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Presence of per- and poly-fluoroalkyl substances (PFAS) in brain samples of marine mammals from the St. Lawrence Estuary and Gulf, Canada

Nejumal Kannankeril Khalid, [a] Amira Aker,[b] Stéphane Lair[c] and Sébastien Sauvé *[a]

This study focused on measuring 80 target per- and poly-fluoroalkyl substances (PFAS) in brain samples from various marine mammals, including: harbor seal, gray seal, harp seal, harbor porpoise, white-sided dolphin, white-beaked dolphin, and True's beaked whale, all collected from the St. Lawrence Estuary and Gulf. A total of 34 PFAS compounds were detected in these mammals. The geometric mean of the detected PFAS levels ranged from 0.02 ng g^{-1} wet weight (ww) to 41 ng g^{-1} ww. Notably, the detection frequency for PFOS was very high at 97.5%. For individual long-chain C9 to C13 perfluorocarboxylic acids (PFCAs), the detection frequencies ranged from 77% to 95%. In contrast, the detection frequencies for C3 to C6 PFCAs specifically PFHxA, PFBA, and PFPrA were much lower, ranging from 2.5% to 5%. This indicates a greater tendency for long-chain PFCAs to accumulate compared to shorter-chain variants. Additionally, a higher occurrence of PFSAs and PFCAs was observed across all species examined. Interesting findings emerged regarding species at higher trophic levels, such as the white-sided dolphin, white-beaked dolphin, and True's beaked whale, which exhibited significant levels of fluorinated alkyl substance acids (FASAs) and their alternatives (FASAAs). In a comparative analysis of PFAS distribution in liver, muscle, and brain tissues, correlation analysis revealed that concentrations in these tissues are positively correlated with each other. This study highlights concerns regarding the impact of PFAS on marine aquatic systems and potential neurocognitive impacts on their brain functions.

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Environmental significance

Marine mammals are at significant risk from PFAS due to their toxicity, ability to bioaccumulate, and persistence in the environment. Biomagnification is the process through which these chemicals accumulate mainly in the fat tissues of organisms, increasing their concentration higher up the food chain. As top predators, marine mammals such as dolphins, seals, and whales are particularly vulnerable because PFAS levels often exceed safe limits and accumulate in their organs, including brain tissues. Prolonged exposure to these compounds can adversely affect their survival, reproduction, and immune function, endangering species that are already at risk. Additionally, PFAS contamination highlights the broader issues of ocean pollution and the long-lasting impact of human activities.

1. Introduction

The world faces significant challenges due to contamination from organic pollutants in various aquatic and terrestrial environments. This contamination largely results from the release of harmful chemicals into the environment through human activities. Such pollutants can have detrimental ecotoxicological effects on both humans and various organisms in aquatic and terrestrial ecosystems.[1–4] One class of chemicals of particular concern is per- and polyfluoroalkyl substances (PFAS). These manufactured chemicals are commonly found in various consumer and commercial products due to their properties, including high stability and strong water and oil repellency.[1,3,5]

The widespread use of PFAS can lead to their excessive detection in various environments, including surface water, groundwater, and sediments.[6–8] PFAS have been found in marine mammals, seabirds, guineafowl, mollusks, and crustaceans.[9–12] Major pathways of PFAS presence in aquatic systems include wastewater treatment plants (WWTPs), landfill leachate, and urban runoff.[5] This has led to high PFAS levels in marine aquatic environments, and ubiquitous PFAS exposure in the food webs of the North Atlantic Ocean.[13] As regulations evolve, significant changes are occurring in the PFAS manufacturing landscape. This has led to an increased production of PFAS alternatives and the formation of byproducts, which also contribute to the contamination.[5,14] A target of

[a]*Department of Chemistry, Université de Montréal, Montréal, Québec H3C 3J7, Canada. E-mail: sebastien.sauve@umontreal.ca*

[b]*School of Public Health, Boston University, Boston, MA, USA*

[c]*Centre québécois sur la santé des animaux sauvages, Canadian Wildlife Health Cooperative, Faculté de médecine vétérinaire, Université de Montréal, Montréal, Québec H3C 3J7, Canada*

30 ng L^{-1} for 25 PFAS compounds, established by Health Canada, was adopted in August 2024 in line with USEPA Method 533. However, the detection of unregulated PFAS in aquatic mammals shows the need for a broader regulatory approach. While existing regulations are in place, stronger measures are required to effectively protect our marine ecosystems. These chemicals and their metabolites are highly persistent in aquatic systems and easily accumulate in various tissues, such as muscle, liver, kidney, blood, and brain of different species.[15–18] PFAS accumulate more effectively in organs with high blood perfusion,[19] and as such, the brain is not recognized as a primary site for the accumulation of PFAS compared to other tissues. However, accumulation can still occur through alternative mechanisms, such as the disruption of the tight junctions in the blood–brain barrier (BBB) and the transport of PFAS across the BBB *via* specific transporters.[20–22]

