Brominated flame retardants are a novel group of global environmental contaminants. Within this group the polybrominated diphenyl ethers (PBDEs) constitute one class of many that are found in electrical appliances, building materials, and textiles. PBDEs are persistent compounds that appear to have an environmental dispersion similar to that of polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDT). Levels of PBDEs are increasing in mother’s milk while other organohalogens have decreased in concentration. We studied for developmental neurotoxic effects two polybrominated diphenylethers, 2,2’,4,4’-tetrabromodiphenyl ether (PBDE 47) and 2,2’,4,4’,5-pentabromodiphenylether (PBDE 99)—congeners that dominate in environmental and human samples—together with another frequently used brominated flame retardant, tetrabromo-bis-phenol-A (TBBPA). The compounds were given to 10-day-old NMRI male mice, as follows: PBDE 47, 0.7 mg (1.4 μmol), 10.5 mg (21.1 μmol)/kg body weight (bw); PBDE 99, 0.8 mg (1.4 μmol), 12.0 mg (21.1 μmol)/kg bw; TBBPA, 0.75 mg (1.4 μmol), 11.5 mg (21.1 μmol)/kg bw. Mice serving as controls received 10 mL/kg bw of the 20% fat emulsion vehicle in the same manner. The present study has shown that neonatal exposure to PBDE 99 and PBDE 47 can cause permanent aberrations in spontaneous behavior, evident in 2- and 4-month-old animals. This effect together with the habituation capability was more pronounced with increasing age, and the changes were dose–response related. Furthermore, neonatal exposure to PBDE 99 also affected learning and memory functions in adult animals. These are developmental defects that have been detected previously in connection with PCBs. Key words: adult, brominated flame retardants, developmental neurotoxicology, memory and learning, neonatal, polybrominated diphenyl ethers, spontaneous behavior. Environ Health Perspect 109:903–908(2001). [Online 20 August 2001] http://ehpnet1.niehs.nih.gov/docs/2001/109p903-908eriksson/abstract.html

Brominated flame retardants are a novel group of global environmental contaminants (1,2). Within this group the polybrominated diphenyl ethers (PBDEs) are used in large quantities as flame-retardant additives in polymers, especially in the manufacture of a variety of electrical appliances, including television and computer casing, building materials, and textiles (3,4). Because PBDEs are mixed, not chemically bound, into the material they are used in, they may migrate from the material. One of the earliest reports of PBDEs in our environment came in 1981 (5). Since then, PBDEs have been shown to be persistent compounds that appear to have an environmental dispersion similar to that of polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDT) (2,6). PBDEs have been found in various wildlife species and in human tissues (1,7). The PBDE congeners that dominate in environmental and human samples are 2,2’,4,4’-tetrabromodiphenyl ether (PBDE 47) and 2,2’,4,4’,5-pentabromodiphenyl ether (PBDE 99). These agents together with tetrabromo-bis-phenol-A (TBBPA) have also been detected in human plasma samples (6,8) and in mother’s milk (9,10). A study on certain organochlorine and organobromine contaminants in milk from native Swedish mothers showed that the concentration of PBDEs has increased continuously from 1972 to 1997, whereas those of organochlorine compounds such as PCB and DDT have decreased (9,10). The concentration of PBDEs in 1972 was still lower than those of PCBs and DDT. The concentration for PCBs was 324 ng/g lipid, for DDT 14 ng/g lipid, and for PBDEs 4 ng/g lipid. However, of special concern is that the PBDEs show an exponential increase from 1972 to 1997 with a rate of increase that doubled in 5-year increments (9,10).

In mammals, the fetus can be directly exposed during gestation via maternal intake of toxic agents. During the neonatal period, offspring may be contaminated by ingesting mother’s milk or by direct exposure to xenobiotics (11). In many mammalian species a rapid growth of the brain occurs during perinatal development—the so-called brain growth spurt (12). In humans, this period begins during the third trimester of pregnancy and continues throughout the first 2 years of life. In mouse and rat this period is neonatal, spanning the first 3–4 weeks of life, during which the brain undergoes several fundamental phases, such as axonal and dendritic outgrowth and the establishment of neural connections. During this period, animals also acquire many new motor and sensory abilities (13) and their spontaneous motor behavior peaks (14). The brain growth spurt is associated with numerous biochemical changes that transform the fetal/neonatal brain into that of the mature adult (15,16). One of the major transmitter systems that undergo rapid development is the cholinergic system, which is involved in many behavioral phenomena and cognitive functions (17,18).

