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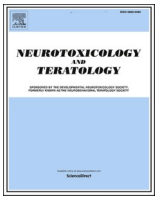

Perfluorinated chemicals (PFOA) can, by interacting with highly brominated diphenyl ethers (PBDE 209) during a defined period of neonatal brain development, exacerbate neurobehavioural defects

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ABSTRACT

Perfluorinated compounds (PFCs) and polybrominated diphenyl ethers (PBDEs) are ubiquitous persistent environmental compounds, present in humans and at higher levels in infants/children than in adults.

This study shows that co-exposure to pentadecafluorooctanoic acid (PFOA) and 2,2′,3,3′,4,4′,5,5′,6,6'-decaBDE (PBDE 209) can significantly exacerbate developmental neurobehavioural defects.

Neonatal male NMRI mice, 3 and 10 days old, were exposed perorally to PBDE 209 (1.4 or 8.0 μmol/kg bw), PFOA (1.4 or 14 μmol/kg bw), co-exposed to PBDE 209 and PFOA (at the given doses), or a vehicle (20% fat emulsion) and observed for spontaneous behaviour in a novel home environment when 2 and 4 months old. The behavioural defects observed included hyperactivity and reduced habituation indicating cognitive defects. This interaction appears most likely dependent on the presence of PBDE 209 and/or its metabolites together with PFOA, during a defined critical period of neonatal brain development, corresponding to the perinatal and newborn period in humans.

1. Introduction

Epidemiological studies indicate that exposure to environmental pollutants during early human development can have deleterious effects on cognitive development in childhood and and it is also suggested that environmental toxicants can by interacting induce defective cognitive development (Grandjean et al., 2001; Grandjean and Landrigan, 2006, 2014). Studies have also indicated that perfluorinated compounds (PFCs) and polybrominated diphenyl ethers (PBDEs) can contribute to impaired neurobehavioural development in children (Herbstman et al., 2010; Hoffman et al., 2010; Hoffman et al., 2012; Eskenazi et al., 2013) and that highly brominated PBDEs, PBDE 209, might be of special concern (Gascon et al., 2012).

PFCs (Giesy and Kannan, 2001; Olsen et al., 2003; Viberg and Eriksson, 2017) and brominated flame retardants (BFRs) (de Boer et al., 1998; de Wit, 2002; Kodavanti et al., 2017) are two categories of persistent chemicals of concern as they are found in humans as well as in wildlife. The PFCs are present in applications such as oil/water repellents for clothing fabrics and carpets, paper coatings, food packaging, lubricants, surfactants and fire extinguishers (Viberg and Eriksson, 2017). PFCs are fully fluorinated chemicals, where PFOA is a 8-carbon chain, $C_8HF_{15}O_2$, (2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluorooctanoic acid). The carbon-fluorine bonds are among the strongest in organic chemistry and PFOA are not known to be metabolized. The elimination half-lives of PFOA varies widely between species, from 4 to 6 days in rat up to 3.8 years in humans see (Viberg and Eriksson, 2017). Several reports have been published on measurements of PFCs in blood, plasma and serum and milk, showing that humans are exposed to numerous PFCs, including PFOA (Kannan et al., 2004; Calafat et al., 2006; Harada et al., 2007; Kärrman et al., 2007). Regarding BFRs, the PBDEs are used in large quantities as flame-retardant additives in polymers. PBDEs consists of two phenyl rings where bromine atom/atoms can bind. The chemical formula is $C_{12}H_{(n-1)}Br_nO$ ($n \leq 10$). The commercial decaBDE, manufactured and used worldwide has mainly been added to textiles and to denser plastics used for electrical appliances. After oral administration in adults, PBDE 209 has been shown to be metabolized to debrominated hepta-, octa- and nona PBDEs, see (Kodavanti et al., 2017). When found in humans, PBDE 209 is reported to have a short half-life of circa 15 days (Thuresson et al., 2006) which can have consequences when correlating the developmental neurotoxic effects of PBDE 209 to its presence in the body.

Newborns, toddlers, and children can be exposed to PFCs and PBDEs

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via mothers' milk or directly via ingestion and inhalation. It is of particular concern that these compounds are seen at the same or even higher levels in children than in adults (Meironyte et al., 1999; So et al., 2006; Kärrman et al., 2007; Toms et al., 2009; Kodavanti et al., 2017).

Direct exposure to chemicals by inhalation and dust ingestion might constitute an important non-dietary exposure route for humans. Infants, especially those who crawl, ingest more dust than adults. PFCs, PFOA is reportedly present in domestic dust (Strynar and Lindstrom, 2008; Bjorklund et al., 2009). In toddlers, house dust can account for 80% of total daily PBDE exposure, compared with 14% in adults (Wilford et al., 2005) and the main PBDE congener in house dust and indoor dust standard reference materials seems to be PBDE 209 (Stapleton et al., 2006; Sjodin et al., 2008; Sahlstrom et al., 2015).

In many mammalian species the newborn period coincides with a period of rapid growth and development of the brain, the 'brain growth spurt' (BGS) (Davison and Dobbing, 1968). In humans, the 'BGS' begins during the third trimester of gestation and continues throughout the first 2 years of *ex utero* life. In mouse and rat this period is neonatal, spanning the first 3–4 weeks of life, during which the brain undergoes several fundamental developmental phases, viz. axonal and dendritic outgrowth, the establishment of neural connections, acquisition of new motor and sensory faculties (Bolles and Woods, 1964; Davison and Dobbing, 1968), and numerous biochemical changes that transform the feto-neonatal brain into that of the mature adult (Coyle and Yamamura, 1976; Abreu-Villaca et al., 2011).

Our earlier studies have shown that low-dose exposure of neonatal mice during the BGS to persistent environmental toxic agents (e.g. PCBs, DDT, BFRs) as well as to known neurotoxic agents (nicotine, organophosphorous compounds, and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)) can lead to disruption of adult brain function (Eriksson, 1997; Eriksson et al., 2001b; Viberg et al., 2003; Eriksson, 2008a), effects induced during a defined critical period of the BGS in mice, namely around postnatal day 10 (Eriksson et al., 1992; Eriksson et al., 2000; Viberg et al., 2003; Eriksson, 2008a). There are some developmental toxicity studies on PFCs (Lau et al., 2003; Johansson et al., 2008a; Butenhoff et al., 2009; Viberg et al., 2013). We have earlier observed that neonatal exposure to just highly brominated diphenyl ethers (e.g. PBDE 209, PBDE 206 and 203) (Viberg et al., 2003; Viberg et al., 2006; Johansson et al., 2008b) or just exposure to the PFCs (e.g. PFOA and PFOS) (Johansson et al., 2008a), caused a similar derangement of spontaneous behaviour and habituation. Furthermore, in these studies we have also seen that PBDEs can affect the amount of nicotinic receptors and that the nicotine induced behaviour is altered in adult mice neonatally exposed to PBDE 209 and PFOA, indicating that the cholinergic system can be affected by both substances. Therefore it is of particular concern to establish if these environmental toxicants can interact at low doses, as earlier reported for PCB, PBDE and MeHg (Eriksson et al., 2006, Fischer et al., 2008b, a), and at doses where each individual compounds lacks the capacity to induce developmental neurotoxic effects when present during this critical period of brain development.

In view of the increasing levels of PFCs and PBDEs in our environment and the presence of both PFOA and PBDE 209 in domestic dust and in newborns and toddlers, and with regard to our earlier findings that both PBDE 209 and PFOA had similar developmental neurotoxic effects, the present study was carried out to ascertain whether PBDE 209 and PFOA, by interacting during neonatal brain development, exacerbate developmental neurotoxic effects on spontaneous behaviour and habituation and whether such effects can be time dependent.

