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Absorption, distribution, and toxicity of per- and polyfluoroalkyl substances (PFAS) in the brain: a review†

Yuexin Cao [a] and Carla Ng *[ab]

Per- and polyfluoroalkyl substances (PFAS) are a class of synthetic chemicals colloquially known as "forever chemicals" because of their high persistence. PFAS have been detected in the blood, liver, kidney, heart, muscle and brain of various species. Although brain is not a dominant tissue for PFAS accumulation compared to blood and liver, adverse effects of PFAS on brain functions have been identified. Here, we review studies related to the absorption, accumulation, distribution and toxicity of PFAS in the brain. We summarize evidence on two potential mechanisms of PFAS entering the brain: initiating blood–brain barrier (BBB) disassembly through disrupting tight junctions and relying on transporters located at the BBB. PFAS with diverse structures and properties enter and accumulate in the brain with varying efficiencies. Compared to long-chain PFAS, short-chain PFAS may not cross cerebral barriers effectively. According to biomonitoring studies and PFAS exposure experiments, PFAS can accumulate in the brain of humans and wildlife species. With respect to the distribution of PFAS in specific brain regions, the brain stem, hippocampus, hypothalamus, pons/medulla and thalamus are dominant for PFAS accumulation. The accumulation and distribution of PFAS in the brain may lead to toxic effects in the central nervous system (CNS), including PFAS-induced behavioral and cognitive disorders. The specific mechanisms underlying such PFAS-induced neurotoxicity remain to be explored, but two major potential mechanisms based on current understanding are PFAS effects on calcium homeostasis and neurotransmitter alterations in neurons. Based on the information available about PFAS uptake, accumulation, distribution and impacts on the brain, PFAS have the potential to enter and accumulate in the brain at varying levels. The balance of existing studies shows there is some indication of risk in animals, while the human evidence is mixed and warrants further scrutiny.

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Environmental significance

Per- and polyfluoroalkyl substances (PFAS) are a group of persistent man-made chemicals used in products to impart hydrophobic and lipophobic properties. The wide application of these compounds in numerous products has led to ubiquitous exposure. Therefore, they have been detected in multiple tissues, including the brain, of various species. The accumulation and distribution of PFAS in the brain highlight their potential to cause toxic effects. Our review integrates current evidence from multiple perspectives (epidemiological, *in vivo*, and *in vitro*) on PFAS accumulation and their potential toxic effects on the brain. More data are needed to specify the mechanisms by which different PFAS enter the brain, and to more concretely link PFAS accumulation in the brain to neurotoxic mechanisms.

1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are synthetic chemicals with useful properties such as water and oil repellence and extreme temperature resistance.[1] These properties led to wide industrial and commercial applications, including in semiconductors, firefighting foams, non-stick cookware and food packaging, resulting in exposure of humans and wildlife.[2–5] PFAS are very persistent in the environment, and once in the body, some PFAS accumulate in tissues.[5,6] Studies have detected PFAS in the blood, liver, kidney, heart, muscle and brain of various species.[7–10] Based on previous studies, PFAS accumulate in the blood due to binding between PFAS and serum albumin.[6,11–13] The brain ensures its normal functions through uptake of oxygen, nutrients and other required substances from the blood.[14] Substance exchange in the cerebral circulation creates the opportunity for PFAS to enter the brain. However,

[a]Department of Civil & Environmental Engineering, University of Pittsburgh, Pittsburgh, PA 15261, USA. E-mail: carla.ng@pitt.edu; Fax: +1-412-624-0135; Tel: +1-412-383-4075

[b]Department of Environmental and Occupational Health, University of Pittsburgh, Pittsburgh, PA 15261, USA

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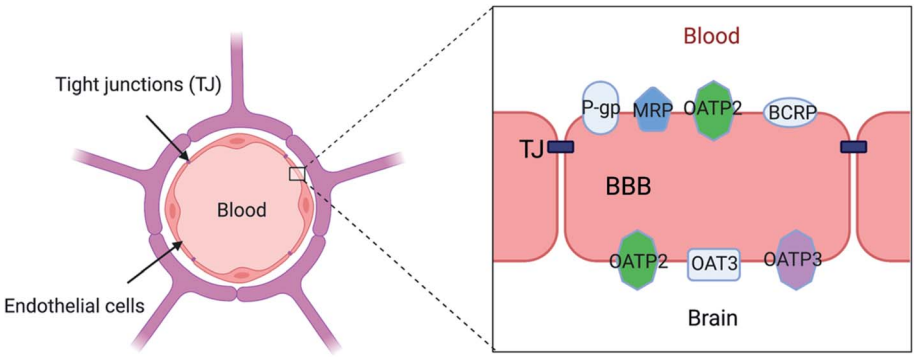

Fig. 1 The structure of the blood–brain barrier (BBB), the biochemical boundary of endothelial cells between the bloodstream and brain. The link between endothelial cells (dark blue rectangles in inset) are tight junctions (TJ), responsible for limiting paracellular leakage during substance transport. Various transporters located at the surface of the BBB exchange chemicals across the cell membrane (abbreviations refer to different transporters as discussed in the text).

xenobiotics cannot usually move freely into the brain because of cerebral barriers, such as the blood–brain barrier (BBB) and blood-cerebrospinal fluid barrier (BCSFB), that protect the central nervous system (CNS, composed of the brain and spinal cord) by allowing needed chemicals in but not toxins and pathogens.[15,16] This barrier function has been shown to also apply to some PFAS.[17]

The BBB is the biochemical boundary of endothelial cells that mediates the exchange of substances between the bloodstream and brain.[18] The link between endothelial cells, known as tight junctions, are responsible for limiting paracellular leakage during substance transport.[19] Various transporters located at the surface of endothelial cells exchange chemicals across the cell membrane, such as P-glycoprotein (P-gp), breast cancer resistance protein (BCRP/Bcrp), multidrug resistance proteins (MRPs/Mrps), organic anion transporting polypeptides (OATPs/Oatps), organic anion transporters (OATs/Oats) and organic cation transporters (OCTs/Octs) (Fig. 1). Chemicals bind to transporters and achieve transmembrane transport through the transporters' conformational changes.[20] Based on previous studies, PFAS could enter the brain by disrupting tight junctions to permeate into the brain[21–23] or binding to transporters to cross the plasma membrane.[24–26] However, studies related to the interaction of PFAS and transporters mainly focus on renal transporters,[27] while the transport of PFAS through similar transporters at the BBB has yet to be verified. In addition, the specific mechanisms by which different PFAS enter the brain is still unclear, but a number or studies have reported the presence of PFAS in the brain.[7,8,10,17,28–37]

Biomonitoring studies have detected a broad array of PFAS, including perfluoroalkyl carboxylic acids (PFCAs), perfluoroalkane sulfonic acids (PFSAs) and PFAS precursors (compounds that have the potential to be degraded to terminal PFAS, including sulfonamides and fluorotelomer substances[38]) in the brain and cerebrospinal fluid (CSF) of humans, and in the brain of wildlife species.[8,17,28,37] The CSF is the fluid surrounding the brain and spinal cord, and PFAS content in this fluid has been used in a small number of studies as a surrogate for PFAS

Yuexin Cao is a graduate student in the Department of Civil and Environmental Engineering at the University of Pittsburgh. She works with Dr Carla Ng and her current focus is on using computational methods and zebrafish models to evaluate the potential hazards of per- and polyfluoroalkyl substances (PFAS) used in the photolithography industry.

Dr Carla Ng is an Assistant Professor in the Department of Civil and Environmental Engineering at the University of Pittsburgh, with a secondary appointment in Environmental and Occupational Health. Her group's research focuses on the development of models for the fate of legacy and emerging chemicals in organisms and ecosystems. Current areas of active research include development of toxicokinetic models for PFAS in organisms and simulating protein–PFAS interactions to understand PFAS fate and toxicity. She is particularly interested in developing mechanistic models to understand and predict the interactions between emerging chemicals, human activity, and ecological systems.

content in the brain interstitial fluid.[17,37] In addition, various wildlife biomonitoring and animal exposure studies have also detected the accumulation of PFAS, most frequently PFOA and PFOS, in the brain of various species.[7,10,29–36,39] In terms of the PFAS distribution data related to specific brain areas, the brain stem, hippocampus, hypothalamus, pons/medulla and thalamus are dominant for PFAS accumulation.[29,30] These brain-region-specific studies are critical for connecting the dominant areas in the brain for PFAS accumulation to the toxic effects of PFAS on the brain, but the available studies are limited. The absorption and accumulation of PFAS in the brain highlight the potential for these substances to cause toxic effects. Studies have reported associations between PFAS exposure and behavioral[40–46] and cognitive[47–52] disorders in both animals and humans, but conflicting results of the direction of the association are present in these studies, and the mechanisms underlying PFAS-induced neurotoxicity remain poorly understood. Various *in vitro* studies proposed two main potential mechanisms, including PFAS-induced intracellular calcium alteration in neurons[53–63] and the impacts of PFAS on neurotransmitters.[64–73] However, most of these *in vitro* studies focus on PFOA and PFOS. The neurotoxicity of other perfluoroalkyl acids (PFAAs) and emerging PFAS still needs to be evaluated.

In this critical review, we surveyed studies related to the absorption, accumulation, distribution and toxicity of a broad array of PFAS in the brain (Table 1 lists those that are the focus of this review; a more exhaustive list for all PFAS analyzed in the reviewed papers can be found in Table S1 in the ESI†). Based on current understanding, we summarized two potential mechanisms for PFAS to enter the brain, including (1) initiating BBB disassembly through the disruption of tight junctions and (2) relying on membrane transporters. To understand the accumulation and distribution of PFAS in the brain of various species, we surveyed studies of PFAS distributions in collected brain samples from biomonitoring studies and controlled exposure experiments. The brain region-specific PFAS distribution may provide links to observed adverse effects. Finally, we reviewed papers discussing the potential neurotoxicity of PFAS, in terms of effects on calcium homeostasis and neurotransmitters, as well as neurobehavioral and cognitive disorders as outcomes of PFAS exposure.

2. Review scope

In this critical review, we used the Web of Science to search for studies using the following search terms: PFAS, brain, blood–brain barrier (or BBB), transporter, accumulation, distribution, exposure and neurotoxicity. This resulted in 65 papers published between 2005 and 2020, which we categorized into three major subcategories corresponding to the review sections to follow: (1) absorption of PFAS in the brain, (2) accumulation and distribution of PFAS in the brain, and (3) potential neurotoxicity of PFAS.

In the absorption section, we identified and reviewed 11 papers. In the accumulation and distribution section, we identified and reviewed 25 papers, classified into PFAS in collected brain samples (15 papers, see Table 2) and controlled PFAS exposure experiments (10 papers, see Table 3). We calculated PFAS brain-to-blood (or brain-to-serum) ratios where paired brain and blood (or serum) data were available and/or PFAS brain-to-liver ratios if paired brain and liver (but not blood) concentrations were available. These ratios are useful to understand PFAS uptake and/or retention rates in the brain relative to overall exposure, since accumulation for many PFAS is greatest in blood and liver. Several of the studies reviewed did not report their raw data or substance-specific PFAS

Table 1 The list of PFAS discussed in this review[a]

Class	Name	Acronym	Carbon chain length
PFCAs	Perfluorobutanoic acid	PFBA	4
	Perfluoropentanoic acid	PFPeA	5
	Perfluorohexanoic acid	PFHxA	6
	Perfluoroheptanoic acid	PFHpA	7
	Perfluorooctanoic acid	PFOA	8
	Perfluorononanoic acid	PFNA	9
	Perfluorodecanoic acid	PFDA	10
	Perfluoroundecanoic acid	PFUnDA (PFUDA)	11
	Perfluorododecanoic acid	PFDoDA (PFDoA)	12
	Perfluorotridecanoic acid	PFTrDA (PFTrA)	13
	Perfluorotetradecanoic acid	PFTeDA (PFTeA, PFTA)	14
	Perfluoropentadecanoic acid	PFPeDA	15
PFSAs	Perfluorobutane sulfonic acid	PFBS	4
	Perfluorohexane sulfonic acid	PFHxS	6
	Perfluorooctane sulfonic acid	PFOS	8
	Perfluorodecane sulfonic acid	PFDS	10
Ethers	6:2 chlorinated polyfluoroalkyl ether sulfonate	6:2 Cl-PFESA (F-53B)	8
	8:2 chlorinated polyfluoroalkyl ether sulfonate	8:2 Cl-PFESA	10
Precursors	Perfluorooctane sulfonamide	PFOSA	8
	8:2 polyfluoroalkyl phosphate diester	8:2 diPAP	20

[a] Note: acronyms in parenthesis are alternative versions used in some reviewed papers.

