HIGHLY BROMINATED DIPHENYL ETHERS (PBDE 209) CAN INTERACT WITH PERFLUORINATED CHEMICALS (PFOA) DURING NEONATAL BRAIN DEVELOPMENT IN ENHANCING DEVELOPMENTAL NEUROBEHAVIOURAL DEFECTS

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Introduction

In our environment there are present known persistent environmental toxicants, such as polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDT). To these older persistent chemicals we need to add more recently detected environmental contaminants that also can be of a persistent nature.

The brominated flame retardants (BFR)[1,2,3] and the perfluorinated compounds (PFC)[4,5,6] are two new groups that have been identified as emerging classes of persistent environmental contaminates, and found to be present in humans as well as wildlife. Of special concern is that both these compounds have, in a recent report from WWF[7], been shown to be present at a higher level in the children, compared to the mother´s and the grandmother's generation. This was the opposite of older and banned persistent chemicals, such as organ chlorine pesticides and PCBs.

Regarding BFR, the polybrominated diphenyl ethers (PBDEs) are used in large quantities as flame-retardant additives in polymers, especially in the manufacture of a wide variety of electrical appliances, including casing for television, computer and other electronic products, building materials, and textiles[8]. PFC is found in consumer applications such as stain resistant treatment coatings for clothing fabrics, carpets and oil-resistant coatings for paper products for food contact[9]. They are also used in foam fire extinguishers.

We have earlier reported that low-dose exposure to environmental toxicants like PCBs, DDT and PBDEs (polybrominated diphenyl ethers), during a critical period of the neonatal brain development; can lead to disruption of the adult brain function, manifested as deranged spontaneous behaviour, lack of/or reduced habituation, defect learning and memory faculties and changes in the cholinergic system [10,11,12,13,14,15,16]. This period, known as the brain growth spurt (BGS), is a phase during the development when the maturational processes of CNS are at a stage of critical vulnerability. In humans, this period begins during the third trimester of pregnancy and continues throughout the first 2 years of life, while in mice and rats this period is neonatal, spanning the first 3-4 weeks of life[17].

In a recent study we have shown that the full brominated PBDE, 2,2',3,3',4,4',5,5',6,6'-decaBDE (PBDE 209) can be taken up during neonatal life and induce developmental neurotoxic effects in adult mice[16]. The neurotoxic recordings showed that the effects only occurred in mice exposed on postnatal day 3 (PND 3) and not in mice exposed on postnatal day 10 (PND 10). This suggests that the developmental neurobehavioural disturbances are caused by a metabolite of PBDE 209 (possibly de-brominated), present around neonatal day 10. Of the PFC we have observed that PFOA can cause developmental neurobehavioural defects when given to neonatal mice on PND 10[18].

A primary route for contaminant exposure of highly lipophilic chemicals to children is through mother´s milk[19]. This is seen for several of the persistent environmental toxicants like DDT, PCBs and several brominated diphenyl ethers. However, there are now reports that suggest highly brominated PBDEs[20], and also PFC[21], are present in dust and therefore infants and young children can be susceptible to exposure. A further support to this is that both BFR and PFC are present in newborns and children, at a higher degree than compared to mother´s and grandmother's generation[7].

With regard to: 1) BFR and PFC are present in children at a higher level than compared to the mother´s and the grandmother's generation, 2) in our earlier studies we have seen that POPs can interact during neonatal life in enhancing developmental neurotoxic effects, and 3) both deca PBDE and PFOA have been shown to cause developmental neurotoxic effects, the present study was carried out to see whether deca-PBDE and PFOA can interact during neonatal brain development to enhance neurobehavioural defects, and whether this occurs during a defined critical phase of neonatal brain development.

Materials and Methods
The 2,2',3,3',4,4',5,5',6,6'-decabromo diphenyl ether (PBDE 209) was synthesized at the Department of Environmental Chemistry, University of Stockholm, Sweden. Perfluorooctanoic acid (PFOA) was purchased from Sigma-Aldrich. Neonatal NMRI male mice, 3 and 10 days of age (PND 3/PND 10), 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, or a vehicle (20% fat emulsion) as follows: 1) vehicle/vehicle, 2) vehicle/PFOA 1.4, 3) vehicle/PFOA 14, 4) PFOA 1.4/vehicle, 5) PFOA/vehicle, 6) PBDE 209 1.4/vehicle, 7) PBDE 209 8/vehicle, 8) PBDE 209 1.3 + PFOA 1.4/vehicle, 9) PBDE 209 1.4 + PFOA 14/ vehicle, 10) PBDE 209 8.0 + PFOA 1.4/vehicle, 11) PBDE 209 8.0 + PFOA 14/vehicle, 12) PBDE 209 1.4/PFOA 1.4, 13) PBDE 209 1.4/PFOA 14) PBDE 209 8.0/PFOA 1.4, 15) PBDE 209 8.0/PFOA 14. Each treatment group comprised mice from 3-4 different litters.

Spontaneous behaviour was tested in the male mice at the age of 2 months. Motor activity was measured over 3x20 min in an automated device consisting of cages (40x25x15 cm) placed within two series of infrared beams (low level and high level. The test measures locomotion: horizontal movement, rearing: vertical movement, and total activity: all types of vibrations within the test cage, i.e. those caused by mouse movements, shaking (tremors) and grooming[10-16]

Results and Discussion
An important endpoint to study when evaluating the effects of interaction between environmental toxicants in mammals is to analyse the behaviour of affected animals. Behaviour is a major function whereby animals adapt to changes in the environment and changes in behaviour may reveal effects on the nervous system caused by the influence of a toxicant. Spontaneous behaviour is especially meaningful as it reflects a function dependent on the integration of a sensoric input into a motoric output, and reveals the ability of animals to habituate to a novel environment and thereby integrate new information with earlier attained.

The present study shows that PBDE 209 and PFOA, at low doses, can interact during neonatal brain development to enhance developmental neurobehavioural defects in mice. This interaction is dependent on both the metabolism of PBDE 209 and the presence of its metabolites (de-brominated ones) together with PFOA during a defined critical period of the neonatal brain development, namely around PND 10. Neonatal exposure to single oral dose of PBDE 209 on PND 3 and later single oral exposure to PFOA on PND 10 caused impaired spontaneous behaviour in 2-month-old mice, an effect significantly changed from single exposure to PBDE 209 or PFOA. Furthermore, neonatal exposure to PFOA on PND 10, but not on PND 3, significantly impaired spontaneous behaviour in the 2-month-old mice, and no effects of interaction between PBDE 209 and PFOA were seen when both compounds were given on PND 3. We have earlier reported that neonatal exposure to PBDE 209 on PND3 can cause developmental neurotoxic effects in mice[16]. The PBDE 209 study suggested that the developmental neurotoxic effects might have been induced by metabolites of PBDE 209, e.g. debrominated products, since no effects were seen after neonatal exposure on PND 10. In a recent study we also have shown that octa- and nona-PBDEs (PBDE 203 and PBDE 206) can cause developmental neurotoxic effects when administered to neonatal mice on PND 10[22]. Taken together this suggests that highly brominated diphenyl ethers can interact with perfluorinated chemicals (PFOA) in enhancing developmental neurobehavioral defects during neonatal brain development.

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