NEONATAL EXPOSURE TO DECABROMINATED DIPHENYL ETHER (PBDE 209) IMPAIRED MORPHOLOGY OF HEPATOCYTES BUT DID NOT AFFECT HEPATIC ENZYME ACTIVITIES AND THYROID HORMONE HOMEOSTASIS IN THE ADULT MOUSE

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Abstract
The brominated flame retardants (BFRs) decabrominated diphenyl ether (PBDE 209) is found in the environment, e.g., in sediments and organisms, in food items, human blood samples and breast milk. Many studies have shown some of the BFRs act as endocrine disruptors via alterations in thyroid hormone homeostasis. The purpose of this study is to clarify whether postnatal exposure to PBDE 209 is entirely responsible for the perturbation in thyroid hormone homeostasis and induced hepatic enzymes activities. Weanling CD-1 mice were gavaged with 10, 100, 500 and 1500 mg/kg PBDE 209 or corn oil for controls per day. After 50 days of treatment, the animals were sacrificed then the serum and liver were collected. No significant effects on S9 7-ethoxyresorufin-O-deethylase (EROD), 7-pentoxyresorufin O-depentylase (PROD) activity, 4-nitorphenol uridinediphosphate-glucuronosyltransferase (UDPGT), serum total triiodothyronine (T3), and thyroxine (T4) in any of the treatment groups. However, histopathological examination revealed that PBDE 209 might cause acute cell swelling of hepatocytes in all treated groups and show dose-related. Our finding suggested that oral exposure to PBDE 209 might not disrupt thyroid hormone homeostasis and not induce hepatic enzymes activities in male mice.

Introduction
Decabrominated diphenyl ether (PBDE 209) is a flame retardant that mostly used in high-impact polystyrene plastic used to produce housings for televisions, computers, stereos and other small electronics as well as in upholstery textiles.[1] PBDE 209 has been detected worldwide in human milk, blood, indoor environment and the food at a fairly high level.[2] PBDEs show an even closer structural relationship to serum thyroxine (T4) than PCBs, allowing them to bind competitively to thyroid hormone transfer proteins and act as endocrine disrupters.[3] Some of the BFRs might elicit of uridinediphosphate-glucuronosyltransferase (UDGPT) and ethoxy-resorufin-O-deethylase (EROD) *in vivo.* Reduced serum T4 levels accompanied by induced cytochrome P450 activities have been reported in rats prenatally exposed to DE-71 (tetra- and penta-bromodiphenyl ether mixtures).[4] One previous study reported that the effects of penta BDE on T4 and the thyroid gland could be principally caused by the induction of liver enzymes.[5] However, little is known the second most used brominated flame retardant (BFRs) affects the thyroid hormone homeostasis and hepatic enzyme activity postnatal exposure to PBDE 209. The aim of this study was to determine whether postnatal exposure to PBDE 209 affects the thyroid hormone homeostasis and hepatic enzyme levels in male mice.

Materials and Methods
A total number of 45 male pups were divided into one control and four exposure groups. Animals were daily gavaged with 10, 100, 500 or 1500 mg/kg of PBDE 209 dissolved in corn oil from PND 21 to PND 70. All mice were anesthetized by CO_2 on PND 71 and the organ were removed and weighed. A part of liver and thyroid gland tissues were taken for histopathology assays. The blood samples were taken by heart puncture for thyroid hormones analysis. Liver samples were collected for hepatic enzyme activity assay. Serum total T3 and T4 levels were measured using Coat-a-Count Total T3 and Total T4 radioimmunoassay kits (Diagnostic Products Corporation, Los Angeles, CA, USA) which were adapted for use with mouse according to the manufacturer's instructions. Liver tissues were perfused *in situ* with ice-cold 0.05 M Tris-0.15 M KCl buffer (pH 7.4), excised, blotted on tissue paper, and weighed. The livers were then homogenized in the same Tris-KCl buffer. The crude homogenate was centrifuged at 10,000 x g for 15 min at 4 ℃ and the supernatant (S9) was conserved at