Table 2 Studies of PFAS accumulation in collected samples[a]

Organisms	Species	*n*	Year	Sample collection area	Reference
Human (autopsy)	—	7	—	Northern Italy	Maestri *et al.*, 2006 (ref. 28)
Human (autopsy)	—	20	2008	Spain, Tarragona county	Pérez *et al.*, 2013 (ref. 8)
Polar bear	*Ursus maritimus*	19	2006	East Greenland	Greaves *et al.*, 2012 (ref. 39)
Polar bear	*Ursus maritimus*	19	2006	East Greenland	Greaves *et al.*, 2013 (ref. 29)
Polar bear	*Ursus maritimus*	9	2011 & 2012	East Greenland	Pedersen *et al.*, 2015 (ref. 30)
Harbor seals	*Phoca vitulina*	4	2007	German Bight	Ahrens *et al.*, 2009 (ref. 31)
Pilot whale	*Globicephala melas*	7	2016	North Atlantic	Dassuncao *et al.*, 2019 (ref. 7)
Glaucous gulls	*Larus hyperboreus*	7	2004	Svalbard & Bear Island, Norwegian Arctic	Verreault *et al.*, 2005 (ref. 10)
Pelicans	*Pelecanus occidentalis*	5	2004	Cartagena Bay	Olivero-Verbel *et al.*, 2006 (ref. 32)
Red-throated divers	—	4	2005	Usedom, Mecklenburg-West Pomerania, Germany	Rubarth *et al.*, 2011 (ref. 33)
Herring gulls	—	8	2020	Chantry Island, Lake Huron	Gebbink & Letcher., 2012 (ref. 34)
Common carp	*Cyprinus carpio*	10	2009	Beijing, China (market)	Shi *et al.*, 2012 (ref. 35)
Crucian carp	*Carassius auratus*	13			
Grass carp	*Ctenopharyngodon idellus*	10			
Bighead	*Aristichthys nobilis*	12			
Snakehead	*Ophicephalus argus*	8			
Tilapia	*Tilapia*	7			
Crucian carp	*Carassius carassius*	28	2014	Drainage systems of Beijing International Airport	Wang *et al.*, 2016 (ref. 36)
Patients*	—	7	—	—	Harada *et al.*, 2007 (ref. 37)
In-patients*	—	223	2017–2018	Jiangsu Province, China	Wang *et al.*, 2018 (ref. 17)

[a] Note: — indicates that the information is not provided in the study; * indicates that CSF, not brain tissue, was sampled.

concentrations, only the sum of analyzed PFAS, summary statistics, or ranges. For those studies, we contacted the authors and requested the raw data. We received raw data from Dr Gebbink and Dr Letcher from their 2012 study[34] and Dr Verreault from the Verreault *et al.* 2005 study.[10] Finally, in the section on potential neurotoxicity, we reviewed 34 papers, including 13 on associations of PFAS exposure with behavioral and cognitive disorders, 11 papers on PFAS effects on calcium

Table 3 Parameters of exposure experiments exploring the accumulation of PFAS in the brain[a]

Organism	Species	Sample size	Age	Sex	Reagents	Variables	Exposure dose	Exposure time	References
Gilthead bream	*Sparus aurata*	70	—	—	8:2 diPAP	Exposure time	29 μg per g of diet	2, 4, & 7 days	Zabaleta *et al.*, 2017 (ref. 95)
Rainbow trout	*Oncorhynchus mykiss*	200	15 months	F, M	PFHxS, PFOS	Water temperature, PFAS type	500 μg per kg of water	80 days	Vidal *et al.*, 2019 (ref. 96)
Zebrafish	*Danio rerio*	—	—	F, M	^{14}C-PFOA	Sex, exposure time	10 μg per L of water; 0.3–30 μg per L of water	40 days	Ulhaq *et al.*, 2015 (ref. 98)
Zebrafish	*Danio rerio*	300	Fully mature	—	PFOS	Exposure time, single-wall carbon nanotubes concentration	200 μg per L of water	24, 48, 72 & 96 hours	Li *et al.*, 2017 (ref. 97)
Zebrafish	*Danio rerio*	300	4 months	—	PFAAs	Exposure time, PFAA chain length	10 μg per L of water	28 days	Wen *et al.*, 2019 (ref. 99)
Common carp	*Cyprinus carpio*	31	2 years	F, M	PFOA	PFOA concentration	200 ng per L of water & 2 mg per L of water	56 days	Giari *et al.*, 2016 (ref. 101)
Crucian carp	*Carassius auratus*	150	Half-year old	M	PFOA	PFOS concentration	0.2–25 000 μg per L of water	7 days	Dong *et al.*, 2019 (ref. 100)
Rat	*Rattus norvegicus*	50	2 months	M	PFOA, PFOS	Exposure time	5 & 20 mg per kg of body weight per day	28 days	Cui *et al.*, 2009 (ref. 102)
Rat	—	—	Pups	F, M	PFOS	Sex, postnatal age	5 mg per mL of subcutaneous injection	24 hours	Liu *et al.*, 2009 (ref. 104)
Rat	*Rattus norvegicus*	40	8 weeks	F, M	PFOA, PFNA, PFOS	Sex, PFAS type	0.05–5 mg per L of drinking water	90 days	Gao *et al.*, 2015 (ref. 103)

[a] Note: — indicates that the information is not provided in the study.

homeostasis, and 10 papers covering effects of PFAS on neurotransmitters. Since this is an emerging area, we did not perform a systematic review that restricted or characterized studies by quality, but rather reported all available evidence.

3. The absorption of PFAS in the brain

Several studies have mentioned barrier functions preventing PFAS from entering the brain.[17,28,37] Specifically, Harada *et al.* (2007) found that, compared to the transport of PFAS from the serum to the bile, the transport of PFAS from the serum to the CSF is relatively limited in patient samples. The substantial difference in PFOA and PFOS levels between the CSF and serum (the median concentrations of PFOA and PFOS in CSF samples: 0.06 and 0.10 ng mL^{-1}, in serum samples: 2.6 and 18.4 ng mL^{-1}) suggests that PFOA and PFOS may not cross the BBB freely and/or they are efficiently pumped out from the brain by transporters.[37] Similar findings, that brain-to-blood ratios of PFOA and PFOS are low in humans, based on post-mortem examinations, were reported in the study of Maestri *et al.* (2006).[28] In these two early studies, the sample sizes were limited, and the PFAS analysis mainly focused on PFOA and PFOS. Therefore, the results may not necessarily represent the general population, and may not be generalizable to other PFAS given known differences in their toxicokinetics based on animal studies.[74,75]

In 2018, Wang *et al.* pointed out the barrier effect is one potential factor influencing the penetration of PFAS from the serum into CSF, since PFAS concentrations in CSF are 2 to 3 orders of magnitude lower than in the serum. In addition, they mentioned inflammation could increase the permeability of the brain barriers. Albumin CSF-to-serum ratios are strongly correlated with PFAS CSF-to-serum ratios, which may provide an alternative explanation to the barrier theory, since PFAS are known to bind to albumin in the blood. While their study had a relatively large sample size (223 serum-CSF pairs), and analyzed a broad array of PFAS, including PFCAs, PFSAs and emerging alternatives, such as 8:2 Cl-PFESA and 6:2 Cl-PFESA (trade name: F-53B), Wang *et al.* (2018) noted that the results might not represent the general population, since the paired serum and CSF samples were collected from hospital patients in the Neurological Department. They also mentioned the bias that may come from using the PFAS content in the CSF to represent the PFAS level in the brain interstitial fluid.[17] Obviously, measuring chemicals within the brain interstitial fluid is challenging. Using the drug content of CSF as a surrogate for the drug content of brain interstitial fluid has been demonstrated as feasible, by showing the generated error is less than 3-fold.[76]

With regard to specific barrier functions, studies indicate different PFAS cross the BBB with varying efficiencies.[7,17] For instance, a pilot whale study by Dassuncao *et al.* (2019) suggested that certain long-chain PFAS, specifically PFDoA, PFTrA, PFTeA and PFDS, may cross the BBB through a process related to the significantly higher phospholipid levels measured in the brain (though the specific mechanism was not evaluated), while short-chain PFAS may not penetrate the BBB effectively.[7] The current understanding of the potential pathways for chemicals to enter the brain includes (1) initiating BBB disassembly mainly through disrupting tight junctions, and (2) binding to transporters to complete transmembrane transport. However, although PFAS have been detected in the brain and CSF, the mechanisms by which PFAS enter and remain in the brain are unclear. Understanding the possible mechanisms is critical, both for investigating strategies to block PFAS entering the brain so as to limit their adverse effects, and for understanding how to select and design safer replacements for these chemicals. In this section, we review what is known about the uptake of PFAS in the brain and CSF.

Several studies have reported PFOS-induced endothelial discontinuity in the brain.[22,23,77] More specifically, PFOS may disrupt tight junctions in brain endothelial cells by triggering the PI3K/Akt signaling pathway. PI3K is a critical regulator of the permeability of endothelial cells. This signaling pathway has been demonstrated *via in vitro* experiments with the PI3K inhibitor, which blocks PFOS-induced endothelial disassembly.[77] In another *in vitro* human microvascular endothelial cell model, PFOS provokes the production of reactive oxygen species (ROS). The existence of ROS induces actin filament remodeling, which is directly associated with increased endothelial permeability.[22] Most recently, Yu *et al.* (2020) reported that PFOS can penetrate the BBB by disrupting the structure of tight junctions and/or decreasing the expression of tight junction proteins (*e.g.*, claudin-5 and occludin). The disrupted tight junctions could then initiate BBB disassembly. Astrocyte hypertrophy and damage have also been found to exacerbate the disassembly of the BBB, as the interaction of endothelial cells and astrocytes is critical for regulating the BBB. PFOS disrupts these interactions and promotes the disruption of the BBB.[23] However, these studies only focused on PFOS. It is still unknown whether other PFAS enter the brain through disrupting the integrity of brain barriers.

The other potential pathway for PFAS entering the brain is by interacting with transport proteins. The traffic of many toxic substances across brain barriers relies on active transport mediated by transporters.[78] Various efflux and influx transporters are expressed at brain barriers, including P-gp, BCRP/Bcrp, MRPs/Mrps, OATPs/Oatps, OATs/Oats and OCTs/Octs, as illustrated in Fig. 1.[20] While the transport of PFAS through transporters at the BBB has yet to be verified, previous studies related to the interaction of PFAS and similar transporters expressed in other tissues may provide useful insight. For example, several studies have indicated that PFAS renal clearance is mediated by OATs/Oats,[24,25] and PFAS renal reabsorption is moderated by Oatps.[26] In addition, previous *in vitro* research has investigated the impacts of PFAS on the P-gp transporter, which is one of the most studied efflux transporters at the BBB.[79] Specifically, PFOA and PFOS could significantly inhibit human P-gp, and this inhibition increased with PFAS dose in an *in vitro* experiment, while the interaction of P-gp with other compounds of low molecular weight (less than 300 Da) was not observed.[80] Another *in vitro* study on the marine mussel (*Mytilus californianus*) found that PFOA, PFNA, PFDA and PFHxS have inhibitory effects on P-gp in a chain-length-dependent manner.

That is, longer-chain PFAS caused more severe inhibition of P-gp than shorter-chain PFAS under the same PFAS exposure dose. The mechanism by which PFNA inhibits P-gp is indirect, which means PFNA disrupts the transporter function rather than competing for binding sites with P-gp substrates. But the inhibitory effect of PFNA and PFDA on P-gp is reversible, and exposure of P-gp to PFNA induces the synthesis of new P-gp transporters.[81] Furthermore, an *in vitro* experiment investigating the interaction of PFOA and PFOS with four types of transporters located at the blood-testis barrier[82] showed both PFOA and PFOS inhibited the activity of the BCRP, P-gp, MRP1 and MRP4, among which the BCRP transporter could transport PFOA as its substrate, while P-gp did not transport any of the PFAS analyzed.[83] This finding of P-gp is in line with the *in vitro* P-gp study by Stevenson *et al.* (2006) mentioned previously.[81] When the PFAS acts as an inhibitor of an efflux transporter, such as with P-gp, it reduces the ability of the transporter to effectively remove xenobiotics (including PFAS) from the tissue where it is expressed. Alternatively, when the PFAS acts as a substrate of a transporter, it could compete for binding sites with the normal substrates of the transporter, and thereby limit the transport of the normal substrates, as with the BCRP and PFOA mentioned here. If the transporter is an efflux transporter, then any PFAS that acts as a substrate will be eliminated as would an endogenous substrate.[84]

Fatty acid transporters are another potential PFAS transporter group. Greaves *et al.* (2013) first found a correlation between long-chain PFCAs (C10–C15) and nonpolar free fatty acids in the brain of polar bears. However, the method they used could not isolate the specific fatty acid types.[29] A recent study in pilot whales demonstrated that phospholipid (one type of fatty acid) concentrations were predictive of the distribution of long-chain PFAS (C12–C14 PFCAs and PFDS) in the brain.[7] The brain takes up the majority of its needed fatty acids from the blood. In order to enter the brain, long-chain fatty acids rely on transporters to cross the BBB.[85] Long-chain PFCAs (C10–C15) may have similar mechanisms to long-chain fatty acids to penetrate the BBB due to their similar structures.[29]

Based on the papers discussed in this section, the existence of cerebral barriers prevents xenobiotic chemicals from entering and accumulating in the CNS, but PFAS may enter the brain by initiating BBB disassembly mainly through disrupting tight junctions and/or by relying on transporters to complete transmembrane transport. PFAS are amphiphilic substances composed of a hydrophilic "head' and a hydrophobic carbon-fluorine "tail", potentially leading to their ability to cross the BBB. Based on quantitative structure–activity relationship (QSAR) modelling of the relationship between chemical structures and their ability to cross the BBB, molecular weight less than 400 to 600 Da, lipophilicity and protein binding affinity are major factors in CNS penetration.[86] Different PFAS may match this description: the "smaller molecular weight" that makes it easier to penetrate tight junctions falls within the molecular weight range of PFAAs with chain length between C4 and C11. Moreover, previous studies have emphasized that the hydrophobic interactions between fluorinated carbon "tails" and the binding pocket of proteins allow PFAS to be substrates of membrane transporters.[87] Compared to long-chain PFAS, short-chain PFAS (which are less hydrophobic) may enter the brain less effectively and/or may be efficiently pumped out from the brain by transporters. For long-chain PFCAs, especially C10–C15 PFCAs are likely to enter the brain through interacting with transporters.[7,29] However, the specific mechanisms of PFAS–transporter interactions in the brain are not well understood, and it is also possible that some PFAS may enter the brain through other as yet unidentified mechanism(s). Given the phaseout of many long-chain PFAS and the advent of emerging PFAS alternatives, it is necessary to extend the PFAS types being investigated with respect to uptake in the brain to understand the factors that regulate the absorption of diverse PFAS in the brain. In addition, while *in vivo* and biomonitoring studies are limited due to the invasive nature of sampling the brain, other methods may be complementary to the current focus on *in vitro* experiments. For example, computational simulations are an increasingly powerful tool to provide us a better understanding of the uptake of xenobiotic chemicals[88] at the BBB that could be applied to PFAS.

