Investigations of Strain and/or Gender Differences in Developmental Neurotoxic Effects of Polybrominated Diphenyl Ethers in Mice

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Polybrominated diphenyl ethers (PBDEs), one class of flame retardants used to suppress or inhibit the risk of fire, are regularly found in the environment and in human milk. The present study shows that neonatal exposure to a widely, environmentally found PBDE, 2,2',4,4',5-pentaBDE (PBDE 99), can induce developmental neurotoxic effects, such as changes in spontaneous behavior (hyperactivity), effects that are dose-response related and worsen with age. These changes are seen in C57/Bl mice of both sexes. Neonatal C57/Bl male and female mice were orally exposed on day 10 to 0.4, 0.8, 4.0, 8.0, or 16 mg PBDE 99/kg body weight. Spontaneous behavior (locomotion, rearing, and total activity) was observed in two-, five-, and eight-month-old mice. The behavior tests showed that the effects were dose-response and time-response related for both male and female mice. The observed developmental neurotoxic effects seen for PBDE 99, in C57/Bl mice, are similar to effects seen for 2,2',4,4'-tetraBDE (PBDE 47), PBDE 99, 2,2',4,4',5,5'-hexaBDE (PBDE 153), 2,2',3,3',4,4',5,5',6,6'-decaBDE (PBDE 209) and for certain PCBs, in male NMRI mice. Furthermore, the effects of PBDEs appear to be as potent in female mice as in male mice, and as potent in C57/Bl mice as in NMRI mice, concerning developmental neurotoxicity.

Key Words: spontaneous behavior; neonatal; flame retardants; polybrominated diphenyl ethers; gender comparison.

Textiles, rubber, high impact polystyrenes, polyamides, and polyolefins (often used in electronic equipment) contain polybrominated diphenyl ethers (PBDEs) for flame retardation. PBDEs are a group of chemical substances with the chemical formula C_{12}H_{10-n}Br_{10-n}O, the theoretical number of possible congeners 209, and a high lipophilicity (log K_{ow} ranges between 4.28 and 9.9) (WHO, 1994). These characteristics are similar to those of the polychlorinated biphenyls (PCBs) (WHO, 1993). PBDEs are not fixed in the polymer product through chemical binding and can thus leak into the environment (Hutzinger et al., 1976; Hutzinger and Thoma, 1987). Recent studies have shown that PBDEs are present in the global environment (de Boer et al., 1998; Johnson and Olson, 2001; Manchester-Neesvig et al., 2001; Strandberg et al., 2001) and that levels of PBDEs are increasing in the Swedish environment (Andersson and Blomkvist, 1981; Nylund et al., 1992; Sellström et al., 1993) and the North American environment (Ikonomou et al., 2002; Law et al., 2003; Luoss et al., 2002; Norstrom et al., 2002; She et al., 2002). PBDEs have also been found in human diet (Domingo, in press) and in human blood (Klasson-Wehler et al., 1997), including in workers in the electrical dismantling industry who show levels of PBDEs in their blood (Sjödin et al., 1999), and in human milk (Erdogru et al., 2004; Meironytė et al., 1999; Norén and Meironytė, 2000; Ohta et al., 2002; Ryan et al., 2002). A Swedish report has shown an exponential increase of PBDEs in human milk from 1972 to 1997, whereas PCBs are steadily decreasing (Meironytė et al., 1999). In human milk from the U.S. the levels seem to be higher than in Sweden and other European countries (Schechter et al., 2003). The most commonly found PBDEs in the environment are 2,2',4,4'-tetraBDE (PBDE 47), 2,2',4,4',5,5'-pentaBDE (PBDE 99), 2,2',4,4',6-pentaBDE (PBDE 100), and 2,2',4,4',5,5'-hexaBDE (PBDE 153) (Darnerud et al., 2001) and, in human milk, PBDE 47 and PBDE 99 (Meironytė et al., 1999; Norén and Meironytė, 2000). This indicates that humans are exposed to PBDEs both as infants and as adults.