In several reports we have shown that low-dose exposure to both persistent and nonpersistent environmental agents during the neonatal period disrupts adult brain function. Among the toxicants that induce such neurotoxic effects are DDT (19,20), pyrethroids (20), organophosphate (21), nicotine (22), paraquat and MPTP (23), and certain PCBs (24). The induction of behavioral and cholinergic disturbances in the adult animal has often been limited to a short period during neonatal development, around postnatal day 10 (19,21,22). These studies also showed that the induction of these disturbances occurs at doses that apparently have no permanent effect when administered to the adult animal. Furthermore, the exposure level for nicotine, PCB, and DDT were also in the same order of magnitude to which humans can be exposed (20,22,24). Exposure to PCB, DDT, or nicotine during this phase of development can also lead to an increased susceptibility to toxic agents at adult age, indicating that neonatal exposure to toxic agents can potentiate and/or modify the reaction to adult exposure to xenobiotics (25–27).

In view of an increasing amount of PBDEs and TBBPA in the environment and in mother’s milk, we undertook the present study to investigate possible behavioral effects of PBDEs and TBBPA when given during the rapid development of the neonatal mouse brain.

Address correspondence to P. Eriksson, Department of Environmental Toxicology, Uppsala University, Norbyvägen, Uppsala, Sweden; or Eva Jakobsson, Department of Environmental Chemistry, Stockholm University, Stockholm, Sweden.
Materials and Methods

Animals and Chemicals

We used male NMRI mice to make this study comparable with our earlier studies, which were performed on male mice. Pregnant NMRI mice were purchased from Charles River, Uppsala, Sweden. Following parturition, each litter, adjusted within 48 hr to eight to ten mice by euthanasia of remaining pups, was kept together with its respective mother in a plastic cage in a room with an ambient temperature of 22°C and a 12 hr light:12 hr dark cycle. At an age of 10 days, pups were exposed to the vehicle or the test compounds. To keep litters and conditions standardized and as close to normal as possible during the neonatal period, we exposed both sexes. At 4 weeks male mice were weaned and were placed and raised in groups of four to seven in a room for male mice only. The animals were supplied with standardized pellet food (Lactamin, Stockholm, Sweden) and tap water ad libitum.

The polybrominated diphenyl ethers PBDE 47 and PBDE 99 were synthesized at the Wallenberg Laboratory (28,29), Uppsala University, Stockholm, Sweden. Tetrabromo-bis-phenol-A (TBBPA) was purchased from Aldrich (Steinheim, Germany) and was recrystallized from chloroform. The purity of the compounds exceeded 98%. The substances were 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% weight:water (w:w) fat emulsion vehicle containing various concentrations of the compounds. The substances were administered orally, at a volume of 10 mL/kg body weight (bw), via a PVC tube (diameter 1.0 mm) as one single dose on postnatal day 10. The amounts of the different compounds given were as follows: 2,2',4,4'-tetrabromodiphenyl ether (PBDE 47), 0.7 mg (1.4 μmol), 10.5 mg (21.1 μmol)/kg bw; 2,2',4,4',5-pentabromodiphenyl ether (PBDE 99), 0.8 mg (1.4 μmol), 12.0 mg (21.1 μmol)/kg bw; tetrabromo-bis-phenol-A (TBBPA), 0.75 mg (1.4 μmol), 11.5 mg (21.1 μmol)/kg bw. Mice serving as controls received 10 mL/kg bw of the 20% fat emulsion vehicle in the same manner. Each treatment group comprised mice from 3–4 different litters.