–80°C.until assayed. EROD and PROD in the liver S9 suspension was used as a measurement of cytochrome P450 1A and P450 2B activity and was determined by a modification of the method of Pohl and Fouts[6], as previously described in Li et al.[7] UDPGT activities in the liver S9 suspension toward 4-nitrophenol were determined by a modification of the method of Watanaba et al.[8] as described in Seo et al.[9] Protein content was determined by the Bradford method using bovine serum albumin as a standard.[10]

Results and Discussion

Body and organ weights: We did not find any significant differences in absolute and relative weights of body, liver, kidney, adrenal glands, and spleen in any of the male mice treated with PBDE 209 as compared to the controls (data not shown). *Liver Enzymes:* Liver S9 EROD (associated with CYP1A1), PROD (associated with CYP2B1) and 4-nitrophenol UDPGT activities were not significantly increased in PBDE 209-treated mice (Fig. 1). *Thyroid hormone levels:* There were no significant changes in serum total T3 and total T4 in any of the PBDE 209-treated groups (Fig. 2). *Histopathology of liver:* The treatment groups were found to have acute accompanied associated with pressure occlusion of hepatic sinusoids. This change was prominent and dose dependent. In the 10 and 100 mg/kg groups, the degenerative change was mainly concentrated on the peripheral areas of hepatic lobules (Fig. 3). In the 500mg/kg and 1500 mg/kg groups, the lobular hepatocytes were found to be diffusely swollen with or without vacuolar degeneration. The morphology of liver was normal in control group. A study has shown that prenatal exposure to tetra-penta BDE congeners resulted in a decrease in serum thyroid levels in both adults and pups.[11]. There are only a very limited number of studies on the effects of PBDE 209 on hepatic enzyme activities. Carlson[5] found no hepatic enzyme induction in rats after 14-day exposure to a PBDE 209. In addition, Zhou et al.[12] reported that no significant induction in UDPGT and EROD in weanling rats following a 4-day exposure to a commercial PBDE 209 mixture and suggested there could be species difference to hepatic enzyme inducing ability exposed to PBDE 209. It seems that mice might have lower EROD induction compared to rats. Our findings are consistent with previous short-term exposure studies. PBDE 209 was poorly absorbed, rapidly eliminated and marginally distributed to adipose tissue.[13, 14] Especially, PBDE 209 has a molecular weight of 959 and is not readily absorbed by the gastrointestinal tract via passive diffusion, thus oral absorption was only between 1 – 10 % of the PBDE 209 dose in rats.[13, 14] Therefore, the lack of effects of PBDE 209 obtained from the present study may be due to very limited absorption of this fully brominated PBDE 209 congener. Our result has offered another demonstrate that postnatal exposure to PBDE 209 might not cause the thyroid hormone disruption and hepatic enzyme levels in male mice.

Acknowledgements

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References

1. Hardy ML, *Chemosphere* 2002; 46:717.
2. Darnerud PO, Eriksen GS, Jóhannesson T, Larsen PB, Viluksela M. *Environ. Health Perspect* 2001;109: 49.
3. McDonald T A. *Chemosphere* 2002; 46:745.
4. Zhou T, Taylor MM, DeVito MJ, Crofton KM. *Toxicol Sci* 2002; 66:105.
5. Carlson GP. *Toxicol Lett* 1980; 5:19.
6. Pohl RJ, Fouts JR, 1980. *Anal Biochem* 107; 150.
7. Li M-H, Zhao Y-D, Hansen LG. *Bull Environ Contam Toxicol* 1994; 35:973.
8. Watanaba HK, Hoskind B, Ho IK. *Biochem Pharmacol* 1986; 35:455.
9. Seo BY, Li MH, Hansen LG, Moore RW, Peterson RE, Schantz SL *Toxicol Lett* 1995; 78:253.
10. Bradford MM. *Anal Biochem* 1976; 72:248.
11. Darnerud PO. *Environ Int* 2003; 29:41.
12. Zhou T, Ross DG, DeVito MJ, Crofton KM. *Toxicol Sci* 2001; 61:76.
13. El Dareer SM, Kalin JR, Tillery K F, Hill DL, *J Toxicol Environ Health* 1987; 22:405.
14. Mörck A, Hakk H, Örn U, Klasson W. E, *Drug Metab Dispos* 2003; 31:900.