2. Materials and methods

2.1. Animals and chemicals

Pregnant NMRI (Naval Medical Research Institute) mice, obtained from B&K, Sollentuna, Sweden, were housed individually in plastic cages (40 × 25 × 15 cm) in a room with an ambient temperature of 22 °C and a normal 12/12-h cycle of light and dark. The animals were fed standardized pellets (Lactamin, Stockholm, Sweden) and tap water ad libitum. The pregnant NMRI mice were checked for parturition twice daily (08.00 and 18.00 h). Day of birth was designated day 0 (birth during the preceding night was designated day 0 when checked at 08.00 h). Litter sizes were adjusted and standardized to 8–12 pups within the first 48 h after birth, by euthanizing excess pups (Irvine and Timiras, 1966). The litters contained pups of both sexes, in about equal numbers, during the neonatal period; no separation with regard to sex was made in the pre-weanlings (Eriksson, 2008b). At round 4 weeks of age, males and female mice were separated and the males of one litter were kept together and raised in groups of 4–7, in a room for males only, and under conditions as detailed above. Only males were used in order to compare with our earlier developmental neurotoxicological studies on PBDE 209 and PFOA (Viberg et al., 2003; Viberg et al., 2006; Viberg et al., 2007; Johansson et al., 2008a; Johansson et al., 2008b; Johansson et al., 2009), and the number of male mice in each treatment group ranged from 15 to 22.

2,2′,3,3′,4,4′,5,5′,6,6'-decaBDE (PBDE 209) was kindly donated by Dr. Åke Bergman, Department of Environmental Chemistry, Stockholm University, Sweden. The purity of the compound exceeded 98%, and PBDE 209 was purified from dioxin-like contaminants via activated charcoal and the 1–2% left are isomerides of PBDEs. PFOA (pentadecafluorooctanoic acid, purity ≥96%) was purchased from Sigma-Aldrich. The substances were dissolved in a mixture of egg lecithin (Merck, Darmstadt, Germany) and peanut oil (Oleum arachidis) (1:10) and then sonicated together with water to yield a 20% (w:w) fat emulsion vehicle containing various concentrations of the compounds. Neonatal mice, of both sexes, were orally exposed to PBDE 209 (1.4 or 8.0 μmol/kg bw), PFOA (1.4 or 14 μmol/kg bw), co-exposed to PBDE 209 and PFOA (PBDE 209 1.4 μmol/kg bw + PFOA 1.4 μmol/kg bw, PBDE 209 1.4 mg/kg bw + PFOA 14 μmol/kg bw, PBDE 209 8.0 μmol/kg bw + PFOA 1.4 μmol/kg bw, PBDE 209 8.0 μmol/kg bw + PFOA 14 μmol/kg bw), or vehicle (20% fat emulsion), at a volume of 10 ml/kg bw, via a metal gastric-tube as a single dose on PND 3 and PND 10, making up 15 different treatment groups according to the schedule shown in Table 1. The doses were selected with regard to our earlier dose-response studies on PBDE 209 (Johansson et al., 2008b) and PFOA (Johansson et al., 2008a), including a borderline dose and one with known developmental neurotoxic effects. Furthermore, PBDE 209 was only given on PND 3 as an earlier study has shown that no developmental neurotoxic effects were seen when PBDE 209 was given on PND 10 or PND 19 (Viberg et al., 2003). Each treatment group comprised mice from 3 to 4 different litters, and the number of pups per group varied between 15 and 22 pups (see Table 1). The purpose of the 20% fat

Table 1
Treatment table for mice exposed to an oral dose of PBDE 209, PFOA, PBDE 209 + PFOA, or vehicle on PND 3 and 10.

Group	Treatment (μmol/kg body weight)[n]	Presented in Figure	
No.	PND 3	PND 10	
1	vehicle [22]	vehicle	1, 2, 3
2	vehicle [22]	PFOA 1.4	1, 3
3	vehicle [15]	PFOA 14	1, 3
4	PFOA 1.4 [21]	vehicle	2, 3
5	PFOA 14 [22]	vehicle	2, 3
6	PBDE 209 1.4 [18]	vehicle	1, 2
7	PBDE 209 1.4 [17]	PFOA 1.4	1
8	PBDE 209 1.4 [20]	PFOA 14	1
9	PBDE 209 1.4 + PFOA 1.4 [18]	vehicle	2
10	PBDE 209 1.4 + PFOA 14 [19]	vehicle	2
11	PBDE 209 8.0 + PFOA 1.4 [19]	vehicle	2
12	PBDE 209 8.0 + PFOA 14 [17]	vehicle	2
13	PBDE 209 8.0 [16]	vehicle	1, 2
14	PBDE 209 8.0 [21]	PFOA 1.4	1
15	PBDE 209 8.0 [16]	PFOA 14	1

emulsion vehicle was to give a more physiologically appropriate absorption and hence distribution of the compounds (Keller and Yeary, 1980), since the fat content of mouse milk is around 14%.

2.2. Behavioural tests

For our neurotoxic recordings we used male NMRI mice to make this study comparable to our earlier studies on PBDE 209 (Viberg et al., 2003) and PFOA (Johansson et al., 2008a).

Spontaneous behaviour, in a novel home environment, was tested in the males at age 2 and 4 months. The animals were tested between 08.00 and 13.00 h, under the same light and temperature conditions as in their housing cages. Ten mice, randomly picked from three to four different litters in each treatment group, were tested once only on each test occasion. The test was conducted during four consecutive days and the groups were run in a counterbalanced order. Motor activity was measured during a 60-min period, divided into three 20-min intervals, in an automated device consisting of cages (40 × 25 × 15 cm, same size as the home cage) placed within two series of infrared beams (low and high level) (Rat-O-Matic, ADEA Elektronik AB, Uppsala, Sweden) (Fredriksson, 1994). Twelve cages, placed in individual sound-proof boxes with separate ventilation, were used.

2.2.1. Locomotion

Movement was registered when a mouse moved horizontally through the low-level grid of infrared beams, aimed 10 mm above the bedded floor (same bedding material as in the housing cage).

2.2.2. Rearing

Movement in the vertical plane was registered at a rate of four counts per sec, when a single high-level beam was intercepted, i.e. number of counts was proportional to time spent rearing. Infrared beams were aimed 80 mm above the bedded floor.

2.2.3. Total activity

All types of vibration within the test cage, i.e. those caused by mouse movements, shaking (tremors), and grooming, were registered by a sensor (a needle mounted on a horizontal arm with a counterweight), connected to the test cage.

All data were collected electronically through a computer interface.

The experiment was carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) after approval from the local ethical committee (Uppsala University and Agricultural Research Council), and by the Swedish Committee for Ethical Experiments on Laboratory Animals.

2.3. Statistical analysis

In analysis of the spontaneous behaviour variables, the locomotion, rearing and total activity data for three consecutive 20-min periods (Treatment, Time, and Treatment × Time; between subjects, within-subjects and interaction factors, respectively) was submitted to a split-plot ANOVA design and pairwise testing between the different treatment groups was performed using a Tukey HSD (honestly significant difference) test (Kirk, 1968) and earlier described (Eriksson, 2008b). Split-plot design was used in order to achieve good statistical power and to keep false positive and negative outcome balanced (Eriksson, 2008b; Lazic and Essioux, 2013).

In analyses of body weight, data from 3-day-old, 10-day-old and 4-week-old mice were subjected to one-way ANOVA, and pairwise testing using Tukey HSD test.

3. Results

There were no overt signs of toxicity in the treated mice throughout the experimental period. No changes in body weight gain or body weight at the end of the experimental period were seen as a result of the exposure to PBDE 209, PFOA, or PBDE 209 + PFOA (data not shown).

Our findings from the spontaneous behaviour variables 'locomotion', 'rearing', and 'total activity' in 2-, and 4-month-old male NMRI mice exposed to dosages reported in Table 1 are presented in Figs. 1A,B, 2A,B, and 3A,B.

3.1. Co-exposure to PBDE 209 and PFOA

Figure 1A, B concerns exposure to PBDE 209 alone on PND 3, PFOA alone on PND 10, and the co-exposed group PBDE 209 on PND 3 and PFO on PND 10. Fig. 2A, B concerns exposure to PBDE 209 alone on PND 3, PFOA alone on PND 3, and the co-exposed group PBDE 209 and PFOA on PND 3. In order to have a correct evaluate the effect of co-exposure and interaction between PBDE 209 and PFOA in mice exposed on PND 3 and PND 10, all 15 treatment groups were used in the statistical evaluation. Two months after exposure, the significant treatment × time interactions were: 'locomotion' [$F_{28,270} = 47.42$, $P \leq 0.0001$], 'rearing' [$F_{28,270} = 60.25$, $P \leq 0.0001$], and 'total activity' [$F_{28,270} = 34.39$, $P \leq 0.0001$], (Figs. 1A and 2A). Four months after the neonatal exposure to PBDE 209, PFOA, or the combination of PBDE 209 + PFOA, mice continued to display significant treatment × time interactions for 'locomotion' [$F_{28,270} = 52.03$, $P \leq 0.0001$], 'rearing' [$F_{28,270} = 94.41$, $P \leq 0.0001$], and 'total activity' [$F_{28,270} = 47.98$, $P \leq 0.0001$], (Figs. 1B and 2B).