4. The accumulation and distribution of PFAS in the brain

4.1 The accumulation of PFAS in collected brain samples

To understand the accumulation and distribution of PFAS in the CNS, we reviewed studies reporting PFAS concentration in human brains and CSF, and in the brains of wildlife species. Basic information related to the samples in these studies is listed in Table 2. The mean PFAS concentrations in the brains, blood and livers in these studies are in (Table S2 in the ESI†).

PFAS have been detected in the brain and CSF of humans. Based on these studies, PFAS content in human CSF is relatively lower than in human brain.[8,17,28,37] The mean concentrations of PFAS in these human samples show a consistent trend, namely that PFCA concentrations decrease with their chain length.[8,17] Another study by Pérez *et al.* (2013) detected higher mean concentration of PFHxA in the brain of cadavers compared to other PFAS analyzed in their study,[8] but this observation is not supported by the patterns we found in other studies for humans and wildlife. Furthermore, a recently published study suggests these observations may need to be taken with some caution due to potential for the analytical method employed and contamination to generate erroneous results for short-chain PFAS like PFBA.[89] In general, the number and sample sizes of studies related to the distribution of PFAS in the human brain are limited, and some of these studies only focused on PFOA and PFOS.[28,37] Further, the experimental data in these studies are either from autopsy or hospital patients. It is therefore not clear whether PFAS distribution in these samples can represent the distribution in the general healthy population.

In addition to humans, PFAS have also been detected in the brain of various wildlife species.[7,10,29–36,39] The dominant detected PFAS are C6–C14 PFCAs, and C6, C8 and C10 PFSAs. The concentration of PFCAs with 6 to 11 carbons increases with chain length in the brain.[7,29–31,34,36] The concentration of PFCAs

with 11 to 15 carbons shows a fluctuating trend wherein the concentration of PFCAs with an odd number of carbons are higher than those with an even number of carbons.[7,29,31,33] According to Greaves *et al.* (2013), the difference between odd and even chain length PFAS may indicate the presence of precursors of PFAS in biota, such as fluorotelomer alcohols (FTOHs), which degrade to both odd and even carbon chain length PFAS.[29] However, this could also be related to the PFAS source: electrochemical fluorination (ECF) and telomerization are the two primary methods of PFAS manufacturing, the former yielding both odd and even chain length PFAS, and the latter producing PFAS with an even number of carbons.[90] Thus, sources containing more ECF-derived PFAS would also have a higher proportion of odd chain-length PFAS. Among the PFSAs, PFOS is always dominant in the brain of wildlife,[10,29–31,33] likely due to the wide application of PFOS historically. Additionally, several studies also detected perfluorooctane sulfonamide (PFOSA), a PFOS precursor, in brain samples.[7,29,31,35] The existence of PFOSA could increase the content of PFOS in brains.

Among all the papers reviewed that were associated with PFAS distribution in the CNS, several of them reported paired blood (or serum) and brain PFAS concentrations and paired liver and brain PFAS concentrations (see ESI, Table S2†). The calculated PFAS brain-to-blood (or serum) ratios in wildlife species increased with chain length, suggesting PFAS with longer chain length can enter or remain in the brain more easily.[29,31,33,34] This trend is consistent with the study of Wen *et al.* (2017) on zebrafish (*Danio rerio*) that long-chain PFAS can outcompete short-chain PFAS for transporters and binding positions, suggesting long-chain PFAS bind to transporters and are transported more effectively than short-chain PFAS.[91] This is in contrast with the more variable data reviewed for humans. This may be because we don't have enough human brain samples to see the trends, or because the mechanisms of accumulation and distribution of PFAS in humans and wildlife species are different, but the former is more likely.

4.2 Brain region-specific PFAS distribution

Among the studies found on PFAS distribution in collected brain samples, Greaves *et al.* (2013) and Pedersen *et al.* (2015) focused on the brain region-specific PFAS distribution in polar bears (*Ursus maritimus*).[29,30] Polar bears are the top predators in their food web, and therefore have higher exposure to bioaccumulative chemicals such as long-chain PFAS.[92] Polar bear samples in these two studies were collected in similar geographical locations in East Greenland, although at different

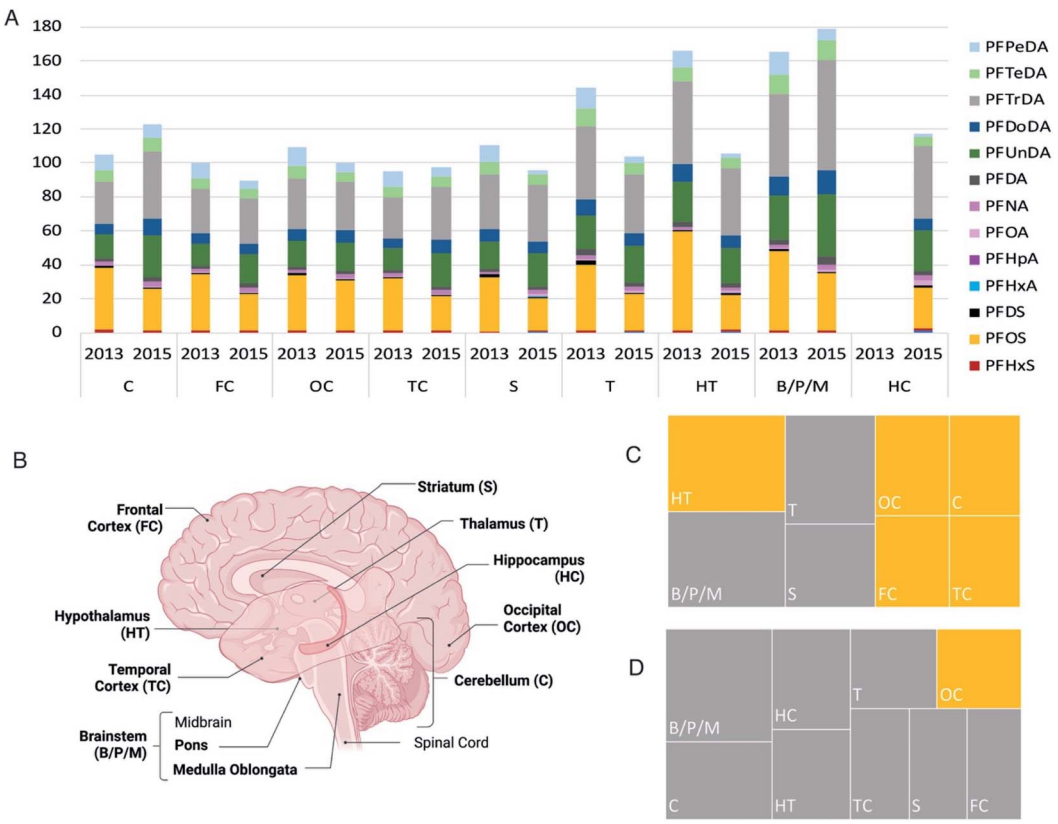

Fig. 2 (A) Brain region-specific PFAS distribution in polar bears extracted from Greaves *et al.* (2013)[29] and Pedersen *et al.* (2015).[30] These two studies are denoted by 2013 and 2015, respectively, according to the year the study was published. The brain regions are represented by abbreviations corresponding to the brain regions shown in (B). The size of the boxes in (C) for Greaves *et al.* (2013) and (D) for Pedersen *et al.* (2015) represent the total amount of PFAS in each brain region (corresponding to the total height of the columns in (A)), while the color represents the dominant PFAS in each brain region (orange: PFOS, grey: PFTrDA).

times (see Table 2). Compared to the polar bears hunted in 2006, the mean concentration of PFCAs in the brains of polar bears collected from 2011 to 2012 increased, while the mean concentration of PFSAs decreased. This trend was also reflected in the dominant PFAS in each brain region. PFOS was the dominant PFAS in four of eight brain regions in 2006 harvested polar bears, but in only one of the brain regions of polar bears collected from 2011–2012 (Fig. 2C and D).[29,30] This decline likely results from the phase-out of PFOS production in the early 2000s, as was posited in the study by Rigét *et al.* (2013), who detected the annual average PFAS concentration in the liver of East Greenland polar bears from 1984 to 2011, and found liver PFOS content decreased since 2006.[93]

Long-chain C11–C15 PFCAs and PFOS are the major PFAS detected in polar bear brains (Fig. 2A).[29,30] According to Smithwick *et al.* (2009) and Greaves *et al.* (2012), C9–C11 PFCAs and PFOS are the major PFAS in polar bear livers.[39,94] Compared to other tissues, the dominant PFAS in polar bear brains have longer chain lengths. Greaves *et al.* (2013) mentioned that the high concentration of longer-chain PFCAs may result from unique transport mechanisms into the brain. Their study was the first to explore the relationship between PFAS concentration and nonpolar free fatty acids content. They found a positive correlation between long-chain PFCAs (mainly C11–C15 PFCAs) and lipid content. The brain is a lipid-rich tissue, providing a more nonpolar environment for the accumulation of long-chain PFCAs, which are more hydrophobic.[29]

In terms of the total PFAS content in each brain region, the brain stem, hippocampus, hypothalamus, pons/medulla and thalamus have higher PFAS content than other brain areas.[29,30] These regions are closer to the incoming bloodstream and receive the freshest blood, providing the PFAS in the blood opportunity to accumulate in these brain regions first.[29] The accumulation of PFAS in these brain regions may have implications for neurotoxicity, as we will discuss in Section 5. Further studies are needed to explore the distribution and accumulation of a broad array of PFAS in the brain and connect them to the neurotoxicity of PFAS.

4.3 PFAS exposure experiments

Various short-term and long-term exposure experiments at a wide range of PFAS concentrations have been conducted on gilthead bream,[95] rainbow trout,[96] zebrafish,[97–99] carp[100,101] and rats[102–104] to investigate the accumulation and distribution of PFAS in the brain and other tissues. PFAS exposure time, dosage, chain length, functional groups and the age of the test organism have all been shown to affect the accumulation of PFAS in the brain (Table 3).

