# Chapter 9
# Neurotoxicity

**Henrik Viberg and Espen Mariussen**

**Abstract** The developing central nervous system is susceptible to exposure to many different classes of chemicals and environmental pollutants and this is also true for the PFCs. In epidemiological studies it has been seen that kids from mothers with high PFOS and PFOA concentrations show delayed motor and cognitive development and the prevalence of ADHD is higher in these children. The epidemiological findings are supported by several studies in laboratory animals, where it has been seen that PFOS, PFOA and PFHxS exposures during the gestational period increased the locomotor activity and caused an inability to habituate to new environments. These chemicals also affects molecular targets in the brain of test animals after gestational exposure and in the newborn period and the cholinergic system may be a possible target for the PFCs. Also in cell culture studies PFCs have been shown to be neurotoxic and affect different subtypes of PKC, strengthening the animal studies. All these possible effects of PFCs are similar to what earlier have been seen for PCBs and PBDEs and there may be possible problems with co-exposures from these different groups of chemicals.

**Keywords** Central nervous system • Brain development • Behavioral toxicity • Neuromotor maturation • Calcium-dependent signaling

H. Viberg (✉)
Department of Organismal Biology, Division of Environmental Toxicology, Uppsala University, Norbyvägen 18A, 75236 Uppsala, Sweden
e-mail: henrik.viberg@ebc.uu.se

E. Mariussen
Protection and Societal Security Division, Norwegian Defence Research Establishment (FFI), P.O. Box 25, NO-2027 Kjeller, Norway
e-mail: espen.mariussen@ffi.no



J.C. DeWitt (ed.), *Toxicological Effects of Perfluoroalkyl and Polyfluoroalkyl Substances*, Molecular and Integrative Toxicology,
DOI 10.1007/978-3-319-15518-0_9

## Abbreviations

| | |
|---|---|
| FTOH | Fluorotelomer alcohol |
| PFBA | Perfluorobutyric acid |
| PFBS | Perfluorobutane sulfonic acid |
| PFC | Perfluoroalkylated compounds |
| PFDA | Perfluorododecanoic acid |
| PFHpA | Perfluoroheptanoic acid |
| PFHSor PFHxS | Perfluorohexanesulfonic acid |
| PFHxA | Perfluorohexanoic acid |
| PFNA | Perfluorononanoic acid |
| PFOA | Perfluorooctanoic acid |
| PFOC | 1H-perfluorooctane |
| PFOS | Perfluorooctanesulfonic acid |
| PFOSA | Perfluorooctanesulfonamide |
| PFPA | Perfluoropropionic acid |
| PFTA | Perfluorotetradecanoic acid |
| TFAA | Trifluoroacetic acid |

## 9.1 Introduction and Background

From the fertilization of the egg, through gestation and during the first years after birth, the brain is subjected to a continuously development and disturbances during susceptible periods can induce many different types of negative alterations. Transfer of xenobiotics occurs from the mother to the fetus through the umbilical cord, via mother's milk to the newborn and via direct inhalation and ingestion to the newborn and toddler. Knowledge about the exposure situation in fetuses, newborns, toddlers in addition to adults is therefore important in order to predict toxic effects. Exposure to environmental contaminants have been suspected as agents for an increased prevalence of attention deficit hyperactivity disorder (ADHD) and susceptibility of dementia disorders, such as Parkinson's disease (Barkley 1998; Brown et al. 2005; Hardell et al. 2002; Hoffman et al. 2010; Lai et al. 2002; Rice 2000; Schettler 2001). It has been hypothesized that environmental contaminants can affect cognitive functions, such as learning and behavior, and motor skills (Grandjean and Landrigan 2006; Grandjean et al. 2014; Mariussen and Fonnum 2006). Human exposures to PFCs are reviewed in Part I of this book, but it is worth repeating that certain tissues and fluids are of extra interest concerning the possible developmental neurotoxic effects of PFCs. Generally it can be said that the concentrations of PFCs are significantly higher in maternal serum than in umbilical cord serum (Apelberg et al. 2007; Inoue et al. 2004; Midasch et al. 2007; Monroy et al. 2008). Unlike PFOS, PFOA appears to cross the placental barrier unhindered (Midasch et al. 2007). Only a few studies have analyzed the levels of PFCs in brain tissue. The PFC found in the

highest concentration in brain tissue is PFOS, followed by PFOA, which probably reflects their historical use, persistency and accumulation potential, and rate of elimination (Mariussen 2012). In general the levels of the PFCs are lower in the brain than in other organs, such as the liver and kidney, and even in developmentally exposed laboratory animals (Kawamoto et al. 2011; Mariussen 2012; Sato et al. 2009). Both the adult and developing brain are protected by the so-called blood brain barrier (BBB) reducing the access of both exogenous and endogenous compounds into the brain (Ek et al. 2012). The properties of PFCs as surfactants may, however, modulate the membrane fluidity of the cells (Levitt and Liss 1986) and there are *in vitro* studies implicating that PFOS can increase the permeability of the blood brain barrier. Wang and co-workers showed that PFOS induced disassembly of endothelial tight junctions in brain endothelial cells (Wang et al. 2011) and later it was also observed that PFOS reduced mRNA expression of cellular adhesion markers in neuronal cells (Choi et al. 2013). Newborns, toddlers and children are the most exposed part of the population, on a body weight basis, since they tend to inhale and ingest more than the adult population (Trudel et al. 2008). Furthermore, it is a well-known fact that many environmental pollutants end up in the mother's milk, exposing the nursing neonate to a cocktail of chemicals, including several of the PFCs, such as PFOS, PFOA, PFHxS, PFBA, PFHxA, PFHpA and PFNA (Antignac et al. 2013; Mondal et al. 2014; Sundstrom et al. 2011). The concentrations of the PFCs in breast milk tend to be highest in mothers who is nursing for the first time compared to mothers who have previously nursed (Tao et al. 2008). One study have compared concentrations of PFOS between the adult and juvenile brain showing a higher relative concentration in brain of the rat fetuses compared with the brains from their mothers (Chang et al. 2009), indicating that the BBB of the fetus has increased permeability of PFOS. Bearing in mind that the PFCs are only one group of toxicants reaching potential targets in the brain it is of importance to both screen the extent of exposure and to evaluate their hazardous potential.

## 9.2 Epidemiological Studies

It is always hard to study toxicological effects in humans especially when it comes to reproductive and developmental effects. Despite that, effort has been put into epidemiological studies to investigate if there are connections or correlations between levels/concentrations of PFCs in maternal serum, umbilical cord serum and birth weight, size and other markers of development in humans. This is summarized in Chap. 14, but some of the effects seen in these epidemiological studies indicate that PFCs can contribute to developmental neurotoxic effects in human. In 2007, reports came from Maryland, U.S., that both PFOS and PFOA concentrations in umbilical cord serum were negatively associated with birth weight and head circumference. When looking at maternal concentrations of PFOS and PFOA in relation to motor and mental developmental in children, it can be seen that children from mothers with high PFOS concentration are slightly delayed in time of sitting

without support (Fei et al. 2008) and also self-reported birth defects were associated with high PFOA exposures (Stein et al. 2009). The same research group used the same cohort to look into the correlation between the serum levels of PFCs in children and teenagers and the prevalence of ADHD, and found indications that high levels of PFCs, especially PFOA and possibly also PFHxS could be factors behind the induction of ADHD (Stein and Savitz 2011). Higher risk of ADHD in relation to PFC levels has also been proposed by other researchers and in that study it was not only the usual suspects mentioned, but also PFNA (Hoffman et al. 2010).

