TOXICOLOGICAL SCIENCES **92(1)**, 211–218 (2006)
doi:10.1093/toxsci/kfj196
Advance Access publication April 12, 2006

Neonatal Exposure to Higher Brominated Diphenyl Ethers, Hepta-, Octa-, or Nonabromodiphenyl Ether, Impairs Spontaneous Behavior and Learning and Memory Functions of Adult Mice

Henrik Viberg,* Niclas Johansson,* Anders Fredriksson,* Johan Eriksson,† Göran Marsh,† and Per Eriksson*,[1]

*Department of Environmental Toxicology, Uppsala University, Uppsala, Sweden; and †Department of Environmental Chemistry, Stockholm University, Stockholm, Sweden

Received December 20, 2005; accepted March 17, 2006

Polybrominated diphenyl ethers (PBDEs), used as flame retardants, have been shown to be increasing in the environment and in human mother's milk. We have earlier reported that lower brominated PBDEs, such as tetra-, penta-, and hexa-brominated diphenyl ethers, can cause developmental neurotoxic effects in mice. Recently, this was also observed with the full-brominated PBDE, deca-brominated diphenyl ether (PBDE 209), although it was suggested that the effects were caused by a (possibly debrominated) metabolite thereof. The present study revealed that 2,2′,3,3′,4,4′,5,5′,6-nonabromodiphenyl ether (PBDE 206), 2,2′,3,4,4′,5,5′,6-octabromodiphenyl ether (PBDE 203), and to a minor extent also 2,2′,3,4,4′,5′,6′-heptabromodiphenyl ether (PBDE 183) can induce developmental neurotoxic effects. Neonatal Naval Medical Research Institute male mice were exposed on postnatal day 3 or 10 to PBDE 206, PBDE 203, or PBDE 183, given as a single oral dose of 21 μmol/kg body weight. At the adult age of 2–3 months, the mice were observed for performance in a spontaneous behavior test and the Morris water maze test. PBDE 203 and PBDE 206, when administered on neonatal day 10, caused disturbances in spontaneous behavior, leading to disrupted habituation and a hyperactive condition in adults at the age of 2 months. These behavioral changes were also seen in 2-month-old mice exposed to PBDE 203 on neonatal day 3. Furthermore, exposure to PBDE 203 on neonatal day 10 affected learning and memory functions in adult mice. The developmental neurotoxic effects were most pronounced in mice exposed to PBDE 203. These developmental neurobehavioral defects were in agreement with those we observed previously with lower brominated PBDEs and with PBDE 209. It is important to consider the fact that different PBDE congeners can have differing degrees of potency, when comparing levels of PBDEs in the environment and in mother's milk.

Key Words: **habituation; learning and memory; Morris water maze test; polybrominated diphenyl ether; spontaneous neonatal behavior.**

Brominated flame retardants are a diverse group of industrial compounds used to retard, suppress, or inhibit combustion processes in an effort to reduce the risk of fire, mainly in polymer products (WHO, 1994). Polybrominated diphenyl ethers (PBDEs), which constitute one group of chemical substances used as additive flame retardants, have the chemical formula $C_{12}H_{(n-1)}Br_{(n)}O$ ($n \leq 10$). The theoretical number of possible congeners is 209. Products that contain flame retardants are electric appliances, such as computers and television sets, textiles, and building materials (WHO, 1994). PBDEs are not bound in the polymer products and thus can leach into the environment (Hutzinger and Thoma, 1987; Hutzinger *et al.*, 1976). PBDEs are demonstrably present in the global environment and have been found in samples taken from aquatic and terrestrial compartments, e.g., sediments, fish, reindeer, and humans (Allchin *et al.*, 1999; Asplund *et al.*, 1999a,b; Hale *et al.*, 2002; Klasson-Wehler *et al.*, 1997; Schecter *et al.*, 2005; Sellström *et al.*, 1993; Sjödin *et al.*, 1999). Of the PBDEs analyzed, the most commonly found congeners in the environment today are 2,2′,4,4′-tetrabromodiphenyl ether (PBDE 47), 2,2′,4,4′,5-pentabromodiphenyl ether (PBDE 99), 2,2′,4,4′,6-pentabromodiphenyl ether (PBDE 100) and 2,2′,4,4′,5,5′-hexabromodiphenyl ether (PBDE 153) (Darnerud *et al.*, 2001).

There are several reports of PBDEs in human milk. A breast milk–monitoring program in Sweden has shown that over the course of 20–30 years (1972–1997), organochlorines decreased to half the original concentration, whereas PBDE levels doubled every 5 years (Norén and Meironyté, 2000). The same kind of increase was seen in a time-trend study in Japan (1973–2000), where the sum of PBDEs in human milk was of a magnitude similar to that in the Swedish study (Akutsu *et al.*, 2003). Occupational exposure to PBDEs has been demonstrated in workers in the electrical dismantling industry, who had increased levels of PBDEs in their blood (Sjödin *et al.*, 1999).

There is now an increase in the number of reports of higher brominated PBDEs in humans. 2,2′,3,4,4′,5′,6′-Heptabromodiphenyl ether (PBDE 183) was reported in blood serum from

[1] To whom correspondence should be addressed at Department of Environmental Toxicology, Uppsala University, Norbyvägen 18 A, S-752 36 Uppsala, Sweden. Fax: +46 18 518843. E-mail: per.eriksson@ebc.uu.se.

American blood donors (Sjödin *et al.*, 2001) and in blood from Swedish workers (Sjödin *et al.*, 1999). PBDE 183 was also found in blood from fetal-maternal pairs, where the fetal blood concentration did not differ substantially from that of the mother, ranging between 15 and 580 ng/g lipid in mothers and from 14 to 460 ng/g lipid in fetal serum (Mazdai *et al.*, 2003). PBDE 183 is also reportedly present in mothers' milk in the United States, Japan, The Netherlands, and Sweden (Akutsu *et al.*, 2003; Baumann *et al.*, 2003; Meironyté Guvenius *et al.*, 2003; Schecter *et al.*, 2003). The full-brominated PBDE 2,2′,3,3′,4,4′,5,5′,6,6′-deca-brominated diphenyl ether (PBDE 209) was found in human blood (Sjödin *et al.*, 2001), and higher levels have been found in computer technicians (Jakobsson *et al.*, 2002) and workers handling flame-retarded rubber (Thuresson *et al.*, 2005), compared with nonexposed reference groups. This indicates that humans have been exposed to the higher brominated PBDEs both as infants and as adults.

