# Spontaneous Behavior and Learning and Memory of the Adult Mice Offspring after Gestational and Lactation Exposure to the Decabrominated diphenyl ether

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## Introduction

Researches have found that exposure to polybrominated diphenyl ethers (PBDEs) in animals can cause toxic effects on neuronal behavior in the animals' offspring. Single exposure to PBDEs in the neonate results in motor impairments (Branchi et al. 2002; Eriksson et al. 2006; Viberg et al. 2004, 2006) and learning deficits in the Morris water maze test (Viberg et al. 2003, 2006). Similarly, both single exposure (Viberg et al. 2003, 2008) and chronic exposure in the neonatal period (Rice et al. 2007) to decabrominated diphenyl ether (PBDE 209) have been reported to show developmental neurotoxicity. Further, maternal exposure to PBDEs has been demonstrated a contamination occurs in the fetal cord blood (Mazdai et al. 2003; Bi et al. 2006), where 30% of breast milk samples are found containing PBDE 209, and the concentrations are as high as 8 ng/g lipid weight (Schecter et al. 2003). Thus, PBDE 209 might be able to enter the fetus through the placenta and breast-feed. Most of the previous studies report the data of exposures to PBDE 209 in neonatal or postnatal period, but few studies have been performed to evaluate the effects of PBDE 209 exposure in gestation and lactation on neuronal development. Therefore, this study was aimed at clarifying the effects of different processes of exposures to PBDE 209 on the function of neuronal behavior in mice.

## Materials and Methods

***Animals and treatments*:** In females, the day of vaginal plug was considered gestation day (GD) 0. Twenty-four pregnant CD-1 mice were used and treated with daily oral gavage of PBDE 209 (0, 10, 500 or 1500 mg/kg) from GD 0 to postnatal day 21 (PND 21). The control group received sham treatments with vehicle, corn oil. The number (10-12 pups) and ration of both sexes in each litter were adjusted on PND 4. Spontaneous behavior and learning and memory were tested in the open field and Morris water maze test on PND 70 and PND 86-90, respectively. All the observations were taken between 20:00 and 04:00 h. ***Open-Field Test:*** This test was conducted for 10 min in an automated

device (40 cm × 40 cm × 25 cm; Auto-Track/Opto-Varimex-4 System, Columbus, USA) for measuring distance traveled, vertical counts, and center vertical counts. ***Morris Water Maze Test:*** This test was performed in a black circular pool (100 cm diameter × 50 cm height) filled with water at 25±1℃. The pool was divided into four quadrants. A hidden platform (9 cm diameter) was set at the middle of the northeast quadrant of the pool and was submerged 1 cm below the surface of the water. The ability of the mice to locate the platform was observed in the consecutive 5 days, 5 trials each day. Prior to the first trial, the mice were placed on the platform for 30s, then, the mice were put on the south position facing the wall of pool and were allowed to locate the platform in 60s (learning trail). If the mice failed to find the platform within 60 s, they were replaced on the platform to go through the procedure again. Probe test was immediately carried out on day 5 at the end of the $20^{th}$ training trial, and the platform was removed. Each mouse was tested to find the location of the platform for 60 s. The time spent in the northeast (target) quadrant was recorded. Thereafter, the platform was removed to a new place, the center of the southwest quadrant, for reversal trials (re-learning trail), and the same procedures with the before were taken again. ***Statistical analysis:*** Statistics analysis was performed by using JMP 4.0. Behavioral data were analyzed using ANOVA and followed by Tukey post hoc test. The data are expressed as the means ± SEM.

**Results and Discussion**

No differences concerning dams' body weight on GD 0, gestational length, number of pups per litters, sex ratio, and average weights for male and female of offspring in weaning (PND 21) were observed between the control and PBDE 209-treated groups (data not shown). Maternal exposure to PBDE 209 (0, 10, 500, 1500 mg/kg) from GD 0 to PND 21 did not affect the distance traveled, vertical counts and center vertical counts of the offspring male mice in the open field test (Table 1). Also, no changes in the percentage of time spent in the target quadrant in the probe trial were found after the PBDE 209 treatment (Table 2). No differences in the latency to find the platform were observed in the re-learning in adult offspring mice between PBDE 209-treated and control groups (figure 1), which may indicate neither learning nor memory impairments after maternal exposure to PBDE 209 (0, 10, 500, 1500 mg/kg) from GD 0 to PND 21. Interestingly, in the first ($21^{st}$) trial on day 5, both control and PBDE 209 (10 mg/kg)-treated mice spent longer time in the target quadrant, compared with that in the last ($20^{th}$) trial of day 4 ($P < 0.05$) (Table 3). This result was same as that reported by Viberg et al. (2003) showing normal behavior during re-learning as the mouse initially searches the previous location of the platform. However, no such phenomena were observed in mice treated with PBDE 209 at the dosages of 500 and 1500 mg/kg. Taken together, our data indicate that maternal exposure may

deteriorate learning ability in the offspring. This study provided valuable information on the characteristics of PBDE 209 by different exposure scenario.