## 9.3 Animal Studies (*In Vivo* and *Ex Vivo* Studies)

Despite few studies it is plausible to believe that PFCs have toxic effects in humans, even though there are limited methods of measuring both exposure and effects. Instead animal studies are used to investigate neurotoxicity and there is a number of different experimental methods that can measure a variety of endpoints from several different exposure paradigms, with different doses, in several different species. Results generated from animal studies can be used to extrapolate and predict human toxicity and are therefore of great importance. Generally, it can be said that PFC neurotoxicity (mainly PFOS and PFOA) have been studied in all types of animals including fishes, birds and mammals, but the vast majority of studies have been done on rodents (Table 9.1). Usually, in order to exert an effect a compound has to be present in the target organ. In the section about the toxicokinetics of PFCs it was described that these compounds can reach the brain, both during development and in adults, which indicates that neurotoxic effects may arise. There are several known neurotoxic effects of PFCs and here we will look into some of them, starting with neuropsychiatric and neuromotoric effects. Effects that can be linked to the above mentioned epidemiological findings. An overview of studies on neurobehavioral effects of different PFCs are, in addition, presented in Table 9.1.

PFOS exposure in mice during the gestational period (6 mg/kg bw/day) delayed a couple of landmarks of neuromotor maturation, such as decreased resistance to backward pull on postnatal days 10 and 11 and decreased climb ability and forelimb strength on postnatal day 11. These effects were transient and not seen later during the postnatal period (Fuentes et al. 2007b). In a more recent study dams were exposed to different doses (0.1, 0.3, and 1.0 mg/kg bw/day) of PFOS from gestational day 0 through postnatal day 20. PFOS treatments had no effect during the postnatal period when looking at the auditory startle response and learning and memory in a swim maze. However, locomotor activity increased in PFOS treated animals (0.3 and 1.0 mg/kg bw/day) on postnatal day 17, which ultimately leads to the inability of the animals to habituate to the novel test environment (Butenhoff et al. 2009a).

The behavioral effects of PFC exposure, such as negative impact on memory, learning, and motor functions, may involve effects on several neurochemical targets. A major challenge is to link the behavioral effects to changes in the nervous tissue,

**Table 9.1** Summary of neurobehavioral studies of PFCs* on rodents, birds and fish

| Animal | Exposure and chemical | Behavioral effects | References |
|---|---|---|---|
| Neonatal rats | Dams exposed orally to 3 mg/kg PFOS*, from GD 2 to GD 21 | No effects | Lau et al. (2003) |
| Neonatal rats | Dams exposed orally to 0.1, 0.4, 1.6 and 2 mg/kg/day PFOS 42 days prior to mating until lactation day 20 | Delay in surface and air righting among offspring in the 1.6 mg/kg group | Luebker et al. (2005) |
| Neonatal rats | Dams exposed orally to 0.1, 0.3 and 1.0 mg/kg PFOS from GD 0 to PND 20 | Male displayed increased motor activity and reduced habituation in high dose group | Butenhoff et al. (2009a) |
| Adult rats | Oral exposure to 0.3, 1, 3 and 10 mg/kg PFHxS for 40–50 days | No effects in the functional observational battery or motor activity | Butenhoff et al. (2009b) |
| Adult rats | Oral exposure to 0, 6, 30 and 150 mg/kg/day PFBA for 28 days | No effects in hearing, static rightning, grip strength or motor activity. Delayed pupillary reflex in the high dose group | Butenhoff et al. (2012) |
| Adult rats | Oral exposure to 0, 1.2, 6 and 30 mg/kg/day PFBA for 90 days | No effects in hearing, static rightning, grip strength or motor activity. Delay in pupillary reflex in the high dose group | Butenhoff et al. (2012) |
| Adult rat | Oral exposure to 30 mg/kg/day PFOA for 28 days | Small decrease in motor activity | Butenhoff et al. (2012) |
| Adult male mice | Adult exposure by gavage to 3 and 6 mg/kg/day PFOS for 4 weeks | Small effect on activity in open-field and on retention tests | Fuentes et al. (2007a) |
| Neonatal mice | Dams exposed orally to 6 mg/kg/day PFOS from GD 12 to 18 | Delayed neuromotor maturation | Fuentes et al. (2007b) |
| Neonatal mice | Dams exposed orally to 6 mg/kg/day PFOS from GD 12 to 18 | Combination of PFOS and restraint stress reduced mobility in the open-field test | Fuentes et al. (2007c) |
| Neonatal male mice | Mice exposed to a single oral dose of 0.75 and 11.3 mg/kg PFOS at PND 10 | Effects on spontaneous behavior and habituation in 2 – and 4 month old mice in the high dose group | Johansson et al. (2008) |
| Neonatal male mice | Mice exposed to a single oral dose of 0.58 and 8.7 mg/kg PFOA at PND 10 | Effects on spontaneous behavior and habituation in 2- and 4 month old mice in all the groups | Johansson et al. (2008) |
| Neonatal male mice | Mice exposed to a single oral dose of 0.72 and 10.8 mg/kg PFDA at PND 10 | No effects | Johansson et al. (2008) |

(continued)

**Table 9.1** (continued)

| Animal | Exposure and chemical | Behavioral effects | References |
|---|---|---|---|
| Neonatal mice | Dams exposed orally to 6 mg/kg/day PFOS from GD 12 to 18. Half of the exposed animals were attributed to restraint stress | Prenatally exposed to mice spent more time in the center of the open-field device. Stress counteracted the effect | Ribes et al. (2010) |
| Neonatal male and female mice | Mice exposed to a single oral dose of 0.61, 6.1 and 9.2 mg/kg PFHxS at PND 10 | Effects on spontaneous behavior in high dose group in 2 and 4 month mice | Viberg et al. (2013) |
| Chicken | Fertilized chicken eggs injected on embryonic day 0–5 mg/kg or 10 mg/kg PFOS | Reduced imprinting performance at hatching day 1 | Pinkas et al. (2010) |
| Chicken | Fertilized chicken eggs injected on embryonic day 0–5 mg/kg or 10 mg/kg PFOS | Reduced imprinting performance at hatching day 1 | Pinkas et al. (2010) |
| Zebrafish | 0.5 μM PFOS in water | Effects on swimming speed after stimulus | Chen et al. (2013) |
| Zebrafish larvae | 0.03–3,000 mg/L of TFAA, PFBA, PFOA, PFNA, PFDA, PFBS, PFOS | Effects on locomotor behavior. Longer carbon chain PFCs were more potent than shorter chain PFCs. PFOS most potent | Ulhaq et al. (2013) |
| Zebrafish larvae | 0.1 and 1 mg/L PFOS | Persistent hyperactivity in the high dose group | Spulber et al. (2014) |

**PFBA* perfluorobutyric acid, *PFOA* perfluorooctanoic acid, *PFNA* Perfluorononanoic acid, *PFDA* perfluorododecanoic acid, *PFBS* perfluorobutane sulfonic acid, *PFHxS* perfluorohexanesulfonic acid, *PFOS* perfluorooctanesulfonic acid, *TFAA* Trifluoroacetic acid

which often probably result from effects on several neurochemical targets. In some of the studies of which neurobehavioral effects of PFCs have been elucidated, efforts have been performed to reveal neurochemical effects *ex vivo* (Table 9.2). In a series of studies it has been shown that different PFCs may affect spontaneous behavior and cognitive functions after administration of a single dose at specific time points (Johansson et al. 2008, 2009; Viberg et al. 2013). Here the spontaneous behavior, locomotion (horizontal movement), rearing (vertical movement) and total activity, was measured for an hour. In the beginning of the 60-min test period the activity was decreased in animals exposed to PFOS, PFOA, and PFHxS, but in the end these animals had not habituated to the novel environment and the activity was higher than in the control animals. This type of behavior was observed both in 2 and 4 months old animals and these behavioral effects were persistent and actually worsened with age. A fourth perfluorinated compound, PFDA (perfluorodecanoic acid) had no effects on adult behavior. So not all PFC have the potency to induce behavioral and cognitive disturbances. When looking at the neurochemical targets it was showed that mice exposed to single doses of PFOS (11.3 mg/kg), PFOA