During their development, mammals may be exposed to toxicants, as fetuses via maternal ingestion of toxicants, during the newborn period via intake of mother's milk, or by direct ingestion of, or contact with, toxicants. In recent studies, we have shown that neonatal exposure to certain PBDEs, during the period of rapid brain development (Davison and Dobbing, 1968), can cause disturbances in spontaneous motor behavior, habituation problems, and dysfunction in learning and memory in adult animals as well as changes in the cholinergic system that persist into adulthood (Eriksson *et al.*, 2001b, 2002; Viberg *et al.*, 2003a,b). The effects of PBDEs have been shown to be inducible during a restricted period of neonatal life, and behavioral effects worsen with age (Eriksson *et al.*, 2001b, 2002; Viberg *et al.*, 2003a,b). Recently, we reported that neonatal exposure to PBDE 209 can cause developmental neurotoxic effects in mice. These effects were similar not only to those reported earlier following developmental exposure to lower brominated PBDEs but also to certain Polychlorinated biphenyls (PCBs) (Eriksson, 1998). The PBDE 209 study suggested that the developmental neurotoxic effects might have been induced by metabolites of PBDE 209, e.g., debrominated products. There are studies showing that PBDE 209 can indeed be metabolized since metabolites such as hepta-, octa-, and nona-brominated diphenyl ethers have been found after oral administration of PBDE 209 to adult rats (Morck *et al.*, 2003; Sandholm, 2003; Sandholm *et al.*, 2003).

In view of the accumulating evidence of higher brominated PBDEs in the environment and in human milk, together with reports on developmental neurotoxicity of PBDE 209 and/or its metabolites, the present study was undertaken to investigate developmental neurotoxic effects of three highly brominated PBDE congeners, namely, PBDE 183, 2,2′,3,4,4′,5,5′,6-octabromodiphenyl ether (PBDE 203), and 2,2′,3,3′,4,4′,5,5′,6-nonabromodiphenyl ether (PBDE 206), when given to neonatal mice. The neurotoxic effects were analyzed by measuring spontaneous behavior and learning and memory functions in adult mice.

MATERIALS AND METHODS

Chemicals and Animals

All three PBDEs, viz, PBDE 183, PBDE 203, and PBDE 206, were synthesized at the Department of Environmental Chemistry, Stockholm University, Sweden (Christiansson *et al.*, in press). The purity of the compounds exceeded 98%. The substances were dissolved in a mixture of egg lecithin and peanut oil (*Oleum arachidis*) (1:10 wt/wt) and then sonicated together with water to yield a 20% (wt/wt) fat emulsion vehicle containing 1.52 mg PBDE 183/ml (2.1 μmol/ml), 1.68 mg PBDE 203/ml (2.1 μmol/ml), and 1.85 mg PBDE 206/ml (2.1 μmol/ml), respectively. The use of a 20% fat emulsion vehicle was to give a more physiologically appropriate absorption and hence distribution of the compounds (Keller and Yeary, 1980; Palin *et al.*, 1982).

Pregnant Naval Medical Research Institute (NMRI) mice, obtained from B&K, Sollentuna, Sweden, 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 pregnant NMRI mice were checked for birth twice daily (0800 h and 1800 h). The day of birth was designated day 0. The size of the litters was adjusted to 10–12 mice, within 48 h of birth, by euthanizing excess pups. The litters contained pups of both sexes during the neonatal period, and no separation with regard to sex was made in the preweanling mice. At the age of 4–5 weeks, all females were sacrificed and the males were kept in litters (in treatment groups) with their siblings and were raised in groups of four to seven, in a room for males only, and under conditions detailed above. In order to compare with our earlier studies and the studies on lower and middle brominated PBDEs and PBDE 209, both male and females were exposed to the PBDEs, but only male mice were used for the neurotoxicological recordings.

Treatment of Neonatal Mice with PBDE 183, PBDE 203, or PBDE 206

The substances were administered orally, in a volume of 10 ml/kg body weight, via a metal gastric tube as one single dose on either postnatal day (PND) 3 or 10. The amounts of the different compounds given were selected to be on the same molar basis as previously used in the studies on PBDE 209, PBDE 153, PBDE 99, and PBDE 47 (Eriksson *et al.*, 2001b, 2002; Viberg *et al.*, 2002, 2003a,b), namely, 21 μmol/kg body weight, i.e., PBDE 206 18.5 mg/kg body weight, PBDE 203 16.8 mg/kg body weight, and PBDE 183 15.2 mg/kg body weight. Control mice, in each age category, were given 10 ml/kg body weight of the 20% fat emulsion vehicle. In this neonatal animal model, each of the different treatment groups comprises mice from three to five different litters. Randomly selecting animals from at least three different litters will have the same statistical effect and power compared to the use of litter-based studies to evaluate developmental neurotoxicity in neonatal mice (Eriksson and Viberg, 2005; Eriksson *et al.*, 2005).

Behavioral Tests

Spontaneous Behavior Tests

Spontaneous behavior was observed in the male mice when 2 months old, as described earlier (Eriksson *et al.*, 2001, 2002; Viberg *et al.*, 2003a,b). The animals were tested between 0800 h and 1300 h, under the same ambient light and temperature conditions. Altogether 10 males were randomly picked from three to five different litters in each treatment group. Motor activity was measured for 60 min, divided into three 20-min intervals, 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 *et al.*, 1997).

Locomotion. Counting took place when the mouse moved horizontally through the low-level grid of infrared beams. Infrared beams were placed 10 mm above the bedded floor.

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. Infrared beams were placed 80 mm above the bedded floor.

Total activity. All types of vibrations within the test cage, i.e., those caused by mouse movements, shaking (tremors), and grooming were registered by a pickup (gramophone-like arm balanced with a counterweight), connected to the test cage.

Swim Maze

Mice exposed either on PND 3 to PBDE 203 or on PND 10 to PBDE 203 and PBDE 206 were observed at the age of 3 months for performance in a swim maze. These treatment groups were selected with regard to significantly altered spontaneous behavior, when tested at the age of 2 months. Some 15–16 mice, from three to five litters, from each treatment group were tested.