In terms of PFAS dosage, some studies reported a positive relationship with PFAS brain accumulation.[100,101] Giari *et al.* (2016) exposed common carp (*Cyprinus carpio*) to 200 ng L^{-1} and 2 mg L^{-1} PFOA, respectively, for 56 days. They found PFOA concentration in carp brain is lower than the limit of detection (0.4 ng g^{-1} wet weight) at 200 ng L^{-1} exposure, while they detected PFOA accumulation in brain samples at 2 mg L^{-1} exposure with the mean concentration of 0.45 ng g^{-1} wet weight.[101] Dong *et al.* (2019) also did not detect PFOA in the crucian carp (*Carassius auratus*) brain at 0.2 μg L^{-1} exposure at the seventh exposure day.[100] However, another study on rats (*Rattus norvegicus*) found no obvious difference in PFOA concentration in the rat brain after 28 days of exposure to either 5 mg kg^{-1} day^{-1} or 20 mg kg^{-1} day^{-1} PFOA, indicating the saturation of PFOA–protein binding sites at low exposure concentration. PFOA could bind to various proteins in the brain, but increased PFOA elimination through urine or feces will occur when binding sites are saturated. This study also tested the accumulation of PFOS in the brain and found high level of PFOS in the rat brain (146 μg g^{-1}) at 20 mg kg^{-1} day^{-1} PFOS exposure, while the increase in PFOS bioconcentration was not proportional to the increase in PFOS exposure concentration. Cui *et al.* (2009) suggested that higher PFOS concentration may cause more serious impacts on the integrity of the BBB, leading to more PFOS penetration into the brain. In addition, the concentration of PFOS in the brain is higher than that of PFOA under the same exposure dose and time, indicating the elimination rate of PFOS might be lower than that of PFOA.[102]

The different findings for PFOA and PFOS might result from their different acid functional groups (carboxylate *vs.* sulfonate) or the presence of an additional fluorinated carbon in PFOS. The study by Wen *et al.* (2019) pointed out that PFAA accumulation in the zebrafish brain is associated with PFAA chain length and functional group. Specifically, the accumulation of PFAAs in the brain increases with the perfluorinated carbon chain length. This trend may be due to the greater hydrophobic forces that enhance longer chain PFAA binding to proteins.[99] In addition, according to Wen *et al.* (2017), longer-chain PFAAs might compete for protein binding sites and transporters with shorter-chain PFAAs so as to lead to the observed differences in their bioconcentration potentials.[91] With regard to functional group, compared to PFCAs, PFSAs with the same perfluorinated carbon chain length are more accumulative in zebrafish brain, since more hydrogen bonds can be formed between amino acid residues and the sulfonate functional group than with the carboxylate functional group.[99]

In addition to PFAS dosage and functional groups, differences among individuals in PFAS exposure experiments could also affect the results of PFAS accumulation in the brain. For example, mice at different postnatal ages were exposed at the same dosage of PFOS (50 mg kg^{-1} body weight). Liu *et al.* (2009) found higher level of PFOS in younger mice after the same PFOS exposure, suggesting the development of the BBB function with age provides added protection from xenobiotic accumulation.[104] However, no obvious sex differences in PFAS brain accumulation has been reported in these PFAS exposure studies.

Exposure experiments with PFAS precursors have also been conducted. For example, the study by Zabaleta *et al.* (2017) explored the exposure of gilthead bream (*Sparus aurata*) to 29 μg g^{-1} 8:2 polyfluoroalkyl phosphate diester (8:2 diPAP), which is a precursor of PFOA, and detected a high level of PFOA (mean concentration 3.7 ng g^{-1}) in their brain after 7 days exposure. However, further studies on the accumulation of PFAS precursors in the brain are needed to investigate whether some PFAS

precursors may be more toxic than their degradation products,[105] and to improve the understanding of emerging PFAS.

In nature, organisms are exposed to various chemical contaminants through multiple pathways. The presence of other substances may affect the bioaccumulation and distribution of PFAS in organisms. An *in vivo* study on the impacts of single-walled carbon nanotubes on the bioaccumulation of PFOS in zebrafish tissues found the bioaccumulation of PFAS declines with the increase of nanotube dose, because the adsorption of PFOS to the carbon nanotubes reduces the bioavailability of PFOS to zebrafish.[97] This suggests various other environmental contaminants could impact the bioaccumulation of PFAS in organisms, but our understanding of this field is still not well-established.

Experiments associated with PFAS accumulation and distribution in the brain should be done with particular care. Various studies used aquatic organisms to explore the accumulation and distribution of PFAS in the brain. As mentioned by Vidal *et al.* (2019) water temperature is critical in the design of studies on aquatic organisms since the distribution and accumulation of PFAS may be affected by metabolic rates, which in aquatic organisms is often closely tied to water temperature. Specifically, they found the brain-to-blood ratios of PFOS increases with water temperature in rainbow trout (*Oncorhynchus mykiss*).[96] Indeed, ectotherms are very sensitive to temperature, which affects their rates of respiration, consumption, and growth and thereby affect most key toxicokinetic parameters.[106] Ulhaq *et al.* (2015) mentioned that the brain is a tissue with complex blood vessels, leading to the mixing of PFAS in the blood with PFAS in the brain during experiments, which could also affect the interpretation of brain data.[98] In order to reduce invasive experiments, studies have proposed alternative non-invasive biomonitoring methods to measure internal PFAS exposure.[103,107–110] For example, Gao *et al.* (2015) used hair as an indicator of PFAA exposure, indicating the correlation between average concentrations of PFAAs in hair and brain can reach up to 0.86 or more for PFNA and PFOS.[103] However, studies of using hair as a biomarker of PFAS exposure are still quite limited, and results vary by PFAS types,[103,110] subject population[110] and gender.[103] More studies are needed to explore whether hair can be a reliable biomarker for PFAS exposure by testing different PFAS in more species and optimizing the analytical methods for PFAS detection and quantification in the hair. Taken together, differences in analytical techniques in different studies and challenges associated with experiments may impact our ability to compare results across studies.

5. The potential neurotoxicity of PFAS

The studies reviewed in the previous sections demonstrate that PFAS accumulate in and distribute through the brain, which highlights the importance to better understand the toxicity of PFAS in the CNS.[29,30] In this review, we surveyed 13 studies related to the associations of PFAS exposure with behavioral and cognitive disorders, mainly including attention deficit hyperactivity disorder (ADHD), fetal congenital cerebral palsy, learning disorders, memory dysfunction, and intellectual disability.[40–52]

In addition, various *in vitro* PFAS exposure experiments have been conducted, mainly on hippocampal neurons, to further explore the mechanisms of PFAS toxicity in the brain. Hippocampal neurons are promising subjects, since the hippocampus is one of the dominant brain areas for PFAS accumulation as discussed above. Also, the hippocampus is related to learning and memory.[111] Here we reviewed 21 papers related to the two most studied potential mechanisms of PFAS neurotoxicity: (1) PFAS-induced intracellular calcium alteration in neurons,[53–63] and (2) the impacts of PFAS on neurotransmitters.[64–73]

5.1 Associations of PFAS exposure with behavioral and cognitive disorders

PFAS have been identified as potential neurobehavioral toxicants, *e.g.* as inducers of behavioral disorders. Multiple studies have explored the prevalence of PFAS exposure and ADHD, but conflicting results exist in these studies, including positive, negative and no associations.[40–45] Specifically, a Norwegian birth cohort study with 1199 mother-child pairs found that higher PFOS concentration in breast milk (collected before infants reached 2 months) increased the odds of ADHD in children (around 13 years old; odd ratio = 1.77, 95% confidence interval: 1.16, 2.72). The positive association between early-life PFOS exposure and ADHD was sex-specific, showing stronger association in girls than boys.[40] Negative associations of PFAS prenatal exposure with ADHD were also reported in multiple studies. For example, a questionnaire-based study with 282 subjects found prenatal PFNA exposure was negatively related to ADHD in 7 year-old children.[41] The study by Stein and Savitz (2011) reported the negative prevalence of PFOA exposure and ADHD in 5-to-18 year-old children living in areas where drinking water was contaminated by PFOA.[42] Stein *et al.* (2014) indicated that the negative association might be because PFOA could slightly activate peroxisome proliferator-activated receptor (PPAR) gamma, acting like PPAR-gamma agonists, which harbors neuroprotective and anti-inflammatory functions. Similar functions of activating PPAR-gamma between PFOA and PPAR-gamma agonists suggests PFOA might also have neuroprotective function.[43] This explanation of negative association between PFOA exposure and ADHD might be extended to other PFCAs due to their similar structures. In addition, no significant association was found between prenatal PFAS exposure and parent-reported ADHD in 18 month-old children, but the sample size ($n = 59$) was small in that study.[44] Another study with 4826 mother-child pairs also did not find the prevalence of PFOS and PFOA prenatal exposure and ADHD (odds ratios ranging from 0.96 to 1.02), but in their stratified analyses, increased association of PFAS exposure and ADHD were found in female infants, and in infants from nulliparous or low-educated mothers. The sex-dependent results might result from different endocrine-disrupting effects of PFAS on estrogen, that thereby cause different impacts on males and females.[45] Besides these prenatal PFAS exposure studies, researchers also explored the relationship between PFAS levels

in children's blood and their ADHD symptoms. For example, the study by Stein *et al.* (2014) found sex-specific prevalence of serum PFOA content and ADHD in 6-to-12 year-old children. That is, serum PFOA level was positively associated with ADHD in boys, but negatively in girls.[43] In addition to ADHD, Liew *et al.* (2014) conducted a case-cohort study and found higher concentrations of PFOA, PFOS and PFHpS in maternal plasma could increase the risk of cerebral palsy only in male infants, which might result from the limited sample size of female infants and/or the existence of the sex-related mechanisms, which need to be further explored.[46] Evidence is mixed in these human data, indicating further replications is needed to better understand the associations of PFAS exposure with human behavioral disorders.

According to animal exposure experiments, both short-chain and long-chain PFAS could induce cognitive disorders.[47–49] The neonatal exposure of mice to PFHxS affected cognitive function in a long-lasting or even persistent manner.[47] PFDoA decreased the ability of adult rats to recognize novel objects in a dose-dependent manner; the cognitive deficit became more severe as PFDoA concentration increased in the brain.[48] Another PFOS exposure study indicated that both prenatal and postnatal PFOS exposure decreased the spatial learning and memory abilities in rat offspring, and the reduction induced by prenatal PFOS exposure was more severe.[49] In addition, PFAS-induced cognitive deficits have also been reported in humans.[50–52] In the study by Skogheim *et al.* (2020) with 944 mother-child samples, the PFAS concentration in maternal plasma was used to represent child prenatal PFAS exposure. They observed weak negative associations between non-verbal working memory in preschool children and their prenatal exposure to PFAS, including PFOS, PFOA and PFHpS; and weak positive prevalence of verbal working memory in preschool children and their prenatal exposure to PFAS, including PFNA, PFDA and PFUnDA.[50] Similarly, positive association between higher PFAS serum concentrations and cognition limitations (self-reported difficulty remembering) in 1766 adults between 60–85 years old was reported by Power *et al.* (2013).[51] However, Vuong *et al.* (2019) did not observe significant associations between either prenatal or childhood PFOS and PFHxS exposure and the alteration of cognitive functions based on the Full Scale Intelligence Quotient (FSIQ) measurement of 8 year-old children. After stratified analyses, they found positive associations between prenatal PFOA exposure and higher IQ in females, and between childhood PFOS exposure and higher IQ in males.[52] Their findings may reflect some other indicators, rather than a causal relationship.

To sum up, we found conflicting results in these studies focusing on the association of PFAS exposure with behavior and cognitive disorders, especially in humans. This is probably because these studies have diverse sample sizes, and/or diverse experimental subjects with different ages, living areas and health conditions. It is also worth noting that most of the studies related to ADHD use parentally-reported symptoms, which might compromise the accuracy of the results. Several studies indicated sex-specific associations in stratified analyses, but it is difficult to determine the mechanism underlying these associations since the number of existing studies and sample sizes are limited and their results are inconsistent. Further studies are needed in this field to validate these findings. It is worth further exploring the mechanisms underlying such PFAS-induced neurotoxicity. In the following sections, we reviewed two potential mechanisms of PFAS neurotoxicity.

5.2 Effects on calcium homeostasis and calcium-dependent signaling molecules

Calcium (Ca^{2+}) is responsible for mediating multiple neuronal processes, such as proliferation, synaptogenesis, apoptosis, and neurotransmitter secretion.[112,113] Various PFAS exposure studies have reported effects of PFAS on calcium homeostasis in neurons, which is considered to be one potential mechanism of PFAS neurotoxicity.[53–57] The PFAS-induced calcium increase in neurons is either from extracellular calcium influx or calcium store release (Fig. 3).[113] Liao *et al.* (2008) found PFOS could induce the influx of extracellular calcium through L-type voltage-gated calcium channels (L-VGCCs) in rat hippocampal neurons.[53] Another study by Liu *et al.* (2011) found both PFOA and PFOS could significantly increase the calcium concentration in cultured rat hippocampal neurons. The increased calcium was mainly released from intracellular calcium storage organs, such as mitochondria and the endoplasmic reticulum (ER),[112] and mediated by inositol 1,4,5-trisphosphate receptors (IP_3Rs) and ryanodine receptors (RyRs) at the surface of calcium stores.[54] Studies have linked calcium overload to neuron dysfunction and even to cell apoptosis.[53,54] Specifically, after acute exposure of hippocampal neurons and brain slices to PFOS, the increased intracellular calcium potentiated synaptic

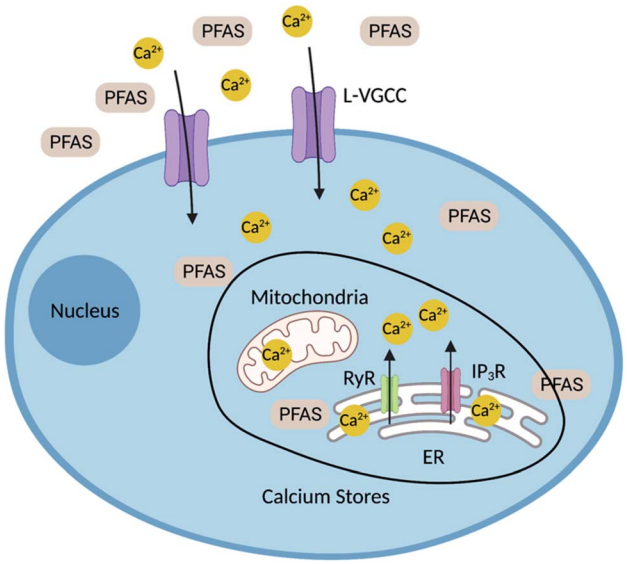

Fig. 3 Proposed mechanisms of PFAS-induced intracellular calcium increase, based on extracellular calcium influx and/or calcium store release. The extracellular calcium influx is mediated by L-VGCCs at the surface of neurons. Intracellular PFAS could induce the release of calcium from intracellular calcium storage organs, such as mitochondria and the ER. Intracellular calcium release is mediated by IP_3Rs and RyRs at the surface of calcium stores.