Exposure to low doses of persistent (nondegradable) environmental agents, e.g., some PCBs and DDT, during neonatal brain development in mice, has been shown to induce irreversible disruption in adult brain function (Eriksson, 1997). In recent studies we have shown that neonatal exposure to three different PBDEs, PBDE 47, PBDE 99, and PBDE 153, on postnatal day 10, can induce persistent dysfunction in adult male NMRI mice, manifested as deranged spontaneous behavior (Eriksson et al., 2001, 2002; Viberg et al., 2002, 2003a, 2004). The observed effects, altered spontaneous motor behavior, and reduced habituation capabilities, were also seen to worsen with increasing age. BrANCHi and coworkers have also found that PBDEs can cause developmental neurotoxic effects (Branchi et al., 2002). Our studies have shown that the disturbances are induced during the period of rapid brain growth. During the brain growth spurt (BGS) (Davison and Dobbing, 1968) the brain undergoes several fundamental phases, such as dendritic and axonal outgrowth and the establishment of neural connections (Davison and Dobbing,
Pregnant C57/Bl mice were purchased from B&K, Sollentuna, Sweden, and were housed individually in plastic cages in a room with an ambient temperature of 22°C and a 12/12 h cycle of light and dark. The animals were supplied with standardized pellet food (Lactamin, Stockholm, Sweden) and tap water ad libitum. The size of the litters was adjusted to 10–12 mice, within the first 48 h after birth, by killing excess pups. The litters contained pups of both sexes. At the age of 4–5 weeks, the offspring were weaned and the males were placed in groups of 4–7 littersmates, in a room for male mice only, and the females were placed in groups of 4–7 littersmates, in a room for female mice only and raised under the same conditions as detailed above.

Male and female mice, at the age of 10 days, were given 0.4, 0.8, 4.0, 8.0, or 16.0 mg PBDE 99/kg body weight (0.7, 1.4, 7.0, 14, or 28 μmol PBDE 99/kg body weight) via a metal gastric tube, as one single po dose. Control mice received 10 ml/kg body weight of the 20% fat emulsion vehicle. Each of the different dosage categories contained three to five litters.

Behavior Tests

Spontaneous behavior test. Spontaneous behavior was tested in the both male and female mice, with 10 days in between testing of male and female mice, at age two, five, and eight months, as earlier described (Eriksson et al., 1992, 2001, 2002; Viberg et al., 2002, 2003a,b, 2004). The investigator was blinded to the different treatments given the mice. The animals were tested between 0800 and 1200 h under the same ambient light and temperature conditions as their housing conditions. A total of eight mice were randomly picked from the three to five different litters in each treatment group, at each testing occasion (i.e., at two, five, and eight months of age). Motor activity was measured for a 60-min period, divided into 3 × 20-min spells, in an automated device consisting of cages (40 × 25 × 15 cm) placed within two series of infrared beams (low and high level) (Rat-O-Matic, ADEA Elektronik AB, Uppsala, Sweden) (Fredriksson, 1994).

Locomotion: Counting took place when the mouse moved horizontally through the low-level grid of infrared beams.

Rearing: Movement in the vertical plane was registered at a rate of four counts per second, when a single high-level beam was interrupted, i.e., the number of counts obtained was proportional to time spent rearing.

Total activity: All types of vibration within the cage, i.e., those caused by mouse movements, shaking (tremors), and grooming, were registered by a pick-up (mounted on a lever with a counterweight), connected to the test cage.

Statistical Analysis

Spontaneous behavior. The data were subjected to a split-plot ANOVA (analysis of variance), and pairwise testing between PBDE 99-treated groups and the control group was performed using a Tukey HSD (honestly significant difference) test (Kirk, 1968).

Habituation capability. In order to study time-dependent changes in habituation (two-month-old vs. eight-month-old mice), data from the spontaneous behavior tests was used. A ratio was calculated between the performance period 40–60 min and 0–20 min for two of the variables, locomotion, and rearing. The following equation was used: 100 × (locomotion counts 40–60 min/locomotion counts 0–20 min) and 100 × (rearing counts 40–60 min/rearing counts 0–20 min). These data were subjected to a two-way ANOVA, and pairwise testing between the habituation ratio from two- and eight-month-old mice within each treatment group was performed using a Tukey HSD (honestly significant difference) test (Kirk, 1968).