The swim maze, of Morris water maze type (Morris, 1981), was a circular gray tub, 102 cm in diameter, filled with water at 23°C to a depth of 15 cm from the brim. In the center of the ''northeast'' quadrant of the tub, a platform was submerged 1 cm beneath the water surface. The platform consisted of a metal mesh, 12 cm in diameter. The relative positions of the pool and observer were the same on all days when tests were performed. Each mouse's ability to locate the submerged platform was observed on 5 consecutive days. The mouse was given five trials each day, and the latency to locate the platform was measured. Testing was conducted between 0900 h and 1400 h. Before the first trial each day, the mouse was placed on the submerged platform for 30 s. It was then released in the ''south'' position, facing the wall of the tub, and was allowed 30 s to locate the platform. If the mouse failed to find the platform within 30 s, it was gently replaced on the platform. After each trial, the mouse remained on the platform for 30 s. The mice were subjected to five trials each day, and the tests were performed on 4 consecutive days, trials 1–20 (days 1–4). On day 5, the platform was moved to the center of the tub's ''northwest'' quadrant for reversal trials; otherwise the procedure was identical. Latencies to reach the platform were measured by the observer; total search time for the five trials was set to 150 s. The first 20 trials (days 1–4) measured the mouse's spatial learning ability and the last five trials (day 5) its relearning ability. The experimental design of the swim maze test was as used earlier in experiments where mice were exposed to PBDEs and PCBs (Eriksson and Fredriksson, 1996a, 1998; Eriksson *et al.*, 2001b; Viberg *et al.*, 2003a).

Statistical Analysis

Body Weight

The data from each age category were subjected to one-way analysis of variance (ANOVA) and pairwise testing using Dunnett, comparing all pairs of treatment groups.

Spontaneous Behavior

The data were subjected to a split-plot ANOVA, and pairwise testing between treated groups and their corresponding control groups was performed with the Tukey honestly significant difference) test (Kirk, 1968).

Swim Maze

The data from days 1 to 4 of the test were subjected to general linear model (GLM) with a split-plot design and to GLM with Duncan's multiple range test. Comparisons between the performance of the last trial on day 4 versus the first trial on day 5 and the comparison between the first and last trial on day 5 were submitted to a paired *t*-test and Kruskal-Wallis with pairwise testing, using Duncan's test.

RESULTS

Neither were there clinical signs of dysfunction in the vehicle- or PBDE-exposed mice throughout the experimental period nor was there any significant deviation in body weight in the PBDE 183–, PBDE 203–, or PBDE 206–treated mice, compared with the vehicle-exposed mice (adult weights of 3-day-old mice, mean ± SD: controls, 42.21 ± 4.51; PBDE 183, 43.97 ± 6.18; PBDE 203, 45.62 ± 4.05; and PBDE 206, 42.48 ± 3.94; adult weights of 10-day-old mice, mean ± SD: controls, 45.65 ± 3.35; PBDE 183, 43.86 ± 4.05; PBDE 203, 44.12 ± 3.59; and PBDE 206, 43.36 ± 4.19).

Effects on Spontaneous Behavior of Adult Mice

The results from the spontaneous behavior variables, locomotion, rearing, and total activity, for 2-month-old male NMRI mice after exposure to a single oral dose of 21 μmol/kg body weight of PBDE 206 (18.5 mg), PBDE 203 (16.8 mg), or PBDE 183 (15.2 mg) on either PND 3 or PND 10 are shown in Figure 1. There were significant group × period interactions ($F_{14,144} = 33.59$, $F_{14,144} = 44.92$, and $F_{14,144} = 10.82$) for locomotion, rearing, and total activity, respectively.

The control mice exposed to the 20% fat emulsion vehicle on PND 3 or PND 10 showed a normal decrease in activity during the 60-min observation period. Pairwise testing between the two control groups revealed no significant difference in the three test variables. This decrease in activity is a normal spontaneous behavior profile, as reported in our earlier studies (see Eriksson, 1997, 1998; Eriksson *et al.*, 1991, 2000, 2001a,b, 2002; Viberg *et al.*, 2002, 2003a,b).

Adult mice neonatally exposed to BDE 183 on PND 3 or PND 10. The results from pairwise testing of mice exposed to PBDE 183 on PND 3 showed a significant ($p \leq 0.01$) decrease in activity in the locomotion and total activity variables during the first 20-min period, compared with the corresponding vehicle-treated animals. The results from pairwise testing of mice exposed to PBDE 183 on PND 10 showed no significant change in activity (in locomotion, rearing, and total activity vs. controls).

Adult mice neonatally exposed to PBDE 203 on PND 3 or PND 10. The results from pairwise testing of mice exposed to PBDE 203 on PND 3 showed a significant ($p \leq 0.01$) decrease in activity during the first 20-min period for the locomotion, rearing, and total activity variables versus their controls. A significant ($p \leq 0.05$) decrease in activity was also seen in the rearing variable during the second 20-min period versus their controls. During the third period, 40–60 min, a significant ($p \leq 0.01$) increase in activity was observed in the locomotion, rearing, and total activity variables versus their controls. The results from pairwise testing of mice exposed to PBDE 203 on PND 10 revealed a significant ($p \leq 0.01$) decrease in activity in locomotion, rearing, and total activity variables versus their controls during the first 20-min period. During the second period (20–40 min), locomotion was significantly ($p \leq 0.01$) increased and rearing was significantly ($p \leq 0.05$) decreased versus their controls. During the last 20-min period (40–60