transmission, which represents the communications between neurons. In addition, PFOS-induced intracellular calcium overload also provoked neuronal excitement, which could lead to neuronal injury. In terms of long-term implications, the exposure to PFOS affected the normal structure and functions of neurons.[53] A further study by Liao *et al.* in 2009 pointed out that the effects of PFAS on rat hippocampal neurons depend on the chain-length, the degree of fluorination and functional groups of PFAS. Specifically, the disturbance of neuronal activities by PFAS increased with the fluorinated carbon chain length and the fluorination level. Compared with perfluorinated carboxylates, perfluorinated sulfonates had stronger effects on neurons.[58] Additionally, Liu *et al.* (2011) also observed the increase of ROS in calcium-overloaded neurons. ROS could induce oxidative stress events, which may eventually lead to cell death.[54] Furthermore, Dusza *et al.* (2018) pointed out the rise of calcium release depends on age, since they found exposure to PFOS increased the calcium release in brain microsomes in adult rats, but not in neonatal rats.[56]

Studies have also reported PFAS-induced alteration of calcium-dependent signaling molecules, a potential molecular mechanism of PFAS-induced neurotoxicity since these molecules are critical for determining the structure and functions of neurons.[54,57,59–61] Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), cAMP-response element binding protein (CREB) and calcineurin (CaM) are critical calcium signaling down-stream molecules.[54,59] CaMKII participates in synaptogenesis and plays important roles in learning and memory.[59,61] CREB is critical in neuronal growth and the formation of long-term memory.[114] CaM is important for neuron survival and cognition.[54] PFOS was shown to increase the expression of CaMKIIα and phosphorylated CREB in adult male rat cortex and hippocampus.[59] The expression of CaM significantly increased in both PFOA and PFOS treated rat hippocampal neurons.[54] Furthermore, to probe the developmental neurotoxicity of PFAS, studies investigated various calcium-dependent signaling molecules in different developmental stages of mice after PFAS exposure.[60,61] Liu *et al.* (2010a) detected that the expression of *N*-methyl-D-aspartate receptor subtype-2B (NR2B), CaM, CaMKIIα and CERB changed both under prenatal and postnatal PFOS exposure in mice. NR2B expression is related to learning ability and memory; CaM can respond to calcium concentration changes, which has been detected in a PFAS exposure study we mentioned previously;[54] the change of CaM will further impact its downstream molecule CaMKIIα; and CERB is related to neuronal growth. They suggested the relationship between the alterations of the expression of these calcium-related signaling molecules and cognitive deficits. Based on their results, PFOS could reach the brain at the embryo stage, and further induce adverse effects to the CNS postnatally.[60]

In addition to these molecules, PFOS and PFOA were also shown to increase the level of growth-associated protein-43 (GAP-43), synaptophysin and tau in mouse hippocampus and cerebral cortex after neonatal exposure. These proteins play important roles in synaptogenesis, neuronal development, and growth. The neonatal stage is a critical brain development period, and PFAS-induced overexpression of these proteins at the neonatal stage affects the healthy development of the mouse brain.[61] Finally, PFNA could also induce increased intracellular calcium concentration and CaMKII expression in rat pheochromocytoma-12 (PC12) cells. These alterations could result in oxidative stress in cells and ultimately lead to cell apoptosis.[57] This observation is in line with Wang *et al.* (2015), who found prenatal and postnatal PFOS exposure could increase hippocampal neuron apoptosis in rat offspring. The increase of apoptosis is in a similar manner to the calcium increase in neurons, suggesting the rise of intracellular calcium is one of the potential mechanisms of neuron apoptosis. Specifically, Wang *et al.* (2015) indicated that PFOS-induced calcium disturbance in neurons injured calcium signaling pathways, then induced neuronal apoptosis, and eventually could cause behavioral deficits, such as ADHD and response inhibition.[55] However, studies also found approaches to reduce PFAS-induced neuronal dysfunctions. For example, Oh *et al.* (2018) pointed out phycoerythrin-derived peptide of *Pyropia yezoensis* (PYP) could alleviate PFOS-induced calcium disorder.[62] A recent study by Zhang *et al.* (2020) found blueberry anthocyanins (ANT) could reduce PFOA-induced neurotoxicity in *Dugesia japonica* in terms of locomotion reduction, oxidative stress and neurotransmitter dysregulation.[63] These studies provide insights for alleviating PFAS-induced neuronal toxic effects, but the mechanisms underlying these protective strategies remain to be explored.

5.3 Effects on neurotransmitters

The second most studied potential mechanism of PFAS neurotoxicity is neurotransmitter dysfunction. Neurotransmitters are chemicals generated by neurons that are responsible for signal transmission.[115] Neurotransmitter levels in the brain are related to the activation of neurons and signal transmission among neurons.[64] Studies have reported the implications of PFAS on neurotransmitters in the brain, mainly dopamine,[64–70] glutamate,[64,66,68,70–72] acetylcholine and the cholinergic system.[66,68,69,73]

According to various exposure experiments, PFOS and PFOA could alter dopamine concentration in the brains of rat, mouse and frog, but the direction of the alteration was not consistent across studies.[64–66] Yu *et al.* (2016) applied a high-throughput targeted metabolomics approach to analyze the PFOA-induced neurotoxicity in male mice, and found the increase of dopamine concentrations in 0.5 mg PFOA kg^{-1} body weight day^{-1} exposure group.[64] In terms of different brain regions, PFOS increased the dopamine concentration in the prefrontal cortex and hippocampus in adult mice after 28 days PFOS exposure, but the alteration of dopamine content in amygdala was not significant.[65] However, another PFAS exposure study on Northern leopard frog (*Lithobates pipiens*) found PFOS and PFOA decreased dopamine in the brain. In addition, this study suggested long-term developmental PFAS exposure could reduce the amount of dopaminergic neurons. Leopard frogs can be more relevant for the study of these neurons compared to rodents, since leopard frogs have neuromelanin-containing dopaminergic neurons, similar to those affected by Parkinson's disease in humans. Therefore, Foguth *et al.* (2019)

contended that further studies on frogs are needed to explore the relationship between PFAS-induced dopamine alteration and Parkinson's disease.[66] In addition to monitoring dopamine content in the brain, detecting alterations of the gene expression of dopamine receptors further helps to explore the potential molecular mechanism of PFAS neurotoxicity.[65,67] To understand the effects of PFOS on the development of CNS, neonatal mice were exposed to PFOS during development. After 24 hours of PFOS exposure, the transcription of dopamine receptor-D5 decreased in mouse cerebral cortex. At 2 months post exposure, the transcription of dopamine receptor-D2 was reduced in mouse hippocampus.[67] Similar findings have been reported in the study by Salgado *et al.* (2016), namely the gene and protein expression of D1 and D2 receptors in rat prefrontal cortex and hippocampus changed after exposure to PFOS.[65] Both D1 and D2 receptors play important roles in cognition and memory. Another study by Hallgren and Viberg (2016) considered the decreased transcription of dopamine receptor-D2 in hippocampus may be related to cognition disorders in adult mice. However, they did not explain the reduced D5 receptor in cerebral cortex due to the lack of developmental roles of the D5 receptor in the cerebral cortex among literature studies.[67]

In addition to decreasing dopamine, Long *et al.* (2013) found the exposure of adult mice to PFOS could increase hippocampal glutamate, which is another critical neurotransmitter related to learning and memory.[70] Another study found the glutamate concentration in the brain decreased after exposing mice to 2.5 mg PFOA kg^{-1} body weight day^{-1} for 28 days.[64] Similar results have been reported by Foguth *et al.* (2020), who included both PFOS alone (10 ppb) and PFAS mixture (4 ppb PFOS, 3 ppb PFHxS, 1.25 ppb PFOA, 1.25 ppb PFHxA and 0.5 ppb PFPeA) exposure groups in their study and found both of these exposures resulted in significantly decreased glutamate concentrations in the brains of Northern leopard frogs in a similar degree.[68] The low glutamate level in the brain could cause adverse effects on synaptic plasticity and memory.[116] Furthermore, an *in vitro* study on rat cerebellar granule neurons mentioned that PFOS and PFOA increased glutamate concentration, and in turn induced glutamate excitotoxicity, which means excessive glutamate leads to excessive stimulation of its receptors, and even to cell injury and eventually death.[71,117] However, the degree of excitotoxicity induced by PFOS and PFOA were different, which the authors suggest may be due to the different mechanisms of neurotoxicity caused by PFOS and PFOA. In addition, the glutamate excitotoxicity also varied from the developmental stages of cultured neurons.[71] Liao *et al.* (2009) found PFOS ranging from 0.1 to 100 μM altered the glutamate-activated current in rat hippocampal neurons.[72] However, the exposure of leopard frog to PFOA and PFOS did not significantly change the glutamate level in the brain.[66]

The study by Foguth *et al.* (2020) detected the alteration of diverse neurotransmitters in Northern leopard frogs exposed to PFAS, among which, they found PFAS could alter these tested neurotransmitters, especially acetylcholine. Specifically, PFOS and the PFAS mixture described above significantly increased acetylcholine level in the later developmental stage of frogs, but the mechanism behind this acetylcholine rise was not clear.[68] In addition, the exposure of neonatal mice to PFOS and PFOA damaged the adult cholinergic system, even at low PFAS exposure dose (1.4 mmol kg^{-1} body weight).[73] However, Foguth *et al.* (2019) did not observe significant change of acetylcholine levels in leopard frog brain after PFOA and PFOS exposure.[66] Based on these observations, the effects of PFAS on neurotransmitters are complex. As Slotkin *et al.* (2008) mentioned, the mechanism of PFAS impacts on neurotransmitters vary by PFAS types.[69] In addition to PFAS type, the alterations of PFAS to neurotransmitters may also depend on PFAS exposure time and dosage, animal species, and brain/neuro-developmental stages, and these factors are important to consider when comparing across studies.

To sum up, PFAS-induced intracellular calcium alteration in neurons and the impacts of PFAS on neurotransmitters are two major potential mechanisms of PFAS neurotoxicity. There is also a potential link between the effects of PFAS on calcium homeostasis and its effects on neurotransmitters because it is known that the increase of intracellular calcium can trigger neurotransmitter secretion.[54] With respect to all of these PFAS neurotoxicity studies, the majority of them focus on PFOS and PFOA, but researchers found long-chain PFAS can enter the brain more effectively than short-chain PFAS, as was also highlighted in the preceding sections on PFAS absorption, accumulation, and distribution in the brain. Although long-chain PFAS have been phased out and replaced by diverse emerging PFAS, they are still present in organisms and in the environment. Information on the neurotoxicity of long-chain and emerging PFAS is still lacking. In addition, many studies have mentioned the different adverse effects resulting from diverse PFAS types, but it is necessary to further explore the specific mechanisms behind observed differences.[54,69] This review has focused primarily on observations of direct effects of PFAS on the brain and associated outcomes, but there are additional, potentially important, indirect impacts of PFAS, for example through disruption of thyroid hormone function in pregnant women which could affect neurodevelopment of the fetus.[118–120] Such indirect effects also merit further consideration. Furthermore, more studies are needed to explore PFAS neurotoxicity at the molecular level. Currently, it is still difficult to connect the potential neurotoxic mechanisms to specific brain diseases. Critical data gaps remain not only for neurotoxicity, but also in the whole field of PFAS toxicology. Exploring these toxicological issues faces similar dilemmas: the existence of thousands of untested PFAS, and the lack of the quantification of the potential health effects associated with PFAS exposure. Data and tools are needed to establish the link between PFAS exposure and toxicity. The framework of adverse outcome pathways (AOPs), for example, which identify the specific molecular events required to cause a toxic effect, could help to analyze the risk of more PFAS more accurately with fewer resources, since *in vitro* experiments and *in silico* approaches can be alternatives to *in vivo* studies to test and screen molecular events linked to specific toxic effects.[121]