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**References**

Bi X, Qy W, Sheng G, Zhang W, Mau B, Chen D, Yu L, Fu J. 2006. Environ Pollut 144:1024.

Branchi I, Alleva E, CostaLG. 2002. Neurotoxicology 23:375.

Eriksson P, Fischer C, Fredriksson A. 2006. Toxicol Sci 94:302.

Mazdai A, Dodder NG, Abernathy MP, Hites RA, Bigsby RM. 2003. Environ Health Perspect 111:1249.

Rice DC, Reeve EA, Herlihy A, Zoeller RT, Thompson WD, Markowski VP. 2007. Neurotoxicol Terato 29:511.

Viberg H, Fredriksson A, Jakobsson E, Örn U, Eriksson P. 2003. Toxicol Sci 76:112.

Viberg H, Fredriksson P, Eriksson P. 2004. Toxicol Sci 81:344

Viberg H, Jakobsson N, Fredriksson A, Eriksson J, Marsh G, Eriksson P. 2006. Toxicol Sci 92:211.

Viberg H, Fredriksson A, Eriksson P. C. 2007. Neurotoxicology 28:136.

Viberg H, Mundy W, Eriksson P. 2008. Neurotoxicology 29:152.

Schecter A, Pavuk M, Papke O, Ryan JJ, Birnbaum L, Rosen R. 2003. Environ Health Perspect 111:1723.

Table 1. Effects of PBDE 209P on behavior in the open field test in male mice by dams treated with PBDE 209.

| | PBDE 209 (mg/kg) | | | |
|---|---|---|---|---|
| | 0 (n=18) | 10 (n=18) | 500 (n=18) | 1500 (n=18) |
| Distance traveled (cm) | 4474.1±307.3 | 4442.0±301.6 | 3777.7±348.2 | 4415.3±276.2 |
| Vertical counts (no.) | 315.8±63.1 | 268.8±26.4 | 406.4±108.5 | 266.9±22.3 |
| Center vertical counts (no.) | 10.7±2.6 | 10.4±2.4 | 9.6±2.6 | 7.7±1.8 |

Data are expressed as the mean ± S.E.M.

Table 2. Effects of PBDE 209Pon percentage of time spent in the target quadrant in probe test in the Morris water maze test in male mice by dams treated with PBDE 209.

| Parameters | PBDE 209 (mg/kg) | | | |
|---|---|---|---|---|
| | 0 (n=18) | 10 (n=18) | 500 (n=18) | 1500 (n=18) |
| Latency (%) | 30.0±3.4 | 32.1±3.1 | 30.1±3.0 | 35.7±3.6 |

Data are expressed as the mean ± S.E.M.

Table 3. Effects of PBDE 209Pon percentage of time spent on searching the platform in the target quadrant in the last learning trail and in the first re-learning trail in the Morris water maze test in male mice by dams treated with PBDE 209.

| Group | Trial 20 (s) | Trial 21 (s) | Trial 20 v.s. 21 |
|---|---|---|---|
| Control | 24.0±5.1 | 41.7±4.9 | P≦0.0178 |
| PBDE 209 (10 mg/kg) | 25.7±4.6 | 43.3±4.9 | P≦0.0142 |
| PBDE 209 (500mg/kg) | 25.3±5.1 | 38.4±5.0 | P≧0.0805 |
| PBDE 209 (1500mg/kg) | 26.0±4.1 | 29.5±4.7 | P≧0.5799 |

Data are expressed as the mean ± S.E.M.

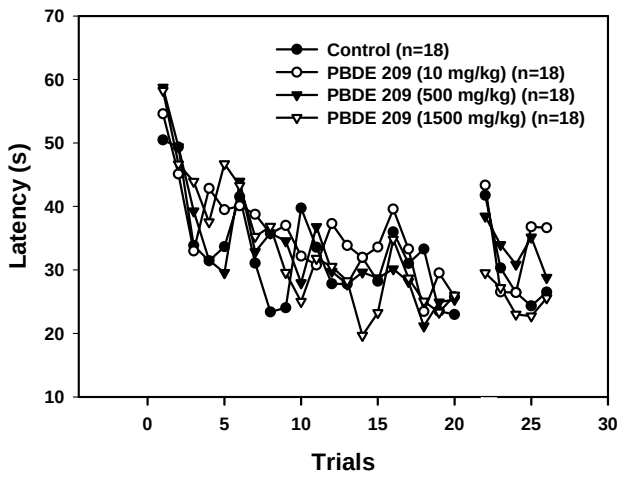


Fig. 1. Effects of PBDE 209 on the re-learning in the Morris water maze test in male mice by dams treated with PBDE 209. Latency to find the platform was recorded in male mice whose mother were treated with PBDE 209 (0, 10, 500, 1500 mg/kg) from GD 0 to PVD 21. Trials 1-20 are learning phase, trials 22-26 are re-learning phase.