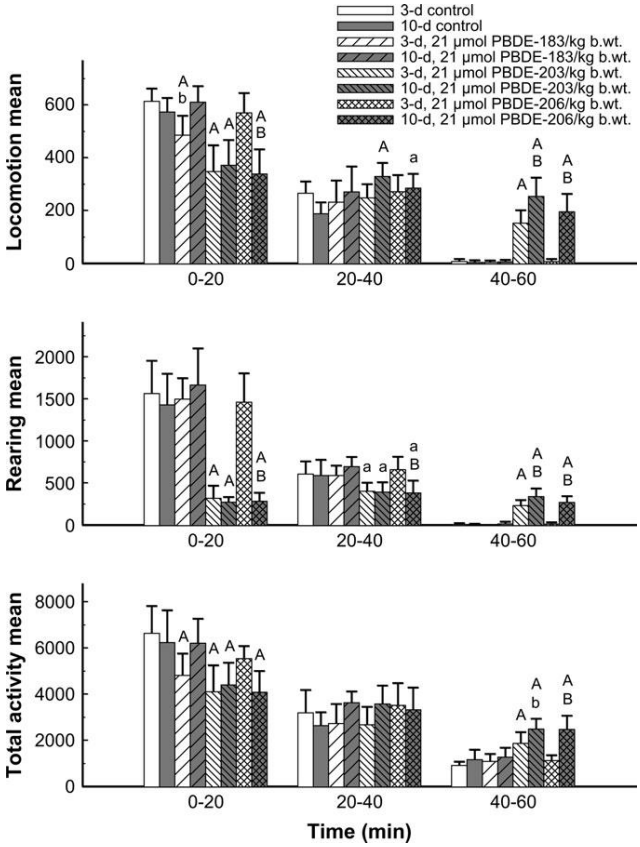

FIG. 1. Spontaneous behavior of 2-month-old NMRI male mice exposed to a single oral dose, 21 µmol/kg body weight, of PBDE 183 (15.2 mg), PBDE 203 (16.8 mg), or PBDE 206 (18.5 mg) on either PND 3 or PND 10. The control animals received in the same manner 10 ml/kg body weight of the 20% fat emulsion vehicle on either PND 3 or PND 10. The data were subjected to an ANOVA with split-plot design, and there were significant group × period interactions ($F_{14,144} = 33.59$, $F_{14,144} = 44.92$, and $F_{14,144} = 10.82$) for locomotion, rearing, and total activity variables, respectively. Pairwise testing between PBDE 183–, PBDE 203–, PBDE 206–exposed animals and control animals was performed with Tukey honestly significant difference tests. The statistical differences are indicated as follows: (A) significantly different versus their respective controls, $p \leq 0.01$; (a) significantly different versus their respective controls, $p \leq 0.05$; (B) significant difference between PND 3 and PND 10 within each treatment group, $p \leq 0.01$; (b) significant difference between PND 3 and PND 10 within each treatment group, $p \leq 0.05$. Bar height represents mean value ± SD.

min), all three variables were significantly increased versus their controls. The results from pairwise testing between mice exposed to PBDE 203 on PND 3 and PND 10 showed a significant difference between the two different treatment days. During the last 20-min period (40–60 min), all three variables were significantly ($p \leq 0.01$) increased in mice given PBDE 203 on PND 10, compared with PND 3.

Adult mice neonatally exposed to PBDE 206 on PND 3 or PND 10. The results from mice exposed to PBDE 206 on PND 3 showed no significant difference in the spontaneous behavior variables, locomotion, rearing, and total activity, compared with the corresponding controls. The results from pairwise testing of mice exposed to PBDE 206 on PND 10 showed a significant ($p \leq 0.01$) decrease in activity in all three variables during the first 20-min period versus their controls. During the second period (20–40 min), locomotion was significantly ($p \leq 0.05$) increased while rearing was significantly ($p \leq 0.05$) decreased versus their controls. During the last period (40–60 min), a significant ($p \leq 0.01$) increase in activity was seen in all three variables versus their controls.

Effects on Learning and Memory in Adult Mice

Mice exposed to PBDE 203 and the vehicle on PND 3 and mice exposed to PBDE 203, PBDE 206, and the vehicle on PND 10 were tested for swim maze performance. Figure 2 shows the performance of 3-month-old mice exposed on PND 3. During the acquisition period (days 1–4) of spatial learning ability, all mice, regardless of treatment, improved their ability to locate the platform ($F_{3,84} = 61.11$, $p \leq 0.01$). Neither did split-plot GLM reveal significant treatment × day interactions among PBDE 203– and vehicle-treated animals ($F_{6,84} = 0.68$) nor were any treatment effects observed. Figure 3 shows the performance of 3-month-old mice exposed on PND 10. During the acquisition period (days 1–4), all mice, regardless of treatment, improved their ability to locate the platform ($F_{3,135} = 35.28$, $p \leq 0.01$). Split-plot GLM revealed no significant treatment × day interactions among PBDE 203–, PBDE 206–, and vehicle-treated animals ($F_{6,135} = 0.1522$). However, a treatment effect ($F_{2,135} = 7.82$) was seen during the

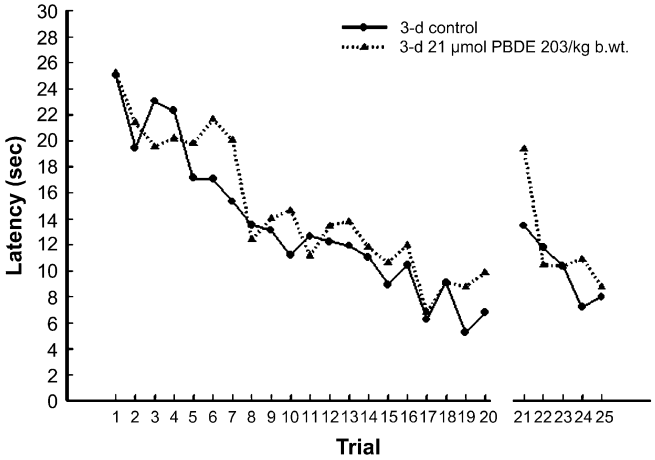

FIG. 2. Swim maze performance in 3-month-old NMRI male mice exposed to a single oral dose of either 16.8 mg PBDE 203/kg body weight (21 µmol/kg body weight) or a 20% fat emulsion vehicle (control) at the age of 3 days. Latencies in locating the platform were measured during the acquisition period, trials 1–20 (days 1–4), and during the relearning period, trials 21–25 (day 5). Statistical analysis: the behavioral data, trials 1–20, were submitted to a GLM and Duncan's multiple range test. There are no significant treatment × day interactions or treatment effects. Trial 20 versus 21 was submitted to a *t*-test, as was trial 21 versus 25, but no significant changes were observed.

