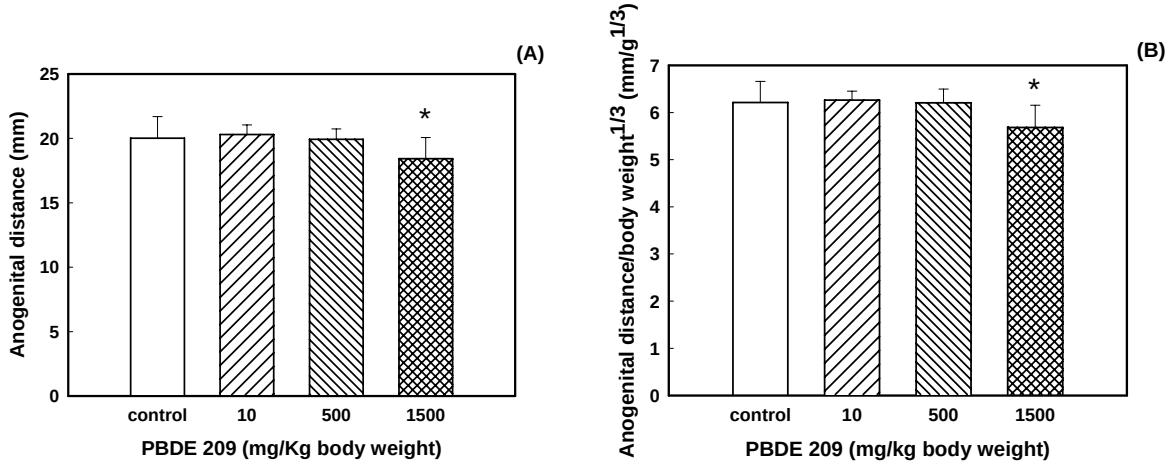

Fig. 2. AGD (A) and AGD/body weight$^{1/3}$ (B) at PND71 in F_1 male mice *in utero* (from GD0 to GD17) exposure to decabrominated diphenyl ether (PBDE 209) daily. Data was expressed in means ± S.E.M

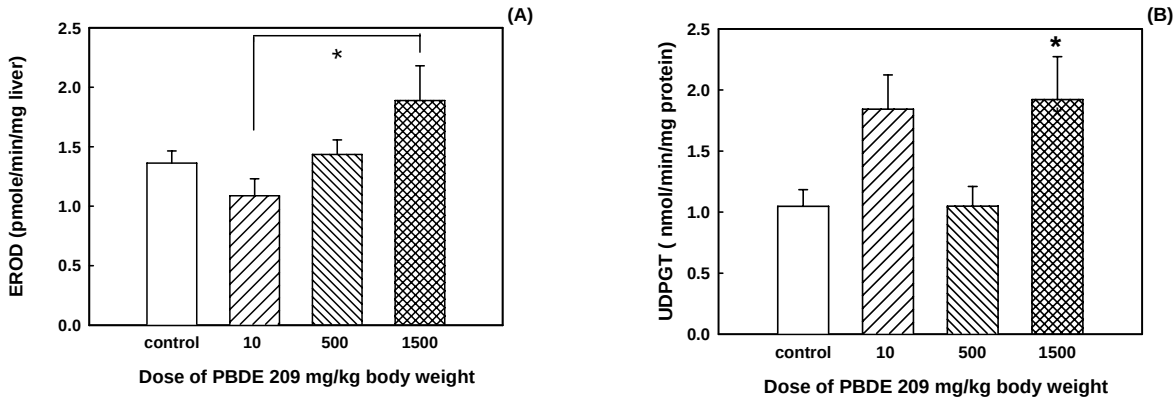

Fig. 3. PBDE 209 induced EROD and UDPGT activity in PND 71 male offspring mice in utero (from GD 0 to GD 17) exposure to decabrominated diphenyl ether (PBDE 209). Data was expressed in means ± S.E.M

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