3.1.1. Neonatal mice exposed to PBDE 209 (PND 3), PFOA (PND 10) or PBDE 209 (PND 3) + PFOA (PND 10) (Fig. 1A + B)

Two months after exposure (Fig. 1A): Pair-wise testing between PBDE 209, PFOA, combination PBDE 209 + PFOA and control groups showed a significant difference between the different treatment groups for all three-test variables (Fig. 1A). For controls, the activity level decreased for all variables throughout the 60-min period, consistent with a normal spontaneous behaviour profile (Fredriksson, 1994; Eriksson, 1997) and also seen in control mice in experiments conducted on PBDE 209 and PFOA (Viberg et al., 2003; Johansson et al., 2008a).

Mice given PBDE 209 (1.4 μmol) + PFOA (14 μmol) in combination were significantly ($P \leq 0.01$) less active during the first 20-min period (0–20 min) whereas during the third period (40–60 min) they were significantly ($P \leq 0.01$) more active than the controls or mice with single exposure to PBDE 209 (1.4 μmol) or PFOA (14 μmol) in all three test variables. Mice just given PBDE 209 (1.4 and 8 μmol) or PFOA (14 μmol) displayed significantly less activity during the first 20-min period, compared with controls, but during the third period (40–60 min) mice given the higher doses of PBDE 209 (8 μmol) or PFOA (14 μmol) were significantly ($P \leq 0.01$) more active than the controls and mice given the lower doses of PBDE 209 (1.4 μmol) and PFOA (1.4 μmol), in all three test variables.

Four months after exposure (Fig. 1B): Pair-wise testing between PBDE 209, PFOA, combination PBDE 209 + PFOA and control groups showed similar significant differences between these treatment groups for all three test variables, observed 2 months after exposure, (Fig. 1B). Control mice continued to display normal spontaneous behaviour, being more active during the first 20 min but with decreasing activity over time. The most pronounced additional change was seen in mice co-exposed to the lower dose of PBDE 209 (1.4 μmol) + PFOA (1.4 μmol). In the last 20-min period (40–60 min) these mice were significantly ($P \leq 0.01$) more active in all three test variables than the controls or mice with single exposure to PBDE 209 (1.4 μmol) or PFOA (1.4 μmol).

3.1.2. Neonatal mice exposed to PBDE 209 (PND 3), PFOA (PND 3) or PBDE 209 + PFOA (PND 3) (Fig. 2A + B)

Two months after exposure (Fig. 2A): Pair-wise testing between PBDE 209, PFOA, combination PBDE 209 + PFOA and control groups revealed a significant difference between the different treatment groups

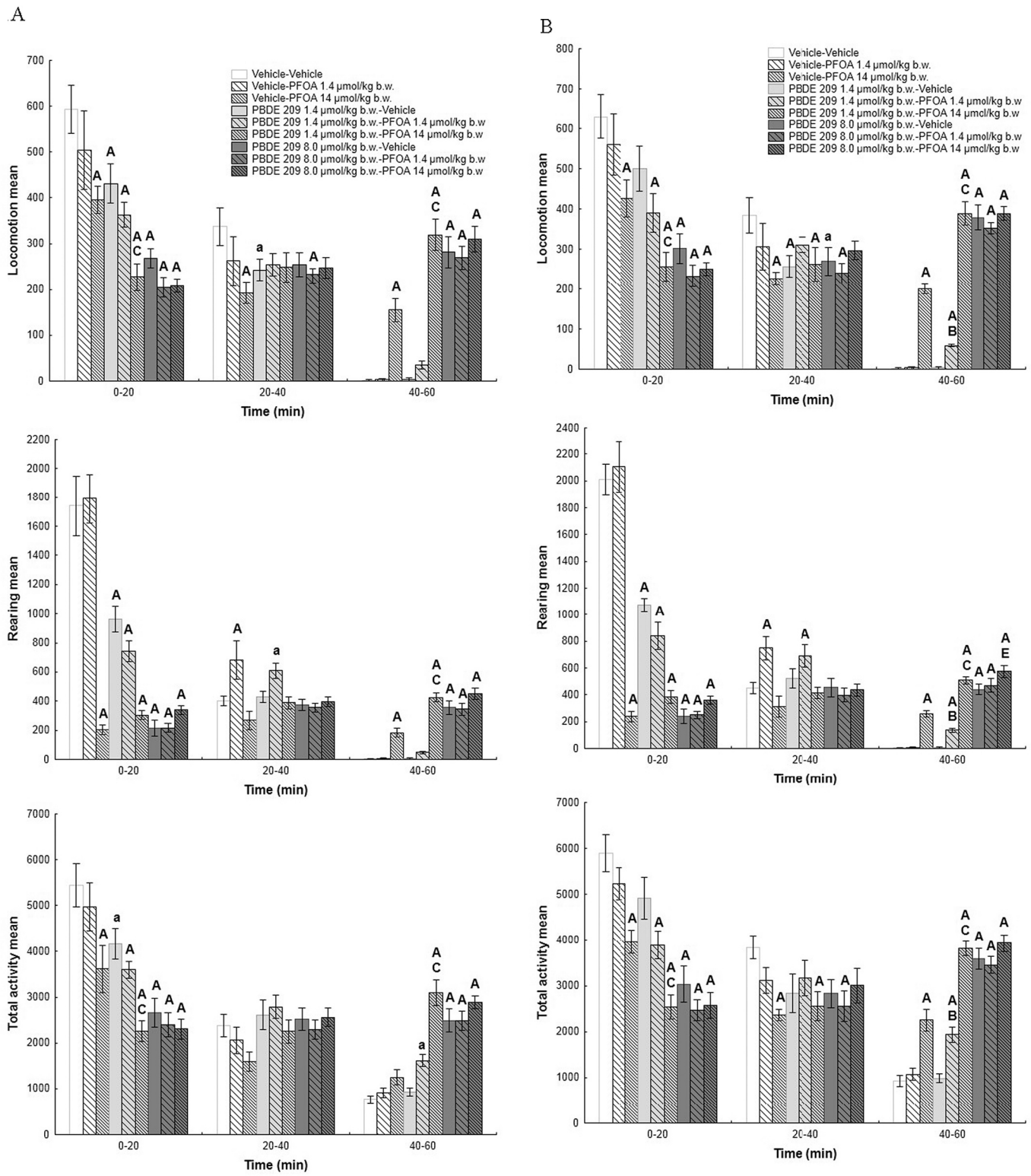

Fig. 1. Spontaneous behaviour of 2-month-old (A) and 4-month-old (B) male NMRI mice orally exposed to PBDE 209 on PND 3 and PFOA on PND 10, or to a 20% fat emulsion vehicle, as shown in Table 1. Statistical analysis: ANOVA with split-plot design, and pair-wise testing with Tukey HSD test. The height of each bar represents the mean ± SD of 10 animals.

Statistical differences:

A = $P \leq 0.01$, a = $P \leq 0.05$, vs control.

B = $P \leq 0.01$, b = $P \leq 0.05$, vs PBDE 209 (1.4 μmol, PND 3) and PFOA (1.4 μmol, PND 10).

C = $P \leq 0.01$, c = $P \leq 0.05$, vs PBDE 209 (1.4 μmol, PND 3) and PFOA (14 μmol, PND 10).

D = $P \leq 0.01$, d = $P \leq 0.05$, vs PBDE 209 (8 μmol, PND 3) and PFOA (1.4 μmol, PND 10).

E = $P \leq 0.01$, e = $P \leq 0.05$, vs PBDE 209 (8 μmol, PND 3) and PFOA (14 μmol, PND 10).