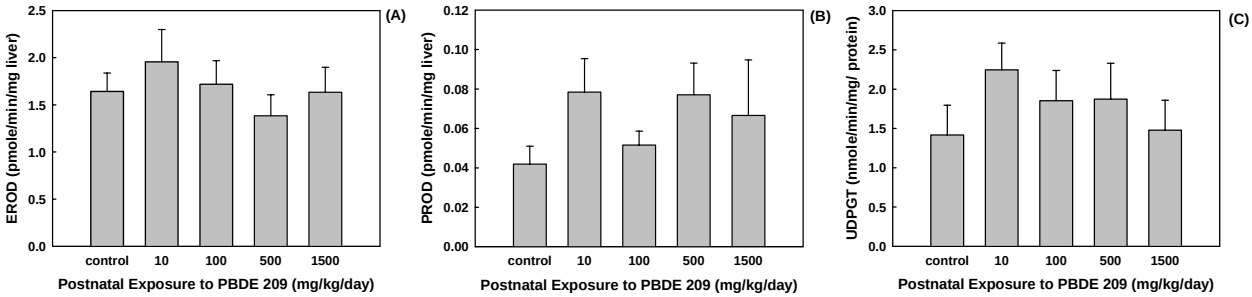

Fig 1. Lack of effect of postnatal exposure to 2,2',3,3',4,4',5,5',6,6'- decabrominated diphenyl ether (10, 100, 500, and 1500 mg/kg) and unexposed controls on liver microsomal enzyme activity, uridinediphosphate-glucuronosyltransferase (UDGPT) activity (A); 7-pentoxyresorufin O-dependentylase (PROD) activity (B) and S9 4-nitorphenol 7-Ethyoxyresorufin O-deethylase (EROD) activity (C). Data was presented as group means; Error bars represent the SEM.

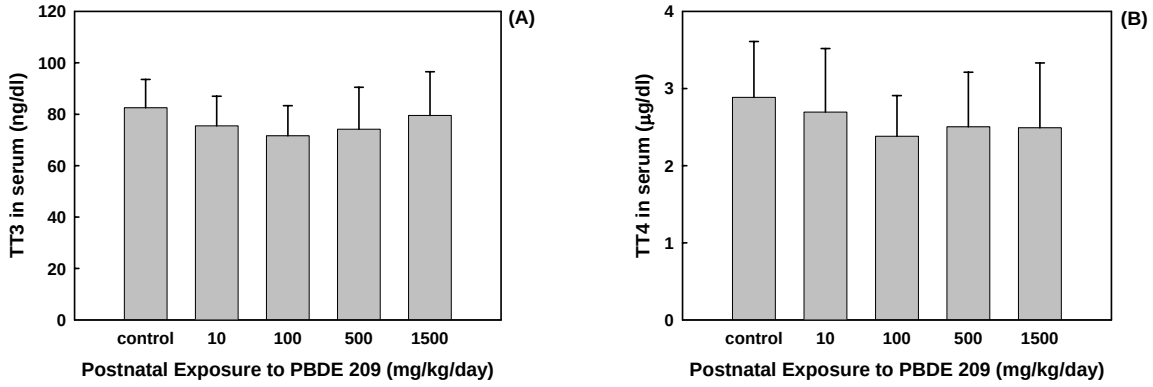

Fig. 2. Lack of effect of serum total triiodothyronine (T3) (A) and total thyroxine (T4) (B) levels in male mice following postnatal exposure to 10, 100, 500, 1500 mg/kg of PBDE 209 or corn oil on postnatal day 21 (PND 21) during PND 70. Data was presented as group means; Error bars represent the SEM.