Marine mammals absorb PFAS largely through diet, leading to increased concentrations in higher trophic species within the food web. Moreover, this has potential hazardous effects on marine organisms, particularly affecting body size, immunotoxicity, growth rate, and fecundity.[23] PFAS concentrations in marine mammals vary significantly by species, tissue type, and geographical location.[5,24,25] The concentration of PFAS in 15 different marine mammal species was first reported by Kannan *et al.* in 2001, where they found PFAS levels in liver and blood samples.[26] PFAS presence has been reported in various marine mammals worldwide.[27–34] Spaan *et al.* reported that cetaceans and killer whales from Greenland exhibit the highest concentration of $\sum$36 PFAS in their liver, with concentrations up to 614 ng g^{-1} wet weight.[35] Interestingly, the occurrence of 7 : 3 fluorotelomer carboxylic acid (7 : 3 FTCA) was first reported in polar bears, with a concentration of approximately 1000 ng g^{-1} wet weight, and also in cetaceans, where the concentration ranged from <6 to 190 ng g^{-1} wet weight.[35] The widespread occurrence of PFAS has also been reported in tropical marine mammals, indicating significant concentrations in the Indo-Pacific humpback dolphin and finless porpoise.[36] These exposures have also been linked to wildlife health. For example, a substantial concentration of PFAS, at 923 ng g^{-1} lipid weight, can affect the immune system in male walruses.[37] Additionally, PFAS can negatively impact the endocrine systems of marine mammals, which affects the neurological health of these species.[31,38]

Recent studies have found that PFAS are present in the aquatic environments of the St. Lawrence River and its estuary in Quebec. Notably, the concentration of PFBA in the river water exceeds that of the more commonly monitored substances, PFOA and PFOS. Additionally, both established and newly identified PFAS, including betaines and sulfonamides, have been detected in water, sediment, and the aquatic food web, affecting everything from plants to apex predator fish such as Smallmouth bass.[39,40] While most sediments in Quebec showed low levels of PFAS, typically below toxicity criteria for aquatic organisms, sediment samples from areas impacted by firefighting foam (AFFF) displayed significantly higher amounts of PFAS, particularly PFOS, compared to reference sites.[41] Concerns have been raised regarding apex predators and potential ecological hazards due to the discovery that PFAS, especially long-chain PFAS such as PFOS and C10–C13 perfluorocarboxylates (PFCAs), accumulate and magnify within the food web. The concentration of PFAS in the river significantly increases due to wastewater discharge from Montreal. Fish located downstream of these effluent outfalls show higher levels of PFAS concentration.[42] According to a previous study, the concentration of $\sum$29PFAS in tap water from the Great Lakes and St. Lawrence River was significantly higher at 14 ng L^{-1}, compared to only 5 ng L^{-1} in other Canadian sources. The study identified several important substances, including PFCAs (C4–C14), perfluoroalkane sulfonates (PFSAs C4, C6, C8), and precursors such as the 5 : 3 fluorotelomer carboxylate (5 : 3 FTCA). Additionally, suspect screening revealed the presence of cyclic PFECHS ($\leq$4 ng L^{-1}), FBSA, FHxSA, and ultra-short chain PFSAs (C2–C3).[43]

We previously documented the accumulation of PFAS in liver and muscle samples from various species collected from the St. Lawrence Estuary and Gulf in Quebec, Canada, including harbor seals, gray seals, harp seals, hooded seals, harbor porpoises, white-sided dolphins, white-beaked dolphins, and True's beaked whales.[5] Our results showed greater accumulation of PFAS in liver compared to muscle samples, with long-chain perfluorocarboxylic acids (PFCAs) such as PFNA, PFDA, PFUnA, PFTrDA, and PFHxDA being particularly dominant. This current study is a continuation of our previous work, and the aim is to analyze the occurrence of PFAS in brain samples from the same species and specimens.

2. Materials and methods

2.1 Chemical and standards

Certified standards of PFAS were obtained from Wellington Laboratories in Guelph, Ontario, Canada; Synquest Laboratories in Alachua, Florida, USA; and DuPont in Wilmington, Delaware, USA. Additional details regarding PFAS, internal standards, and other chemicals used in this study can be found in the SI.

2.2 Sample collections

Brain samples were collected from archived subsamples from the same specimen for which muscle and liver concentrations of PFAS have already been reported.[5] Such samples were initially collected from marine mammals (arranged in the order of trophic levels, harbor seal (*Phoca vitulina*, $n = 11$), gray seal (*Halichoerus grypus*, $n = 1$), harp seal (*Pagophilus groenlandicus*, $n = 1$), harbor porpoise (*Phocoena phocoena*, $n = 22$), white-sided dolphin (*Lagenorhynchus acutus*, $n = 1$), white-beaked dolphin (*Lagenorhynchus albirostris*, $n = 1$), and True's beaked whale (*Mesoplodon mirus*, $n = 1$)) located dead by the shoreline along the St. Lawrence Estuary and Gulf, Canada. These carcasses were transported, usually frozen, by the *Réseau québécois d'urgences pour les mammifères marins* to the Canadian Wildlife Health Cooperative Quebec Regional Center where sampling and a full necropsy were performed.

The sample collection for this study presented significant challenges, primarily due to our reliance on marine mammals

that had died of natural causes and were subsequently recovered from the estuary. After the expert veterinarian from the Faculté de médecine vétérinaire at the Université de Montréal meticulously conducted necropsies. Following this thorough examination, tissues were collected from the brain, liver, and muscle and used later for the analysis of PFAS. Due to the random occurrence of sea mammals that naturally or accidently die on the shores of the St-Lawrence, the numbers of some species are higher, while others are lower.