**Table 9.2** Summary of *ex vivo* studies of PFCs*

| Animal | Chemical and concentrations | Effects | Ref |
|---|---|---|---|
| Mice and rats | Peroral adult exposure to one single dose to 125, 250 and 500 mg/kg PFOS* | No effects on brain neurotransmitter levels of norepinephrine, dopamine, serotonin, glutamate, GABA or glycine, 24 and 48 h after exposure of 250 mg/kg. No brain histopathological changes detected | Sato et al. (2009) |
| Rats | Maternal peroral exposure to 3.2 mg/kg PFOS in food from GD 1 to PND 35. Pups exposed after weaning (PND21) to PND 35 by cross-fostering model | Effects on mRNA expression of calcium related signalling molecules (NR2B, CaM, CaMKIIα, CREB). At PND 1 an increase in NR2B, CaM, CaMKIIα. At PND 7 increase in CREB. At PND 35 a decrease in NR2B | Liu et al. (2010a) |
| Rats | Maternal peroral exposure to 3.2 mg/kg PFOS in food from GD 1 to PND 21. Pups exposed to 3.2 mg/kg in food to PND35 | At PND 1 and 7 micro-arrays study showed effects on genes involved in neurodevelopment and synaptic plasticity (ligand receptor interaction, calcium signalling, cell communication, long term potentiation/depression). Less effects on PND 35 | Wang et al. (2010) |
| Rats | 0, 2, 8 and 32 and 128 ppm PFOS in the diet for 13 weeks (approximately 0.12, 0.5, 2.1 and 8.5 mg/kg/day) | No brain histopathological changes detected | Kawamoto et al. (2011) |
| Rats | Maternal peroral exposure to 0.1, 0.6 and 2.0 mg/kg/day PFOS from gestational day (GD) 0–20 | Dose dependent decrease in mRNA expression of synaptophysin and synapsin (Syn1 and 2) in hippocampus in pups at postnatal day 0 (PND 0) and 21. Ultrastructural changes in hippocampus at PND 21 | Zeng et al. (2011a) |
| Rats | Maternal peroral exposure to 0.1, 0.6 and 2.0 mg/kg/day PFOS from gestational day (GD) 0–20 | At PND 0 and 21 an increase in glial brain fibrillary acidic protein and S100 calcium binding protein B. An increased mRNA expression of TNF-α, IL-1β, AP-1, CREB, NF-kappa-B. A reduction in brain synapsin and synaptophysin | Zeng et al. (2011b) |
| Rats | Maternal peroral exposure to 3.2 mg/kg PFOS in food from GD 1 to PND 7 | Reduction in expression of miRNA involved in neurodevelopment and synaptic transmission. Reduction in synapse-associated proteins, vGlut, NGRF and TrKC | Wang et al. (2011) |

(continued)

**Table 9.2** (continued)

| Animal | Chemical and concentrations | Effects | Ref |
|---|---|---|---|
| Rats | Adult rats administered 1.7, 5.0, and 15 mg/L PFOS in drinking water for 91 days. | Increase in expression of CaMKII and pCREB in cortex and hippocampus. Upregulation of transcription factors c-jun in hippocampus and and cortex, and c-fos in hippocampus | Liu et al. (2010b) |
| Mice | One subcutaneous administration of 50 mg/kg PFOS at (PND 7, 14, 21, 28 and 35 | 24 h after exposure a reduction in brain superoxide dismutase (SOD) activity in male rats exposed at PND 7 and 21. A reduction in brain antioxidant capability in male rats exposed at PND 21 | Liu et al. (2009) |
| Mice | Peroral exposure, administered once, of 22 µmol/kg (11.3 and 8.7 mg/kg PFOS and PFOA) to 10 days old mice | 24 h after exposure, both compounds increased the concentrations of CaMKII, GAP-43 and synaptophysin in hippocampus. PFOA increased concentration of Tau in hippocampus. Both compounds increased the concentration of synaptophysin and Tau in cerebral cortex | Johansson et al. (2009) |
| Mice | Peroral exposure, administered once, of 14 or 21 µmol/kg (6.1 or 9.2 mg/kg) PFHxS to 10 days old mice | 24 h after exposure a reduction in levels of BDNF and GAP-43 in cerebral cortex in (9.2 mg/kg). An increase in CAMKII and Tau in hippocampus in both groups. An increase in synaptophysin in hippocampus (9.2 mg/kg) | Lee and Viberg (2013) |
| Chicken | Administration of one dose (5 mg and 10 mg/kg) PFOA and PFOS in egg at incubation day 0 | At hatching day 1 an overall increase in brain cytosolic PKC (PKC αβγ) in animals exposed to PFOA, and an overall decrease in cytosolic PKC in animals exposed to PFOS | Pinkas et al. (2010) |
| Chicken | Administration of one dose (8.9, 94, 890, and 9,300 ng/egg PFHxS and 9.7, 94, 1,000, and 9,700 ng/egg PFHxA) in egg at incubation day 0 | Upregulation of neurogranin mRNA in chicks exposed to 890 and 38,000 ng/egg PFHxS | Cassone et al. (2012) |

* *PFHxA* perfluorohexanoic acid, *PFOA* perfluorooctanoic acid, *PFHxS* perfluorohexanesulfonic acid, *PFOS* perfluorooctanesulfonic acid

(8.7 mg/kg) and PFHxS (6.1 mg/kg) 10 days after birth, had increased levels of the proteins CaMKII, synaptophysin and tau in hippocampus 1 day after the exposure (Johansson et al. 2009; Lee and Viberg 2013). It was, in addition, shown that PFOA and PFOS induced increased levels of synaptophysin in the mice cerebral cortex (Johansson et al. 2009). The effects on CaMKII by PFOS, PFOA and PFHxS are supported by changes in the gene expression of calcium-dependent signaling molecules in rat hippocampus after perinatal PFOS exposure. The expression of calcium-related signaling molecules, which are critical to the function of the central

nervous system, such as N-methyl-D-aspartate receptors, calmodulin, $Ca^{(2+)}$/calmodulin-dependent kinase II alpha and cAMP-response element-binding, were increased in the PFOS exposure group on postnatal day 1 (PND 1). In some cases these changes lasted for only a short period in postnatal life, but calmodulin and the N-methyl-D-aspartate receptor subtype-2B were still reduced on postnatal day 35 (Liu et al. 2010a). Furthermore these proteins are involved in neuronal growth, synaptogenesis and mediation of neurotransmitter release and indicate that the exposure of PFCs may influence the development of the juvenile mouse brain related to cognitive functions. Synaptophysin, which is a synaptic vesicle associated protein, has for example also been shown to be involved in modulation of cognitive functions such as learning and memory, and novelty exploration (Schmitt et al. 2009). A similar study was performed by (Zeng et al. 2011a, b) who exposed pregnant rats daily from GD2 to GD21 for 0.1, 0.6 and 2.0 mg PFOS/kg/day. The levels of synaptophysin and synapsin in hippocampus were analyzed at PND 0 and 21 showing a reduction in the levels in hippocampus, and an increase in the levels of synaptophysin and a decrease in the levels of synapsin in the cerebral cortex. Synapsin are synaptic vesicle associated proteins involved in the regulation of neurotransmitter release, and in the study by Zeng et al. (2011a) it was also claimed that PFOS induced morphological changes in the synaptic structure and reduced numbers of synaptic vesicles. The discrepancy from the findings by Johansson et al. (2009), who observed an increase in the levels of synaptophysin in the mice brain, may be due to the different administration procedure. The juvenile rats in the study by Zeng et al. (2011a, b) were exposed chronically during pregnancy and the juvenile mice in the study by Johansson et al. (2009) were administered a single oral acute dose 10 days after birth. These studies, therefore, indicate that the PFCs might influence synaptic plasticity, which may have consequences for neuronal development.

When the neonatally animals, which showed effects on cognitive function after exposure to PFOS, PFOA, and PFHxS, were challenged with nicotine in adulthood, their response was changed compared to normal animals.

