DEVELOPMENTAL NEUROTOXICITY OF PERFLUOROOCTANE SULFONATE (PFOS) AND PERFLUOROOCTANOIC ACID (PFOA) IN THE NEONATAL MOUSE; DERANGED BEHAVIOUR AND ALTERED SUSCEPTIBILITY OF THE CHOLINERGIC SYSTEM AS ADULTS

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Introduction
Perfluorinated compounds (PFC) have been identified as an emerging class of persistent environmental contaminates, and found to be present in humans as well as wildlife [1, 2, 3, 4]. 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. It is also used in foam fire extinguishers [5]. The PFC is stable and practically nonbiodegradable, and has been found to be persistent in the environment [6].

A recent report from WWF, indicate a higher level of PFC in the children's generation, compared to mother's and grandmother's generation [7]. This is the opposite of older, banned persistent chemicals, such as organochlorine pesticides and PCBs. 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 [8, 9,10,11,12,13,14,15]. 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 [16].

The present study was undertaken to investigate developmental neurotoxic effects of perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA) and perfluorodecanoic acid (PFDA), on the spontaneous behaviour, anxiety-like behaviour and the cholinergic system of adult mice.

Materials and Methods
Perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA) and perfluorodecanoic acid (PFDA), where purchased from Sigma-Aldrich. Neonatal NMRI male mice were given one single oral dose of either PFOS (perfluorooctanesulfonic acid; 0.75 or 11.3 mg/kg bw), PFOA (perfluorooctanoic acid; 0.58 or 8.70 mg/kg bw), or PFDA (perfluorodecanoic acid; 0.72 or 10.8 mg/kg bw), via a metal gastric-tube at the age of 10 days. The two doses are equal to 1.4 or 21 µmol/kg bw, doses used in our earlier studies regarding developmental neurotoxicity of PCBs and PBDEs. Mice serving as control animals received a 20% fat emulsion vehicle. At an age of 2 and 4 months, the mice were observed for spontaneous behaviour. The test measures locomotion (horizontal movement), rearing (vertical movement), and total activity (all types of vibration in the test cage, including movement, shaking (tremors) and grooming) of the mice.

In order to elucidate effects on the cholinergic system, nicotine-induced behaviour was studied, directly after recording of the spontaneous behaviour. The mice were picked up from the test cage and were directly given a single sc injection of 80 µg nicotine base/kg bw. This amount of nicotine is known to cause an increased activity in normal adult NMRI mice [17]. Directly after the nicotine injection, the mice were replaced in the test cage and observed for another 60-min period.

Four month-old mice were also subjected to performance in an elevated plus-maze. This procedure gives a measure of anxiety-like behaviour. The test was performed based on the method of Lister 1987 [18].

Results and Discussion
The present study has shown that neonatal exposure to PFOS and PFOA can induce neurotoxic effects in the adult mice. The spontaneous behaviour data showed that PFOS and PFOA cause a deranged spontaneous behaviour, in 2- and 4-month-old male NMRI mice neonatally exposed to 1.4 or 21 µmol/kg bw. PFOS and PFOA, on postnatal day 10. The animals displayed a reduced activity in the beginning of the 60 min observational period, while toward the end they became hyperactive, compared to control mice. Neonatal exposure to PFOS and PFOA also affected habituation, here defined as a decrease in locomotion, rearing and total activity variables in response to the diminished novelty of the test chamber, over the 60-min test period.

This habituation capability was also seen to worsen with age, as the habituation was less pronounced in the 4-month-old mice compared to 2-month-old.

The nicotine-induced behaviour revealed a hypoactive response to nicotine, in mice neonatally exposed to 21 µmol/kg bw. PFOS or PFOA, whereas in controls and mice exposed to PFDA a hyperactive response was seen. This indicates that the cholinergic system is affected in adult mice neonatally exposed to PFOS or PFOA.

The results from the performance in the elevated plus-maze of 4-month-old mice, revealed no significant difference between the different treatment groups, neither regarding percent entries made into open arms, nor percent time spent in open arms. This indicates that the decreased activity during the first 20-min period of the spontaneous behaviour was not due to any anxiety-like behaviour.

The present study has shown that neonatal exposure to PFOS and PFOA can cause developmental neurotoxic effects, manifested as deranged spontaneous behaviour and lack of habituation, effects that worsen with age. It was also shown that PFOS and PFOA can affect the cholinergic system. These developmental neurotoxic effects are similar to those we earlier have reported for PCBs and PBDEs. This suggests that PFOS and PFOA can be included in the group of POPs causing developmental neurotoxic effects.

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