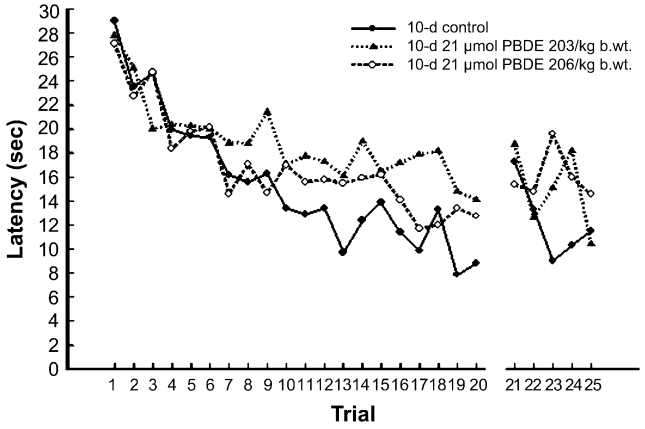

FIG. 3. Swim maze performance in 3-month-old NMRI male mice exposed to a single oral dose of 16.8 mg PBDE 203/kg body weight (21 μmol/kg body weight), 18.5 mg PBDE 206/kg body weight (21 μmol/kg body weight), or 20% fat emulsion vehicle (control) at the age of 10 days. Latencies in locating the platform were measured during the acquisition period, trials 1–20 (days 1–4), and during the relearning period, trials 21–25 (day 5). Statistical analysis: the behavioral data, trials 1–20, were submitted to the GLM and Duncan's multiple range test. A significant treatment effect ($F_{2,135} = 7.82$) is seen during the acquisition period (days 1–4) where mice exposed to PBDE 203 on PND 10 significantly differed from vehicle-treated mice ($p \leq 0.01$) and from those exposed to PBDE 206 ($p \leq 0.05$). Trial 20 versus 21 was submitted to a *t*-test, as was trial 21 versus 25, but no significant changes were observed.

acquisition period, where mice exposed to PBDE 203 on PND 10 differed significantly from control mice ($p \leq 0.01$) and from PBDE 206 mice ($p \leq 0.05$).

On day 5, the platform was relocated for relearning by reversal trials. In the first trial on day 5, the control mice in each age category displayed significantly longer latencies than those in the last trial on day 4, control mice exposed on PND 3 $p \leq 0.01$ (Fig. 2), and those exposed on PND 10, $p \leq 0.05$ (Fig. 3). This is normal behavior during relearning because, initially, the mouse searches near the previous platform location (Morris *et al.*, 1982). Mice exposed to PBDE 203 on PND 3 or to PBDE 203 and PBDE 206 on PND 10 did not differ significantly ($p \geq 0.05$) from their controls. Furthermore, on the fifth day, mice exposed to the vehicle on PND 3 or on PND 10 significantly improved their ability to find the new location of the platform (paired *t*-test trial 21 vs. trial 25), $p \leq 0.05$, indicating normal relearning by the control animals. Mice exposed to PBDE 203 on PND 3 or to PBDE 203 and PBDE 206 on PND 10 did not significantly ($p \geq 0.05$) differ from their controls.

DISCUSSION

In this study, the administration of PBDE 203 or PBDE 206 to mice on neonatal day 10 caused disturbances in spontaneous behavior, leading to disrupted habituation and a hyperactive condition in the mice at an adult age of 2 months. This change in spontaneous behavior was also observed in 2-month-old mice exposed to PBDE 203 on neonatal day 3. Furthermore, neonatal exposure to PBDE 203 on neonatal day 10 affected learning and memory functions in adult mice (see Table 1).

The spontaneous motor behavior data showed a disruption of habituation in adult mice exposed neonatally to PBDE 203 (21 μmol/kg body weight) and to PBDE 206 (21 μmol/kg body weight) on PND 10. Normal habituation, defined here as a decrease in the variables locomotion, rearing, and total activity, in response to the diminished novelty of the test chamber over a 60-min test period, divided into three 20-min periods, was observed in control mice exposed to the 20% fat emulsion vehicle on PND 10. Mice exposed to PBDE 203 and PBDE 206 on PND 10 were obviously hypoactive early in the 60-min test period, whereas toward the end, they became hyperactive. This nonhabituating behavior profile has also been seen in adult NMRI male mice exposed neonatally to PBDE 47 (21 μmol/kg body weight), PBDE 99 (1.4–21 μmol/kg body weight), and PBDE 153 (0.7–14 μmol/kg body weight) at the age of 10 days (Eriksson *et al.*, 2001b, 2002; Viberg *et al.*, 2002, 2003a, 2004). This indicates that, in addition to the lower brominated PBDEs, highly brominated PBDEs such as PBDE 203 and PBDE 206 can disrupt spontaneous behavior and impair habituation capability in adult mice when the exposure occurs around neonatal day 10.

Developmental neurobehavioral effects were also seen in mice exposed to PBDE 203 on PND 3. In all the three spontaneous behavioral variables, locomotion, rearing, and total activity, these mice displayed a hypoactive condition early in the 60-min test period, while being hyperactive toward the end. This change in spontaneous behavior is similar to the changes observed after neonatal exposure to PBDE 203 on PND 10. However, the effects were significantly more pronounced in mice exposed on PND 10 versus PND 3, which indicates an increased susceptibility in 10-day-old mice compared with 3-day-old mice.

The ability of adult mice to learn and memorize was observed in a swim maze of the Morris water maze type, after exposure to PBDE 203 on PND 3 or PND 10 and to PBDE 206 on PND 10. During the 4-day acquisition period, all animals reduced the time needed to locate the submerged platform. However, this test showed that mice exposed to PBDE 203 on PND 10 performed significantly worse during the acquisition phase of learning and memory than both controls and mice given PBDE 206. Furthermore, in mice given PBDE 203 on PND 3, there were no significant deviations from their own controls. This test also confirms that the neurobehavioral defects are more pronounced when PBDEs are present in the brain around PND 10 of neonatal brain development.