Control animals became significantly hyperactive by the adult nicotine injection, while the neonatally exposed animals reacted totally opposite with very little activity, displaying a clear hypoactivity. This indicates that PFC could affect the cholinergic transmitter system during the neonatal brain development, because the cholinergic system is involved in many physiological functions, including cognitive capacity (Johansson et al. 2008; Viberg et al. 2013). Other studies support that the cholinergic system could be a target for developmental PFC exposure. For example choline acetyltransferase, a very important enzyme in the cholinergic system of mammals, is involved in the recycling of the neurotransmitter acetylcholine by joining of Acetyl-CoA and choline to reform acetylcholine. In utero exposure to 3 mg PFOS/kg bw/day, during the gestational period, in rats resulted in decreased activity of choline acetyltransferase in prefrontal cortex at different postnatal ages (Lau et al. 2003). Interestingly these effects on cognitive function, behavior and motor activity, are similar to developmental neurotoxicological effects seen after gestational or neonatal exposure to other persistent organic pollutants, such as PCBs and PBDEs (Eriksson 1998; Eriksson et al. 2001; Viberg et al. 2003a, b). In addition, the

mechanistic background to these disturbances are also similar, meaning that they seem to affect the same types of proteins and the same transmitter systems (Eriksson 1998; Viberg et al. 2002, 2007).

Pinkas and co-workers exposed chicks prenatally to single doses of PFOS and PFOA at incubation day 0 for 5 and 10 mg/kg (Pinkas et al. 2010). The chicks were subjected to behavioral testing at hatching day 1 and showed impaired imprinting behavior. An *ex vivo* examination of the brains showed that the PFOS exposed birds had an overall reduction in the levels of different cytosolic PKC isoforms (PKC -α, -β, -γ), whereas PFOA induced an overall increase in the levels of cytosolic PKC. No effects on membrane bound PKC were found. According to the authors, translocation of cytosolic PKC to the membrane is required for imprinting and plays a role in the transfer of cholinergic input involved in learning and memory. Different PKC isoforms have previously been postulated as possible targets following both adult and developmental exposure to halogenated aromatic hydrocarbons, such as the PCBs (Kodavanti et al. 1994, 1998; Yang et al. 2003). The doses used in the experiment by Pinkas et al. (2010) lead, however to high mortality. Between 30 and 50 % of the exposed eggs did not develop embryos indicating that the doses used were detrimental to the chicks leading to other substantial non-neurotoxic effects. Additional studies have been done by Sean Kennedy's research group, who exposed chickens *in ovo* to high doses of PFHxS and PFHxA. They saw that PFHxS induced increases in mRNA levels of neurogranin in cerebral cortex (Cassone et al. 2012). Neurogranin is expressed solely in central nervous system, particularly in dendrites, and is a calmodulin-binding protein, participating in the protein kinase C signaling pathway. PFHxA on the other hand did not have an effect on the mRNA levels of neurogranin.

PFCs can affect the nervous system of mammals and birds, but other studies have also shown that fish are susceptible to PFC exposure during their development. PFOS and PFOA are the most studied (Shi et al. 2009; Spulber et al. 2014; Zhang et al. 2011), and there is one particular interesting study out showing that water exposure to several different PFCs, in zebrafish, can cause behavioral disturbances in locomotor activity. Among the PFCs inducing behavioral disturbances were TFAA, PFNA, PFBS and PFOS. When looking at the structure of the PFCs, PFCs with longer carbon chain length and with attached sulfonic groups showed larger potential to affect locomotor behavior in zebrafish larvae (Ulhaq et al. 2013).

## 9.4 Cell Cultures (*In Vitro* Studies)

In order to look into the developmental neurotoxicity of PFCs and to get a better understanding of the potential mechanisms behind the neurotoxic effects, *in vitro* experiments have been conducted and investigated such as on cell differentiation and synaptic plasticity (Table 9.3). It has been shown that PFOSA and PFOS (50–250 μM) promote differentiation of the PC12 cell into the cholinergic phenotype at the expense of the dopaminergic phenotype (Slotkin et al. 2008). At the highest

**Table 9.3** Summary of *in vitro* studies of PFCs*

| Preparation | Chemical and concentrations | Effects | Ref |
|---|---|---|---|
| PC 12 cells | 10–250 μM PFOS*, PFOA, PFOSA, PFBS | PFOS promoted differentiation of ACh phenotype at the expense of DA phenotype. Induction of lipid peroxidation and ROS, reduction in cell viability | Slotkin et al. (2008) |
| PC12 cells | 100–500 μM PFHxS | Reduced cell viability and caspase-3 activation; activation of ERK (pro-apoptotic), JNK (anti-apoptotic) and p38MAPK. Protection by NMDA receptor antagonist and ERK-antagonist | Lee et al. (2014a) |
| Rat cerebellar granule cells | 3 and 30 μM PFOS | Reduced cell viability and caspase-3 activation; activation of ROS and PKC. PKC antagonists and antioxidant (N-acetylcysteine) were protective | Lee et al. (2012) |
| Rat cerebellar granule cells | 12–100 μM PFOS, PFOSA, PFOA, FTOH 8:2 | Reduced cell viability (EC50 PFOS, PFOA, PFOSA and FTOH: 61, >100, 13 and 15 μM respectively) and induction of ROS (EC50 PFOS, PFOA, PFOSA and FTOH: 27, 25, 57 and >100 μM respectively) | Reistad et al. (2013) |
| Rat cerebellar granule cells | 10 and 30 μM PFOS | Reduced cell viability and caspase-3 activation; activation of ERK (pro-apoptotic). PKC antagonist was protective | Lee et al. (2013) |
| Rat cerebellar granule cells | 100–500 μM PFHxS | Reduced cell viability and caspase-3; activation of ERK (pro-apoptotic) and JNK (anti-apoptotic). Activation of ROS | Lee et al. (2014b) |
| Rat cerebellar Purkinje cells | 30 μM PFOS | PFOS decreased action potential frequency. Influenced Ca, Na and K-currents toward a hyperpolarized state | Harada et al. (2006) |
| Rat primary hippocampal neurons and slices | 10–100 μM PFOS | Increased frequency of miniature postsynaptic currents (mPSCs) and the amplitude of field excitatory postsynaptic potentials. Increased inward Ca-currents and intracellular Ca, inhibited by L-type Ca-channel inhibitor. Suppression of synaptogenesis in cultured neurons | Liao et al. (2008) |
| Rat primary hippocampal neurons | 50 and 100 μM PFPA, PFBA, PFOA, PFDA, PFTA, PFBS, PFHS, PFOS, PFOC | Increased frequency of mPSCs. The increase was proportional to carbon chain length, and the carboxylates were less potent than the sulfonates | Liao et al. (2009b) |

(continued)

**Table 9.3** (continued)

| Preparation | Chemical and concentrations | Effects | Ref |
|---|---|---|---|
| Rat primary hippocampal neurons | 10–100 μM PFOS | Increase in K currents and glutamate activated currents | Liao et al. (2009a) |
| Rat primary hippocampal neurons | 30–300 μM PFOS<br>100–300 μM PFOS | Elevation of intracellular Ca from intracellular Ca stores and induction of ROS. A role of ryanodine and inositol triphosphate receptors | Liu et al. (2011) |
| Neural stem cells | 25–100 nM PFOS | Decreased cell viability (100 nM). Lower number of proliferating cells (50 nM) and an increase in neuronal differentiation. Upregulation of PPARγ | Wan Ibrahim et al. (2013) |
| N2a neuronal cells | EC50 PFOS 196–471 μM<br>EC50 PFOA 389–632 μM | Decreased cell viability. Decreased expression levels of mRNA of differentiation markers (NSE, GFAP, and CNP). Reduction in expression of mRNA of cell adhesion markers (E-cadherin and connexion) | Choi et al. (2013) |
| HBMEC brain endothelial cells | 50–160 μM PFOS | Disassembly of endothelial tight junctions, via the phosphatidylinositol-3 kinase/Akt-pathway, increasing the permeability of PFOS | Wang et al. (2011) |

