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PFASS IN THE ENVIRONMENT AND HUMANS: A NEW THREAT?

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PFASs in the environment and humans: a new threat?

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Introduction

Perfluorinated alkyl substances (PFASs) have emerged as global environmental pollutants widely distributed in wildlife and humans 1. PFASs are synthetic compounds which have been applied in the last decades in a broad spectrum of commercial products and industrial processes because of their physico-chemical properties. They are heat stable, extremely resistant to degradation and environmental breakdown, and repel both water and oil 2. They are mainly known for being components of polytetrafluoroethylene (PTFE)™ and are used as repellents in pans, as waterproof and breathable materials, in construction products, as surfactants and as flame retardants. Their numerous uses and unique characteristics make it difficult to develop an understanding on how they are distributed in the environment and how people and animals become exposed 3, 4.

Exposure and risks to PFASs is mainly attributed to their environmental persistence and bioaccumulative properties. The accumulation potential and inherent toxicity depend on the chemical structure. The greater the number of carbon-fluorine bonds and the carbon-chain length, the better their surfactant properties. However, longer-chain PFAS have lower water solubility and thus, possess higher bioaccumulation potential and become potentially harmful to biota and humans 5.

High concentration of PFASs have typically been documented in areas with direct industrial inputs, non-treated discharges, or receiving waste water treatment (WWTP) effluents, run-off or accidental spills that have impacted receiving waters. However, despite the low concentrations detected in water, both perfluoroalkyl sulfonates (PFSAs) and perfluoroalkyl carboxylates (PFCAs) are accumulated in aquatic organisms and can have a serious impact in the ecosystems, including food chain biomagnification6. In addition, their presence in human populations and wildlife species from remote locations suggests a widespread global distribution of these pollutants 7.

Concerns about PFASs toxicity have risen not only due to its widespread distribution and persistence and in the environment, but due to their ability to act as endocrine disrupters and obesogens in living organisms 8. In addition, they are suspected to interfere in the lipid metabolism and to affect the oxidative metabolism of cells 9. Adverse hepato-histological effects in the amphibian Xenopus laevis at high concentrations (100-1000 µg/L) 10 have been reported, as well as affections in the membrane properties (e.g. membrane fluidity, mitochondrial membrane potential) 11.

Within this context, the aim of this study starting in 2009 was triple:

• To determine the occurrence of PFSAs and PFCAs in a wide array of aquatic organisms including insect larvae, mussels, crabs, turtle's blood, birds' blood and gull eggs collected in Catalonia (NE Spain);

• To determine the distribution of PFSAs and PFCAs in human blood of the population from the Barcelona area;

• To elucidate the differential uptake of PFASs in JEG-3 cells and further to investigate the cytotoxicity of PFASs and their capacity to interfere with cellular P450 aromatase (CYP19) in these cells. Moreover, a comparative analysis of lipid profiles of non-exposed and exposed cells permitted to determine the effects of PFASs on JEG-3 cell lipidome.

Materials and methods

Compounds studied

Native compounds studied were perfluorobutane sulfonate (PFBS), perfluorohexane sulfonate (PFHxS), perfluorooctane sulfonate (PFOS), perfluorodecane sulfonate (PFDS), perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnA), perfluorododecanoic acid (PFDoA), perfluorotridecanoic acid (PFTriDA), perfluorotetradecanoic acid (PFTeDA), perfluorohexadecanoic acid (PFHxDA), perfluorooctadecanoic acid (PFODA), supplied by Wellington Laboratories (Ontario, Canada).