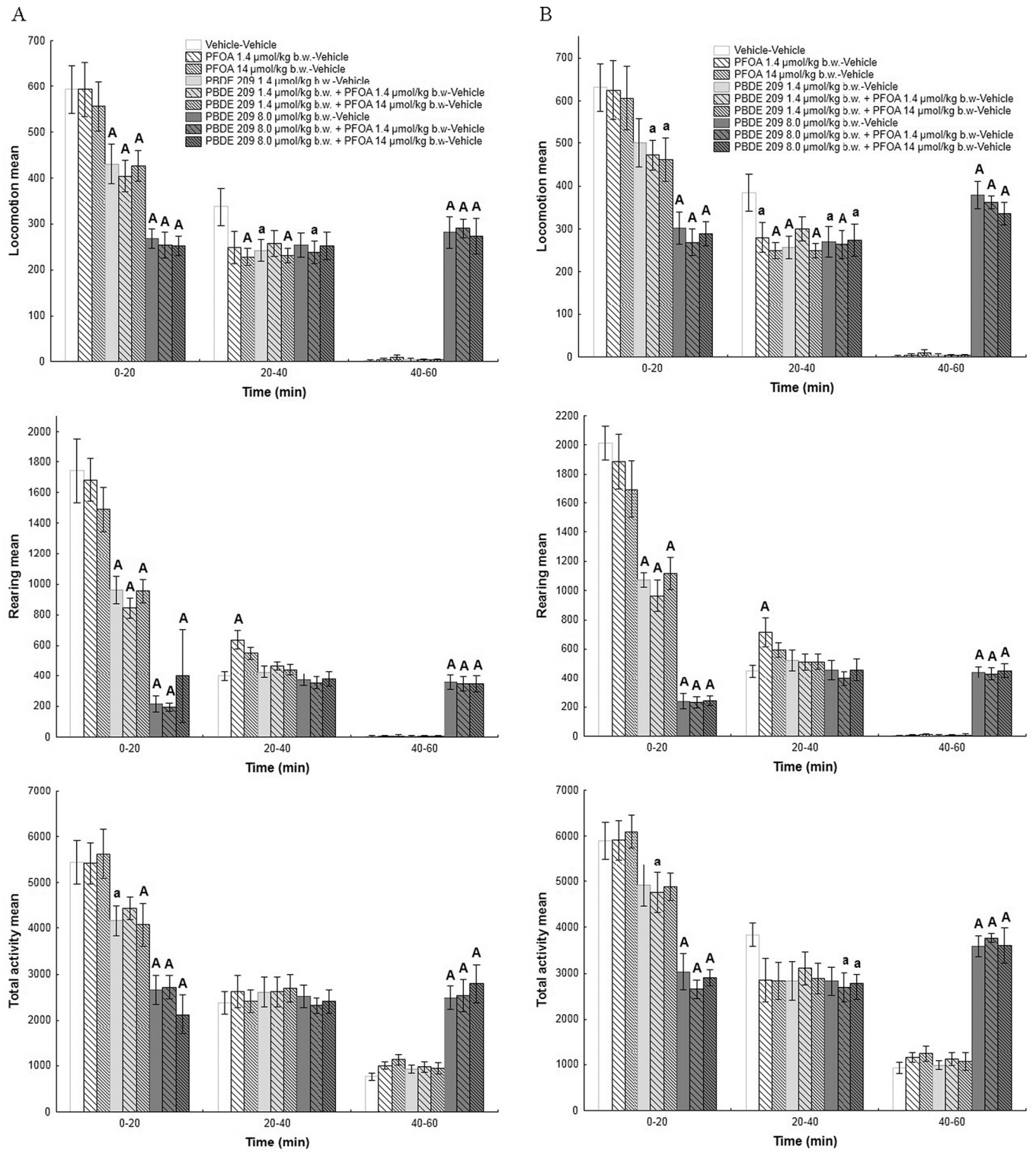

Fig. 2. Spontaneous behaviour of 2-month-old (A) and 4-month-old (B) male NMRI mice orally exposed to PBDE 209 on PND 3 and PFOA on PND 3, or to a 20% fat emulsion vehicle, as shown in Table 1. Statistical analysis: ANOVA with split-plot design, and pair-wise testing with Tukey HSD test. The height of each bar represents the mean ± SD of 10 animals.
Statistical differences:
A = P ≤ 0.01, a = P ≤ 0.05, vs vehicle.

for all three-test variables. Mice given PBDE 209 (1.4 µmol) + PFOA (1.4 µmol) in combination, PBDE 209 (1.4 µmol) + PFOA (14 µmol) in combination, and just PBDE 209 (1.4 µmol) were all significantly ($P \leq 0.01$) less active during the first 20-min period (0–20 min) than controls or mice with single exposure to PFOA (1.4 µmol and 14 µmol) in the locomotion and rearing teste variables. Mice given PBDE 209 (8 µmol) + PFOA (1.4 µmol) in combination, PBDE 209 (8 µmol) + PFOA (14 µmol) in combination, and mice with single exposure to PBDE 209 (8 µmol) were significantly ($P \leq 0.01$) less active during the first 20-min period (0–20 min) than controls or mice with single exposure to PBDE

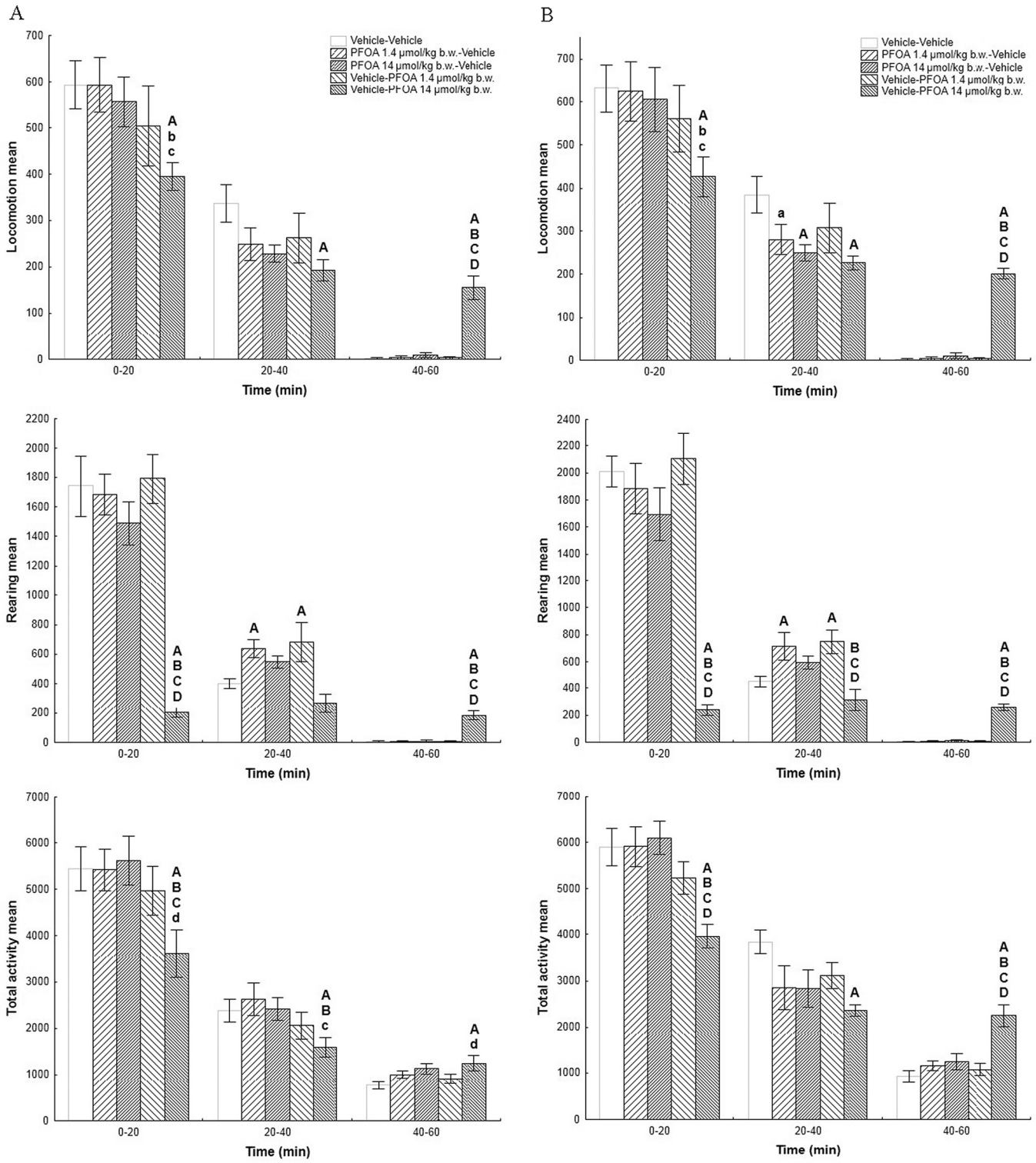

Fig. 3. Spontaneous behaviour of 2-month-old (A) and 4-month-old (B) male NMRI mice following neonatal exposure to PFOA or 20% fat emulsion on PND 3 or PND 10, as shown in Table 1. Statistical analysis: ANOVA with split-plot design, and pair-wise testing with Tukey HSD test. The height of each bar represents the mean ± SD of 10 animals.