In earlier studies, we reported that neonatal exposure to some PBDEs, such as PBDE 99 and PBDE 153, on PND 10 affected the performance of adult animals in the Morris water maze test (Eriksson *et al.*, 2001b; Viberg *et al.*, 2003a). Impaired performance in Morris water maze have also been

TABLE 1
Summary of Effects on Spontaneous Behavior, Habituation, Learning, and Memory after Neonatal Exposure to PBDE 183, PBDE 203, and PBDE 206

	Behavioral effects in adults					
Neonatal treatment	Spontaneous behavior				Morris water maze	
Age/PBDE congener	Locomotion	Rearing	Total activity	Habituation	Learning	Relearning
3-Days old						
PBDE 183	S	NS	S	NS	NA	NA
PBDE 203	S	S	S	S	NS	NS
PBDE 206	NS	NS	NS	NS	NA	NA
10-Days old						
PBDE 183	NS	NS	NS	NS	NA	NA
PBDE 203	S	S	S	S	S	NS
PBDE 206	S	S	S	S	NS	NS

Note. NMRI male mice exposed to a single oral dose, 21 μmol/kg body weight, of PBDE 183 (15.2 mg), PBDE 203 (16.8 mg), or PBDE 206 (18.5 mg) on either PND 3 or PND 10. The control animals received in the same manner 10 ml/kg body weight of the 20% fat emulsion vehicle on either PND 3 or PND 10. S, significant; NS, not significant; NA, not analyzed.

seen in adult mice neonatally exposed to an *ortho*-substituted PCB, 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB 52) (Eriksson and Fredriksson, 1996a). These studies revealed that the cholinergic system was affected, manifested as an altered response to nicotine at adult age as well as a decrease in cholinergic nicotinic receptors (Eriksson and Fredriksson, 1996a,b; Viberg *et al.*, 2002, 2003a, 2004). The swim maze of Morris water maze type, with a submerged platform, is designed to measure spatial learning. Spatial learning tasks, dependent on external cues for their solution, have been found to be highly sensitive to central cholinergic dysfunctions (Levin, 2002; Riekkinen *et al.*, 1990; Sutherland *et al.*, 1982). Many studies in rodents have also shown that different cholinergic agonists and antagonists affect memory and learning (see Levin, 2002). Whether highly brominated PBDEs can affect the cholinergic system, as earlier seen for the lower and middle brominated PBDEs, remains to be studied as a possible mechanism for the deranged behavior.

In several developmental neurotoxicity studies in mice, we have shown that the presence of persistent or nonpersistent compounds around neonatal day 10 caused persistent developmental neurotoxic disturbances, manifested as changes in spontaneous behavior, reduced habituation capability, impaired learning and memory faculties in maze tests, and alterations in the cholinergic transmitter system (Eriksson, 1997, 1998; Eriksson *et al.*, 2001a,b; Viberg *et al.*, 2002, 2003a,b). However, this study indicated that disturbances can also be induced when mice are exposed to PBDE 203 and PBDE 183 on neonatal day 3. When mice were exposed to PBDE 183 (21 μmol/kg body weight), effects on spontaneous behavior were only seen following exposure on PND 3. There was a significant reduction in locomotion and total activity variables during the first 20-min period, but habituation was not affected. Interestingly, exposure to PBDE 183 on PND 10 did not affect the spontaneous behavior, as was seen in mice given PBDE 203 or PBDE 206. The reasons for the less pronounced neurotoxic effect of PBDE 183 and also lack of effect when given on PND 10, compared with PBDE 203 and PBDE 206, remain unknown. There may be differences in uptake and/or metabolism among the different PBDE congeners. We have recently shown that neonatal exposure to different PBDEs—PBDE 99 and PBDE 209—can alter spontaneous behavior and reduce the habituation capability of adult mice neonatally exposed on PND 3. The behavioral disturbances seen after neonatal exposure to PBDE 99 on PND 3 were attributed to the amount left 7 days after the exposure and thereby present on PND 10, which was suggested to be enough to induce adult behavioral disturbances (Eriksson *et al.*, 2002). Regarding neonatal exposure to PBDE 209 (21 μmol/kg body weight), the developmental neurotoxic effects on spontaneous behavior and habituation capability were seen only in mice exposed on PND 3 but not in mice given PBDE 209 on PND 10. It is known that the amount of a toxic agent present in the brain at different neonatal ages can vary. Earlier studies, using radiolabeled compounds, have shown a pronounced retention of lipophilic chlorinated hydrocarbons or their metabolites (e.g., PBDE 99, DDT, PCB 52, PCB 153, and chlorinated paraffins) in the brain when administered on neonatal day 10 (Eriksson, 1984, 1998; Eriksson and Darnerud, 1985; Eriksson *et al.*, 2002). During the course of time, the amount of radioactivity from these substances was the same or decreased over the 7-day period. In contrast, the radioactivity from ^{14}C-PBDE 209 increased significantly in animals exposed to ^{14}C-labeled PBDE 209 on PND 3 or PND 10 (Viberg *et al.*, 2003b). That neonatal mice are capable of metabolizing persistent organic compounds, e.g., PCBs, has been reported by Vodicnik and Lech, 1980.

The developmental neurotoxic effects of PBDE 209 were therefore suggested to be caused by one or more metabolites of the parent compound. Recent studies have also shown that PBDE 209, after oral administration to rats, can be metabolized to several metabolites, including PBDE 183, PBDE 203, and PBDE 206 (Morck *et al.*, 2003; Sandholm, 2003; Sandholm *et al.*, 2003). Whether or not the metabolites of PBDE 209 in the neonatal brain of 10-day-old mice constitute debrominated products like PBDE 203 and PBDE 206, after administration to neonatal mice on PND 3, the present study has shown that congeners such as PBDE 203 and PBDE 206 are capable of inducing developmental neurotoxic effects when given on PND 10.

In conclusion, the present investigation has shown that neonatal exposure to higher brominated PBDEs, such as the PBDE 203 and PBDE 206, can cause developmental neurotoxic effects, manifested as defective spontaneous behavior, reduced or lack of habituation, and impaired learning and memory functions, when present during a critical period of the neonatal brain development. Furthermore, from both the spontaneous behavior test and the maze learning/memory test, it is clear that PBDE 203 is more potent in causing developmental neurotoxic effects than are PBDE 206 and PBDE 183. This indicates differences in neurotoxicity among PBDE congeners. That different PBDEs differ in their ability to induce developmental neurotoxicity was also reported in our earlier studies (Eriksson *et al.*, 2001b; Viberg *et al.*, 2003a,b), and different degrees of potency in causing neurotoxic effects are important when comparing the levels of PBDEs in the environment and in mother's milk.

ACKNOWLEDGMENTS

Financial support was provided by the Swedish Research Council for Environmental, Agricultural Sciences and Spatial Planning and the Foundation for Strategic Environment Research.

REFERENCES

Akutsu, K., Kitagawa, M., Nakazawa, H., Makino, T., Iwazaki, K., Oda, H., and Hori, S. (2003). Time-trend (1973–2000) of polybrominated diphenyl ethers in Japanese mother's milk. *Chemosphere* **53,** 645–654.