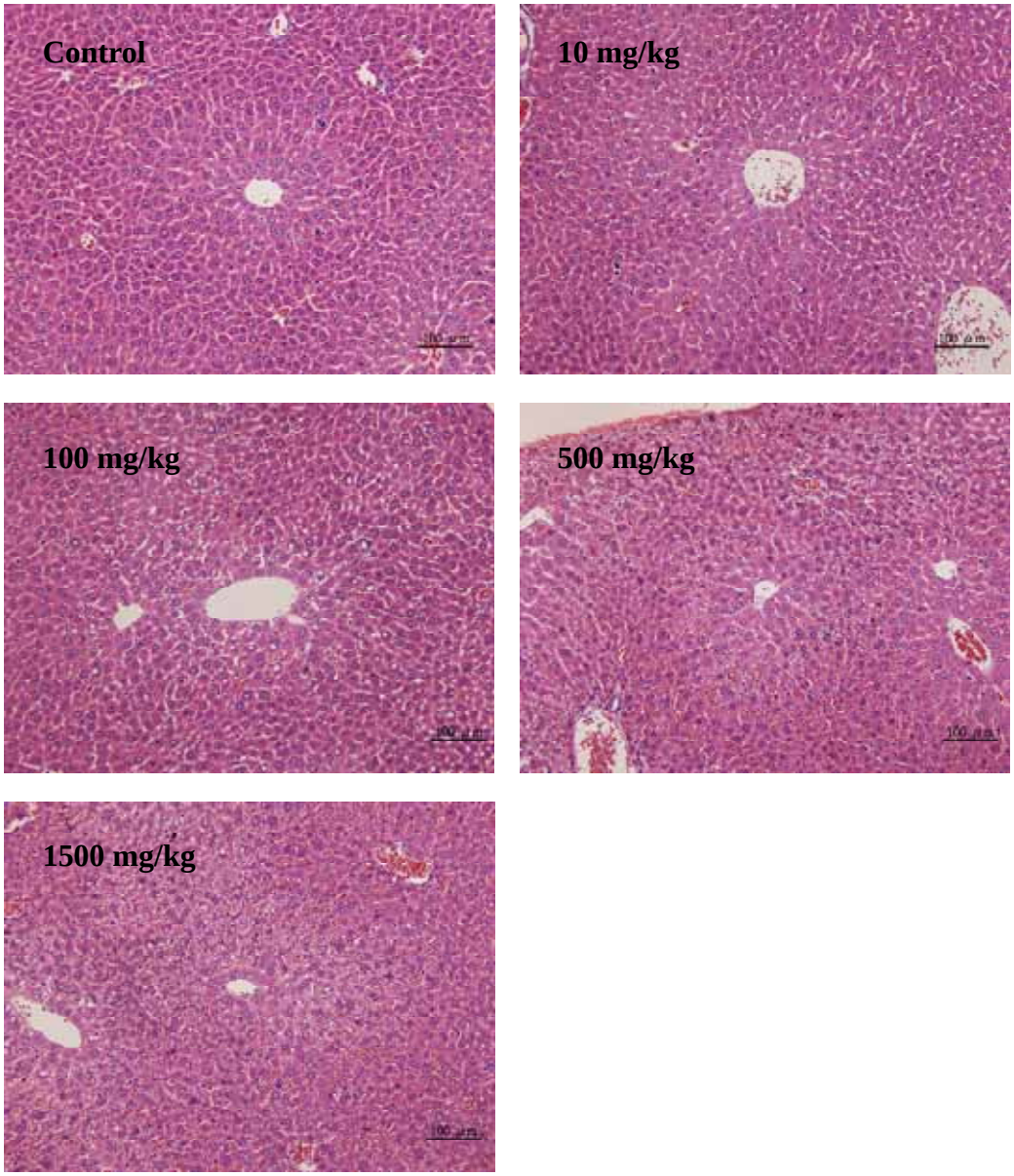

Fig 3. Normal architecture of liver was found in a male mouse of the control group. Acute cell swelling of hepatocytes accompanied by occlusion of blood sinusoids was found mainly in the peripheral zone of liver lobules on the 10 and 100 mg PBDE 209/kg treated mice. The hepatocytes shown diffuse cell swelling without any particular distribution pattern in case of 500mg PBDE 209/kg and 1500mg PBDE 209/kg treated mice and show dose-related.