Statistical differences:

A = $P \leq 0.01$, a = $P \leq 0.05$, vs vehicle.

B = $P \leq 0.01$, d = $P \leq 0.05$, vs 1.4 μmol PND 3.

C = $P \leq 0.01$, e = $P \leq 0.05$, vs 14 μmol PND 3.

D = $P \leq 0.01$, b = $P \leq 0.05$, vs 1.4 μmol PND 10.

209 (1.4 μmol), PFOA (1.4 μmol), or PFOA (14 μmol), whereas during the third period (40–60 min) they were significantly ($P \leq 0.01$) more active than the controls or mice with single exposure to PBDE 209 (1.4 μmol), PFOA (1.4 μmol) or PFOA (14 μmol) in all three test variables.

Four months after exposure (Fig. 2B): Pair-wise testing between PBDE 209, PFOA, combination PBDE 209 + PFOA and control groups revealed no additional significant difference between the different treatment groups for the three-test variables.

This indicates a lack of additional effect of PFOA together with PBDE 209 when both are given together on PND 3.

3.2. Neonatal mice exposed to PFOA on PND 3 or PND 10

Figure 3A, B concerns exposure to PFOA alone on PND 3 or PND 10 describing the time-window for induction of behavioural effects of PFOA. In this statistical evaluation only mice exposed on PND 3 and PND 10 to PFOA or the vehicle were included (5 groups).

Two months after neonatal exposure to PFOA (1.4 μmol or 14 μmol), when either 3- or 10-days old, there were significant treatment × time interactions [$F_{8,90} = 9.60$, $P \leq 0.0001$; $F_{8,90} = 40.57$, $P \leq 0.0001$; $F_{8,90} = 5.50$, $P \leq 0.0001$], for 'locomotion', 'rearing', and 'total activity', variables (Fig. 3A). Pair-wise testing between PFOA given on PND 3 and on PND 10 and the control group showed a significant difference between the different treatment groups for all three-test variables. Mice given PFOA (14 μmol) on PND 10 were significantly less active during the first 20-min period (0–20 min) than controls ($P \leq 0.01$) and mice given PFOA (1.4 μmol or 14 μmol) on PND 3 ($P \leq 0.05$), whereas during the third period (40–60 min) they were significantly ($P \leq 0.01$) more active on locomotion and rearing than the controls, and mice given PFOA (1.4 μmol or 14 μmol) on PND 3, and the low dose of PFOA (1.4 μmol) on PND 10, in all three test variables. Mice given PFOA on PND 10 were also significantly more active for the 'total activity' variable during the third period (40–60 min) than controls ($P \leq 0.01$) and mice given the low dose of PFOA (1.4 μmol).

Four months after neonatal exposure to PFOA (1.4 μmol or 14 μmol) at an age of either 3 or 10 days, the mice continued to display significant treatment × time interactions for 'locomotion' [$F_{8,90} = 11.57$, $P \leq 0.0001$], 'rearing' [$F_{8,90} = 47.27$, $P \leq 0.0001$], and 'total activity' [$F_{8,90} = 17.04$, $P \leq 0.0001$] (Fig. 3B). Pair-wise testing between PFOA given on PND 3 and on PND 10 and the control group revealed significant differences between the different treatment groups in all three-test variables. For 'locomotion', 'rearing' and 'total activity' variables, mice given PFOA (14 μmol) on PND 10 were significantly ($P \leq 0.01$) less active during the first 20-min period (0–20 min) than controls, than mice given PFOA (1.4 μmol or 14 μmol) on PND 3, and mice given PFOA (1.4 μmol) on PND 10, whereas during the third period (40–60 min) they were significantly ($P \leq 0.01$) more active than the controls, mice given PFOA (1.4 μmol or 14 μmol) on PND 3, and PFOA (1.4 μmol) on PND 10.

No significant behavioural changes during the first or last 20-min periods were observed in mice given PFOA when 3 days old.

4. Discussion

The present study shows that co-exposure to a PFC, PFOA, and to a highly brominated diphenyl ether, PBDE 209, at low doses during neonatal brain development, can lead to exacerbation of developmental neurobehavioural defects in mice. This type of interaction appears most likely dependent on both the metabolism of PBDE 209 and the presence of its metabolites (debrominated ones) together with PFOA during a defined critical period of the neonatal brain development, namely around PND 10. Neonatal exposure to a single low oral dose of PBDE 209 (1.4 μmol/kg bw) on PND 3 and later a single oral exposure to PFOA (14 μmol/kg bw) on PND 10 impaired spontaneous behaviour in a novel home environment in 2- and 4-month-old mice, an effect differing significantly from single exposure to PBDE 209 and PFOA. Furthermore, these behavioural defects were also persistent and at low doses also time-dependent as significant defects were observed in 4-month-old mice (but not in 2-month-old mice) in mice co-exposed to PBDE 209 (1.4 μmol/kg bw) + PFOA (1.4 μmol/kg bw).

Mice exposed on PND 3 to PBDE 209 (1.4 μmol) and on PND 10 to PFOA (14 μmol) displayed significantly disrupted spontaneous behaviour in a novel home environment and defective habituation at the age of 2 and 4 months. Spontaneous behaviour depends on the integration of sensory input into motor output. It also reveals the ability of animals to habituate to a novel home environment and to integrate new information with that previously attained. It can thereby be a measure of cognitive function as habituation is known to be connected to cognitive function (Groves and Thompson, 1970; Daenen et al., 2001; Wright et al., 2004). Habituation, defined here as a decrease in 'locomotion', 'rearing', and 'total activity' variables in response to the diminishing novelty of the test chamber over 60 min, was evident in the control animals, whereas mice co-exposed to PBDE 209 (1.4 μmol) + PFOA (14 μmol) were obviously hypoactive early in the 60-min test period, but becoming hyperactive toward the end. It is worth noting that the developmental neurotoxic effects were as pronounced in mice given the combined dose of PBDE 209 + PFOA (PND 3: 1.4 μmol + PND 10: 14 μmol), as in mice given the six-fold higher dose of PBDE 209 (PND 3: 8 μmol), and the behavioural deviation was about twice as high as in mice given only PFOA (PND 10: 14 μmol). The combination PBDE 209 + PFOA (PND 3: 1.4 μmol + PND 10: 14 μmol) and the six-fold higher dose of PBDE 209 both caused a similar hypoactive condition during the first 20 min and also a hyperactive condition during the last 20-min period. Of special significance was the observed significant increase in activity (during the last 20-min period) in 4-month-old mice neonatally co-exposed to the low doses of PBDE 209 and PFOA (PND 3: 1.4 μmol + PND 10: 1.4 μmol), at which the dose of PFOA had no significant effects and PBDE 209 is a borderline dose. This indicates that the behavioural disorder is dependent on dosages during neonatal brain development and the time after exposure for its appearance in the adult animals.