Behavioral Tests

Spontaneous behavior. We tested spontaneous behavior in the male mice at ages 2 and 4 months, as described previously (19,24). We tested eight mice, randomly selected from three to four different litters, once only, and the tests were performed between 0800 and 1200 hr under the same ambient light and temperature conditions. We measured motor activity over 3 × 20 min in an automated device consisting of cages (40 × 25 × 15 cm) placed within two series of infrared beams (low level and high level) (Rat-O-Matic; ADEA Elektronik AB, Uppsala, Sweden) (30,31). Locomotion was registered when the mouse moved horizontally through the low-level grid of infrared beams. For rearing, vertical movement was registered at a rate of 4 counts per second, whenever and as long as a single high-level beam was interrupted—the number of counts obtained was proportional to time spent rearing up. To measure total activity, a pick-up (mounted on a lever with a counter-weight) with which the test cage was in contact registered all types of vibration within the test cage, such as mouse movements, shaking (tremors), and grooming.

Swim maze. We administered a behavior test to male mice at the age of 5 months. We tested 16–18 mice, randomly selected from three to four different litters, for swim maze performance. The swim maze was of M orris water maze type (32); a circular gray tub, 73 cm in diameter, filled with water at 23°C to a depth of 13 cm from the brim. In the center of the northwest quadrant of the pool, a platform was submerged 1 cm beneath the water surface. The platform was formed of metal mesh and had a diameter of 12 cm. We observed the mouse’s ability to locate the submerged platform for 5 days, the animals being given five trials each day between 0900 and 1400 hr. Before the first trial each day, the mouse was placed on the submerged platform for 30 sec. It was then released from the south position with its head toward the side of the tub and allowed 30 sec to locate the platform. If the mouse failed to find the platform within 30 sec, it was placed on the platform. After each trial, the mouse remained on the platform for 30 sec. The mice received five trials per day on 4 consecutive days. On the fifth day the platform was moved to the center of the northeast quadrant for reversal trials; otherwise the procedure was the same. Latency to locate the platform constituted the total search time of five trials, maximum 150 sec. Trials 1–20 (days 1–4) measured the mouse’s spatial learning ability and trials 21–25 (day 5) its relearning ability. The experimental design of the swim maze test was the same as used earlier in the experiment where mice were exposed to the PCBs (24).

Statistical Analysis

Spontaneous behavior. The data were subjected to a split-plot analysis of variance (ANOVA). Pairwise testing between PBDE 47-, PBDE 99-, TBBPA-, and vehicle-treated groups was performed with Tukey’s honestly significant difference (HSD) test (33).

Habituation capability. From the spontaneous behavior test we calculated a ratio between the performance period 40–60 min and 0–20 min for the three different variables: locomotion, rearing, and total activity. We used the following calculation: 100 (counts locomotion 40–60 min/counts locomotion 0–20 min), 100 (counts rearing 40–60 min/counts rearing 0–20 min), and 100 (counts total activity 40–60 min/counts total activity 0–20 min). This ratio was used to analyze alteration in habituation between 2-month-old and 4-month-old mice. These data were subjected to a split-plot ANOVA.

Swim maze. The data from day 1 to day 4 were subjected to a split-plot ANOVA. Pairwise testing between PBDE 47-, PBDE 99-, TBBPA-, and vehicle-treated groups was performed with Tukey’s HSD test. Statistical analysis for the behavioral data of day 5 was submitted to paired t-test (difference between trial 1 and trial 5) and one-way ANOVA with pairwise testing between PBDE 47-, PBDE 99-, TBBPA-, and vehicle-treated groups, using Duncan’s test.

Results

There were no clinical signs of dysfunction in the treated mice throughout the experimental period, nor were there any significant deviations in body weight gain in the PBDE 47-, PBDE 99-, and TBBPA-treated mice, compared with the vehicle-treated mice.