Analysis of PFASs in aquatic organisms and humans

One gram of biological sample (whole fish, crab, eggs, etc.) or 0.3 to 0.5 mL of whole blood was weighted in polypropylene tubes and internal standards (m-PFOS and m-PFOA) were added at a concentration of 50 ng/g-ww. Samples were extracted using 9 mL of acetonitrile in an ultrasonic bath for 10 min at room temperature (3 times), centrifuged at 2500 rpm for 5 min and extracts were purified by adding 25 mg of activated carbon and 50 µL of glacial acetic acid. Centrifuged extracts were dried and reconstituted with 500 µL acetonitrile:10 mM NH4OAc HPLC water (1:1). Analysis was carried out by Ultra High Performance Liquid Chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS ACQUITY TQD from Waters, Massachusetts, USA) with an ACQUITY UPLC BEH C18 (1.7 µm particle size, 2.1 mm x 100 mm, from Waters). Acquisition was performed by Multiple Reaction Monitoring (MRM) and internal standard quantification was used.

Toxicological analysis

Several tests were performed using JEG-3 cells, used as a model cell line, at an exposure level of 0.6 and 6 µM. JEG-3 cell line, derived from a placental carcinoma in humans, was obtained from American Type Culture Collection (ATCC HTB-36). JEG-3 cells were grown in Eagle's Minimum Essential Medium supplemented with 5% fetal bovine serum, 2 mM L-glutamine, 1 mM sodium pyruvate, 0.1 mM nonessential amino acids, 1.5 g/L sodium bicarbonate and 50 U/mL penicillin G/ 50 µg/mL streptomycin in a humidified incubator with 5% CO2 at 37 ºC. Cells were routinely cultured in 75 cm2 polystyrene flasks. When 90% confluence was reached, cells were dissociated with 0.25% (w/v) trypsin and 0.9 mM EDTA for subculturing and experiments. Experiments were carried out on confluent cell monolayers. Assays and toxicological endpoints tested were:

- Uptake of PFASs by cells was tested by exposing cells to a mixture of PFAS. The amount of compound in the cell and in the culture medium was assessed by UHPLC-MS/MS.
- Cell viability assay was conducted by applying Alamar BlueTM (AB) to monitor the metabolic activity and 5-carboxyfluorescein diacetate (CFDA-AM) to evaluate the membrane integrity.
- Cellular P450 aromatase (CYP19) activity measures the amount of 3H2O formed during the aromatization of [1β-3H] androstenedione (3H-AD) by JEG-3 cells. PFASs test solutions ranged from 3 nM to 500 µM.
- Lipid disruption was performed by analyzing the lipid profiles of exposed and non-exposed JEG-3 cells by HPLC-Time of flight MS under positive ESI. The comparative study of lipid profiles was focused on phosphatidylcholines (PC), plasmalogen PC, lyso plasmalogen PC, diacylglycerols (DAG), triacylglycerols (TAG) and cholesterol esters (CE). Positive identification of lipids was based on the accurate mass measurement with an error <5 ppm and its LC relative retention time, compared to that of standards (±2%).

Results and discussion

Occurrence of PFASs in aquatic organisms

The most significant result of the survey carried out is that PFASs were detected in all samples, despite belonging to different species, occupy a different trophic level and having a distinct biology and habitat. This is a clear demonstration that PFASs are widespread in the aquatic environment of Catalonia. Among studied compounds, PFOS was the most ubiquitous compound and the one detected at the highest concentrations, followed in a much lesser extent by PFUnA and PFDoA PFOA and PFNA. Figure 1 shows PFOS levels in different species and in human blood, distributed according to low and high concentrations. Levels below 2 ng/g ww were observed in many species which were not suspected to contain traces of these compounds, such as turtles' blood, American crab, fish and mussels. When analyzed as whole organism, all Mediterranean fish species contained PFOS at low levels, and according to the analysis of only sardines' heads, PFOS were mainly accumulated in this part of the body. PFASs were detected in all human blood samples at relatively low levels, in comparison to other studies, and this was attributed to the non-exposed population, although among 39 samples analyzed, young people