It is known that exposure of mice and rats to PBDE 209 during development can cause neurobehavioural defects at adult age (Viberg et al., 2003; Rice et al., 2007; Viberg et al., 2007; Johansson et al., 2008b; Rice et al., 2009). We have also suggested in earlier studies that these defects are probably attributable to metabolites of PBDE 209 when present during a critical period of brain development, namely around PND 10 (Viberg et al., 2003). This supposition was corroborated by an oral neonatal administration of [U-^{14}C]PBDE 209 on PND 3, 10, or 19, showing that the radioactivity dispersed throughout the body and increased in the brain, from 24 h after administration to 7 days after, in both 3-day-old and 10-day-old mice. Disturbances in spontaneous behaviour, habituation, occurred only in mice exposed on PND 3, even though [U-^{14}C]PBDE 209 when given on PND 10 the amount of radioactivity present in the brain indicated a sufficient amount of PBDE 209 on PND 10 to induce neurotoxic disturbances. Although the metabolism of xenobiotics can be low in young animals it is known that POPs such as PCBs can be metabolized in neonates (Vodicnik and Lech, 1980). That PBDE 209 can be metabolized and debrominated to nona-, octa-, and hepta-PBDEs has been shown in adult rats orally exposed to PBDE 209 (Morck et al., 2003; Sandholm et al., 2003). Although the amounts of nona- and octa-PBDE in the neonatal brain following oral exposure to PBDE 209 on PND 3 remains to be studied, we recently reported that following neonatal exposure to octa-PBDE (PBDE 203) and nona-PBDE (PBDE 206) on PND 3 or PND 10, the most pronounced neurobehavioural aberrations were observed in adult mice exposed on PND 10 (Viberg et al., 2006). The early postnatal period was also found to be vulnerable in a study by Xing and co-workers (Xing et al., 2009) where the most pronounced effects for induction of effects on synaptic plasticity was observed when exposure to PBDE 209 occurred during the lactational period. A critical window for induction of persistent developmental effects is also suggested in animals exposed to PFOA. The aberration in habituation over 60 min was seen only in mice neonatally exposed to PFOA (14 μmol) on PND 10 but not when given on PND 3.

Taken together, these earlier reported developmental neurotoxic effects of PBDE 209, with the present window of developmental neurotoxicity of PFOA on PND 10, and the lack of additional behavioural defects when both PBDE 209 and PFOA were given on PND 3, suggest that it is the presence of PFOA and possible metabolite(s) of PBDE 209 around PND 10 that causes and exacerbates the neurobehavioural defects in adult mice. In other studies, we have also shown that this period of rapid brain development is vulnerable to insult by xenobiotics and that the presence of the compound in the brain during this defined period of maturational processes is a critical factor (Eriksson et al., 1992; Eriksson et al., 2000; Viberg et al., 2003; Eriksson, 2008a).

In our earlier studies we have seen the cholinergic system to be a target for POPs (Eriksson, 1997; Eriksson et al., 2001a) and also observed increased susceptibility of the cholinergic system in adult mice when neonatally exposed to PBDE 209 (Johansson et al., 2008b) as well as to PFOA (Johansson et al., 2008a), a system closely related to cognitive function (Contestabile, 2011; Woolf and Butcher, 2011). We found recently that the amount of certain important developmental proteins, such as CaMKII, GAP-43 and synaptophysin, could be modified in the developing brain by neonatal exposure to PBDE 209 (Viberg et al., 2008) and PFOA (Johansson et al., 2009) at a dose of 21 μmol/kg body weight. It is known that the levels of these proteins increases during the BGS in the neonatal mouse, with the most pronounced increase taking place around PND 7–14 (Viberg et al., 2008; Viberg, 2009) and that these proteins are known biochemical substrates for cellular processes such as neurite outgrowth and synaptogenesis. Whether or not modified levels of these proteins could inhibit normal brain development and be part of the mechanisms behind the observed behavioural defects remains a topic for further studies.

Reviews by Grandjean and co-workers (Grandjean et al., 2001; Grandjean and Landrigan, 2006), including epidemiological studies, indicated that environmental toxicants can by interacting cause defective cognitive development. We have earlier reported that neonatal co-exposure to low doses of another PBDE, PBDE 99, together with an ortho-substituted PCB, PCB 52 (Eriksson et al., 2006), or together with MeHg (Fischer et al., 2008a), as well as the co-exposure to PCB153 and MeHg (Fischer et al., 2008b) can exacerbate developmental neurobehavioural defects at doses where the individual dose is without effect. The study on neonatal co-exposure to PCB153 and MeHg (Fischer et al., 2008b) support a hypothesis that the neuropsychological deficits in children from the Faeroes (exposed to both PCB and MeHg) but not in children from the Seychelles (exposed to MeHg) (Grandjean et al., 2001; Davidson et al., 2006) may be attributed to the presence of PCB as well as MeHg. Furthermore, the neurobehavioural defects in adult mice neonatally co-exposed to PCB and MeHg occurred at doses that could be approximated to those reported for exposed children (Fischer et al., 2008b). It is worth noting that the doses of PBDE 209 and PFOA used in the present study are comparable at the molar level to the doses used in those reported co-exposure studies conducted in experimental animals on PBDE 99, PCBs, and MeHg (Eriksson et al., 2006, Fischer et al., 2008b, a).

Epidemiological studies indicate that developmental exposure to PBDEs (Herbstman et al., 2010; Eskenazi et al., 2013), PBDE 209 (Gascon et al., 2012) and PFCs (Hoffman et al., 2010) can lead to neurodevelopmental defects, including increased risk of ADHD. Furthermore, reports also indicate increasing levels of both PBDE 209 and PFOA in domestic dust (Stapleton et al., 2006; Strynar and Lindstrom, 2008) and that the levels of PBDE 209 and PFC can be higher in newborns and toddlers (Olsen et al., 2004; Fischer et al., 2006; Kärrman et al., 2007; Sahlstrom et al., 2015). It is also known that PBDE 209 has a rather short half-life in humans (Thuresson et al., 2006) and the exposure to and developmental neurotoxic effects of such toxicants can be difficult to ascertain from epidemiological studies, as the compound may not be present at adult age. Thus, direct exposure to certain POPs by inhalation and/or ingestion of dust can constitute an important exposure route for newborns and infants, with possible consequences for brain development. It is therefore not only chemicals present in food and mother's milk we need to consider, but also direct exposure to chemicals such as PBDE 209 and PFOA, present in dust, that can be inhaled and/or ingested by newborns and infants when the brain is in a stage of rapid development and vulnerable to insults.

In conclusion, the present study shows that low doses of PFOA and PBDE 209 can, by co-operating during neonatal brain development, exacerbate developmental neurobehavioural defects in mice when the co-exposure occurs during neonatal brain development. This type of interaction is suggested to be dependent on both the metabolism of PBDE 209 and the presence of its metabolites (possible debrominated ones) together with PFOA during a defined critical period of neonatal brain development, namely around PND 10. In view of the increasing number of reports suggesting that PFC and highly brominated PBDEs are present in dust, the present study indicates that infants and young children can be susceptible to direct exposure during brain development, which calls for further investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The authors do not have permission to share data.