RESULTS

There were no clinical signs of toxicity in the PBDE 99-treated mice, regardless of sex, at any given time during the experimental period, nor was there any significant difference in body weight gain or adult weight between the PBDE 99-treated and

MATERIALS AND METHODS

Chemicals and animals. To conduct the study, 2,2',4,4',5-pentaBDE (PBDE 99) (purity > 99%) was obtained from Eva Jakobsson at Wallenberg Laboratory, Stockholm. First, it was dissolved in a mixture of egg lecithin (Merck, Darmstadt, Germany) and peanut oil (Oleum arachidis) (1:10) and then sonicated with water to yield a 20% (w:w) fat emulsion vehicle containing 0.04, 0.08, 0.4, 0.8, or 1.6 mg PBDE 99/ml (0.07, 0.14, 0.7, 14, or 2.8 μmol/ml, respectively). The use of a 20% fat emulsion vehicle was to give a more physiologically appropriate absorption and hence distribution (Keller and Yeary, 1980; Palin et al., 1982), since the fat content of mouse milk is around 14%.
the vehicle-treated mice, regardless of sex, in the five different dosage categories.

**Effects on Spontaneous Behavior in Adult Male C57/Bl Mice**

The results from the spontaneous behavioral variables locomotion, rearing, and total activity in two-, five-, and eight-month-old male C57/Bl mice, after exposure to a single po dose of 0.4, 0.8, 4.0, 8.0, or 16.0 mg PBDE 99/kg body weight at an age of 10 days, are shown in Figures 1, 2, and 3, respectively.

Neonatal exposure to PBDE 99 showed that there were significant group × period interactions after two months \(F_{10,84} = 237.15; F_{10,84} = 82.56; F_{10,84} = 414.74\), after five months \(F_{10,84} = 249.71; F_{10,84} = 134.04; F_{10,84} = 293.76\), and after eight months \(F_{10,84} = 835.13; F_{10,84} = 157.22\).

FIG. 1. Spontaneous behavior of two-month-old male C57/Bl mice exposed to a single po dose of either 20% fat emulsion vehicle or 0.4, 0.8, 4.0, 8.0, or 16 mg PBDE 99/kg body weight (0.7, 1.4, 7.0, 14, or 28 μmol PBDE 99/kg body weight) at 10 days of age. The data were subjected to an ANOVA with split-plot design and there were significant group × period interactions \(F_{10,84} = 237.15; F_{10,84} = 82.56; F_{10,84} = 414.74\) for the variables locomotion, rearing, and total activity, respectively. Pairwise testing between PBDE 99-exposed and control animals was performed using Tukey HSD tests. The statistical differences are indicated as (A) significantly different vs. controls, \(p \leq 0.01\); (a) significantly different vs. controls, \(p \leq 0.05\); (B) significantly different vs. 0.4 mg PBDE 99/kg body weight, \(p \leq 0.01\); (C) significantly different vs. 0.8 mg PBDE 99/kg body weight, \(p \leq 0.01\); (D) significantly different vs. 4.0 mg PBDE 99/kg body weight, \(p \leq 0.01\); (E) significantly different vs. 8.0 mg PBDE 99/kg body weight, \(p \leq 0.01\); (c) significantly different vs. 8.0 mg PBDE 99/kg body weight, \(p \leq 0.05\). The height of the bars represents the mean value ± SD.

FIG. 2. Spontaneous behavior of five-month-old male C57/Bl mice exposed to a single po dose of either 20% fat emulsion vehicle or 0.4, 0.8, 4.0, 8.0, or 16 mg PBDE 99/kg body weight (0.7, 1.4, 7.0, 14, or 28 μmol PBDE 99/kg body weight) at 10 days of age. The data were subjected to an ANOVA with split-plot design and there were significant group × period interactions \(F_{10,84} = 249.71; F_{10,84} = 134.04; F_{10,84} = 293.76\) for the variables locomotion, rearing, and total activity, respectively. Pairwise testing between PBDE 99-exposed and control animals was performed using Tukey HSD tests. The statistical differences are indicated as (A) significantly different vs. controls, \(p \leq 0.01\); (a) significantly different vs. controls, \(p \leq 0.05\); (B) significantly different vs. 0.4 mg PBDE 99/kg body weight, \(p \leq 0.01\); (b) significantly different vs. 0.4 mg PBDE 99/kg body weight, \(p \leq 0.05\); (C) significantly different vs. 0.8 mg PBDE 99/kg body weight, \(p \leq 0.05\); (D) significantly different vs. 0.8 mg PBDE 99/kg body weight, \(p \leq 0.01\); (c) significantly different vs. 0.8 mg PBDE 99/kg body weight, \(p \leq 0.05\); (d) significantly different vs. 4.0 mg PBDE 99/kg body weight, \(p \leq 0.05\); (E) significantly different vs. 4.0 mg PBDE 99/kg body weight, \(p \leq 0.01\); (e) significantly different vs. 8.0 mg PBDE 99/kg body weight, \(p \leq 0.05\). The height of the bars represents the mean value ± SD.
Effects on Spontaneous Behavior in Adult Female C57/Bl Mice