6. Conclusion

Research on PFAS absorption, accumulation, distribution and toxicity in the brain is increasing, but many knowledge gaps remain. PFAS may enter the brain through initiating BBB disassembly and/or relying on transporters located at the BBB, but diverse PFAS with different chain-length and functional groups have different abilities to enter the brain. Future studies are needed to specify the mechanism of each PFAS entering the brain, and how the uptake efficiencies are affected by differences in PFAS structure and properties. After entering the brain, PFAS have the potential to distribute to and accumulate in different areas of the brain. The available studies related to PFAS distribution in various brain regions are quite limited, as are PFAS accumulation data in human brains. Indeed, experimenting on the brain is invasive and should be done with particular care since the brain is a vulnerable tissue with complex blood vessels. As a result, to reduce the invasive experiments and to make the PFAS-related brain studies more accessible, it will be helpful to find surrogates (such as the CSF and hair) that can represent PFAS concentration in the brain. In addition to *in vivo* methods, 3-D tri-culture models have been used for drug screening and disease modeling in the brain.[122,123] Although this technology has not yet been used in the study of PFAS, it is a potentially powerful path forward to explore the absorption, accumulation and effects of PFAS in the brain with an *in vitro* system that more closely mimics *in vivo* activity. Computational methods may likewise be useful alternatives or complements to experiments to explore PFAS toxicokinetics in the brain. More studies are needed to explore the characteristics of the accumulation of PFAS in the brain and the brain region-specific PFAS distribution. These studies help to understand the specific toxic effects to the CNS induced by PFAS since the brain is composed of various regions which are responsible for mediating different functions, such as learning, memory, emotions and movement. In this review, we summarized PFAS-induced toxic effects including behavioral and cognitive deficits. Although learning and memory disorders have been observed, the link between PFAS exposure to specific diseases, such as Alzheimer disease and Parkinson's disease, remains to be explored. Two primary mechanisms of PFAS-induced neurotoxicity have been proposed: disrupting calcium homeostasis and the alteration of neurotransmitters. However, the existence of disconnects across studies on the toxicity of PFAS in the brain and on potential neurotoxicity mechanisms makes interpretation difficult. For example, studies related to the prevalence of prenatal/postnatal PFAS exposure and behavioral and cognitive disorders and *in vitro* studies exploring mechanisms of PFAS neurotoxicity do not evaluate consistent PFAS types, exposure concentrations, or model organisms. Finally, to understand PFAS in the brain more comprehensively, we expect future studies to be better aligned between the accumulation of PFAS and PFAS toxicity in the brain. Currently, PFOA and PFOS are overrepresented in the literature. Given that other PFAS have been shown to accumulate in the brain, and the general lack of studies on emerging PFAS, it is important to identify the neurotoxicity of these other ubiquitous environmental contaminants.

In this review, we show the existing evidence from multiple perspectives (epidemiological, *in vivo*, and *in vitro*) that PFAS do enter and accumulate in the brain, and there are indications they may have an effect. The importance of addressing gaps in our understanding is that there are potentially thousands of PFAS (with at least hundreds in active use) that haven't been tested: the lack of toxicity of some of them does not mean that the others will be safe. It is important to determine whether "emerging" or replacement PFAS may have more profound neurological effects than others, and to connect the understanding of the absorption, distribution and toxicity of PFAS in the brain.

Conflicts of interest

The authors declare no competing financial interest.

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References

1 R. C. Buck, J. Franklin, U. Berger, J. M. Conder, I. T. Cousins, P. de Voogt, A. A. Jensen, K. Kannan, S. A. Mabury and S. P. van Leeuwen, Perfluoroalkyl and polyfluoroalkyl substances in the environment: terminology, classification, and origins, *Integrated Environ. Assess. Manag.*, 2011, **7**, 513–541.

2 S. Fiedler, G. Pfister and K.-W. Schramm, Poly- and perfluorinated compounds in household consumer products, *Toxicol. Environ. Chem.*, 2010, **92**, 1801–1811.

3 J. Glüge, M. Scheringer, I. T. Cousins, J. C. DeWitt, G. Goldenman, D. Herzke, R. Lohmann, C. A. Ng, X. Trier and Z. Wang, An overview of the uses of per- and polyfluoroalkyl substances (PFAS), *Environ. Sci.: Processes Impacts*, 2020, **22**, 2345–2373.

4 D. Herzke, E. Olsson and S. Posner, Perfluoroalkyl and polyfluoroalkyl substances (PFASs) in consumer products in Norway – a pilot study, *Chemosphere*, 2012, **88**, 980–987.

5 K. Kannan, Perfluoroalkyl and polyfluoroalkyl substances: current and future perspectives, *Environ. Chem.*, 2011, **8**, 333.

6 C. A. Ng and K. Hungerbühler, Bioaccumulation of Perfluorinated Alkyl Acids: Observations and Models, *Environ. Sci. Technol.*, 2014, **48**, 4637–4648.

7 C. Dassuncao, H. Pickard, M. Pfohl, A. K. Tokranov, M. Li, B. Mikkelsen, A. Slitt and E. M. Sunderland, Phospholipid Levels Predict the Tissue Distribution of Poly- and Perfluoroalkyl Substances in a Marine Mammal, *Environ. Sci. Technol. Lett.*, 2019, **6**, 119–125.
8 F. Pérez, M. Nadal, A. Navarro-Ortega, F. Fàbrega, J. L. Domingo, D. Barceló and M. Farré, Accumulation of perfluoroalkyl substances in human tissues, *Environ. Int.*, 2013, **59**, 354–362.
9 K. I. Van de Vijver, L. Holsbeek, K. Das, R. Blust, C. Joiris and W. De Coen, Occurrence of Perfluorooctane Sulfonate and Other Perfluorinated Alkylated Substances in Harbor Porpoises from the Black Sea, *Environ. Sci. Technol.*, 2007, **41**, 315–320.
10 J. Verreault, M. Houde, G. W. Gabrielsen, U. Berger, M. Haukås, R. J. Letcher and D. C. G. Muir, Perfluorinated Alkyl Substances in Plasma, Liver, Brain, and Eggs of Glaucous Gulls (*Larus hyperboreus*) from the Norwegian Arctic, *Environ. Sci. Technol.*, 2005, **39**, 7439–7445.
11 M. Houde, J. W. Martin, R. J. Letcher, K. R. Solomon and D. C. G. Muir, Biological Monitoring of Polyfluoroalkyl Substances: A Review, *Environ. Sci. Technol.*, 2006, **40**, 3463–3473.
12 P. C. Hebert and L. A. MacManus-Spencer, Development of a Fluorescence Model for the Binding of Medium- to Long-Chain Perfluoroalkyl Acids to Human Serum Albumin Through a Mechanistic Evaluation of Spectroscopic Evidence, *Anal. Chem.*, 2010, **82**, 6463–6471.
13 L. A. MacManus-Spencer, M. L. Tse, P. C. Hebert, H. N. Bischel and R. G. Luthy, Binding of Perfluorocarboxylates to Serum Albumin: A Comparison of Analytical Methods, *Anal. Chem.*, 2010, **82**, 974–981.
14 D. Attwell, A. M. Buchan, S. Charpak, M. Lauritzen, B. A. MacVicar and E. A. Newman, Glial and neuronal control of brain blood flow, *Nature*, 2010, **468**, 232–243.
15 L. Sanchez-Covarrubias, L. M. Slosky, B. J. Thompson, T. P. Davis and P. T. Ronaldson, Transporters at CNS Barrier Sites: Obstacles or Opportunities for Drug Delivery?, *Curr. Pharm. Des.*, 2014, **20**, 1422–1449.
16 R. Daneman, The blood–brain barrier in health and disease, *Ann. Neurol.*, 2012, **72**, 648–672.
17 J. Wang, Y. Pan, Q. Cui, B. Yao, J. Wang and J. Dai, Penetration of PFASs Across the Blood Cerebrospinal Fluid Barrier and its Determinants in Humans, *Environ. Sci. Technol.*, 2018, **52**, 13553–13561.
18 P. Ballabh, A. Braun and M. Nedergaard, The blood–brain barrier: an overview, *Neurobiol. Dis.*, 2004, **16**, 1–13.
19 S. M. Stamatovic, R. F. Keep and A. V. Andjelkovic, Brain Endothelial Cell-Cell Junctions: How to "Open" the Blood–Brain Barrier, *Curr. Neuropharmacol.*, 2008, **6**, 179–192.
20 L. Sanchez-Covarrubias, L. M. Slosky, B. J. Thompson, T. P. Davis and P. T. Ronaldson, Transporters at CNS Barrier Sites: Obstacles or Opportunities for Drug Delivery?, *Curr. Pharm. Des.*, 2014, **20**, 1422–1449.
21 J. Wang, Y. Pan, Q. Cui, B. Yao, J. Wang and J. Dai, Penetration of PFASs Across the Blood Cerebrospinal Fluid Barrier and its Determinants in Humans, *Environ. Sci. Technol.*, 2018, **52**, 13553–13561.
22 Y. Qian, A. Ducatman, R. Ward, S. Leonard, V. Bukowski, N. L. Guo, X. Shi, V. Vallyathan and V. Castranova, Perfluorooctane Sulfonate (PFOS) Induces Reactive Oxygen Species (ROS) Production in Human Microvascular Endothelial Cells: Role in Endothelial Permeability, *J. Toxicol. Environ. Health, Part A*, 2010, **73**, 819–836.
23 Y. Yu, C. Wang, X. Zhang, J. Zhu, L. Wang, M. Ji, Z. Zhang, X.-M. Ji and S.-L. Wang, Perfluorooctane sulfonate disrupts the blood–brain barrier through the crosstalk between endothelial cells and astrocytes in mice, *Environ. Pollut.*, 2020, **256**, 113429.
24 H. Nakagawa, T. Hirata, T. Terada, P. Jutabha, D. Miura, K. H. Harada, K. Inoue, N. Anzai, H. Endou, K. Inui, Y. Kanai and A. Koizumi, Roles of Organic Anion Transporters in the Renal Excretion of Perfluorooctanoic Acid, *Basic Clin. Pharmacol. Toxicol.*, 2008, **103**, 1–8.
25 Y. M. Weaver, D. J. Ehresman, J. L. Butenhoff and B. Hagenbuch, Roles of Rat Renal Organic Anion Transporters in Transporting Perfluorinated Carboxylates with Different Chain Lengths, *Toxicol. Sci.*, 2010, **113**, 305–314.
26 C.-H. Yang, K. P. Glover and X. Han, Organic anion transporting polypeptide (Oatp) 1a1-mediated perfluorooctanoate transport and evidence for a renal reabsorption mechanism of Oatp1a1 in renal elimination of perfluorocarboxylates in rats, *Toxicol. Lett.*, 2009, **190**, 163–171.
27 X. Han, D. L. Nabb, M. H. Russell, G. L. Kennedy and R. W. Rickard, Renal Elimination of Perfluorocarboxylates (PFCAs), *Chem. Res. Toxicol.*, 2012, **25**, 35–46.
28 L. Maestri, S. Negri, M. Ferrari, S. Ghittori, F. Fabris, P. Danesino and M. Imbriani, Determination of perfluorooctanoic acid and perfluorooctanesulfonate in human tissues by liquid chromatography/single quadrupole mass spectrometry, *Rapid Commun. Mass Spectrom.*, 2006, **20**, 2728–2734.
29 A. K. Greaves, R. J. Letcher, C. Sonne and R. Dietz, Brain region distribution and patterns of bioaccumulative perfluoroalkyl carboxylates and sulfonates in East Greenland polar bears (Ursus maritimus), *Environ. Toxicol. Chem.*, 2013, **32**, 713–722.
30 K. Eggers Pedersen, N. Basu, R. Letcher, A. K. Greaves, C. Sonne, R. Dietz and B. Styrishave, Brain region-specific perfluoroalkylated sulfonate (PFSA) and carboxylic acid (PFCA) accumulation and neurochemical biomarker Responses in east Greenland polar Bears (Ursus maritimus), *Environ. Res.*, 2015, **138**, 22–31.
31 L. Ahrens, U. Siebert and R. Ebinghaus, Total body burden and tissue distribution of polyfluorinated compounds in harbor seals (Phoca vitulina) from the German Bight, *Mar. Pollut. Bull.*, 2009, **58**, 520–525.

32 J. Olivero-Verbel, L. Tao, B. Johnson-Restrepo, J. Guette-Fernández, R. Baldiris-Avila, I. O'byrne-Hoyos and K. Kannan, Perfluorooctanesulfonate and related fluorochemicals in biological samples from the north coast of Colombia, *Environ. Pollut.*, 2006, **142**, 367–372.

33 J. Rubarth, A. Dreyer, N. Guse, J. W. Einax and R. Ebinghaus, Perfluorinated compounds in red-throated divers from the German Baltic Sea: new findings from their distribution in 10 different tissues, *Environ. Chem.*, 2011, **8**, 419–428.

34 W. A. Gebbink and R. J. Letcher, Comparative tissue and body compartment accumulation and maternal transfer to eggs of perfluoroalkyl sulfonates and carboxylates in Great Lakes herring gulls, *Environ. Pollut.*, 2012, **162**, 40–47.

35 Y. Shi, J. Wang, Y. Pan and Y. Cai, Tissue distribution of perfluorinated compounds in farmed freshwater fish and human exposure by consumption, *Environ. Toxicol. Chem.*, 2012, **31**, 717–723.

