ENVIRONMENTAL CONCENTRATIONS, TOXICOLOGY, AND DEVELOPMENT OF NEW METHODS FOR EXTRACTION AND MASS BALANCE ANALYSIS OF PERFLUORINATED COMPOUNDS IN ENVIRONMENTAL SAMPLES

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ENVIRONMENTAL CONCENTRATIONS, TOXICOLOGY, AND DEVELOPMENT OF NEW METHODS FOR EXTRACTION AND MASS BALANCE ANALYSIS OF PERFLUORINATED COMPOUNDS IN ENVIRONMENTAL SAMPLES
環境樣品中有機全氟化物的濃度、毒性、新提取方法以及物質平衡分析的研究

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ABSTRACT

Polyfluorinated and perfluorinated compounds (PFCs) are emerging chemicals of concern. They are widely used in a variety of consumer goods and industrial products because of their unique physico-chemical properties. Concern about fluorinated organic compounds, particularly the fully fluorinated PFCs, has been growing since the late 1990’s because of their ubiquitous occurrence in the environment. Perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) are representative and commonly occurring PFCs.

The aims of the present PhD research project are to extend the current knowledge of PFC distribution in developing countries (i.e. India and South China), as well as to develop a new concept for understanding and explaining PFC pollution patterns by demonstrating the presence of unidentified organic fluorine-containing compounds in environmental samples other than PFCs. As part of this work, a new extraction method using acetonitrile (ACN) and solid phase extraction (SPE) and an ion exchange column to separate possible interferences from target PFCs was developed to improve PFC detection. The final goal of the project was to investigate the toxicity of several PFCs using laboratory studies.

Two monitoring studies were conducted in South China and India. For the first one, concentrations and profiles of PFCs were investigated in surface waters (rivers, lakes, coastal seas and untreated sewage; n=42) including Ganges River water, and biota such as shrimp (n=2), fish (n=28), and the Ganges River dolphin (*Platanista gangetica*; n=15) in India. PFOS was the dominant PFC found in most of the samples analyzed including water samples, with the exception of untreated sewage (water: <0.04-3.91 ng/L; biota: 0.248-27.9 ng/g wet weight (ww)) in India. Long-chain (C11-C18) perfluorocarboxylates (PFCAs) were not detected in the water samples (<0.2 ng/L), although perfluorodecanoate (PFDA) (0.061-0.923 ng/g ww) and perfluoroundecanoate (PFUnDA) (0.072-0.998 ng/g ww) were found in biological samples. Overall, concentrations of PFCs in water and biological samples from India were lower than the concentrations reported for other countries so far. PFC profiles in Indian waters were dominated by PFOS, followed by PFOA, which differ from the pattern reported for other countries such as Korea, Japan and USA, where PFOA was the predominant compound in water samples.

The second study measured the concentrations of 10 PFCs (PFOS, perfluorohexane sulfonate (PFHxS), perfluorooctanesulfonamide (PFOSA), N-ethyl perfluorooctanesulfonamide (N-EtFOSA), N-ethyl perfluorooctanesulfonamidoacetate (N-EtFOSAA), perfluorododecanoate (PFDoDA), PFUnDA, PFDA, perfluorononanoate
(PFNA), PFOA, and perfluoroheptanoate (PFHpA)) in liver samples of Indo-Pacific humpback dolphins (*Sousa chinensis*; n=10) and finless porpoises (*Neophocaena phocaenoides*; n=10) stranded in Hong Kong, South China, between 2003 and 2007. PFOS was found to be the dominant PFC in dolphin and porpoise tissues at concentrations ranging from 26-693 ng/g ww in dolphins and 51.3-262 ng/g ww in porpoises. The PFOS concentrations in liver samples were comparable to those reported in other studies on marine mammals. PFC composition profiles were similar between dolphins and porpoises: PFOS (75-83% of total PFCs), PFUnDA (10%), PFDA (3%) and PFNA (2%). In contrast, PFOSA contributed 5% of total PFCs in dolphin liver samples, whereas PFOSA only accounted for around 1% in porpoise liver samples, possibly due to different habitats and dietary habits between the two species. No significant correlations were found between PFC concentrations and other persistent environmental pollutants, namely total polybrominated diphenyl ethers (PBDEs) (and individual PBDE congeners) and total polychlorinated biphenyls (PCBs), in the dolphin and porpoise samples. These results imply that the sources and the exposure pathways of PCBs/PBDEs and PFCs are likely to be different.