2.3 Sample preparation

Brain samples were stored at −20 °C before extraction. Sample preparation was performed as follows: prior to analysis, a 300 mg of freeze-dried wet weight aliquot of the marine specimen's brain tissue sample was weighed in a 15 mL polypropylene (PP) tube. We did not target any specific brain region for this project and used the archived samples. The samples were spiked with 100 μL of a mix of surrogate internal standards (prepared at 100 ng mL^{-1} in pure MeOH, more internal standards methodological details are included in the SI Table S2), and a 1 hour waiting time was applied. Subsequently, 5 mL of MeOH was added as the extraction solvent, followed by high-speed vortexing (3200 rpm, 0.5 min), ultrasonication in ice-cold water (10 min), centrifugation (6000 rpm, 5 min), and transfer of the supernatant to a fresh 15 mL PP tube (triplicate samples). The combined supernatants were concentrated to 1.8 mL and subjected to cleanup with Supelclean ENVI-Carb cartridges (250 mg). Finally, the prepared samples were aliquoted for analysis in LCMS vials (200 μL). This procedure is based on a protocol we previously validated and published in our previous studies.[5]

QA/QC was conducted for each LC/MS batch sequence using an in-house protocol. Matrix-matched calibration curves all had a determination coefficient (R^2 > 0.99). Method blanks are injected in the sequence and showed low levels of specific PFAS.

3. Results and discussion

A total of 34 out of 80 target PFAS compounds, including PFSAs (perfluoroalkane sulfonic acid), PFCAs (perfluoroalkyl carboxylic acids), cyclic PFASAs (cyclic perfluoroalkane sulfonic acid), FASAs/FASAAs (fluoroalkane sulfonamides/fluoroalkane sulfonamide alcohols), FTCAs/FTUCAs (fluorotelomer carboxylic acids/fluorotelomer unsaturated carboxylic acid), FTSAs (fluorotelomer sulfonic acids), ether-PFAS (ether-linked polyfluoroalkyl substances), and ESI+ ECF precursors (electrospray ionization positive electrochemical fluorination derivatives), were detected. The geometric mean of detected PFAS ranged from 0.02 ng g^{-1} wet weight (ww) to 41 ng g^{-1} ww. The average concentration of individual C9 to C13 perfluorocarboxylic acids (PFCAs) varied from 1.8 ng g^{-1} ww to 23.1 ng g^{-1} ww (see SI), with a detection frequency (DF) of 77% to 95%. In contrast, the concentrations of C3 to C6 PFCAs (specifically PFHxA, PFBA, and PFPrA) ranged from 0.31 ng g^{-1} ww to 0.81 ng g^{-1} ww, with a DF of 2.5% to 5%. This indicates a greater tendency of brain tissues to accumulate long-chain PFCAs relative to shorter ones. The significant presence of C9–C14 PFCAs in the brain can be attributed to differences in the levels and types of binding proteins in cerebrospinal fluid compared to whole blood.[44] Additionally, variations in the transport mechanisms of specific PFAS across the blood–brain barrier also play a role.[44] Our study observed a higher abundance of PFUnA and PFTrDA in brain samples from various marine mammals, with a similar trend noted in juvenile seabirds, where 44% of long-chain PFCAs were found in brain samples.[45]

Among the target PFAS compounds, PFOS was the most prevalent, showing a detection frequency of 97.5% and a geometric mean concentration of 41.3 ng g^{-1} wet weight, and ranged from 0.30 ng g^{-1} ww to 287 ng g^{-1} ww. The concentrations of PFOS in brain samples are lower than those detected in other tissues, such as liver, muscle, plasma, and blubber, of marine mammals across different regions and temporal studies.[5,11,31,45,46] Research indicates that polar bears show a brain concentration of PFAS around 25 ng g^{-1} wet weight, with PFOS comprising nearly 91% of this total.[21] Juvenile seabirds also show significant levels of PFOS detected in their brain samples.[45]

FOSA has a high detection frequency (65%) in the brain of the selected marine mammals, with concentrations ranging from 0.85 ng g^{-1} ww to 34 ng g^{-1} ww, and a median concentration of 2.3 ng g^{-1} ww. FOSA was also detected in 80% of fish samples (liver and muscle) from the Pearl River Delta, China.[47] Many other studies have shown that FOSA is present in mammals at significant concentrations.[48–50] FOSA was also found in plasma (5.4 ng mL^{-1}) in humans, and has been associated with the occurrence or development of glioma.[22] Furthermore, numerous studies have reported a concentration of FOSA in human serum.[51–53]

PFSAs are a type of PFAS characterized by a fully fluorinated carbon chain that is bonded to a sulfonic acid group (–SO_3H). These compounds are highly resistant to degradation due to their strong carbon–fluorine bonds, which provide impressive thermal, chemical, and environmental stability. Furthermore, this durability allows them to persist in the environment and bioaccumulate in living organisms.[54–56] The PFSA class includes substances like PFHxS, PFDS, and PFHpS, while the PFCA class specifically includes PFOA. These substances can exhibit DF ranging from 35% to 57% (Table 1). The environmental behaviour, bioaccumulation, and toxicity of PFAS in marine mammals are significantly influenced by the length of their carbon chains. For instance, in our study, we observed that PFDS (C10) has a DF of 52.5% and a mean concentration of 3.3 ng g^{-1} ww, similarly, PFNA (C9) has a DF of 77.5% and a mean concentration of 1.8 ng g^{-1} ww, these are examples of long-chain PFAS, which tend to be more persistent and bioaccumulative than their shorter-chain equivalent, PFPrA (C3) and PFPrS (C3), both of which have a DF of 2.5%. This variation is primarily due to the higher affinity long-chain PFAS have for binding proteins, particularly albumin and fatty acid transport proteins, leading to greater accumulation in marine mammals.[57–59] For example, PFDS is more hydrophobic and more readily accumulates in marine mammals than PFPrS, which is attributed to its longer perfluoroalkyl chain and sulfonate group. Shorter-chain PFAS, such as PFHpS (C7) with