36 Y. Wang, R. Vestergren, Y. Shi, D. Cao, L. Xu, Y. Cai, X. Zhao and F. Wu, Identification, Tissue Distribution, and Bioaccumulation Potential of Cyclic Perfluorinated Sulfonic Acids Isomers in an Airport Impacted Ecosystem, *Environ. Sci. Technol.*, 2016, **50**, 10923–10932.

37 K. H. Harada, S. Hashida, T. Kaneko, K. Takenaka, M. Minata, K. Inoue, N. Saito and A. Koizumi, Biliary excretion and cerebrospinal fluid partition of perfluorooctanoate and perfluorooctane sulfonate in humans, *Environ. Toxicol. Pharmacol.*, 2007, **24**, 134–139.

38 E. F. Houtz and D. L. Sedlak, Oxidative Conversion as a Means of Detecting Precursors to Perfluoroalkyl Acids in Urban Runoff, *Environ. Sci. Technol.*, 2012, **46**, 9342–9349.

39 A. K. Greaves, R. J. Letcher, C. Sonne, R. Dietz and E. W. Born, Tissue-Specific Concentrations and Patterns of Perfluoroalkyl Carboxylates and Sulfonates in East Greenland Polar Bears, *Environ. Sci. Technol.*, 2012, **46**, 11575–11583.

40 V. Lenters, N. Iszatt, J. Forns, E. Čechová, A. Kočan, J. Legler, P. Leonards, H. Stigum and M. Eggesbø, Early-life exposure to persistent organic pollutants (OCPs, PBDEs, PCBs, PFASs) and attention-deficit/hyperactivity disorder: A multi-pollutant analysis of a Norwegian birth cohort, *Environ. Int.*, 2019, **125**, 33–42.

41 G.-W. Lien, C.-C. Huang, J.-S. Shiu, M.-H. Chen, W.-S. Hsieh, Y.-L. Guo and P.-C. Chen, Perfluoroalkyl substances in cord blood and attention deficit/hyperactivity disorder symptoms in seven-year-old children, *Chemosphere*, 2016, **156**, 118–127.

42 R. Stein Cheryl and A. Savitz David, Serum Perfluorinated Compound Concentration and Attention Deficit/Hyperactivity Disorder in Children 5–18 Years of Age, *Environ. Health Perspect.*, 2011, **119**, 1466–1471.

43 C. R. Stein, D. A. Savitz and D. C. Bellinger, Perfluorooctanoate Exposure in a Highly Exposed Community and Parent and Teacher Reports of Behaviour in 6–12-Year-Old Children, *Paediatr. Perinat. Epidemiol.*, 2014, **28**, 146–156.

44 I. Quaak, M. De Cock, M. De Boer, M. Lamoree, P. Leonards and M. Van de Bor, Prenatal Exposure to Perfluoroalkyl Substances and Behavioral Development in Children, *Int. J. Environ. Res. Publ. Health*, 2016, **13**, 511.

45 F. Joan, V. Marc-Andre, I. Nina, N. Nikola, B. C. Carlsen, V. Martine, O. Costa, A. Ainara, S. Eva, H. Birgit Bjerre, W. Jürgen, L.-E. Maria-Jose, I. Jesus, H.-P. Irva, T. Gunnar, S. Hein, G. Mònica, L. Zeyan and E. Merete, Early Life Exposure to Perfluoroalkyl Substances (PFAS) and ADHD: A Meta-Analysis of Nine European Population-Based Studies, *Environ. Health Perspect.*, 2020, **128**, 057002.

46 Z. Liew, B. Ritz, E. C. Bonefeld-Jørgensen, T. B. Henriksen, E. A. Nohr, B. H. Bech, C. Fei, R. Bossi, O. S. von Ehrenstein, E. Streja, P. Uldall and J. Olsen, Prenatal Exposure to Perfluoroalkyl Substances and the Risk of Congenital Cerebral Palsy in Children, *Am. J. Epidemiol.*, 2014, **180**, 574–581.

47 H. Viberg, I. Lee and P. Eriksson, Adult dose-dependent behavioral and cognitive disturbances after a single neonatal PFHxS dose, *Toxicology*, 2013, **304**, 185–191.

48 K. Kawabata, H. Matsuzaki, S. Nukui, M. Okazaki, A. Sakai, Y. Kawashima and N. Kudo, Perfluorododecanoic Acid Induces Cognitive Deficit in Adult Rats, *Toxicol. Sci.*, 2017, **157**, 421–428.

49 Y. Wang, W. Liu, Q. Zhang, H. Zhao and X. Quan, Effects of developmental perfluorooctane sulfonate exposure on spatial learning and memory ability of rats and mechanism associated with synaptic plasticity, *Food Chem. Toxicol.*, 2015, **76**, 70–76.

50 T. S. Skogheim, G. D. Villanger, K. V. F. Weyde, S. M. Engel, P. Surén, M. G. Øie, A. H. Skogan, G. Biele, P. Zeiner, K. R. Øvergaard, L. S. Haug, A. Sabaredzovic and H. Aase, Prenatal exposure to perfluoroalkyl substances and associations with symptoms of attention-deficit/hyperactivity disorder and cognitive functions in preschool children, *Int. J. Hyg Environ. Health*, 2020, **223**, 80–92.

51 M. C. Power, T. F. Webster, A. A. Baccarelli and M. G. Weisskopf, Cross-Sectional Association between Polyfluoroalkyl Chemicals and Cognitive Limitation in the National Health and Nutrition Examination Survey, *Neuroepidemiology*, 2013, **40**, 125–132.

52 A. M. Vuong, K. Yolton, C. Xie, K. N. Dietrich, J. M. Braun, G. M. Webster, A. M. Calafat, B. P. Lanphear and A. Chen, Prenatal and childhood exposure to poly- and perfluoroalkyl substances (PFAS) and cognitive development in children at age 8 years, *Environ. Res.*, 2019, **172**, 242–248.

53 C. Liao, X. Li, B. Wu, S. Duan and G. Jiang, Acute Enhancement of Synaptic Transmission and Chronic Inhibition of Synaptogenesis Induced by Perfluorooctane Sulfonate through Mediation of Voltage-Dependent Calcium Channel, *Environ. Sci. Technol.*, 2008, **42**, 5335–5341.

54 X. Liu, Y. Jin, W. Liu, F. Wang and S. Hao, 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*, 2011, **25**, 1294–1301.

55 Y. Wang, H. Zhao, Q. Zhang, W. Liu and X. Quan, Perfluorooctane sulfonate induces apoptosis of hippocampal neurons in rat offspring associated with calcium overload, *Toxicol. Res.*, 2015, **4**, 931–938.

56 H. M. Dusza, P. H. Cenijn, J. H. Kamstra, R. H. S. Westerink, P. E. G. Leonards and T. Hamers, Effects of environmental pollutants on calcium release and uptake by rat cortical microsomes, *Neurotoxicology*, 2018, **69**, 266–277.

57 X. Fang, C. Wu, H. Li, W. Yuan and X. Wang, Elevation of intracellular calcium and oxidative stress is involved in perfluorononanoic acid–induced neurotoxicity, *Toxicol. Ind. Health*, 2018, **34**, 139–145.

58 C. Liao, T. Wang, L. Cui, Q. Zhou, S. Duan and G. Jiang, Changes in Synaptic Transmission, Calcium Current, and Neurite Growth by Perfluorinated Compounds Are Dependent on the Chain Length and Functional Group, *Environ. Sci. Technol.*, 2009, **43**, 2099–2104.

59 X. Liu, W. Liu, Y. Jin, W. Yu, L. Liu and H. Yu, Effects of subchronic perfluorooctane sulfonate exposure of rats on calcium-dependent signaling molecules in the brain tissue, *Arch. Toxicol.*, 2010, **84**, 471–479.

60 X. Liu, W. Liu, Y. Jin, W. Yu, F. Wang and L. Liu, Effect of gestational and lactational exposure to perfluorooctanesulfonate on calcium-dependent signaling molecules gene expression in rats' hippocampus, *Arch. Toxicol.*, 2010, **84**, 71–79.

61 N. Johansson, P. Eriksson and H. Viberg, 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.*, 2009, **108**, 412–418.

62 J. H. Oh, E.-Y. Kim and T.-J. Nam, Phycoerythrin Peptide from Pyropia yezoensis Alleviates Endoplasmic Reticulum Stress Caused by Perfluorooctane Sulfonate-Induced Calcium Dysregulation, *Mar. Drugs*, 2018, **16**, 44.

63 J. Zhang, X. Shao, B. Zhao, L. Zhai, N. Liu, F. Gong, X. Ma, X. Pan, B. Zhao, Z. Yuan and X. Zhang, Neurotoxicity of perfluorooctanoic acid and post-exposure recovery due to blueberry anthocyanins in the planarians Dugesia japonica, *Environ. Pollut.*, 2020, **263**, 114471.

64 N. Yu, S. Wei, M. Li, J. Yang, K. Li, L. Jin, Y. Xie, J. P. Giesy, X. Zhang and H. Yu, Effects of Perfluorooctanoic Acid on Metabolic Profiles in Brain and Liver of Mouse Revealed by a High-throughput Targeted Metabolomics Approach, *Sci. Rep.*, 2016, **6**, 23963.

65 R. Salgado, S. López-Doval, N. Pereiro and A. Lafuente, Perfluorooctane sulfonate (PFOS) exposure could modify the dopaminergic system in several limbic brain regions, *Toxicol. Lett.*, 2016, **240**, 226–235.

66 R. M. Foguth, R. W. Flynn, C. de Perre, M. Iacchetta, L. S. Lee, M. S. Sepúlveda and J. R. Cannon, Developmental exposure to perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) selectively decreases brain dopamine levels in Northern leopard frogs, *Toxicol. Appl. Pharmacol.*, 2019, **377**, 114623.

67 S. Hallgren and H. Viberg, Postnatal exposure to PFOS, but not PBDE 99, disturb dopaminergic gene transcription in the mouse CNS, *Environ. Toxicol. Pharmacol.*, 2016, **41**, 121–126.

68 R. M. Foguth, T. D. Hoskins, G. C. Clark, M. Nelson, R. W. Flynn, C. de Perre, J. T. Hoverman, L. S. Lee, M. S. Sepúlveda and J. R. Cannon, Single and mixture per- and polyfluoroalkyl substances accumulate in developing Northern leopard frog brains and produce complex neurotransmission alterations, *Neurotoxicol. Teratol.*, 2020, **81**, 106907.

69 T. A. Slotkin, E. A. MacKillop, R. L. Melnick, K. A. Thayer and F. J. Seidler, Developmental Neurotoxicity of Perfluorinated Chemicals Modeled in Vitro, *Environ. Health Perspect.*, 2008, **116**, 716–722.

70 Y. Long, Y. Wang, G. Ji, L. Yan, F. Hu and A. Gu, Neurotoxicity of Perfluorooctane Sulfonate to Hippocampal Cells in Adult Mice, *PloS One*, 2013, **8**, e54176.

71 H. F. Berntsen, A. Moldes-Anaya, C. G. Bjørklund, L. Ragazzi, T. M. Haug, R. A. U. Strandabø, S. Verhaegen, R. E. Paulsen, E. Ropstad and R. A. Tasker, Perfluoroalkyl acids potentiate glutamate excitotoxicity in rat cerebellar granule neurons, *Toxicology*, 2020, **445**, 152610.

72 C. Liao, L. Cui, Q. Zhou, S. Duan and G. Jiang, Effects of perfluorooctane sulfonate on ion channels and glutamate-activated current in cultured rat hippocampal neurons, *Environ. Toxicol. Pharmacol.*, 2009, **27**, 338–344.

73 N. Johansson, A. Fredriksson and P. Eriksson, Neonatal exposure to perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) causes neurobehavioural defects in adult mice, *Neurotoxicology*, 2008, **29**, 160–169.

74 K. L. Hassell, T. L. Coggan, T. Cresswell, A. Kolobaric, K. Berry, N. D. Crosbie, J. Blackbeard, V. J. Pettigrove and B. O. Clarke, Dietary Uptake and Depuration Kinetics of Perfluorooctane Sulfonate, Perfluorooctanoic Acid, and Hexafluoropropylene Oxide Dimer Acid (GenX) in a Benthic Fish, *Environ. Toxicol. Chem.*, 2020, **39**, 595–603.

75 D. M. Pizzurro, M. Seeley, L. E. Kerper and B. D. Beck, Interspecies differences in perfluoroalkyl substances (PFAS) toxicokinetics and application to health-based criteria, *Regul. Toxicol. Pharmacol.*, 2019, **106**, 239–250.

76 X. Liu, K. Van Natta, H. Yeo, O. Vilenski, P. E. Weller, P. D. Worboys and M. Monshouwer, Unbound Drug Concentration in Brain Homogenate and Cerebral Spinal Fluid at Steady State as a Surrogate for Unbound Concentration in Brain Interstitial Fluid, *Drug Metab. Dispos.*, 2009, **37**, 787–793.

77 X. Wang, B. Li, W.-D. Zhao, Y.-J. Liu, D.-S. Shang, W.-G. Fang and Y.-H. Chen, Perfluorooctane sulfonate triggers tight junction "opening" in brain endothelial cells via phosphatidylinositol 3-kinase, *Biochem. Biophys. Res. Commun.*, 2011, **410**, 258–263.