Allchin, C. R., Law, R. J., and Morris, S. (1999). Polybrominated diphenylethers in sediment and biota downstream of potential sources in the UK. *Environ. Pollut.* **105,** 197–207.

Asplund, L., Athanasiadou, M., Sjödin, A., Bergman, Å., and Börjeson, H. (1999a). Organohalogen substances in muscle, egg and blood from healthy Baltic salmon (*Salmo salar*) and Baltic salmon that produces offspring with the M74 syndrome. *Ambio* **28,** 67–76.

Asplund, L., Hornung, M., Peterson, R., Thuresson, K., and Bergman, Å. (1999b). Levels of polybrominated diphenyl ethers (PBDEs) in fish from the Great Lakes and Baltic Sea. *Organohalogen Compd.* **40,** 351–354.

Baumann, B., Hijman, W., van Beuzekom, S., Hoogerbrugge, R., Houweling, D., and Zeilmaker, M. (2003). PBDEs in human milk from the Dutch 1998 monitoring programme. *Organohalogen Compd.* **61,** 187–190.

Christiansson, A., Teclechiel, D., Eriksson, J., Bergman, Å., and Marsh, G. (2006). Methods for synthesis of nonabromodiphenyl ethers and a chlorononabromodiphenyl ether. *Chemosphere* **63,** 562–569.

Darnerud, P. O., Eriksen, G. S., Johannesson, T., Larsen, P. B., and Viluksela, M. (2001). Polybrominated diphenyl ethers: occurrence, dietary exposure, and toxicology. *Environ. Health Perspect.* **109**(Suppl. 1), 49–68.

Davison, A. N., and Dobbing, J. (1968). *Applied Neurochemistry.* Blackwell, Oxford.

Eriksson, P. (1984). Age-dependent retention of [^{14}C]DDT in the brain of the postnatal mouse. *Toxicol. Lett.* **22,** 323–328.

Eriksson, P. (1997). Developmental neurotoxicity of environmental agents in the neonate. *Neurotoxicology* **18,** 719–726.

Eriksson, P. (1998). *Perinatal Developmental Neurotoxicity of PCBs*, p. 56. Swedish Environmental Protection Agency, Stockholm.

Eriksson, P., Ankarberg, E., and Fredriksson, A. (2000). Exposure to nicotine during a defined period in neonatal life induces permanent changes in brain nicotinic receptors and in behaviour of adult mice. *Brain Res.* **853,** 41–48.

Eriksson, P., Ankarberg, E., Viberg, H., and Fredriksson, A. (2001a). The developing cholinergic system as target for environmental toxicants, nicotine and polybrominated biphenyls (PCBs): Implications for neurotoxicological processes in mice. *Neurotox. Res.* **3,** 37–51.

Eriksson, P., and Darnerud, P. O. (1985). Distribution and retention of some chlorinated hydrocarbons and a phthalate in the mouse brain during the preweaning period. *Toxicology* **37,** 189–203.

Eriksson, P., and Fredriksson, A. (1996a). Developmental neurotoxicity of four ortho-substituted polychlorinated biphenyls in the neonatal mouse. *Environ. Toxicol. Pharmacol.* **1,** 155–165.

Eriksson, P., and Fredriksson, A. (1996b). Neonatal exposure to 2,2′,5,5′-tetrachlorobiphenyl causes increased susceptibility in the cholinergic transmitter system at adult age. *Environ. Toxicol. Pharmacol.* **1,** 217–220.

Eriksson, P., and Fredriksson, A. (1998). Neurotoxic effects in adult mice neonatally exposed to 3,3′,4,4′,5-pentachlorobiphenyl or 2,3,3′,4,4′-pentachlorobiphenyl. Changes in nicotinic receptors and behaviour. *Environ. Toxicol. Pharmacol.* **5,** 17–27.

Eriksson, P., Jakobsson, E., and Fredriksson, A. (2001b). Brominated flame retardants: A novel class of developmental neurotoxicants in our environment? *Environ. Health Perspect.* **109,** 903–908.

Eriksson, P., Lundkvist, U., and Fredriksson, A. (1991). Neonatal exposure to 3,3′,4,4′-tetrachlorobiphenyl: Changes in spontaneous behaviour and cholinergic muscarinic receptors in the adult mouse. *Toxicology* **69,** 27–34.

Eriksson, P., and Viberg, H. (2005). Tiered testing in mammals—The neonatal animal model. In *Science for a Safe Chemical Environment* (S. Hansson and C. Rudén, Eds.), pp. 103–133. A MISTRA Funded Research Programme, Stockholm.

Eriksson, P., Viberg, H., Jakobsson, E., Orn, U., and Fredriksson, A. (2002). A brominated flame retardant, 2,2′,4,4′,5-pentabromodiphenyl ether: Uptake, retention, and induction of neurobehavioral alterations in mice during a critical phase of neonatal brain development. *Toxicol. Sci.* **67,** 98–103.

Eriksson, P., von Rosen, D., Viberg, H., and Fredriksson, A. (2005). Developmental toxicology in the neonatal mouse: The use of randomly selected individuals as statistical unit compared to the litter in mice neonatally exposed to PBDE 99. *Toxicologist* **84,** 219.

Fredriksson, A., Eriksson, P., and Archer, T. (1997). MPTP-induced deficits in motor activity: Neuroprotective effects of the spintrapping agent, alpha-phenyl-tert-butyl-nitrone (PBN). *J. Neural Transm.* **104,** 579–592.

Hale, R. C., La Guardia, M. J., Harvey, E. P., Mainor, T. M., Duff, W. H., and Gaylor, M. O. (2002). Polybrominated diphenyl ether flame retardants in the Virginia freshwater fishes (USA). *Environ. Sci. Technol.* **35,** 4585–4591.

Hutzinger, O., Sundström, G., and Safe, S. (1976). Environmental chemistry of flame retardants. Part I. Introduction and principles. *Chemosphere* **1,** 3–10.

Hutzinger, O., and Thoma, H. (1987). Polybrominated dibenzo-p-dioxins and dibenzofurans: The flame retardant issue. *Chemosphere* **16,** 1877–1880.

Jakobsson, K., Thuresson, K., Rylander, L., Sjodin, A., Hagmar, L., and Bergman, A. (2002). Exposure to polybrominated diphenyl ethers and tetrabromobisphenol A among computer technicians. *Chemosphere* **46,** 709–716.