Table 1 Detection frequency (D.F.), geometric mean (ng g^{-1}), minimum, maximum, median, and % contribution of targeted PFAS

Class	Target PFAS	Detection frequency (D.F., %)	Geometric mean (ng g^{-1}, ww)	Min (ng g^{-1}, ww)	Max (ng g^{-1}, ww)	Median	% contribution
PFSA	PFOS	97.5	41.3	0.30	287	56.0	25.1
	PFHxS	57.5	1.40	0.002	14.8	1.89	
	PFDS	52.5	3.25	0.90	10.9	3.22	
	PFHpS	35	0.43	0.15	2.79	0.44	
	PFBS	7.5	0.04	0.03	0.05	0.03	
	PFEtS	5	0.10	0.04	0.23	0.14	
	PFPrS	2.5	0.23	—	—	—	
	PFNS	2.5	0.19	—	—	—	
PFCA	PFUnA	95	11.0	0.19	47.4	12.9	50.2
	PFTrDA	95	23.1	0.23	102	25.4	
	PFDoA	92.5	5.94	1.19	20.9	5.79	
	PFDA	90	2.80	0.40	10.0	2.75	
	PFNA	77.5	1.81	0.62	10.3	1.76	
	PFOA	35	0.17	0.07	1.13	0.12	
	PFTeDA	15	1.89	1.11	6.25	1.48	
	PFHpA	7.5	0.15	0.11	0.25	0.14	
	PFBA	5	0.81	0.69	0.94	0.82	
	PFHxA	5	0.25	0.18	0.33	0.26	
	PFPrA	2.5	0.31	—	—	—	
FASA	FBSA	65	2.46	0.36	18.3	2.10	15.2
	FOSA	65	2.82	0.85	34.7	2.32	
	FHxSA	27.5	1.34	0.23	7.40	1.12	
FTAB/FTB	6 : 2-FTAB	32.5	0.32	0.13	0.85	0.31	3.6
	5 : 3-FtB	2.5	0.25	—	—	—	
	5 : 1 : 2-FtB	2.5	0.28	—	—	—	
FTSA	6 : 2-FTS	12.5	0.59	0.32	0.84	0.66	1.5
	4 : 2-FTS	2.5	0.31	—	—	—	
FTCAs/FTUCAs	7 : 3-acid	7.5	2.72	0.36	7.74	4.45	1.7
	5 : 3-acid	5	1.40	1.19	1.64	1.42	
	8 : 2-FTCA	5	0.21	0.004	10.0	5.00	
Ether-PFAS	PFMBA	2.5	0.24	—	—	—	0.5
	PFEESA	2.5	0.02	—	—	—	
Cyclic PFSA	PFECHS	22.5	0.62	0.24	2.04	0.57	2.2

a DF of 35 and PFPrA (C3) with a DF of 2.5%, are more environmentally mobile due to their decreased hydrophobicity, which limits their ability to partition into tissues. These differences underscore the critical impact of chain length on the environmental persistence of PFAS.[60] Long-chain PFAS are particularly hazardous to marine ecosystems because of their increased capacity for bioaccumulation.[46] Table 2 illustrates the total concentrations of PFAS observed in the current study. This data are presented to facilitate a comprehensive comparison with previously reported concentrations of PFAS found in various tissues and species, enhancing our understanding of the prevalence and distribution of these substances across different biological contexts.

3.1 Distribution of PFAS in mammals

From Fig. 1, there are no consistent patterns related to the trophic levels. The highest concentrations of PFAS in brain samples were observed in the following order: harbor seal, white-sided dolphin, harbor porpoise, gray seal, white-beaked dolphin, harp seal, and True's beaked whale. The analysis revealed varying concentrations of PFAS across different tissues, including the liver, brain, and muscles shown in Fig. 1b. Notably, the liver samples exhibited significant levels of these substances, highlighting potential concerns regarding their accumulation and impact on this vital organ.

The accumulation of PFAS in brain samples primarily depends on differences in metabolism and excretion specific to each species. Each species has varying blood–brain barrier permeability, alongside different exposure levels and routes.[22] BBB disruption, where PFAS can break down tight junctions to gain access, and transporter-mediated pathways that help them penetrate into brain tissue are some of the causes that contribute to the accumulation of PFAS in the brain.[20,21,63,65,69,70] Another factor for accumulation of PFAS in brain tissue is that their unique interaction with phospholipids in cell membrane. These phospholipids support neurons and maintain the integrity of the BBB. Long chain PFAS, have greater hydrophobicity and stronger attraction to phospholipids, allowing them to penetrate the brain more effectively. The selective permeability of the BBB facilitates the movement of specific chain length PFAS, while shorter chain variants are less able to cross. Consequently, the brain serves as a significant reservoir for long chain PFAS, given its lipid-rich environment and interactions with binding proteins in cerebrospinal fluid.[22,44,69,71]

A higher occurrence of PFSAs and PFCAs was observed across all species. Species at higher trophic levels, such as the white-

Table 2 Comparison of the current study with reported concentrations of PFAS in various tissues and species