**PFPA* perfluoropropionic acid, *PFBA* perfluorobutyric acid, *PFOA* perfluorooctanoic acid, *PFDA* perfluorododecanoic acid, *PFTA* perfluorotetradecanoic acid, *PFBS* perfluorobutane sulfonic acid, *PFHS* or *PFHxS* perfluorohexanesulfonic acid, *PFOS* perfluorooctanesulfonic acid, *PFOC* 1H-perfluorooctane, *PFOSA* perfluorooctanesulfonamide, *FTOH* fluorotelomer alcohol

concentration, the effect of PFOSA (100 μM) switched and promoted differentiation into the dopaminergic phenotype. No mechanisms for the effects were postulated, but it was suggested that the induction of oxidative stress could be a factor. PFOSA was shown to induce lipid peroxidation and was also the most cytotoxic compound. Wan Ibrahim and co-workers examined the effects of low PFOS concentrations (12.5–100 nM) on differentiation of neural stem cell (Wan Ibrahim et al. 2013). It was shown that 100 nM PFOS reduced cell viability, whereas the lower concentrations increased neuronal differentiation, as shown as a lower numbers of proliferating cells and a higher number of neurite bearing cells. The effect was attributed to PPARγ activation.

One strategy to evaluate neurochemical targets of PFCs has been to exploit their effects on cell viability, which may indicate a neurotoxic potential of the compounds. Reduced cell viability may be a response of a range of cellular processes triggered by the toxic agents, such as oxidative stress, disruption of the calcium homeostasis, and effects on neurotransmission and signaling. These are cellular processes that are important for neuronal development and survival. Cerebellar

granule cells (CGCs) have been a convenient model to evaluate the neurotoxic potential and mechanisms of effect of a range of environmental toxicants, such as polychlorinated biphenyls, brominated flame retardants as well as PFCs. In two studies by Lee and co-workers, CGCs were exposed to PFOS (3 and 30 μM) and PFHxS (≥100 μM). It was observed induction of apoptosis as shown by increased caspase-3 activity and induced DNA-fragmentation (Lee et al. 2012, 2014b). The PFOS induced apoptosis was connected to activation of different subtypes of protein kinase C (PKC-α, PKC-βII and PKC-ε). The effect of PFOS and PFOA on PKC-translocation was also observed by Pinkas et al. (2010) on developmentally exposed chicks as described in the previous section. PKC is involved in a range of processes in the brain such as cognitive functions, learning and memory and several of studies have shown that other environmental toxicants, such as PCBs, dioxins and brominated flame retardants also influence PKC activity (Kodavanti et al. 1994). This may have implications on the risk of being exposed to mixtures of contaminants which have effects on similar targets. A later study by Lee and co-workers showed that the PFOS activation of PKC was followed by activation of the ERK-pathway (Lee et al. 2013), which is one of the mitogen-activated protein kinases (MAPKs). Also PFHxS was shown to induce ERK (Lee et al. 2014b). By inhibiting the ERK-pathway, the PFOS and PFHxS induced apoptosis was blocked. The ERK-pathway has also been shown activated in cerebellar granule cells by tetrabromobisphenol A (TBBPA), which is a brominated flame retardant and possibly by hydroxyl-PCBs, which are metabolites of PCBs (Dreiem et al. 2009), and recently by PFHxS in PC12 cells (Lee et al. 2014a).

Reactive oxygen species (ROS) is a collective term for short lived, highly reactive compounds, often including oxygen radicals and non-radical products of oxygen. The brain is especially vulnerable to oxidative damage, partly because of its high oxygen demand, corresponding to about 20 % of the basal oxygen consumption. The membrane lipids of the nerve cells are rich in polyunsaturated fatty acids which are sensitive to attack from ROS. Nerve cells have often a large surface area making them more exposed to attack from ROS. ROS are typically generated as by-products in cellular metabolism, from toxic agents, inflammations and diseases (Halliwell and Guttenridge 1999). Oxidative stress refers to the consequence of a mismatch between the production of ROS and the ability of the cell to defend itself against them. In the study by (Lee et al. 2012) it was shown that PFOS induced production of reactive oxygen species (ROS), and the detrimental effect of PFOS, both the PKC-activation and apoptosis, was blocked by pretreatment of N-acetylcysteine (NAC). NAC is used as a scavenger of ROS-products. Reistad and co-workers exposed CGCs to four different PFCs to evaluate their potential to affect cell viability and induce ROS-formation (Reistad et al. 2013). The effect of the PFCs varied of which PFOSA and FTOH 8:2 were considerably more cytotoxic than PFOS and PFOA. PFOSA and FTOH 8:2 had EC50-values of 13 and 15 μM respectively, whereas PFOS had an EC50-value of 61 μM. PFOA did not induce cell death at concentrations up to 100 μM. Similar to these studies Slotkin et al. (2008) showed that PFOSA was the most potent in reducing the cell viability of PC12 cells followed by PFOS, and PFOA did not induce loss of cell viability. An interesting

observation in the study by Reistad et al. (2013) was the lack of correlation between cytotoxicity and ROS formation. PFOS and PFOA were equally potent ROS inducers, with EC-50 concentrations of 27 μM and 25 μM respectively, but PFOA did not induce cell death. PFOSA induced ROS with an EC50-concentration of 57 μM whereas FTOH had no effect, but they were equally cytotoxic. For PFOS Lee et al. (2012) found a correlation between ROS formation and apoptosis in cerebellar granule cells, whereas this appeared not to be the case in PFHxS induced apoptosis (Lee et al. 2014b). Slotkin et al. (2008) found a correlation between cell viability and lipid peroxidation in PC12 cells exposed to PFOSA and PFOS. Similar correlation has also been found for PCBs, OH-PCBs and TBBPA (Dreiem et al. 2009; Mariussen et al. 2002; Reistad et al. 2007). Cellular damage should probably ultimately lead to increased ROS formation. A possible explanation for the lack of correlation between cell death and ROS formation may be the selectivity of the method used to identify ROS and the time after exposure when the endpoints were measured. In addition, the PFCs also differ with respect to their physical-chemical properties such as water solubility, which probably will reflect their ability to reach targets in cells.

A crucial factor for normal functioning cells is maintenance of the intracellular $Ca^{2+}$-homeostasis. $Ca^{2+}$ is an important second messenger in the cells. However, a sustained increase of the intracellular level of $Ca^{2+}$ may induce formation of reactive oxygen species (ROS) followed by cellular injury. Calcium is also crucial for neurotransmitter release. Upon stimulation of a neuron the transmitter molecules are released from the nerve terminal into the synapse by a $Ca^{2+}$ dependent process. In a study by Harada and co-workers it was shown that 30 μM of PFOS had a modulating effect on ion currents in rat cerebellar Purkinje cells leading towards a hyperpolarized state (Harada et al. 2006). The effect involved voltage gated $Ca^{2+}$, $Na^{+}$ and $K^{+}$ channels. In a later study, Liao and co-workers also showed that PFOS increases $K^{+}$ currents at doses over 10 μM towards a hyperpolarized direction in hippocampal neurons (Liao et al. 2009b) similar as observed by Harada et al. (2006) on cerebellar Purkinje cells. Effects of PFOS on nervous ion currents were also found by Liao, who showed increased $Ca^{2+}$ currents recorded in the CA1 region of hippocampal slices and in cultured hippocampal neurons (Liao et al. 2008). It was also shown that PFOS inhibits neurite growth and synaptogenesis in cultured neurons. The effects could be blocked by the L-type voltage gated $Ca^{2+}$ channel blocker nifedipine. These findings indicate that PFOS may facilitate influx of calcium leading to an increased susceptibility of calcium related effects, which ultimately may lead to reduced cell viability or impairment of cellular growth. A structure activity study showed that the effect on the calcium currents increased with the carbon chain length of the tail moiety of the PFCs, and that the effects of the carboxylated compounds were less pronounced than the sulfonates (Liao et al. 2009a). The effects of PFOS and PFOA on the $Ca^{2+}$-homeostasis in hippocampal neurons have been elucidated in more detail by Liu and co-workers, showing that the PFCs affect several calcium dependent processes (Liu et al. 2011). The sulfonated PFOS (30 μM) was more potent than the carboxylated PFOA (100 μM) to induce elevated intracellular concentrations of $Ca^{2+}$. The increase intracellular $Ca^{2+}$ appeared to be of both