(18-25 years) had the highest levels. In contrast, PFOS had a much higher impact in birds' blood, both the vegetarian and piscivorous species. The swamp hen (Porphyrio porphyrio) dwells in wet areas and eats the tender shoots and vegetable-like matter, mainly Ruppia maritima, Juncus and Phragmites. Traces of PFOS were detected in Ruppia, suggesting that accumulation from food can take place. Much higher levels were found in gull species, both the opportunistic feeder Larus michahellis and the piscivorous species Larus audouinii. Female blood had lower levels than those found in males, attributed to the annual maternal transfer to eggs. Finally, and totally unexpected, Hydropsyche exocellata larvae had a high PFOS concentration. Whereas this compound is not found in extremely polluted sites, it dwells in the water-air interface and has a high potential for bioaccumulation of water contaminants. Overall, 12 species were studied with more than 200 samples analyzed and all of them releveled the presence of PFASs, specially PFOS and other long chain PFASs. This indicates that their presence in the aquatic environment impacts wildlife and humans, and thus, their mode of action and effects that they can produce deserve attention.

Uptake study and toxicological tests

PFBA, PFHxA, PFOA, PFNA, PFDoA and PFHxS, PFBS and PFOS were studied for toxicological analysis to have chain-length representativeness. The differential uptake of PFASs in the in-vitro system was assessed by measuring the fraction retained in the cells right after exposure (time 0) to the PFASs mixture (0.6 and 6 μM), and 1, 3, 5, 8 and 24 h later. Interestingly, the percentage of compounds that entered into the cells was higher on cells exposed to the lowest PFAS dose. As observed in Figure 2, the amount of detected compounds corresponded to 0-16% and 0-5% of the spiked dose in cells exposed to 0.6 and 6 μM PFAS mixture, respectively, indicating that the bioavailable fraction is very small in both cases, but higher under the first conditions. Interestingly, the same trend was observed in both assay conditions, being PFDoA and PFOS the compounds that exhibited the highest concentration in JEG-3 cells exposed to PFAS mixture at 0.6 and 6 μM, with maximum concentrations at 8 and 5 h, respectively. The rest of compounds (i.e., PFBA, PFBS, PFHxS and PFHxA) were detected at very low concentrations (some of them were below detection limit under our assay conditions).

No significant cytotoxicity was observed for the shortest chain length PFASs (PFBA, PFHxA, PFBS and PFHxS) after 24 h incubation with JEG-3 cells. In contrast, cell viability decreased to 55-59% following exposure to 500 μM of PFOA, while PFOS, PFNA and PFDoA caused a decline in cell viability higher than 90% (Figure 3).

Overall, PFASs residues detected in JEG-3 cells after 24 hours exposure were as follows: PFDoA > PFOS >> PFNA > PFOA ~ PFHxA, being for shorter chain PFASs (PFBA, PFBS, PFHxS) below detection limit. The cytotoxic effect of PFASs, although to some extent affected by the absorption of the compounds, did not follow the same pattern: PFOS > PFDoA ~ PFNA > PFOA > PFHxA. Interestingly, the non-cytotoxic compounds (PFBA, PFHxA, PFBS and PFHxS) were the ones that were not detected in JEG-3 cells or that showed the lowest cell residue after 24 h exposure.

Most PFASs significantly inhibited CYP19 aromatase activity at rather low endogenous cellular concentrations. Exposure of JEG-3 cells to the shorter chain PFASs resulted in a concentration dependent inhibition of aromatase activity, where PFBS and PFHxS were stronger inhibitors of aromatase activity (97 and 84% inhibition when tested at 500 μM) than the corresponding acidic compounds (PFBA and PFHxA: 13 and 26% inhibition). IC50 were of 298 ± 29 μM for PFHxS and 68 ± 11 μM for PFBS. PFOS, PFOA and PFBS had a high potential to act as aromatase inhibitors in placental cells. At 100 μM, 80% inhibition of aromatase activity and IC50 (equal to 57 ± 4 μM) was observed for PFOS and 62% with an IC50 of 80 ± 4 μM for PFOA. PFNA and PFDoA were weaker inhibitors of CYP19 aromatase: a 39% and a 31% inhibition were detected.