Species	Study area	Tissue type	PFAS analyte	Concentration range (ng g^{-1} ww)	Reference
Harbour seal	St. Lawrence river, Quebec	Brain	∑PFAS	174	Present study
Gray seal				98	Present study
Harp seal				28	Present study
Harbor porpoise				118	Present study
White sided dolphin				137	Present study
White beaked dolphin				76	Present study
True's beaked whale				15	Present study
North Atlantic pilot whales		Liver	$\sum_{24}$ PFAS	216–295	44
		Brain	$\sum_{24}$ PFAS	54–91	44
Harbor seal	German Bight	Brain	∑PFSAs/∑PFSiAs	101	61
			∑PFCs	106	
			∑PFSAs/∑PFSiAs	1030	61
			∑PFCs	1071	
Human tissues	Northen Italy	Brain	PFOS	1	62
			PFOA	0.5	
		Liver	PFOS	13	62
			PFOA	3	
		Lung	PFOS	7	62
			PFOA	3	
		Kidney	PFOS	6	62
			PFOA	3	
Human (Autopsy tissue)	Spain, Tarragona County	Liver	PFHxA	68	63
		Brain	PFHxA	141	
		Bone	PFOA	20	
Polar bear	East Greenland	Brain	∑PFCA	32.7 ± 1.7	64
		Adipose		649 ± 111	
		Blood		1220 ± 130	
		Liver		10 720 ± 1210	
Glaucous gulls	Norwegian arctic	Egg	∑PFCA	41.8 ± 5.27	65
		Plasma		102 ± 11.6	
White semiknife carp	Gaobeidian Lake	Serum	PFOS	9 ng mL	66
Tilapia	Gaobeidian Lake	Serum	PFOS	5	66
Leather catfish	Gaobeidian Lake	Serum	PFOS	12	66
Common carp	Gaobeidian Lake	Serum	PFOS	32	66
Different fish species	Minnesota lakes and rivers, USA	Fillet	PFOS	<1–2000	67
Perch	Baltic sea, Sweden	Muscle	PFOS	2	68
Burbot	Baltic sea, Sweden	Muscle	PFOS	1	68
Whitefish	Baltic sea, Sweden	Muscle	PFOS	2	68
Salmon	Baltic sea, Sweden	Muscle	PFOS	0.98	68
Brown trout	Baltic sea, Sweden	Muscle	PFOS	1	68

sided dolphin, white-beaked dolphin, and True's beaked whales, had higher levels of fluorinated alkyl substance acids (FASAs) and their alternatives (FASAAs). We analyzed the relationship between PFOS and the total concentration of long-chain PFCAs in two species: the harbor porpoise and the harbor seal. Unfortunately, other species did not have sufficient sample sizes for statistical analysis (Gray seal, Harp seal, White-sided dolphin, White-beaked dolphin, and True's beaked whale).

The following results indicate a statistically significant correlation between PFOS and long-chain PFCAs in both species. For the harbor porpoise, Spearman's correlation coefficient was $r = 0.882$, with a value $p < 0.01$. For the harbor seal, Spearman's r was 0.8545, with a $p < 0.01$. The strong correlation between PFOS and total PFCAs in the brain samples of these marine mammals suggests a close relationship in the distribution and accumulation of these PFAS. The previous studies show significant correlations between specific PFAS and PFCAs in polar bears from East Greenland.[70]

For PFSAs, higher concentrations of PFOS were observed in harbor porpoises, with a maximum level of 287 ng g^{-1} ww. The minimum PFOS concentration recorded for harbor porpoises was 0.30 ng g^{-1} ww. Previous studies have reported PFOS levels in brain samples of harbor porpoises from the Black Sea ranging from 33 to 1790 ng g^{-1} ww;[72] in comparison, our study indicates lower concentrations. A significant concentration of FOSA was found in white-sided dolphins, measuring 35 ng g^{-1} ww, and in white-beaked dolphins, measuring 16 ng g^{-1} ww. Among various species, the highest concentration of FOSA was observed in beluga whales from Canada, with a reported concentration of 214 ± 3 ng g^{-1} ww.[73] In the Faroe Islands, long-finned pilot whale juveniles were found to have a concentration of 43 ng g^{-1} ww, while adult females had a concentration of 162 ng g^{-1} ww.[74]