extracellular origin involving voltage gated $Ca^{2+}$ channels, as shown by Liao et al. (2008), and intracellular origin such as activation of ryanodine receptors and inositol phosphate-3 ($IP_3$)-receptors. The disturbance of the $Ca^{2+}$-homeostasis was followed by an increase in oxidative stress, as measured with DCF, and an increased expression of calcineurin, which is a $Ca^{2+}$ activated protein phosphatase. Another interesting finding by Liao et al. (2009a) was that a low concentration of PFOS (1 μM) increased inward glutamate currents whereas higher concentrations of PFOS (10 and 100 μM) dose-dependently reduced the inward glutamate currents. Glutamate is the quantitatively the most important excitatoric neurotransmitter in the brain. Glutamate is an excitotoxin so a prolonged stimulation of glutamatergic receptors in the brain may cause a sustained elevation of the intracellular $Ca^{2+}$ level in the neuron, which can mobilize $Ca^{2+}$- dependent processes, leading to inflammation, ROS production, and ultimately cause cell death (Fonnum 1998). Hippocampus, which is mainly glutamatergic, is one of the major brain areas concerned with the acquirement of memory, and only minor damage to this area is sufficient to produce memory disturbances (Bliss and Collingridge 1993; Fonnum et al. 1995; Milner 1972; Victor et al. 1961).

Cytotoxicity and oxidative stress may also be induced as a consequence of inflammatory responses, such as immune responses. In prenatally PFOS exposed rats it was observed an increase inflammatory response in the juvenile rat brains as shown by increased levels of the astrocyte markers fibrillary acidic protein and S100 $Ca^{2+}$-binding protein B in hippocampus and cortex (Zeng et al. 2011b). It was also found an increase in the mRNA levels of proinflammatory cytokines, such as interleukin 1β, tumour necrosis factor α, AP-1, NF-kappa-B and CREB. Changes in the mRNA levels as a response on an exposure may not necessarily imply changes or harmful effects on a higher protein or cellular level. There is, however, previously been shown that PFOS and PFOA enhance inflammatory responses of macrophages to lipopolysaccharide in mice, indicating that PFCs may be implicated in stress responses related to the immune system (Qazi et al. 2009).

## 9.5 Summary and Conclusion

There is no doubt that PFCs can induce developmental neurotoxic effects, since research in humans, animals and cell cultures all point in the same direction. Functional effects in animals, such as impaired behavior and cognitive functions, have also been investigated to elucidate the mechanisms behind the effects. Disturbances in the processes of synaptogenesis, dendritic outgrowth and ontogeny of neurotransmitter systems all look as plausible mechanisms and apoptosis, specific proteins, signaling molecules, calcium homeostasis as well as oxidative stress can be the molecular reasons behind the disturbances of these processes. It is important to remember, though, that the real world is much more complicated than exposure to one single compound at the time. Therefore, effects of PFCs in combination and/or in combination with other environmental pollutants need to be investigated.

At the moment it is hard to find good examples of neurotoxic effects after combination exposure to PFCs or PFCs and other types of chemicals, but one study shows that combined exposure to low doses of PFOA and the polybrominated diphenyl ether PBDE 209, during the neonatal period, can interact and exacerbate adult functional neurobehavioral effects, compared to the single compounds alone (Johansson 2009).

## References

Antignac JP, Veyrand B, Kadar H, Marchand P, Oleko A, Le Bizec B, Vandentorren S (2013) Occurrence of perfluorinated alkylated substances in breast milk of French women and relation with socio-demographical and clinical parameters: results of the ELFE pilot study. Chemosphere 91:802–808

Apelberg BJ, Goldman LR, Calafat AM, Herbstman JB, Kuklenyik Z, Heidler J, Needham LL, Halden RU, Witter FR (2007) Determinants of fetal exposure to polyfluoroalkyl compounds in Baltimore, Maryland. Environ Sci Technol 41:3891–3897

Barkley RA (1998) Attention-deficit hyperactivity disorder. Sci Am 279:66–71

Bliss TV, Collingridge GL (1993) A synaptic model of memory: long-term potentiation in the hippocampus. Nature 361:31–39

Brown RC, Lockwood AH, Sonawane BR (2005) Neurodegenerative diseases: an overview of environmental risk factors. Environ Health Perspect 113:1250–1256

Butenhoff JL, Ehresman DJ, Chang SC, Parker GA, Stump DG (2009a) Gestational and lactational exposure to potassium perfluorooctanesulfonate (K+PFOS) in rats: developmental neurotoxicity. Reprod Toxicol 27:319–330

Butenhoff JL, Chang SC, Ehresman DJ, York RG (2009b) Evaluation of potential reproductive and developmental toxicity of potassium perfluorohexanesulfonate in Sprague Dawley rats. Reprod Toxicol 27:331–341

Butenhoff JL, Bjork JA, Chang SC, Ehresman DJ, Parker GA, Das K, Lau C, Lieder PH, van Otterdijk FM, Wallace KB (2012) Toxicological evaluation of ammonium perfluorobutyrate in rats: twenty-eight-day and ninety-day oral gavage studies. Reprod Toxicol 33:513–530

Cassone CG, Vongphachan V, Chiu S, Williams KL, Letcher RJ, Pelletier E, Crump D, Kennedy SW (2012) In ovo effects of perfluorohexane sulfonate and perfluorohexanoate on pipping success, development, mRNA expression, and thyroid hormone levels in chicken embryos. Toxicol Sci 127:216–224

Chang SC, Ehresman DJ, Bjork JA, Wallace KB, Parker GA, Stump DG, Butenhoff JL (2009) Gestational and lactational exposure to potassium perfluorooctanesulfonate (K+PFOS) in rats: toxicokinetics, thyroid hormone status, and related gene expression. Reprod Toxicol 27:387–399

Chen J, Das SR, La Du J, Corvi MM, Bai C, Chen Y, Liu X, Zhu G, Tanguay RL, Dong Q, Huang C (2013) Chronic PFOS exposures induce life stage-specific behavioral deficits in adult zebrafish and produce malformation and behavioral deficits in F1 offspring. Environ Toxicol Chem 32:201–206

Choi SK, Kim JH, Park JK, Lee KM, Kim E, Jeon WB (2013) Cytotoxicity and inhibition of intercellular interaction in N2a neurospheroids by perfluorooctanoic acid and perfluorooctanesulfonic acid. Food Chem Toxicol 60:520–529

Dreiem A, Rykken S, Lehmler HJ, Robertson LW, Fonnum F (2009) Hydroxylated polychlorinated biphenyls increase reactive oxygen species formation and induce cell death in cultured cerebellar granule cells. Toxicol Appl Pharmacol 240:306–313

Ek CJ, Dziegielewska KM, Habgood MD, Saunders NR (2012) Barriers in the developing brain and neurotoxicology. Neurotoxicology 33:586–604