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References

Abreu-Villaca, Y., Filgueiras, C.C., Manhaes, A.C., 2011. Developmental aspects of the cholinergic system. Behav. Brain Res. 221, 367–378.
Bjorklund, J.A., Thuresson, K., De Wit, C.A., 2009. Perfluoroalkyl compounds (PFCs) in indoor dust: concentrations, human exposure estimates, and sources. Environ. Sci. Technol. 43, 2276–2281.
de Boer, J., Wester, P.G., Klamer, H.J., Lewis, W.E., Boon, J.P., 1998. Do flame retardants threaten ocean life? Nature 394, 28–29.
Bolles, R.C., Woods, P.J., 1964. The ontogeny of behaviour in the albino rat. Anim. Behav. 12, 427–441.
Butenhoff, J.L., Ehresman, D.J., Chang, S.C., Parker, G.A., Stump, D.G., 2009. Gestational and lactational exposure to potassium perfluorooctanesulfonate (K(+)PFOS) in rats: developmental neurotoxicity. Reprod. Toxicol. 27, 319–330.
Calafat, A.M., Kuklenyik, Z., Caudill, S.P., Reidy, J.A., Needham, L.L., 2006. Perfluorochemicals in pooled serum samples from United States residents in 2001 and 2002. Environ. Sci. Technol. 40, 2128–2134.
Contestabile, A., 2011. The history of the cholinergic hypothesis. Behav. Brain Res. 221, 334–340.
Coyle, J.T., Yamamura, H.I., 1976. Neurochemical aspects of the ontogenesis of cholinergic neurons in the rat brain. Brain Res. 118, 429–440.
Daenen, E.W., Van der Heyden, J.A., Kruse, C.G., Wolterink, G., Van Ree, J.M., 2001. Adaptation and habituation to an open field and responses to various stressful events in animals with neonatal lesions in the amygdala or ventral hippocampus. Brain Res. 918, 153–165.
Davidson, P.W., Myers, G.J., Weiss, B., Shamlaye, C.F., Cox, C., 2006. Prenatal methyl mercury exposure from fish consumption and child development: a review of evidence and perspectives from the Seychelles child development study. Neurotoxicology 27, 1106–1109.
Davison, A.N., Dobbing, J., 1968. Applied Neurochemistry, pp 178-221, 253-316. Blackwell, Oxford.
Eriksson, P., 1997. Developmental neurotoxicity of environmental agents in the neonate. Neurotoxicology 18, 719–726.
Eriksson, P., 2008a. Developmental neurotoxicity of PCBs in mice: Critical period of brain development and effects of interaction. In: Hansen, L.G., Robertson, L.W. (Eds.), PCBs: Human and Environmental Disposition and Toxicology. University of Illinois Press, Urbana and Chicago, pp. 172–185.