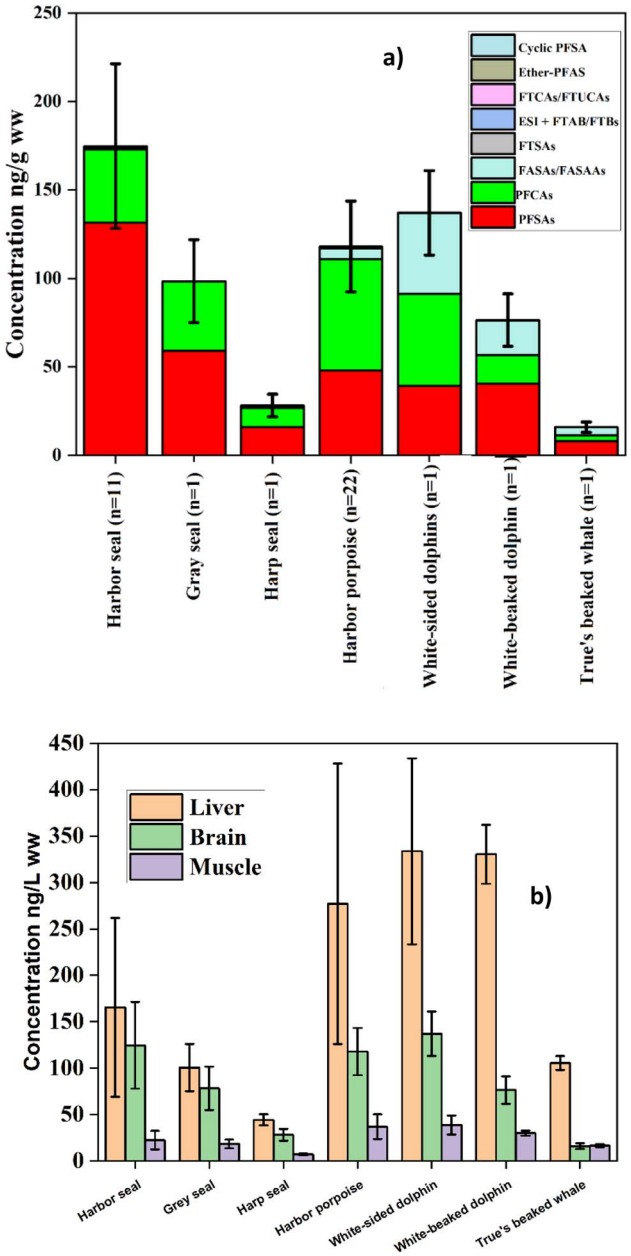

Fig. 1 (a) Geometric mean of targeted PFAS in brain sample of each species. Species are plotted based on trophic level.[5,83] (b) Total concentration of PFAS in samples from the liver, brain, and muscle.

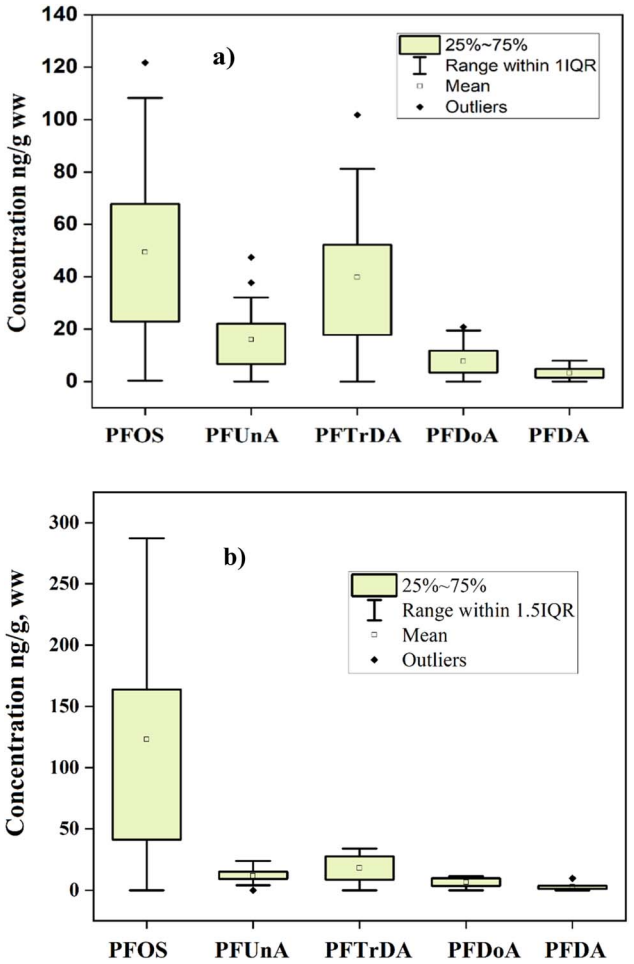

Fig. 2 The distribution pattern of (a) harbor porpoises ($n = 24$) and (b) harbor seals ($n = 11$).

The distribution pattern of harbor porpoises and harbor seals, as shown in Fig. 2, indicates high concentrations for some of the most detected compounds, including PFOS, PFUnA, PFTrDA, PFDoA, and PFDA. The results demonstrate that PFOS is the dominant compound in both species (see details in SI Table S5). A similar trend was observed in harbor porpoise liver samples from the Baltic and North Seas.[75]

The results are consistent with findings from other marine mammals, which show that PFOS is the most commonly identified PFAS in various tissues, including the brain, liver, and other organs.[5,31,45,46] This prevalence suggests that PFOS is widespread in marine environments and can bioaccumulate to hazardous levels.

Regional differences in PFAS profiles are evident. For example, PFUnA is more prevalent in the North Atlantic Ocean,[75] while PFDA makes a significant contribution to the South Pacific Ocean.[76] An increasing trend in several individual PFCAs and PFBS was observed in cetacean samples from 2002 to 2004,[36] suggesting an ongoing and potentially worsening issue of contamination. These differences can be attributed to factors such as localized pollution sources, ocean currents, and specific bioaccumulation patterns.[24,25,77] Additionally, Indo-Pacific humpback dolphins have shown significantly higher levels of PFAS compared to finless porpoises in the South China Sea,[17] highlighting how contamination profiles can vary both by region and species.

Previous studies have revealed that PFAS present in marine aquatic systems can bioaccumulate, leading to significant ecological and health challenges. These substances have been linked to hazardous effects, including oxidative stress, neurotoxicity, and behavioral abnormalities in mammals. The accumulation of PFAS not only poses risks to individual organisms but also disrupts marine ecosystems.[15,78–82] The impacts and

potential toxicological effects of PFAS accumulation in brain tissues have not yet been well studied and even less well understood.

3.2 Clustering of PFAS in the brains of marine mammals

The distribution of PFAS compounds among various marine mammal species is clearly illustrated by the PCA results (Fig. 3), providing valuable insights into possible exposure patterns and bioaccumulation trends. We can focus on the first two major components to understand the underlying structure of the data, as they account for a significant 78.3% of the variance. These species may share similar habitats or food sources, which could contribute to their contamination profiles. This is evident in the distinct positions of species such as the harbor porpoise, gray seal, and harbor seal within the PCA space, which is significantly influenced by chemicals like PFOS, PFDS, and PFHpS. Additionally, the relationship of the white-sided dolphin on the PC2 axis with FBSA and PFTrDA suggests a specific exposure scenario that may be linked to their ecological niche or metabolic mechanisms. The complexity of contaminant processes in marine ecosystems is underscored by these distinctions among species, highlighting the importance of species-specific assessments in environmental monitoring programs.