78 N. J. Abbott, L. Rönnbäck and E. Hansson, Astrocyte–endothelial interactions at the blood–brain barrier, *Nat. Rev. Neurosci.*, 2006, **7**, 41–53.

79 L. Huang, B. Li, X. Li, G. Liu, R. Liu, J. Guo, B. Xu, Y. Li and W. Fang, Significance and Mechanisms of P-glycoprotein in

Central Nervous System Diseases, *Curr. Drug Targets*, 2019, **20**, 1141–1155.

80 A. Georgantzopoulou, E. Skoczyńska, J. H. J. V. den Berg, W. Brand, S. Legay, S. G. Klein, I. M. C. M. Rietjens and A. J. Murk, P-gp efflux pump inhibition potential of common environmental contaminants determined in vitro, *Environ. Toxicol. Chem.*, 2014, **33**, 804–813.

81 C. N. Stevenson, L. A. MacManus-Spencer, T. Luckenbach, R. G. Luthy and D. Epel, New Perspectives on Perfluorochemical Ecotoxicology: Inhibition and Induction of an Efflux Transporter in the Marine Mussel, *Mytilus californianus*, *Environ. Sci. Technol.*, 2006, **40**, 5580–5585.

82 D. D. Mruk and C. Y. Cheng, The Mammalian Blood-Testis Barrier: Its Biology and Regulation, *Endocr. Rev.*, 2015, **36**, 564–591.

83 A. C. A. Dankers, M. J. E. Roelofs, A. H. Piersma, F. C. G. J. Sweep, F. G. M. Russel, M. van den Berg, M. B. M. van Duursen and R. Masereeuw, Endocrine Disruptors Differentially Target ATP-Binding Cassette Transporters in the Blood-Testis Barrier and Affect Leydig Cell Testosterone Secretion In Vitro, *Toxicol. Sci.*, 2013, **136**, 382–391.

84 R. R. Crawford, P. K. Potukuchi, E. G. Schuetz and J. D. Schuetz, Beyond Competitive Inhibition: Regulation of ABC Transporters by Kinases and Protein-Protein Interactions as Potential Mechanisms of Drug-Drug Interactions, *Drug Metab. Dispos.*, 2018, **46**, 567–580.

85 R. W. Mitchell and G. M. Hatch, Fatty acid transport into the brain: Of fatty acid fables and lipid tails, *Prostagl. Leukot. Essent. Fat. Acids*, 2011, **85**, 293–302.

86 H. Pajouhesh and G. R. Lenz, Medicinal chemical properties of successful central nervous system drugs, *Neurotherapeutics*, 2005, **2**, 541–553.

87 L. Zhang, X.-M. Ren and L.-H. Guo, Structure-Based Investigation on the Interaction of Perfluorinated Compounds with Human Liver Fatty Acid Binding Protein, *Environ. Sci. Technol.*, 2013, **47**, 11293–11301.

88 T. S. Carpenter, D. A. Kirshner, E. Y. Lau, S. E. Wong, J. P. Nilmeier and F. C. Lightstone, A Method to Predict Blood–Brain Barrier Permeability of Drug-Like Compounds Using Molecular Dynamics Simulations, *Biophys. J.*, 2014, **107**, 630–641.

89 K. Abraham, A. H. El-Khatib, T. Schwerdtle and B. H. Monien, Perfluorobutanoic acid (PFBA): no high-level accumulation in human lung and kidney tissue, *Int. J. Hyg Environ. Health*, 2021, **237**, 113830.

90 *History and Use of Per- and Polyfluoroalkyl Substances (PFAS)*, https://pfas1.itrcweb.org/fact_sheets_page/PFAS_Fact_Sheet_History_and_Use_April2020.pdf, (accessed May 2021).

91 W. Wen, X. Xia, D. Hu, D. Zhou, H. Wang, Y. Zhai and H. Lin, Long-Chain Perfluoroalkyl acids (PFAAs) Affect the Bioconcentration and Tissue Distribution of Short-Chain PFAAs in Zebrafish (Danio rerio), *Environ. Sci. Technol.*, 2017, **51**, 12358–12368.

92 B. C. Kelly, M. G. Ikonomou, J. D. Blair, B. Surridge, D. Hoover, R. Grace and F. A. P. C. Gobas, Perfluoroalkyl Contaminants in an Arctic Marine Food Web: Trophic Magnification and Wildlife Exposure, *Environ. Sci. Technol.*, 2009, **43**, 4037–4043.

93 F. Rigét, R. Bossi, C. Sonne, K. Vorkamp and R. Dietz, Trends of perfluorochemicals in Greenland ringed seals and polar bears: indications of shifts to decreasing trends, *Chemosphere*, 2013, **93**, 1607–1614.

94 M. Smithwick, D. C. G. Muir, S. A. Mabury, K. R. Solomon, J. W. Martin, C. Sonne, E. W. Born, R. J. Letcher and R. Dietz, Perfluoroalkyl contaminants in liver tissue from East Greenland polar bears (Ursus maritimus), *Environ. Toxicol. Chem.*, 2005, **24**, 981–986.

95 I. Zabaleta, E. Bizkarguenaga, U. Izagirre, N. Negreira, A. Covaci, J. P. Benskin, A. Prieto and O. Zuloaga, Biotransformation of 8:2 polyfluoroalkyl phosphate diester in gilthead bream (Sparus aurata), *Sci. Total Environ.*, 2017, **609**, 1085–1092.

96 A. Vidal, F. Lafay, G. Daniele, E. Vulliet, E. Rochard, J. Garric and M. Babut, Does water temperature influence the distribution and elimination of perfluorinated substances in rainbow trout (Oncorhynchus mykiss)?, *Environ. Sci. Pollut. Res.*, 2019, **26**, 16355–16365.

97 Y. Li, B. Men, Y. He, H. Xu, M. Liu and D. Wang, Effect of single-wall carbon nanotubes on bioconcentration and toxicity of perfluorooctane sulfonate in zebrafish (Danio rerio), *Sci. Total Environ.*, 2017, **607–608**, 509–518.

98 M. Ulhaq, M. Sundström, P. Larsson, J. Gabrielsson, Å. Bergman, L. Norrgren and S. Örn, Tissue uptake, distribution and elimination of 14C-PFOA in zebrafish (Danio rerio), *Aquat. Toxicol.*, 2015, **163**, 148–157.

99 W. Wen, X. Xia, D. Zhou, H. Wang, Y. Zhai, H. Lin, J. Chen and D. Hu, Bioconcentration and tissue distribution of shorter and longer chain perfluoroalkyl acids (PFAAs) in zebrafish (Danio rerio): effects of perfluorinated carbon chain length and zebrafish protein content, *Environ. Pollut.*, 2019, **249**, 277–285.

100 H. Dong, G. Lu, Z. Yan, J. Liu and Y. Ji, Molecular and phenotypic responses of male crucian carp (Carassius auratus) exposed to perfluorooctanoic acid, *Sci. Total Environ.*, 2019, **653**, 1395–1406.

101 L. Giari, F. Vincenzi, S. Badini, C. Guerranti, B. S. Dezfuli, E. A. Fano and G. Castaldelli, Common carp Cyprinus carpio responses to sub-chronic exposure to perfluorooctanoic acid, *Environ. Sci. Pollut. Res.*, 2016, **23**, 15321–15330.

102 L. Cui, Q. Zhou, C. Liao, J. Fu and G. Jiang, Studies on the Toxicological Effects of PFOA and PFOS on Rats Using Histological Observation and Chemical Analysis, *Arch. Environ. Contam. Toxicol.*, 2008, **56**, 338.

103 B. Gao, X. He, W. Liu, H. Zhang, N. Saito and S. Tsuda, Distribution of perfluoroalkyl compounds in rats: indication for using hair as bioindicator of exposure, *J. Expo. Sci. Environ. Epidemiol.*, 2015, **25**, 632–638.

104 L. Liu, W. Liu, J. Song, H. Yu, Y. Jin, K. Oami, I. Sato, N. Saito and S. Tsuda, A comparative study on oxidative

damage and distributions of perfluorooctane sulfonate (PFOS) in mice at different postnatal developmental stages, *J. Toxicol. Sci.*, 2009, **34**, 245–254.

105 M. M. Phillips, M. J. A. Dinglasan-Panlilio, S. A. Mabury, K. R. Solomon and P. K. Sibley, Fluorotelomer Acids are More Toxic than Perfluorinated Acids, *Environ. Sci. Technol.*, 2007, **41**, 7159–7163.

106 T. Sandersfeld, F. C. Mark and R. Knust, Temperature-dependent metabolism in Antarctic fish: Do habitat temperature conditions affect thermal tolerance ranges?, *Polar Biol.*, 2017, **40**, 141–149.

107 J. Li, F. Guo, Y. Wang, J. Zhang, Y. Zhong, Y. Zhao and Y. Wu, Can nail, hair and urine be used for biomonitoring of human exposure to perfluorooctane sulfonate and perfluorooctanoic acid?, *Environ. Int.*, 2013, **53**, 47–52.

108 A. Alves, G. Jacobs, G. Vanermen, A. Covaci and S. Voorspoels, New approach for assessing human perfluoroalkyl exposure via hair, *Talanta*, 2015, **144**, 574–583.

109 Y. Wang, Y. Zhong, J. Li, J. Zhang, B. Lyu, Y. Zhao and Y. Wu, Occurrence of perfluoroalkyl substances in matched human serum, urine, hair and nail, *J. Environ. Sci.*, 2018, **67**, 191–197.

110 Y. Wang, Y. Shi, R. Vestergren, Z. Zhou, Y. Liang and Y. Cai, Using hair, nail and urine samples for human exposure assessment of legacy and emerging per- and polyfluoroalkyl substances, *Sci. Total Environ.*, 2018, **636**, 383–391.

111 L. E. Jarrard, On the role of the hippocampus in learning and memory in the rat, *Behav. Neural. Biol.*, 1993, **60**, 9–26.

112 A. J. Verkhratsky and O. H. Petersen, Neuronal calcium stores, *Cell Calcium*, 1998, **24**, 333–343.

113 M. J. Berridge, P. Lipp and M. D. Bootman, The versatility and universality of calcium signalling, *Nat. Rev. Mol. Cell Biol.*, 2000, **1**, 11–21.

114 E. R. Kandel, The molecular biology of memory: cAMP, PKA, CRE, CREB-1, CREB-2, and CPEB, *Mol. Brain*, 2012, **5**, 14.

115 H. Lodish, A. Berk, S. L. Zipursky, P. Matsudaira, D. Baltimore and J. Darnell, *Neurotransmitters, Synapses, and Impulse Transmission, Molecular Cell Biology*, 2000.

116 W. J. McEntee and T. H. Crook, Glutamate: its role in learning, memory, and the aging brain, *Psychopharmacology*, 1993, **111**, 391–401.

117 D. G. Nicholls and S. L. Budd, Mitochondria and neuronal glutamate excitotoxicity, *Biochim. Biophys. Acta Bioenerg.*, 1998, **1366**, 97–112.

118 G. M. Webster, S. A. Venners, A. Mattman and J. W. Martin, Associations between Perfluoroalkyl acids (PFASs) and maternal thyroid hormones in early pregnancy: a population-based cohort study, *Environ. Res.*, 2014, **133**, 338–347.

119 K. Inoue, B. Ritz, S. L. Andersen, C. H. Ramlau-Hansen, B. B. Høyer, B. H. Bech, T. B. Henriksen, E. C. Bonefeld-Jørgensen, J. Olsen and Z. Liew, Perfluoroalkyl Substances and Maternal Thyroid Hormones in Early Pregnancy; Findings in the Danish National Birth Cohort, *Environ. Health Perspect.*, 2019, **127**, 117002.

120 R. Aimuzi, K. Luo, R. Huang, X. Huo, M. Nian, F. Ouyang, Y. Du, L. Feng, W. Wang and J. Zhang, Perfluoroalkyl and polyfluroalkyl substances and maternal thyroid hormones in early pregnancy, *Environ. Pollut.*, 2020, **264**, 114557.

121 G. T. Ankley, R. S. Bennett, R. J. Erickson, D. J. Hoff, M. W. Hornung, R. D. Johnson, D. R. Mount, J. W. Nichols, C. L. Russom, P. K. Schmieder, J. A. Serrrano, J. E. Tietge and D. L. Villeneuve, Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment, *Environ. Toxicol. Chem.*, 2010, **29**, 730–741.

122 J. Park, I. Wetzel, I. Marriott, D. Dréau, C. D'Avanzo, D. Y. Kim, R. E. Tanzi and H. Cho, A 3D human triculture system modeling neurodegeneration and neuroinflammation in Alzheimer's disease, *Nat. Neurosci.*, 2018, **21**, 941–951.

123 L. Song, X. Yuan, Z. Jones, K. Griffin, Y. Zhou, T. Ma and Y. Li, Assembly of Human Stem Cell-Derived Cortical Spheroids and Vascular Spheroids to Model 3-D Brain-like Tissues, *Sci. Rep.*, 2019, **9**, 5977.