Spontaneous Behavior

Figures 1 and 2 show the results from the spontaneous behavior variables locomotion, rearing, and total activity in 2- and 4-month-old NMRI male mice exposed to a single oral dose of either 1.4 μmol or 21.1 μmol/kg bw of one of the compounds PBDE 47, PBDE 99, TBBPA, with corresponding controls receiving 10 mL/kg bw of the 20% fat emulsion vehicle. Two months after the exposure there were significant treatment × time interactions [F(12,126) = 12.30, F(12,126) = 13.38, F(12,126) = 10.45], for the locomotion, rearing, and total activity variables, respectively. Pairwise testing among PBDE 47, PBDE 99, TBBPA, 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 behavioral variables over 60 min. Mice receiving the higher dose of PBDE 99 (12 mg) or PBDE 47 (10.5 mg) displayed significantly less activity than controls during the first 20-min period (0–20 min), but during the third 20-min period (40–60 min) they were significantly more active than the controls. Mice receiving the lower dose of PBDE 99 (0.8 mg) showed significantly less rearing and total activity than controls during the first 20-min period.
(0–20 min). During the last 20-min period (40–60 min) these mice were significantly more active than the controls in the locomotion variable. In mice receiving TBBPA there were no significant change in the variables locomotion, rearing, and total activity compared with controls.

Four months after neonatal exposure to the different brominated flame retardants there were still significant treatment × time interactions [F (12,126) = 21.41 F (12,126) = 21.09, F (12,126) = 27.12], for the locomotion, rearing, and total activity variables, respectively (Figure 2). Pairwise testing among PBDE 47, PBDE 99, TBBPA and control groups showed a significant dose-related change in locomotion, rearing, and total activity. Pairwise testing between PBDE 99 and control groups showed a significant dose-related change in all three test variables. Mice receiving the lower and the higher dose of PBDE 99 (0.8 mg or 12 mg) were significantly less active than controls during the first 20-min period (0–20 min), but during the last 20-min period (40–60 min) they were significantly more active than the controls. Mice receiving the higher dose of PBDE 47 (10.5 mg) displayed significantly less locomotion, rearing, and total activity than controls during the first 20-min period (0–20 min), but during the last 20-min period (40–60 min) they were significantly more active than the controls. In mice receiving TBBPA there were no significant change in the variables locomotion, rearing, and total activity compared with controls.

Habituation Capability

By analyzing the habituation ratio between performance period 40–60 min and 0–20 min, we obtained information about the ability to habituate to a novel environment which can be used to analyze changes in habituation with age. The results from the habituation ratio, calculated from the spontaneous behavior variables locomotion, rearing, and total activity in 2- and 4-month-old NMRI male mice are given in Table 1. The habituation capability was shown to significantly (*p ≤ 0.001) decrease with age in mice exposed to PBDE 47 [10.5 mg (21.1 µmol)] and PBDE 99 [0.8 mg (1.4 µmol) and 12.0 mg (21.1 µmol)]. In mice neonatally exposed to TBBPA or the vehicle, no significant change in habituation ratio was observed.

Swim Maze Behavior

Mice receiving the higher dose of PBDE 47, PBDE 99, and TBBPA were tested for swim maze performance. Figure 3 shows the performance of 5-month-old mice neonatally exposed to PBDE 47, PBDE 99, TBBPA, or the vehicle. During the acquisition period of spatial learning ability, measured from day 1 to day 4, all mice, regardless of treatment, improved their ability to locate the platform [F (3,161) = 215.46]. Split-plot ANOVA revealed no significant treatment × time interactions among PBDE 47, PBDE 99, TBBPA, and controls [F (6,161) = 0.54]. On day 5 the platform was relocated for relearning by reversal trials. In the first trial on day 5, control mice displayed longer latency than in the last trial on day 4. This is normal behavior during relearning because, initially, the mouse searches near the previous platform location (34). However, controls significantly improved their ability to find the new location (paired t-test 1 vs. trial 5, p ≤ 0.01), indicating normal relearning in...
control animals. In mice receiving the higher dose of PBDE 99 (12.0 mg) the difference in latency between trial 1 and trial 5 differed significantly from controls [F(3,57) = 3.42; Duncan, p ≤ 0.01].

**Discussion**

Behavior is a major function whereby animals adapt to changes in the environment. Changes in behavior may reveal the influence of chemical pollution on our natural environment. Spontaneous behavior is especially meaningful in environmental toxicology because it reflects function that is important for survival of the individual and for the species in the wild—for example, the mobility needed to search for food, to mate, and to elude predators (35).