The results from the spontaneous behavioral variables locomotion, rearing, and total activity in two-, five, and eight-month-old female C57/Bl mice, after exposure to a single po dose of 0.4, 0.8, 4.0, 8.0, or 16.0 mg PBDE 99/kg body weight at an age of 10 days, are shown in Figures 4, 5, and 6, respectively.

Two months after the neonatal exposure to PBDE 99, there were significant group × period interactions \( F_{10,84} = 113.49; F_{10,84} = 137.55; F_{10,84} = 173.73 \) for the locomotion, rearing, and total activity variables, respectively (Fig. 4). Pairwise testing between PBDE 99-treated and control groups showed a significant dose-related change in all three test variables. In control mice, there was a distinct decrease in activity in all three behavioral variables over the 60-min period. Female mice receiving 0.8 to 16 mg PBDE 99/kg body weight showed a dose-response dependent difference in activity for locomotion, rearing, and total activity, where these animals showed a significantly decreased activity during the first 20-min period (0–20 min) and a significantly increased activity during the last 20-min period (40–60 min), compared to the control animals.

An increased activity was seen in the rearing variable in animals receiving 0.4 mg PBDE 99/kg body weight. Whether this effect is a result of the treatment or that controls showed lower rearing activity counts compared to female control animals tested at five and eight months of age, is not clear.

Five months after the neonatal exposure to PBDE 99, there were significant group × period interactions \( F_{10,84} = 415.88; F_{10,84} = 155.25; F_{10,84} = 518.75 \) for the locomotion, rearing, and total activity variables, respectively (Fig. 5). Pairwise testing between PBDE 99-treated and control groups showed a significant dose-related change in all three test variables. In control mice, there was a distinct decrease in activity in all three behavioral variables over the 60-min period. Female mice receiving 0.8 to 16 mg PBDE 99/kg body weight showed a dose-response dependent difference in activity for locomotion, rearing, and total activity, where these animals showed a significantly decreased activity during the first 20-min period (0–20 min) and a significantly increased activity during the last 20-min period (40–60 min) compared to the control animals.

Eight months after the neonatal exposure to PBDE 99, there were significant group × period interactions \( F_{10,84} = 664.28; F_{10,84} = 101.12; F_{10,84} = 874.08 \) for the locomotion, rearing, and total activity variables, respectively (Fig. 6). Pairwise testing between PBDE 99-treated and control groups showed a significant dose-related change in all three test variables. In
control mice, there was a distinct decrease in activity in all three behavioral variables over the 60-min period. Female mice receiving 0.8 to 16 mg PBDE 99/kg body weight showed a dose-response dependent difference in activity for locomotion, rearing, and total activity, where these animals showed a significantly decreased activity during the first 20-min (0–20 min) period and a significantly increased activity during the last 20-min period (40–60 min) compared to the control animals.

**Effects on Habituation Capability in Adult Male and Female C57/Bl Mice**

By analyzing the habituation ratio between the performance periods of 40–60 min and 0–20 min in the spontaneous behavior...
The habituation capability concerning the rearing variable did not show any significant difference between two- and eight-month-old male mice.

The results for the habituation ratio, calculated from the spontaneous behavior variables locomotion and rearing in two- and eight-month-old C57Bl/Bl female mice, are presented in Table 2. The habituation capability concerning the locomotion variable was shown to significantly decrease ($p < 0.01$) from two to eight months of age in female mice exposed neonatally to 4.0 or 16 mg PBDE 99/kg body weight. No other groups showed significant decreases in this variable from two to eight months of age. The habituation capability concerning the rearing variable was shown to significantly decrease ($p < 0.05$) from two to eight months of age in female mice exposed neonatally to 0.8 or 1.4 mg PBDE 99/kg body weight. No other groups showed significant decreases in this variable from two to eight months of age. The habituation capability concerning the rearing variable was shown to significantly decrease ($p < 0.05$) from two to eight months of age in female mice exposed neonatally to 0.4 or 4.0 mg PBDE 99/kg body weight. No other groups showed significant decreases in this variable from two to eight months of age. The habituation capability concerning the rearing variable was shown to significantly decrease ($p < 0.01$) from two to eight months of age in female mice exposed neonatally to 8.0 or 16 mg PBDE 99/kg body weight. No other groups showed significant decreases in this variable from two to eight months of age.