Our understanding of the connections between various PFAS in marine ecosystems is enhanced by the correlation patterns identified in the study. Strong relationships between PFOS, PFHxS and PFDS suggest that these chemicals may have similar environmental fates, transport mechanisms, shared sources and physiological behavior within the animals. Conversely, the lower and sometimes negative correlation of FBSA with other compounds, along with its varied behavior, raises intriguing questions about its origins, degradation pathways, and bioaccumulation or depuration mechanisms.

The clustering of harbor seals and harp seals in the principal component analysis (PCA – Fig. 3) plot indicates that these species are exposed to similar contaminants, likely due to overlapping habitats or food choices. The isolation of the white-sided dolphin in this analysis is particularly noteworthy, as it may indicate specialized exposure pathways or different physiological responses to these contaminants.

Although these findings provide a solid foundation for understanding contaminant burdens specific to each species, incorporating more ecological data and exploring additional sources of variation could enhance our interpretation and offer a more comprehensive view of the dynamics of PFAS in marine environments.

3.3 Comparison of distribution patterns of PFAS in liver, muscle and brain tissues

The Spearman rank correlation was used to assess the total concentration of each PFAS in brain samples in relation to muscle and liver tissues. The results showed a weak yet significant correlation with muscle ($r = 0.0738$, $P > 0.05$) and a strong correlation with the liver ($r = 0.7995$, $P < 0.05$). Both results indicate that the concentrations of each compound in the respective tissues are significantly positively correlated. Comparing the distribution of all three tissues, we observe a higher DF for PFOS, PFNA, PFDA, PFUnA, PFTrDA, and PFDoA. Previous studies also showed that the highest occurrence of PFAS was found in liver samples compared to other tissues, such as those from fish and marine mammals,

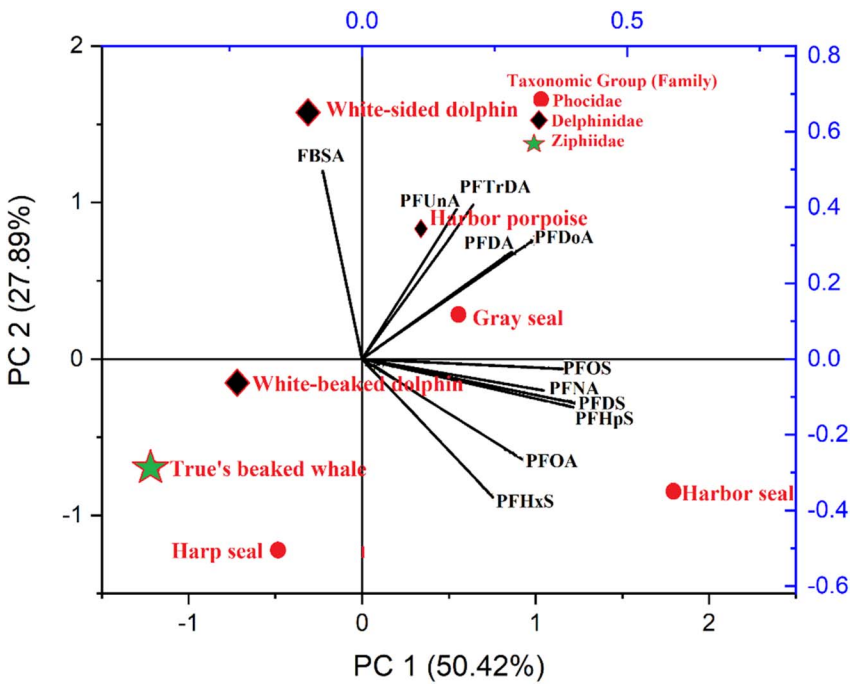

Fig. 3 PCA analysis of each PFAS compound across different species (harbor seal ($n = 11$), gray seal ($n = 1$), harp seal ($n = 1$), harbor porpoise ($n = 22$), white-sided dolphin ($n = 1$), white-beaked dolphin ($n = 1$), and True's beaked whale ($n = 1$).