Our study has shown that neonatal exposure to PBDE 99 and PBDE 47 can cause permanent aberrations in spontaneous behavior. This effect and the habituation capability appear also to worsen with age. Furthermore, neonatal exposure to PBDE 99 also affected learning and memory functions in adult animals. Exposure to TBBPA in the same dose range did not cause any significant change in the investigated behavioral variables. Whether the absence of neurotoxic effects of TBBPA is related to altered uptake or metabolism is not known, but TBBPA is known to have a short half-life (36,37).

The spontaneous motor behavior data showed a dose–response-related disruption of habituation in mice treated with both PBDE 99 and PBDE 47. Habituation—a decrease in locomotion, rearing, and total activity variables in response to the diminishing novelty of the test chamber over 60 min—was demonstrated in the control animals, but mice treated with PBDE 99 and PBDE 47 were obviously hypoactive early in the 60-min test period, whereas toward the end they became hyperactive. This nonhabituating behavior profile has also been reported in adult mice neonatally exposed to ortho-substituted PCBs, such as PCB 28, PCB 52, PCB 153 (24,38,39), and in mice in neonatally exposed to coplanar PCBs, such as PCB 77, PCB 126, and PCB 169 (24,40,41). This indicates that some PBDEs can be potent inducers of behavioral aberrations and that the changes can be similar to those observed earlier for some ortho- and coplanar PCBs. Furthermore, the effects were induced at doses that did not affect the animals' weight gain and at doses comparable to those used in the PCB studies.

The results from the spontaneous behavior tests further indicate that the functional disorder worsens with increasing age: The aberrations were more pronounced in 4-month-old than in 2-month-old mice. In mice receiving the higher dose of PBDE 99 (12.0 mg/kg bw) the difference, compared with controls, in the locomotor variable during the first 20-min period was about 68% in 4-month-old mice compared with about 58% in 2-month-old mice, a difference that was significant (t-test, p ≤ 0.01). This time-dependent effect was evident in mice given the lower dose (0.8 mg/kg bw) where a significant decrease in the locomotor variable was seen in the 4-month-old but not in the 2-month-old mice. The time-dependent effect was also evident in this treatment group during the last 20-min period, where a significant increase in activity in the rearing and total activity variables was seen in the 4-month-old but not in the 2-month-old mice. That this functional disorder can worsen with age is supported by the significantly reduced habituation capability in 4-month-old mice compared to 2-month-old mice. Both the change in spontaneous motor behavior profile, time dependent and dose related, and the reduced habituation capability indicate the advance of brain dysfunction induced at the time of rapid brain development in the neonatal mouse. Significantly, neonatal exposure to certain ortho-substituted and coplanar PCBs can cause this change in spontaneous motor behavior profile, both time dependent and dose related (24,41).

In the present study, the ability of adult mice to learn and memorize was observed in a swim maze of the Morris water-maze type. This maze revealed that mice exposed to the higher dose of PBDE 99 (12 mg/kg bw) performed significantly worse than control animals. The swim maze allowed a 4-day acquisition period followed by reversal trials on the 5th day, when the platform was moved. In control mice and in mice given PBDE 99, PBDE 47, and TBBPA, latency to locate the platform decreased during the acquisition training, and all tested animals performed equally well at the end of the acquisition period. In the reversal trials on the fifth day, however, mice exposed to the higher dose of PBDE 99 did not improve in

![Figure 3](image-url) **Figure 3.** Swim-maze performance of 5-month-old NMRI male mice exposed to a single oral dose of PBDE 47, PBDE 99, TBBPA, or the 20% fat emulsion vehicle at neonatal day 10. Latencies to reach the platform were measured during acquisition (days 1–4) and during reversal trials (day 5). Behavioral data, days 1–4, were analyzed by ANOVA using a split-plot design (33). Statistical analyses of behavioral data for day 5 were done by paired t-test and ANOVA 1-way combined with Duncan's test. Each point represents the mean of 16–18 animals.
finding the new location of the platform, an improvement that was seen in control mice and mice exposed to PBDE 47 and TBBPA. 