Eriksson P (1998) Perinatal developmental neurotoxicity of PCBs. Swedish Environmental Protection Agency, Stockholm, p 56
Eriksson P, Jakobsson E, Fredriksson A (2001) Brominated flame retardants: a novel class of developmental neurotoxicants in our environment? Environ Health Perspect 109:903–908
Fei C, McLaughlin JK, Lipworth L, Olsen J (2008) Prenatal exposure to perfluorooctanoate (PFOA) and perfluorooctanesulfonate (PFOS) and maternally reported developmental milestones in infancy. Environ Health Perspect 116:1391–1395
Fonnum F (1998) Excitotoxicity in the brain. Arch Toxicol Suppl 20:387–395
Fonnum F, Myhrer T, Paulsen RE, Wangen K, Oksengard AR (1995) Role of glutamate and glutamate receptors in memory function and Alzheimer's disease. Ann N Y Acad Sci 757:475–486
Fuentes S, Colomina MT, Vicens P, Domingo JL (2007a) Influence of maternal restraint stress on the long-lasting effects induced by prenatal exposure to perfluorooctane sulfonate (PFOS) in mice. Toxicol Lett 171:162–170
Fuentes S, Colomina MT, Vicens P, Franco-Pons N, Domingo JL (2007b) Concurrent exposure to perfluorooctane sulfonate and restraint stress during pregnancy in mice: effects on postnatal development and behavior of the offspring. Toxicol Sci 98:589–598
Fuentes S, Vicens P, Colomina MT, Domingo JL (2007c) Behavioral effects in adult mice exposed to perfluorooctane sulfonate (PFOS). Toxicology 242:123–129
Grandjean P, Landrigan PJ (2006) Developmental neurotoxicity of industrial chemicals. Lancet 368:2167–2178
Grandjean P, Weihe P, Debes F, Choi AL, Budtz-Jorgensen E (2014) Neurotoxicity from prenatal and postnatal exposure to methylmercury. Neurotoxicol Teratol 43C:39–44
Halliwell B, Guttenridge JMC (1999) Free radicals in biology and medicine. Oxford University Press, New York
Harada KH, Ishii TM, Takatsuka K, Koizumi A, Ohmori H (2006) Effects of perfluorooctane sulfonate on action potentials and currents in cultured rat cerebellar Purkinje cells. Biochem Biophys Res Commun 351:240–245
Hardell L, Lindstrom G, Van Bavel B (2002) Is DDT exposure during fetal period and breastfeeding associated with neurological impairment? Environ Res 88:141–144
Hoffman K, Webster TF, Weisskopf MG, Weinberg J, Vieira VM (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
Inoue K, Okada F, Ito R, Kato S, Sasaki S, Nakajima S, Uno A, Saijo Y, Sata F, Yoshimura Y, Kishi R, Nakazawa H (2004) Perfluorooctane sulfonate (PFOS) and related perfluorinated compounds in human maternal and cord blood samples: assessment of PFOS exposure in a susceptible population during pregnancy. Environ Health Perspect 112:1204–1207
Johansson N (2009) Neonatal exposure to highly brominated diphenyl ethers and perfluorinated compounds. Developmental dependent toxicity and interaction. Acta Univ. Ups., Comprehensive summaries of Uppsala dissertations from the Faculty of Science and Technology., Environmental Toxicology, Uppsala University, Uppsala, p 67
Johansson N, Fredriksson A, Eriksson P (2008) Neonatal exposure to perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) causes neurobehavioural defects in adult mice. Neurotoxicology 29:160–169
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
Kawamoto K, Sato I, Tsuda S, Yoshida M, Yaegashi K, Saito N, Liu W, Jin Y (2011) Ultrasonic-induced tonic convulsion in rats after subchronic exposure to perfluorooctane sulfonate (PFOS). J Toxicol Sci 36:55–62
Kodavanti PR, Shafer TJ, Ward TR, Mundy WR, Freudenrich T, Harry GJ, Tilson HA (1994) Differential effects of polychlorinated biphenyl congeners on phosphoinositide hydrolysis and protein kinase C translocation in rat cerebellar granule cells. Brain Res 662:75–82
Kodavanti PR, Derr-Yellin EC, Mundy WR, Shafer TJ, Herr DW, Barone S, Choksi NY, MacPhail RC, Tilson HA (1998) Repeated exposure of adult rats to Aroclor 1254 causes brain

region-specific changes in intracellular Ca2+ buffering and protein kinase C activity in the absence of changes in tyrosine hydroxylase. Toxicol Appl Pharmacol 153:186–198
Lai BC, Marion SA, Teschke K, Tsui JK (2002) Occupational and environmental risk factors for Parkinson's disease. Parkinsonism Relat Disord 8:297–309
Lau C, Thibodeaux JR, Hanson RG, Rogers JM, Grey BE, Stanton ME, Butenhoff JL, Stevenson LA (2003) Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse. II: postnatal evaluation. Toxicol Sci 74:382–392
Lee I, Viberg H (2013) A single neonatal exposure to perfluorohexane sulfonate (PFHxS) affects the levels of important neuroproteins in the developing mouse brain. Neurotoxicology 37:190–196
Lee HG, Lee YJ, Yang JH (2012) Perfluorooctane sulfonate induces apoptosis of cerebellar granule cells via a ROS-dependent protein kinase C signaling pathway. Neurotoxicology 33:314–320
Lee YJ, Lee HG, Yang JH (2013) Perfluorooctane sulfonate-induced apoptosis of cerebellar granule cells is mediated by ERK 1/2 pathway. Chemosphere 90:1597–1602
Lee YJ, Choi SY, Yang JH (2014a) NMDA receptor-mediated ERK 1/2 pathway is involved in PFHxS-induced apoptosis of PC12 cells. Sci Total Environ 491:227–2234
Lee YJ, Choi SY, Yang JH (2014b) PFHxS induces apoptosis of neuronal cells via ERK1/2-mediated pathway. Chemosphere 94:121–127
Levitt D, Liss A (1986) Toxicity of perfluorinated fatty acids for human and murine B cell lines. Toxicol Appl Pharmacol 86:1–11
Liao CY, Li XY, Wu B, Duan S, Jiang GB (2008) Acute enhancement of synaptic transmission and chronic inhibition of synaptogenesis induced by perfluorooctane sulfonate through mediation of voltage-dependent calcium channel. Environ Sci Technol 42:5335–5341
Liao C, Wang T, Cui L, Zhou Q, Duan S, Jiang G (2009a) Changes in synaptic transmission, calcium current, and neurite growth by perfluorinated compounds are dependent on the chain length and functional group. Environ Sci Technol 43:2099–2104
Liao CY, Cui L, Zhou QF, Duan SM, Jiang GB (2009b) Effects of perfluorooctane sulfonate on ion channels and glutamate-activated current in cultured rat hippocampal neurons. Environ Toxicol Pharmacol 27:338–344
Liu L, Liu W, Song J, Yu H, Jin Y, Oami K, Sato I, Saito N, Tsuda S (2009) A comparative study on oxidative damage and distributions of perfluorooctane sulfonate (PFOS) in mice at different postnatal developmental stages. J Toxicol Sci 34:245–254
Liu X, Liu W, Jin Y, Yu W, Wang F, Liu L (2010a) Effect of gestational and lactational exposure to perfluorooctanesulfonate on calcium-dependent signaling molecules gene expression in rats' hippocampus. Arch Toxicol 84:71–79.
Liu X, Liu W, Jin Y, Yu W, Liu L, Yu H (2010b) Effects of subchronic perfluorooctane sulfonate exposure of rats on calcium-dependent signaling molecules in the brain tissue. Arch Toxicol 84:471–479
Liu X, Jin Y, Liu W, Wang F, Hao S (2011) Possible mechanism of perfluorooctane sulfonate and perfluorooctanoate on the release of calcium ion from calcium stores in primary cultures of rat hippocampal neurons. Toxicol In Vitro 25:1294–1301
Luebker DJ, Case MT, York RG, Moore JA, Hansen KJ, Butenhoff JL (2005) Two-generation reproduction and cross-foster studies of perfluorooctanesulfonate (PFOS) in rats. Toxicology 215:126–148
Mariussen E (2012) Neurotoxic effects of perfluoroalkylated compounds: mechanisms of action and environmental relevance. Arch Toxicol 86:1349–1367
Mariussen E, Fonnum F (2006) Neurochemical targets and behavioral effects of organohalogen compounds: an update. Crit Rev Toxicol 36:253–289