Finally, relative changes in lipid content of JEG-3 cells exposed for 24 h to the mixture of PFASs at 0.6 and 6 μM were observed. A statistically significant increase (2 to 3-fold) of PC, plasmalogen PC and lyso plasmalogen PC was detected together with a minor increase of TAG (30%), and no significant changes in the relative abundance of DAG and CE species. The effects were more evident in cells exposed to 0.6 μM PFASs mixture than in those exposed to 6 μM PFASs, due to the potential capacity of cells to detoxify xenobiotics when exposed to high concentrations or rather, that at low concentrations target compounds exert a higher effect as they may act "unseen" (see Figure 2). These results suggest the PFASs interfere with membrane lipids.

Overall, the research carried out regarding the presence and effects of PFASs in organisms and in humans reveals that these compounds pose a new threat to wildlife and humans. Attention should be paid to the use and release of these chemicals in the environment, given their widespread distribution and potential negative alterations identified at laboratory controlled conditions. Further studies should be aimed to

evaluate the effects directly in wildlife by carrying out integrated chemical and toxicological monitoring studies. Because PFASs represent a family of compounds still in use, accumulative effects might also be expected in the long term.

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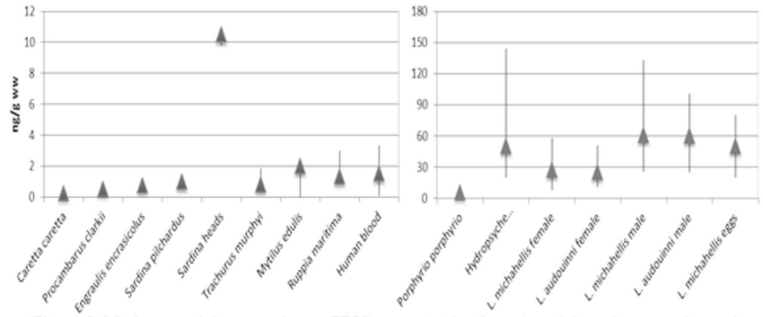
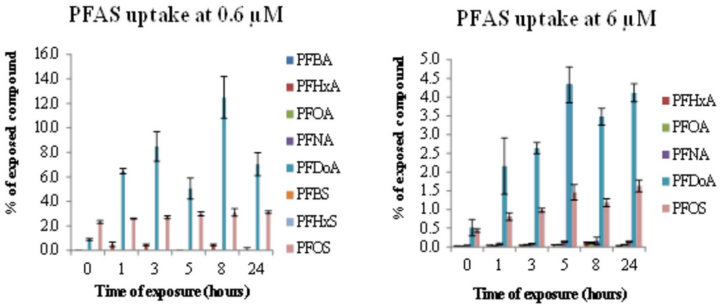


Figure 1. Maximum, minimum and mean PFOS concentration (in ng/g ww) in various aquatic species monitored in Catalonia (NE Spain) and in human blood from Barcelona residents, distributed according to levels detected.



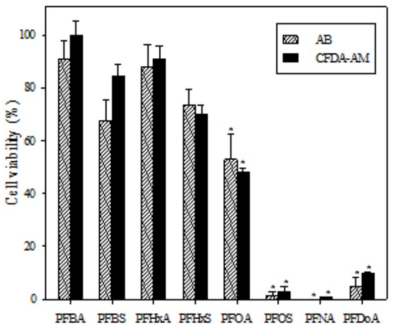


Figure 3. Cytotoxicity of PFASs tested at a concentration of 500 µM in JEG-3 cells. Cell viability expressed as percentage of viable cells referred to control cells (exposed to the solvent). Values are means ± SEM (n= 3). *Statistical significant differences ($P < 0.05$).