The present study shows that exposure to low doses of PBDE 99, 0.4 to 16 mg/kg body weight, on postnatal day 10, can give rise to irreversible disturbances in the spontaneous behavior of adult male and female C57Bl/Bl mice. These disturbances are both dose-response and time-response related, i.e., the disturbances

**TABLE 1**

Habituation Capability in Two- and Eight-Month-Old Male C57Bl/Bl Mice Exposed to PBDE 99 on Postnatal Day 10

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>Two-month-old</th>
<th>Eight-month-old</th>
<th>F value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.47 ± 0.88</td>
<td>0.33 ± 0.68</td>
<td>0.12</td>
<td>n.s.</td>
</tr>
<tr>
<td>0.4</td>
<td>0.79 ± 0.97</td>
<td>0.49 ± 0.70</td>
<td>0.51</td>
<td>n.s.</td>
</tr>
<tr>
<td>0.8</td>
<td>8.22 ± 7.01</td>
<td>7.94 ± 6.16</td>
<td>0.01</td>
<td>n.s.</td>
</tr>
<tr>
<td>4.0</td>
<td>71.3 ± 6.11</td>
<td>74.7 ± 11.3</td>
<td>0.55</td>
<td>n.s.</td>
</tr>
<tr>
<td>8.0</td>
<td>97.7 ± 8.72</td>
<td>105 ± 6.42</td>
<td>4.52</td>
<td>n.s.</td>
</tr>
<tr>
<td>16</td>
<td>247 ± 59.6</td>
<td>458 ± 121</td>
<td>19.5</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Note. Habituation is the ratio between performance in the spontaneous behavior period 40–60 min and 0–20 min in two- and eight-month-old male C57Bl/Bl mice exposed to a single po dose of PBDE 99 or 20% fat emulsion vehicle on postnatal day 10. Statistical evaluation of behavioral data was done by two-way ANOVA (Kirk, 1968). Abbreviation: n.s., not significant.

99/kg body weight. In the lower PBDE 99 treatments and control groups no difference in habituation capability was seen between two and eight months of age. The habituation capability concerning the rearing variable was not shown any significant difference between two- and eight-month-old male mice.

The present study shows that exposure to low doses of PBDE 99, 0.4 to 16 mg/kg body weight, on postnatal day 10, can give rise to irreversible disturbances in the spontaneous behavior of adult male and female C57Bl/Bl mice. These disturbances are both dose-response and time-response related, i.e., the disturbances

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The results for the habituation ratio, calculated from the spontaneous behavior variables locomotion and rearing in two- and eight-month-old C57Bl/Bl female mice, are presented in Table 2. The habituation capability concerning the locomotion variable was shown to significantly decrease ($p < 0.01$) from two to eight months of age in female mice exposed neonatally to 4.0 or 16 mg PBDE 99/kg body weight. No other groups showed significant decreases in this variable from two to eight months of age. The habituation capability concerning the rearing variable was shown to significantly decrease from two to eight months of age in female mice exposed neonatally to 0.8 or 16 mg PBDE 99/kg body weight. No other groups showed significant decreases in this variable from two to eight months of age. The habituation capability concerning the rearing variable was not shown any significant difference between two- and eight-month-old male mice.

The present study shows that exposure to low doses of PBDE 99, 0.4 to 16 mg/kg body weight, on postnatal day 10, can give rise to irreversible disturbances in the spontaneous behavior of adult male and female C57Bl/Bl mice. These disturbances are both dose-response and time-response related, i.e., the disturbances
The spontaneous motor behavior data showed a disruption of spontaneous behavior and habituation in both male and female C57/Bl mice exposed to PBDE 99. Habituation, here defined as a decrease in the locomotion, rearing, and total activity variables in response to the diminishing novelty of the test chamber over a 60-min period, was displayed in the control animals, but the animals exposed to PBDE 99 were clearly hypoactive during the beginning of the 60-min period, while toward the end of the test period they were hyperactive. This derangement in spontaneous behavior tests also indicated that the functional disorder is dose-response related in both male and female mice.