Mariussen E, Myhre O, Reistad T, Fonnum F (2002) The polychlorinated biphenyl mixture aroclor 1254 induces death of rat cerebellar granule cells: the involvement of the N-methyl-D-aspartate receptor and reactive oxygen species. Toxicol Appl Pharmacol 179:137–144

Midasch O, Drexler H, Hart N, Beckmann MW, Angerer J (2007) Transplacental exposure of neonates to perfluorooctanesulfonate and perfluorooctanoate: a pilot study. Int Arch Occup Environ Health 80:643–648

Milner B (1972) Disorders of learning and memory after temporal lobe lesions in man. Clin Neurosurg 19:421–446

Mondal D, Weldon RH, Armstrong BG, Gibson LJ, Lopez-Espinosa MJ, Shin HM, Fletcher T (2014) Breastfeeding: a potential excretion route for mothers and implications for infant exposure to perfluoroalkyl acids. Environ Health Perspect 122:187–192

Monroy R, Morrison K, Teo K, Atkinson S, Kubwabo C, Stewart B, Foster WG (2008) Serum levels of perfluoroalkyl compounds in human maternal and umbilical cord blood samples. Environ Res 108:56–62

Pinkas A, Slotkin TA, Brick-Turin Y, Van der Zee EA, Yanai J (2010) Neurobehavioral teratogenicity of perfluorinated alkyls in an avian model. Neurotoxicol Teratol 32:182–186

Qazi MR, Bogdanska J, Butenhoff JL, Nelson BD, DePierre JW, Abedi-Valugerdi M (2009) High-dose, short-term exposure of mice to perfluorooctanesulfonate (PFOS) or perfluorooctanoate (PFOA) affects the number of circulating neutrophils differently, but enhances the inflammatory responses of macrophages to lipopolysaccharide (LPS) in a similar fashion. Toxicology 262:207–214

Reistad T, Mariussen E, Ring A, Fonnum F (2007) In vitro toxicity of tetrabromobisphenol-A on cerebellar granule cells: cell death, free radical formation, calcium influx and extracellular glutamate. Toxicol Sci 96:268–278

Reistad T, Fonnum F, Mariussen E (2013) Perfluoroalkylated compounds induce cell death and formation of reactive oxygen species in cultured cerebellar granule cells. Toxicol Lett 218:56–60

Ribes D, Fuentes S, Torrente M, Colomina MT, Domingo JL (2010) Combined effects of perfluorooctane sulfonate (PFOS) and maternal restraint stress on hypothalamus adrenal axis (HPA) function in the offspring of mice. Toxicol Appl Pharmacol 243:13–18

Rice DC (2000) Parallels between attention deficit hyperactivity disorder and behavioral deficits produced by neurotoxic exposure in monkeys. Environ Health Perspect 108(Suppl 3):405–408

Sato I, Kawamoto K, Nishikawa Y, Tsuda S, Yoshida M, Yaegashi K, Saito N, Liu W, Jin Y (2009) Neurotoxicity of perfluorooctane sulfonate (PFOS) in rats and mice after single oral exposure. J Toxicol Sci 34:569–574

Schettler T (2001) Toxic threats to neurologic development of children. Environ Health Perspect 109(Suppl 6):813–816

Schmitt U, Tanimoto N, Seeliger M, Schaeffel F, Leube RE (2009) Detection of behavioral alterations and learning deficits in mice lacking synaptophysin. Neuroscience 162:234–243

Shi X, Liu C, Wu G, Zhou B (2009) Waterborne exposure to PFOS causes disruption of the hypothalamus-pituitary-thyroid axis in zebrafish larvae. Chemosphere 77:1010–1018

Slotkin TA, MacKillop EA, Melnick RL, Thayer KA, Seidler FJ (2008) Developmental neurotoxicity of perfluorinated chemicals modeled in vitro. Environ Health Perspect 116:716–722

Spulber S, Kilian P, Wan Ibrahim WN, Onishchenko N, Ulhaq M, Norrgren L, Negri S, Di Tuccio M, Ceccatelli S (2014) PFOS induces behavioral alterations, including spontaneous hyperactivity that is corrected by dexamfetamine in Zebrafish larvae. PLoS One 9:e94227

Stein CR, Savitz DA (2011) Serum perfluorinated compound concentration and attention deficit/hyperactivity disorder in children 5–18 years of age. Environ Health Perspect 119:1466–1471

Stein CR, Savitz DA, Dougan M (2009) Serum levels of perfluorooctanoic acid and perfluorooctane sulfonate and pregnancy outcome. Am J Epidemiol 170:837–846

Sundstrom M, Ehresman DJ, Bignert A, Butenhoff JL, Olsen GW, Chang SC, Bergman A (2011) A temporal trend study (1972–2008) of perfluorooctanesulfonate, perfluorohexanesulfonate, and perfluorooctanoate in pooled human milk samples from Stockholm, Sweden. Environ Int 37:178–183

Tao L, Kannan K, Wong CM, Arcaro KF, Butenhoff JL (2008) Perfluorinated compounds in human milk from Massachusetts, U.S.A. Environ Sci Technol 42:3096–3101

Trudel D, Horowitz L, Wormuth M, Scheringer M, Cousins IT, Hungerbuhler K (2008) Estimating consumer exposure to PFOS and PFOA. Risk Anal 28:251–269

Ulhaq M, Orn S, Carlsson G, Morrison DA, Norrgren L (2013) Locomotor behavior in zebrafish (Danio rerio) larvae exposed to perfluoroalkyl acids. Aquat Toxicol 144–145:332–340

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

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

Viberg H, Fredriksson A, Jakobsson E, Orn U, Eriksson P (2003b) 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, 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, Lee I, Eriksson P (2013) Adult dose-dependent behavioral and cognitive disturbances after a single neonatal PFHxS dose. Toxicology 304:185–191

Victor M, Angevine JB Jr, Mancall EL, Fisher CM (1961) Memory loss with lesions of hippocampal formation. Report of a case with some remarks on the anatomical basis of memory. Arch Neurol 5:244–263

Wan Ibrahim WN, Tofighi R, Onishchenko N, Rebellato P, Bose R, Uhlen P, Ceccatelli S (2013) Perfluorooctane sulfonate induces neuronal and oligodendrocytic differentiation in neural stem cells and alters the expression of PPARgamma in vitro and in vivo. Toxicol Appl Pharmacol 269:51–60

Wang F, Liu W, Jin Y, Dai J, Yu W, Liu X, Liu L (2010) Transcriptional effects of prenatal and neonatal exposure to PFOS in developing rat brain. Environ Sci Technol 44:1847–1853

Wang X, Li B, Zhao WD, Liu YJ, Shang DS, Fang WG, Chen YH (2011) Perfluorooctane sulfonate triggers tight junction "opening" in brain endothelial cells via phosphatidylinositol 3-kinase. Biochem Biophys Res Commun 410:258–263

Yang JH, Derr-Yellin EC, Kodavanti PR (2003) Alterations in brain protein kinase C isoforms following developmental exposure to a polychlorinated biphenyl mixture. Brain Res Mol Brain Res 111:123–135

Zeng HC, Li YY, Zhang L, Wang YJ, Chen J, Xia W, Lin Y, Wei J, Lv ZQ, Li M, Xu SQ (2011a) Prenatal exposure to perfluorooctanesulfonate in rat resulted in long-lasting changes of expression of synapsins and synaptophysin. Synapse 65:225–233

Zeng HC, Zhang L, Li YY, Wang YJ, Xia W, Lin Y, Wei J, Xu SQ (2011b) Inflammation-like glial response in rat brain induced by prenatal PFOS exposure. Neurotoxicology 32:130–139

Zhang L, Li YY, Chen T, Xia W, Zhou Y, Wan YJ, Lv ZQ, Li GQ, Xu SQ (2011) Abnormal development of motor neurons in perfluorooctane sulphonate exposed zebrafish embryos. Ecotoxicology 20:643–652