The maze learning/memory test and the spontaneous behavior test reveal that PBDE 49 is more potent in causing neurotoxic effects than PBDE 47. This indicates differences in neurotoxicity among different PCB congeners—changes such as those seen earlier for different PCB congeners. In previous studies we observed altered new/reversal learning in swin-maze performance in adult mice neonatally exposed to PCB 52 (38) and PCB 126 (41) but not after neonatal exposure to PCB 28 (38). Furthermore, in animals showing defects in new/reversal learning, the nicotinic receptors in the brain were affected. The behavioral performance in tasks requiring attention and rapid processing of information in humans and new/reversal learning and working memory in animals may involve cholinergic transmission (42), and the cholinergic system is one of the major transmitter systems that correlate closely with cognitive function (43,44). Whether neonatal exposure to PBDEs can affect cholinergic receptors in the brain is therefore of special interest and calls for further studies because in neurodegenerative diseases, such as Alzheimer and Parkinson, there is a change in cholinergic nicotinic receptors in the cerebral cortex and hippocampus (45,46).

Do we have a novel class of developmental neurotoxicants in our environment? It is particularly worth noting that neonatal exposure to PBDE 99, and to a lesser degree PBDE 47, affected spontaneous behavior, habituation capability, and learning and memory in adult mice, similar to those observed earlier for PCBs. That different PCB congeners can have different potency in causing neurotoxic effects is important when comparing the levels of PBDEs in the environment and in mother's milk, but also when comparing levels of PBDEs to the levels of certain PCBs. In our earlier studies we have shown that the amount of ortho-substituted PCB congeners, such as PCB 52 and PCB 153, found in the brain 24 hr after a single oral administration to 10-day-old mice is about 3–5 µmol/kg of the administered dose (24). Data on actual tissue levels of PCBs in infants are few. The amounts of these different PCBs given in our studies resulted in a brain tissue concentration (ppb levels) that can be of the same order of magnitude observed in infants less than 1 year of age (47).

In human studies it is difficult to distinguish between exposure of offspring by transplacental or by breast milk transfer. However, both human and animal data from a variety of species suggest that accumulation of highly persistent chemicals ingested via milk far exceeds the contribution made by maternal–fetal transfer (47). Although the total amount of PCB in mother's milk is higher than that of PBDEs, it is important to compare the levels of single congeners. The concentration of PBDEs found in native Swedish mother's milk was 4 ng/g lipid in 1997 (9,10). The amount of PCB 47 was 2.3 ng/g lipid and PBDE 99 0.5 ng/g lipid. The levels of PCB 52 in Swedish mother's milk was 1 ng/g lipid in 1996 and for PCB 153 73 ng/g lipid in 1997 (9,10). From the same study (9) it is also worth noting that the amount of DDT, a well known neurotoxic compound, was about 6 times higher than PBDE 47—14 ng/g lipid. Human epidemiologic studies suggest that perinatal exposure to PCBs can have developmental neurotoxic effects (48–51). Experimental studies in animals have shown that commercial mixtures of PCBs can cause behavioral aberrations and changes in brain neurotransmitter metabolism (52–54). Exposure of mice, rats, and monkeys to commercial mixtures of PCBs during development can produce long-term neurobehavioral changes (55,56). Recently, Rice and H ayward (57) have shown cognitive defects in adult monkeys exposed postnatally, from birth to 20 weeks of age, to a PCB mixture representative of the PCB congeners typically found in human breast milk. In our animal model we have seen that certain PCB congeners, known to be present in the environment and human milk, given during a critical phase of neonatal development, when the maturation of the developing brain and CNS is at a stage of critical vulnerability, induce persistent neurotoxic effects (24). In these studies, the lowest dose of the PCB congeners, PCB 52 and PCB 153, shown to induce developmental neurotoxic effects is the same as the one used in the present study of PBDE 99—1.4 µmol/kg bw.

Our present findings that developmental exposure to PBDEs can cause similarities in behavioral disturbances as seen earlier for PCBs is of special interest, not only for PBDEs as a single agent but for possible interactive effects between these persistent environmental agents and the present background levels of PCBs. Given the increasing concentration of PBDEs in mother's milk, we call for future research into PBDEs as potential neurotoxicants.

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