In male C57/Bl mice, the four highest doses of PBDE 99 (0.8, 4.0, 8.0, and 16 mg/kg body weight) caused a significant change in spontaneous behavior on all three testing occasions. The spontaneous behavior tests indicated that the change in behavioral motor activity worsens with increasing age, as the aberrations appeared to be most pronounced in the eight-month-old male C57/Bl mice, which was also true when looking at the habituation capabilities. This change over time is clearly demonstrated in mice receiving the highest dose of PBDE 99 (16 mg/kg body weight), where the habituation ratio for locomotion increased significantly, when comparing two-month-old mice with eight-month-old mice. This means that the ability to habituate to a novel environment became worse with age after neonatal exposure to PBDE 99. This type of both dose-response and time-response behavioral defects and reduced habituation capability with age have been seen in male NMRI mice neonatally exposed to PBDE 47, PBDE 99, PBDE 153, and PBDE 209 (Eriksson et al., 2001, 2002; Viberg et al., 2002, 2003a,b). It has also been shown that exposure to PBDE 99, via the dam, during gestation and lactation can induce behavioral deficits in male CD-1 Swiss offspring (Branchi et al., 2002). These studies indicate that the developmental neurotoxic effects of PBDEs are not specific for one mouse strain.

In female C57/Bl mice, the four highest doses of PBDE 99 (0.8, 4.0, 8.0, and 16 mg/kg body weight) caused a significant change in spontaneous behavior. The spontaneous behavior tests indicated that the change in behavioral motor activity worsens with increasing age, as the aberrations appeared to be most pronounced in the eight-month-old female C57/Bl mice, which was also true when looking at the habituation capabilities. In female mice this is demonstrated at the doses 4.0 and 16 mg PBDE 99/kg body weight, where the ability to habituate is significantly decreased for the locomotion variable. Also, for the rearing variable the ability to habituate decreased over time in female C57/Bl mice receiving 0.8, 8.0, or 16 mg PBDE 99/kg body weight. This type of both dose-response and time-response behavioral defects and reduced habituation capability with age corresponds well to the effects seen for male C57/Bl mice. When comparing male and female C57/Bl mice both dose-response and time-response effects are similar. However, the time-response effect appears, from this study, to be more pronounced in female mice, where this effect had already been seen in the rearing variable at the dose of 0.8 mg PBDE 99/kg body weight.

Our recent studies on developmental neurotoxic effects of PBDEs have revealed that 0.8 mg PBDE 99/kg body weight is the lowest dose to alter the spontaneous behavior in adult male NMRI mice (Eriksson et al., 2001; Viberg et al., 2004). This dose, 0.8 mg PBDE 99/kg body weight, also altered the spontaneous behavior of male and female C57/Bl mice. In male NMRI mice exposed to PBDE 99 no altered spontaneous behavior was seen for 0.4 mg PBDE 99/kg body weight (Viberg et al., 2004), which is also the case in the present study. This leads to the conclusion that the no observed effect level (NOEL) for developmental neurotoxic effects, manifested on spontaneous behavior, is located somewhere in between 0.4 and 0.8 mg PBDE 99/kg body weight.

The observed developmental neurotoxic effects seen for PBDEs are similar to those observed for PCBs, and especially ortho-substituted PCBs. It is interesting to note that, in comparison with developmental effects after neonatal exposure to PCB, it has been seen that certain ortho-substituted PCBs, such as PCB 52 and PCB 153 (Eriksson, 1998; Eriksson and Fredriksson, 1996a,b), can induce these kinds of dose-response related and time-response related changes in spontaneous behavior, together with reduced habituation capability with increasing age. Furthermore, the capacity of PBDEs to induce behavioral neurotoxic effects in different strains and sexes is not unique.