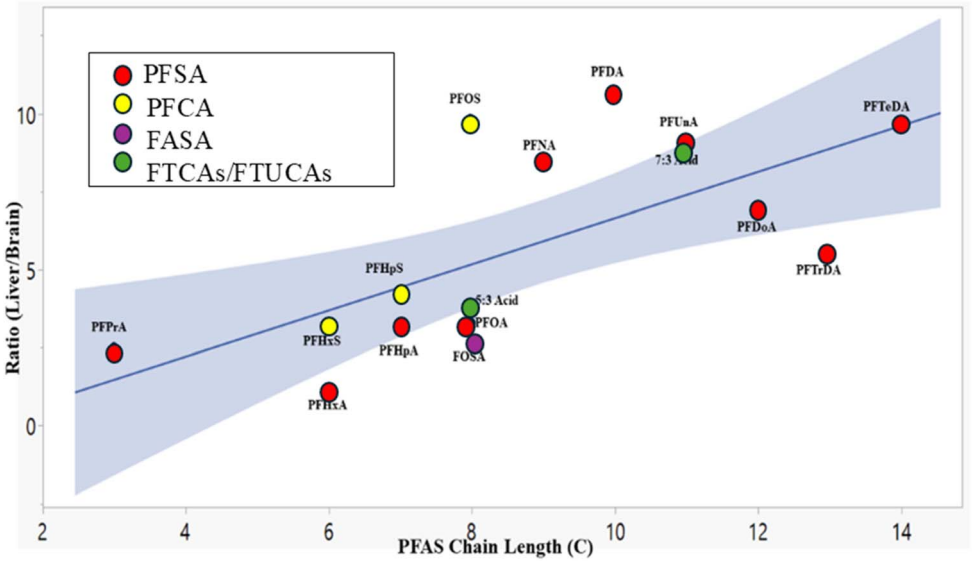

Fig. 4 Mean ratio of concentrations of predominant PFAS in liver/brain samples of marine mammals (calculated wet weight/wet weight).

including the finless porpoise and the Indo-Pacific humpback dolphin.[15–17]

Fig. 4 shows the mean ratio of PFAS in liver and brain samples. As expected, the liver shows the highest concentrations of many PFAS compounds, particularly PFOS, which is known for its strong bioaccumulative properties. These findings align with the liver's role in metabolizing and storing persistent organic compounds. Muscle and brain tissues contain moderate levels of shorter-chain PFAS. PFAS can cross the BBB and accumulate in specific areas of the brain; however, the liver is the primary site of their accumulation. The distribution and accumulation of PFAS in biological tissues are significantly influenced by their chain length. Long-chain PFAS, which contain ten or more carbon atoms, tend to accumulate more in lipid-rich tissues, such as the brain, compared to shorter-chain counterparts.[70,72,84] The molecular weight and chain length of PFAS determine their ability to cross the BBB and accumulate in the brain. Previous research has shown that longer-chain PFAS, like PFOS and PFTrDA, have been detected in brain regions such as the brainstem, hippocampus, and thalamus, which are known for their high lipid content.[69,70]

The distribution of PFAS in the liver, brain, and muscle is influenced by various physiological factors, including the protein and lipid content of these tissues, as well as the binding affinities of PFAS to biomolecules.[44,85–87] Generally, PFAS concentrations are highest in the liver, which serves as a key retention site due to its high protein content and the strong affinity of PFAS, particularly longer-chain PFAS, for liver proteins and phospholipids.[44,85–87] In contrast, muscle tissue accumulates fewer PFAS because it contains less protein and fat. Another important factor is the binding affinity of PFAS.[44] Those with longer carbon chains and higher hydrophobicity tend to bond more firmly to tissue proteins and phospholipids, increasing their accumulation in organs such as the liver and brain.[44,86] Conversely, shorter-chain PFAS, which can be removed more easily and have weaker binding, are less likely to accumulate.[85] Fig. 3 shows nevertheless that brain tissues accumulate significantly more PFAS than muscle tissues, raising potential concerns to better understand the potential neurotoxicological impacts this may cause in the brain.

The distribution of PFAS indicates that PFAS, particularly PFOS, is dominant in terms of tissue accumulation. Other classes, such as PFCA and FTUCA, exhibit lower and more varied distribution across tissues. The differences in PFAS levels across tissues are influenced by the unique properties of each compound, such as chain length and functional groups, which affect their bioaccumulation and affinity for different tissues. This finding highlights the liver as a key organ for PFAS accumulation in marine mammals, raising concerns about the potential toxic effects on these animals and their ecosystems.[5]

4. Conclusions

This study reveals the occurrence of 34 per- and polyfluoroalkyl substances (PFAS) in brain samples from various marine mammals. The highest occurrence was observed for compounds ranging from C9 to C14, with PFOS showing the highest detection frequency. The PFSA class includes substances such as PFHxS, PFDS, and PFHpS, while the perfluorocarboxylic acids (PFCAs) class specifically includes PFOA. These substances can exhibit detection frequencies ranging from 35% to 57%. The liver is the main site for PFAS accumulation, occurring more often than in the brain. However, PFAS can cross the blood–brain barrier (BBB) and gather in certain brain areas. Long-chain PFAS are more likely to accumulate in the brain than short-chain PFAS, as their ability to penetrate the BBB depends on their molecular weight and chain length.

We analyzed the relationship between PFOS and the total concentration of long-chain PFCAs in two species: the harbor porpoise and the harbor seal. The results indicate a statistically

significant correlation between PFOS and long-chain PFCAs in both species. The Spearman rank correlation was used to assess the total concentration of each PFAS in brain samples in relation to muscle and liver tissues. The results showed a correlation with muscle tissue and a strong correlation with liver tissue. The findings indicate that the concentrations of each compound in the respective tissues are significantly positively correlated. This study reveals that action is needed to restrict PFAS in the marine system and to protect these mammals.

Conflicts of interest

There are no conflicts of interest to declare.

Data availability

The data to support the findings of this study are provided in the supplementary material files or if more information is needed, it can be requested from the corresponding author.

The supplementary information file includes additional tables and figures that support the main text. It provides a detailed list of the PFAS compounds quantitatively targeted in this study, information on internal standards, the UHPLC-HRMS acquisition method, and specimen details, including species, weight, location, and sex for brain samples. It also includes a comprehensive table of 80 target PFAS with their retention times and monitored m/z values, method detection limits (mLOD), instrumental detection limits (iLOD), and the distribution patterns of PFAS (ng g^{-1} ww) across different marine mammal species. See DOI: **https://doi.org/10.1039/d5va00061k**.

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