Eriksson, P., 2008b. Response to: use of the pup as the statistical unit in developmental neurotoxicity studies: overlooked model or poor research design? Toxicol. Sci. 103, 411–413.
Eriksson, P., Ahlbom, J., Fredriksson, A., 1992. Exposure to DDT during a defined period in neonatal life induces permanent changes in brain muscarinic receptors and behaviour in adult mice. Brain Res. 582, 277–281.
Eriksson, P., Ankarberg, E., 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., Fredriksson, A., 2001a. The developing cholinergic system as target for environmental toxicants, nicotine and polychlorinated biphenyls (PCBs): implications for neurotoxicological processes in mice. Neurotox. Res. 3, 37–51.
Eriksson, P., Jakobsson, E., Fredriksson, A., 2001b. Brominated flame retardants: a novel class of developmental neurotoxicants in our environment? Environ. Health Perspect. 109, 903–908.
Eriksson, P., Fischer, C., Fredriksson, A., 2006. Polybrominated diphenyl ethers, a group of brominated flame retardants, can interact with polychlorinated biphenyls in enhancing developmental neurobehavioral defects. Toxicol. Sci. 94, 302–309.
Eskenazi, B., Chevrier, J., Rauch, S.A., Kogut, K., Harley, K.G., Johnson, C., Trujillo, C., Sjodin, A., Bradman, A., 2013. In utero and childhood polybrominated diphenyl ether (PBDE) exposures and neurodevelopment in the CHAMACOS study. Environ. Health Perspect. 121, 257–262.
Fischer, C., Fredriksson, A., Eriksson, P., 2008a. Coexposure of neonatal mice to a flame retardant PBDE 99 (2,2′,4,4′,5-pentabromodiphenyl ether) and methyl mercury enhances developmental neurotoxic defects. Toxicol. Sci. 101, 275–285.
Fischer, C., Fredriksson, A., Eriksson, P., 2008b. Neonatal co-exposure to low doses of an ortho-PCB (PCB 153) and methyl mercury exacerbate defective developmental neurobehavior in mice. Toxicology 244, 157–165.
Fischer, D., Hooper, K., Athanasiadou, M., Athanasiadou, I., Bergman, Å., 2006. Children show highest levels of polybrominated diphenyl ethers in a California family of four: a case study. Environ. Health Perspect. 114, 1581–1584.
Fredriksson, A., 1994. MPTP-Induced Behavioural Deficits in Mice: Validity and Utility of a Model of Parkinsonism (vol. Doctoral Thesis). Uppsala University, Uppsala.
Gascon, M., Fort, M., Martinez, D., Carsin, A.E., Forns, J., Grimalt, J.O., Santa Marina, L., Lertxundi, N., Sunyer, J., Vrijheid, M., 2012. Polybrominated diphenyl ethers (PBDEs) in breast milk and neuropsychological development in infants. Environ. Health Perspect. 120, 1760–1765.
Giesy, J.P., Kannan, K., 2001. Global distribution of perfluorooctane sulfonate in wildlife. Environ. Sci. Technol. 35, 1339–1342.
Grandjean, P., Landrigan, P.J., 2006. Developmental neurotoxicity of industrial chemicals. Lancet 368, 2167–2178.
Grandjean, P., Landrigan, P.J., 2014. Neurobehavioural effects of developmental toxicity. Lancet Neurol. 13, 330–338.
Grandjean, P., Weihe, P., Burse, V.W., Needham, L.L., Storr-Hansen, E., Heinzow, B., Debes, F., Murata, K., Simonsen, H., Ellefsen, P., Budtz-Jorgensen, E., Keiding, N., White, R.F., 2001. Neurobehavioral deficits associated with PCB in 7-year-old children prenatally exposed to seafood neurotoxicants. Neurotoxicol. Teratol. 23, 305–317.
Groves, P.M., Thompson, R.F., 1970. Habituation: a dual-process theory. Psychol. Rev. 77, 419–450.
Harada, K., Koizumi, A., Saito, N., Inoue, K., Yoshinaga, T., Date, C., Fujii, S., Hachiya, N., Hirosawa, I., Koda, S., Kusaka, Y., Murata, K., Omae, K., Shimbo, S., Takenaka, K., Takeshita, T., Todoriki, H., Wada, Y., Watanabe, T., Ikeda, M., 2007. Historical and geographical aspects of the increasing perfluorooctanoate and perfluorooctane sulfonate contamination in human serum in Japan. Chemosphere 66, 293–301.
Herbstman, J.B., Sjodin, A., Kurzon, M., Lederman, S.A., Jones, R.S., Rauh, V., Needham, L.L., Tang, D., Niedzwiecki, M., Wang, R.Y., Perera, F., 2010. Prenatal exposure to PBDEs and neurodevelopment. Environ. Health Perspect. 118, 712–719.
Hoffman, K., Webster, T.F., Weisskopf, M.G., Weinberg, J., Vieira, V.M., 2010. Exposure to polyfluoroalkyl chemicals and attention deficit/hyperactivity disorder in U.S. children 12-15 years of age. Environ. Health Perspect. 118, 1762–1767.
Hoffman, K., Adgent, M., Goldman, B.D., Sjodin, A., Daniels, J.L., 2012. Lactational exposure to polybrominated diphenyl ethers and its relation to social and emotional development among toddlers. Environ. Health Perspect. 120, 1438–1442.
Irvine, G.L., Timiras, P.S., 1966. Litter size and brain development in the rat. Life Sci. 5, 1577–1582.
Johansson, N., Fredriksson, A., Eriksson, P., 2008a. Neonatal exposure to perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) causes neurobehavioural defects in adult mice. Neurotoxicology 29, 160–169.
Johansson, N., Viberg, H., Fredriksson, A., Eriksson, P., 2008b. Neonatal exposure to deca-brominated diphenyl ether (PBDE 209) causes dose-response changes in spontaneous behaviour and cholinergic susceptibility in adult mice. Neurotoxicology 29, 911–919.
Johansson, N., Eriksson, P., Viberg, H., 2009. Neonatal exposure to PFOS and PFOA in mice results in changes in proteins which are important for neuronal growth and synaptogenesis in the developing brain. Toxicol. Sci. 108, 412–418.
Kannan, K., Corsolini, S., Falandysz, J., Fillmann, G., Kumar, K.S., Loganathan, B.G., Mohd, M.A., Olivero, J., Van Wouwe, N., Yang, J.H., Aldoust, K.M., 2004. Perfluorooctanesulfonate and related fluorochemicals in human blood from several countries. Environ. Sci. Technol. 38, 4489–4495.
Kärrman, A., Ericson, I., van Bavel, B., Darnerud, P.O., Aune, M., Glynn, A., Lignell, S., Lindstrom, G., 2007. Exposure of perfluorinated chemicals through lactation: levels of matched human milk and serum and a temporal trend, 1996-2004, in Sweden. Environ. Health Perspect. 115, 226–230.
Keller, W.C., 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. Experimental Design: Procedures for the Behavioural Sciences. Belmont, CA.
Kodavanti, P.R., Stoker, T.E., Fenton, S.E., 2017. Brominated flame retardants. In: Gupta, R.C. (Ed.), Reproductive and Developmental Toxicology. Academic Press, New York, pp. 681–710.
Lau, C., Thibodeaux, J.R., Hanson, R.G., Rogers, J.M., Grey, B.E., Stanton, M.E., Butenhoff, J.L., Stevenson, L.A., 2003. Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse. II: postnatal evaluation. Toxicol. Sci. 74, 382–392.
Lazic, S.E., Essioux, L., 2013. Improving basic and translational science by accounting for litter-to-litter variation in animal models. BMC Neurosci. 14, 37.
Meironyte, D., Noren, K., Bergman, A., 1999. Analysis of polybrominated diphenyl ethers in Swedish human milk. A time-related trend study, 1972-1997. J. Toxicol. Environ. Health A 58, 329–341.
Morck, A., Hakk, H., Orn, U., Klasson Wehler, E., 2003. Decabromodiphenyl ether in the rat: absorption, distribution, metabolism, and excretion. Drug Metab. Dispos. 31, 900–907.
Olsen, G.W., Church, T.R., Miller, J.P., Burris, J.M., Hansen, K.J., Lundberg, J.K., Armitage, J.B., Herron, R.M., Medhdizadehkashi, Z., Nobiletti, J.B., O'Neill, E.M., Mandel, J.H., Zobel, L.R., 2003. Perfluorooctanesulfonate and other fluorochemicals in the serum of American red cross adult blood donors. Environ. Health Perspect. 111, 1892–1901.
Olsen, G.W., Church, T.R., Hansen, K.J., Burris, J.M., Butenhoff, J.L., Mandel, J.H., Zobel, L.R., 2004. Quantitative evaluation of perfluorooctanesulfonate (PFOS) and other fluorochemicals in the serum of children. J. Child Health 2, 53–76.
Rice, D.C., Reeve, E.A., Herlihy, A., Zoeller, R.T., Thompson, W.D., Markowski, V.P., 2007. Developmental delays and locomotor activity in the C57BL6/J mouse following neonatal exposure to the fully-brominated PBDE, decabromodiphenyl ether. Neurotoxicol. Teratol. 29, 511–520.
Rice, D.C., Thompson, W.D., Reeve, E.A., Onos, K.D., Assadollahzadeh, M., Markowski, V.P., 2009. Behavioral changes in aging but not young mice after neonatal exposure to the polybrominated flame retardant decaBDE. Environ. Health Perspect. 117, 1903–1911.
Sahlstrom, L.M., Sellstrom, U., de Wit, C.A., Lignell, S., Darnerud, P.O., 2015. Estimated intakes of brominated flame retardants via diet and dust compared to internal concentrations in a Swedish mother-toddler cohort. Int. J. Hyg. Environ. Health 218, 422–432.
Sandholm, A., Emanuelsson, B.-M., Klasson Wehler, E., 2003. Bioavailability and half-life of decabromodiphenyl ether (BDE-209) in rat. Xenobiotica; Fate Foreign Comp. Biol. Syst. 33, 1149–1158.
Sjodin, A., Wong, L.Y., Jones, R.S., Park, A., Zhang, Y., Hodge, C., Dipietro, E., McClure, C., Turner, W., Needham, L.L., Patterson Jr., D.G., 2008. Serum concentrations of polybrominated diphenyl ethers (PBDEs) and polybrominated biphenyl (PBB) in the United States population: 2003-2004. Environ. Sci. Technol. 42, 1377–1384.
So, M.K., Yamashita, N., Taniyasu, S., Jiang, Q., Giesy, J.P., Chen, K., Lam, P.K., 2006. Health risks in infants associated with exposure to perfluorinated compounds in human breast milk from Zhoushan, China. Environ. Sci. Technol. 40, 2924–2929.
Stapleton, H.M., Harner, T., Shoeib, M., Keller, J.M., Schantz, M.M., Leigh, S.D., Wise, S. A., 2006. Determination of polybrominated diphenyl ethers in indoor dust standard reference materials. Anal. Bioanal. Chem. 384, 791–800.
Strynar, M.J., Lindstrom, A.B., 2008. Perfluorinated compounds in house dust from Ohio and North Carolina, USA. Environ. Sci. Technol. 42, 3751–3756.
Thuresson, K., Hoglund, P., Hagmar, L., Sjodin, A., Bergman, A., Jakobsson, K., 2006. Apparent half-lives of hepta- to decabrominated diphenyl ethers in human serum as determined in occupationally exposed workers. Environ. Health Perspect. 114, 176–181.
Toms, L.M., Sjodin, A., Harden, F., Hobson, P., Jones, R., Edenfield, E., Mueller, J.F., 2009. Serum polybrominated diphenyl ether (PBDE) levels are higher in children (2-5 years of age) than in infants and adults. Environ. Health Perspect. 117, 1461–1465.
Viberg, H., 2009. Neonatal ontogeny and neurotoxic effect of decabrominated diphenyl ether (PBDE 209) on levels of synaptophysin and tau. Int. J. Dev. Neurosci. 27, 423–429.
Viberg, H., Eriksson, P., 2017. Perfluorooctane sulfonate and perfluorooctanoic acid. In: Gupta, R.C. (Ed.), Reproductive and Developmental Toxicology. Academic Press, New York, pp. 811–827.
Viberg, H., Fredriksson, A., Jakobsson, E., Orn, U., Eriksson, P., 2003. Neurobehavioral derangements in adult mice receiving decabrominated diphenyl ether (PBDE 209) during a defined period of neonatal brain development. Toxicol. Sci. 76, 112–120.
Viberg, H., Johansson, N., Fredriksson, A., Eriksson, J., Marsh, G., Eriksson, P., 2006. Neonatal exposure to higher brominated diphenyl ethers, hepta-, octa-, or nonabromodiphenyl ether, impairs spontaneous behavior and learning and memory functions of adult mice. Toxicol. Sci. 92, 211–218.
Viberg, H., Fredriksson, A., Eriksson, P., 2007. Changes in spontaneous behaviour and altered response to nicotine in the adult rat, after neonatal exposure to the brominated flame retardant, decabrominated diphenyl ether (PBDE 209). Neurotoxicology 28, 136–142.
Viberg, H., Mundy, W., Eriksson, P., 2008. Neonatal exposure to decabrominated diphenyl ether (PBDE 209) results in changes in BDNF, CaMKII and GAP-43, biochemical substrates of neuronal survival, growth, and synaptogenesis. Neurotoxicology 29, 152–159.
Viberg, H., Lee, I., Eriksson, P., 2013. Adult dose-dependent behavioral and cognitive disturbances after a single neonatal PFHxS dose. Toxicology 304, 185–191.

Vodicnik, M.J., 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.
Wilford, B.H., Shoeib, M., Harner, T., Zhu, J., Jones, K.C., 2005. Polybrominated diphenyl ethers in indoor dust in Ottawa, Canada: implications for sources and exposure. Environ. Sci. Technol. 39, 7027–7035.
de Wit, C.A., 2002. An overview of brominated flame retardants in the environment. Chemosphere 46, 583–624.
Woolf, N.J., Butcher, L.L., 2011. Cholinergic systems mediate action from movement to higher consciousness. Behav. Brain Res. 221, 488–498.
Wright, J.W., Murphy, E.S., Elijah, I.E., Holtfreter, K.L., Davis, C.J., Olson, M.L., Muhunthan, K., Harding, J.W., 2004. Influence of hippocampectomy on habituation, exploratory behavior, and spatial memory in rats. Brain Res. 1023, 1–14.
Xing, T., Chen, L., Tao, Y., Wang, M., Chen, J., Ruan, D.Y., 2009. Effects of decabrominated diphenyl ether (PBDE 209) exposure at different developmental periods on synaptic plasticity in the dentate gyrus of adult rats in vivo. Toxicol. Sci. 110, 401–410.