### TABLE 2

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>Two-month-old</th>
<th>Eight-month-old</th>
<th>F value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Locomotion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.39 ± 0.83</td>
<td>0.36 ± 1.03</td>
<td>0.00</td>
<td>n.s.</td>
</tr>
<tr>
<td>0.4</td>
<td>0.49 ± 0.63</td>
<td>0.30 ± 0.42</td>
<td>0.50</td>
<td>n.s.</td>
</tr>
<tr>
<td>0.8</td>
<td>8.11 ± 4.89</td>
<td>11.9 ± 1.45</td>
<td>4.31</td>
<td>n.s.</td>
</tr>
<tr>
<td>4.0</td>
<td>70.8 ± 7.28</td>
<td>90.4 ± 8.50</td>
<td>24.4</td>
<td>0.01</td>
</tr>
<tr>
<td>8.0</td>
<td>104 ± 14.1</td>
<td>95.9 ± 13.4</td>
<td>1.53</td>
<td>n.s.</td>
</tr>
<tr>
<td>16</td>
<td>263 ± 79.2</td>
<td>511 ± 163</td>
<td>15.0</td>
<td>0.01</td>
</tr>
<tr>
<td>Rearing</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.24 ± 0.69</td>
<td>0.22 ± 0.61</td>
<td>0.01</td>
<td>n.s.</td>
</tr>
<tr>
<td>0.4</td>
<td>0.51 ± 0.95</td>
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<td>n.s.</td>
</tr>
<tr>
<td>0.8</td>
<td>1.49 ± 0.93</td>
<td>2.83 ± 0.56</td>
<td>12.3</td>
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<tr>
<td>4.0</td>
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<td>43.8 ± 7.40</td>
<td>0.20</td>
<td>n.s.</td>
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<td>8.0</td>
<td>94.2 ± 14.0</td>
<td>118 ± 12.2</td>
<td>13.2</td>
<td>0.01</td>
</tr>
<tr>
<td>16</td>
<td>217 ± 49.1</td>
<td>271 ± 41.3</td>
<td>5.72</td>
<td>0.05</td>
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</tbody>
</table>

*Note. Habituation is the ratio between performance in the spontaneous behavior period 40–60 min and 0–20 min in two- and eight-month-old female C57/Bl mice exposed to a single po dose of PBDE 99 or 20% fat emulsion vehicle on postnatal day 10. Statistical evaluation of behavioral data was done by two-way ANOVA (Kirk, 1968). Abbreviation: n.s., not significant.*
Different studies have shown that PCBs can induce neurotoxic effects in both male and rats, and in male and female individuals (Eriksson, 1998; Herr et al., 2001; Holene et al., 1998, 1999; Kremer et al., 1999; Tilson et al., 1990). In some studies though, the effects of PCBs are reported to affect the sexes differently. Postnatal exposure to PCB 153 has been shown to increase the motor activity in male rats, but not in female rats, who instead showed long-lasting effects in operant behavioral testing (Holene et al., 1998, 1999). In our recent studies we have shown that neonatal exposure to PBDEs can affect the cholinergic system in mice, manifested as increased susceptibility to nicotine (Viberg et al., 2002) and decreased density of nicotinic cholinergic receptors in the hippocampus (Viberg et al., 2003a, 2004). The cholinergic system is one of the major transmitter systems that correlate closely to cognitive function (Drachman, 1977; Fibiger, 1992) and behavioral performance in tasks requiring attention and rapid processing of information in humans. Reversal learning and working memory in animals has been suggested to involve cholinergic transmission (Hodges et al., 1991). The cholinergic agent nicotine shows differences between the sexes, in its neurotoxic actions. Usually females are more sensitive to the actions of nicotine, which act through the cholinergic system (Faraday et al., 2003; Pogun, 2001; Popke et al., 1997). Although PBDEs, which also affect the cholinergic system and change motor activity, do not seem to affect the different mouse strains NMRI and C57/Bl differently, the reduced habituation capacity in females, at lower doses compared to males, might reflect a minor gender difference. This needs to be further evaluated.

In conclusion, the present investigation shows that neurotoxic effects of neonatal PBDE 99-exposure are inducible in both male and female C57/Bl mice and therefore there seem to be no major gender differences in the neurotoxic effects. The neurotoxic effects are dose-response related and irreversible; in fact effects get worse with increasing age. Taken together, the present study on C57 Bl mice and our earlier studies on NMRI mice indicate that the neurotoxic effects of PBDE 99 are not confined to one particular strain of mice. These results and earlier reported results about the neurotoxicity of PBDEs, together with the facts that PBDEs are still increasing in the environment, are found in human milk, and have a similar pattern of neurotoxicity as PCBs, calls for further studies of the neurotoxicity of PBDEs. For example, there is still a need to investigate whether the neurotoxicity is limited to mice or if other mammals are also susceptible to the action of PBDEs. Although one of the target systems in the brain involves the cholinergic system, there are further important discoveries to be made about the mechanisms of action behind the neurotoxicity of PBDEs.

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REFERENCES