Keller, W. C., and Yeary, R. A. (1980). A comparison of the effects of mineral oil, vegetable oil and sodium sulfate on the intestinal absorption of DDT in rodents. *Clin. Toxicol.* **16,** 223–231.

Kirk, R. E. (1968). *Procedures for the Behavioural Science.* Brooks/Cole, Belmont, CA.

Levin, E. D. (2002). Nicotinic receptor subtypes and cognitive function. *J. Neurobiol.* **53,** 633–640.

Mazdai, A., Dodder, N. G., Abernathy, M. P., Hites, R. A., and Bigsby, R. M. (2003). Polybrominated diphenyl ethers in maternal and fetal blood samples. *Environ. Health Perspect.* **111,** 1249–1252.

Meironyté Guvenius, D., Aronsson, A., Ekman-Ordeberg, G., Bergman, Å., and Norén, K. (2003). Human prenatal and postnatal exposure to polybrominated diphenyl ethers, polybrominated biphenyls, polychlorobiphenylols, and pentachlorophenol. *Environ. Health Perspect.* **111,** 1235–1241.

Morck, A., Hakk, H., Orn, U., and Klasson Wehler, E. (2003). Decabromodiphenyl ether in the rat: Absorption, distribution, metabolism, and excretion. *Drug Metab. Dispos.* **31,** 900–907.

Morris, R. (1981). Spatial localization does not require the presence of local cues. *Learn. Motiv.* **12,** 239–260.

Morris, R. G., Garrud, P., Rawlins, J. N., and O'Keefe, J. (1982). Place navigation impaired in rats with hippocampal lesions. *Nature* **297,** 681–683.

Norén, K., and Meironyté, D. (2000). Certain organochlorine and organobromine contaminants in Swedish human milk in perspective of past 20–30 years. *Chemosphere* **40,** 1111–1123.

Palin, K. J., Wilson, C. G., Davis, S. S., and Phillips, A. J. (1982). The effects of oil on the lymphatic absorption of DDT. *J. Pharm. Pharmacol.* **34,** 707–710.

Riekkinen, P. J., Sirviö, J., Aaltonen, M., and Riekkinen, P. (1990). Effects of concurrent manipulations of nicotinic and muscarinic receptors on spatial and passive avoidance learning. *Pharmacol. Biochem. Behav.* **37,** 405–410.

Sandholm, A. (2003). *Metabolism of Some Polychlorinated Biphenyl and Polybrominated Diphenyl Ether Congeners in the Rat*, p. 58. Department of Environmental Chemistry, Stockholm University, Stockholm.

Sandholm, A., Emanuelsson, B.-M., and Klasson Wehler, E. (2003). Bioavailability and half-life of decabromodiphenyl ether (BDE-209) in rat. *Xenobiotica* **33,** 1149–1158.

Schecter, A., Papke, O., Tung, K. C., Joseph, J., Harris, T. R., and Dahlgren, J. (2005). Polybrominated diphenyl ether flame retardants in the U.S. population: Current levels, temporal trends, and comparison with dioxins, dibenzofurans, and polychlorinated biphenyls. *J. Occup. Environ. Med.* **47,** 199–211.

Schecter, A., Pavuk, M., Päpke, O., Ryan, J. J., Birnbaum, L., and Rosen, R. (2003). Polybrominated diphenyl ethers (PBDEs) in U.S. mothers' milk. *Environ. Health Perspect.* **111,** 1723–1729.

Sellström, U., Jansson, B., Kierkegaard, A., and de Wit, C. (1993). Polybrominated diphenyl ethers (PBDE) in biological samples from the Swedish environment. *Chemosphere* **26,** 1703–1718.

Sjödin, A., Hagmar, L., Klasson-Wehler, E., Kronholm-Diab, K., Jakobsson, E., and Bergman, Å. (1999). Flame retardant exposure: Polybrominated diphenyl ethers in blood from Swedish workers. *Environ. Health Perspect.* **107,** 643–648.

Sjödin, A., Patterson, D. G., Jr., and Bergman, Å. (2001). Brominated flame retardants in serum from U.S. blood donors. *Environ. Sci. Technol.* **35,** 3830–3833.

Sutherland, R. J., Whishaw, I. Q., and Regehr, J. C. (1982). Cholinergic receptor blockade impairs spatial localization by use of distal cues in the rat. *J. Comp. Physiol. Psychol.* **96,** 563–573.

Thuresson, K., Bergman, A., and Jakobsson, K. (2005). Occupational exposure to commercial decabromodiphenyl ether in workers manufacturing or handling flame-retarded rubber. *Environ. Sci. Technol.* **39,** 1980–1986.

Viberg, H., Fredriksson, A., and Eriksson, P. (2002). Neonatal exposure to the brominated flame retardant 2,2′,4,4′,5-pentabromodiphenyl ether causes altered susceptibility in the cholinergic transmitter system in the adult mouse. *Toxicol. Sci.* **67,** 104–107.

Viberg, H., Fredriksson, A., and Eriksson, P. (2003a). Neonatal exposure to polybrominated diphenyl ether (PBDE 153) disrupts spontaneous behaviour, impairs learning and memory, and decreases hippocampal cholinergic receptors in adult mice. *Toxicol. Appl. Pharmacol.* **192,** 95–106.

Viberg, H., Fredriksson, A., and Eriksson, P. (2004). Neonatal exposure to the brominated flame-retardant, 2,2′,4,4′,5-pentabromodiphenyl ether, decreases cholinergic nicotinic receptors in hippocampus and affects spontaneous behaviour in the adult mouse. *Environ. Toxicol. Pharmacol.* **17,** 61–65.

Viberg, H., Fredriksson, A., Jakobsson, E., Orn, U., and Eriksson, P. (2003b). Neurobehavioural derangements in adult mice receiving decabrominated diphenyl ether (PBDE 209) during a defined period of neonatal brain development. *Toxicol. Sci.* **76,** 112–120.

Vodicnik, M. J., and Lech, J. J. (1980). The transfer of 2,4,5,2′,4′,5′-hexachlorobiphenyl to fetuses and nursing offspring. I. Disposition in pregnant and lactating mice and accumulation in young. *Toxicol. Appl. Pharmacol.* **54,** 293–300.

WHO (1994). *Brominated Diphenyl Ethers.* WHO IPCS Environmental Health Criteria Document, 162. WHO, Geneva, PA.