Mass balance analysis was conducted by measuring total fluorine (TF) and extractable organic fluorine (EOF) in several types of environmental samples by combustion ion chromatography for fluorine. Human blood (n=30) from 6 cities in China was analyzed for TF analyses. Analysis of known PFCs and EOF showed that known PFCs accounted for >70% of EOF in Chinese human blood samples from Beijing, Shenyang and Guiyang, whereas known PFCs only accounted for around 30% of EOF in samples from Jintan. In wild animals, measurement of known PFCs and EOF in the marine mammal from South China (i.e. Indo-Pacific humpback dolphin and finless porpoise) livers showed that a large proportion (~70%) of the organic fluorine in both species is of unknown origin. Wild rat blood was also used to further demonstrate the presence of unidentified organic fluorine-containing compounds in terrestrial wild animals. The contribution of known PFCs to EOF varied from 9 to 89% (mean: 56%), suggesting that wild animals are exposed to a wider range of PFCs than humans. These investigations are critical for a comprehensive assessment of the risks of these compounds to humans and other receptors. Characterization and identification of these unidentified fluorinated compounds will be also instructive in terms of human and environmental risk assessment.
Limitations of the currently used PFC-extraction method and the observation that unidentified organic fluorine-containing compounds were present in environmental samples led to the development of a better extraction method for PFCs in biota samples. A method using ACN extraction with SPE cleanup was developed which can be used to measure more than 28 perfluorinated compounds (perfluoroalkyl sulfonates: C4, C6, C8, C10; perfluoroalkyl sulfinites: C6, C8, C10; PFOSA, N-EtFOSA, N-EtFOSAA, perfluorocarboxylates: C4-C14; fluorotelomer carboxylates: 7:3, 8:2) in whole blood with recoveries ranging from 70-120%. This new method can extract and quantify more individual PFCs in whole blood samples than the concurrent ion pairing extraction method. As part of this method, separation of possible interferences such as taurodeoxycholic acid was accomplished using an ion exchange JJ50-2D column.

In this project, toxicological and toxicokinetic parts are given to chickens because many other studies focused on mammalian species such as rats and monkeys. Health risk is also importance to avian species, however, these sources of information are limited. Two studies were conducted to extend the knowledge of possible health effects on chickens. These studies looked into the gene expression patterns and toxicokinetics of PFCs in domestic chickens (Gallus gallus) exposed in the laboratory. The effects of PFOS and PFOA on the gene expression patterns of chickens that were exposed to either compound at low doses were investigated with the use of microarray techniques. Twelve Genechip Chicken Genome Arrays were used to study hepatic gene expression in six-week-old chickens that were exposed to either PFOA (0.1, 0.5, or 5 mg/mL), PFOS (0.02 or 0.1 mg/mL), or a saline vehicle control (0.9% NaCl in Milli-Q water) via subcutaneous implantation of a 2 mL osmotic pump for four weeks or for four weeks with a further four weeks of depuration. The genes that were affected after four weeks of PFOS exposure were mainly related to the transport of electrons and oxygen and the metabolism of lipids and fatty acids, while the genes that were affected after four weeks of exposure with a further four weeks of depuration were mainly related to the transport of electrons and ions, and protein amino acid phosphorylation and proteolysis. The genes that were affected after four weeks of PFOA exposure were related to the transport of ions, lipids, and electrons and cytochromes, while the genes that were affected after four weeks of exposure with a further four weeks of depuration were related to protein amino acid phosphorylation and proteolysis, the transport of ions, and the metabolism of fatty acids and lipids. The results also showed that the gene expression patterns between chickens that were treated with PFOS and those that were
treated with PFOA were different, which points to the importance of the separate evaluation of the toxicities of PFOS and PFOA.

The other toxicity study examined the effects of exposure of one-day-old male chicks to mixtures of PFOS, PFOA, and PFDA at either a low dose (0.1 mg/kg body weight (bw)) or a high dose (1.0 mg/kg bw), or a saline/ethanol vehicle control via oral gavage thrice a week for three weeks. After three weeks of exposure, half of the chicks were sacrificed and the other half were allowed to depurate for a further three weeks. No dose-dependent statistically significant differences in body/organ weights were observed among treatment and control groups after three weeks of exposure or after three weeks of depuration. Neither 15 histological nor 14 plasma biochemical parameters were significantly different in chicks from the exposed groups and vehicle controls. PFOS and PFDA accumulated at much higher concentrations than PFOA in blood/liver/kidney during the experimental periods. The half-lives for each PFC at the 0.1 mg/kg and 1.0 mg/kg doses were, respectively, approximately 15 and 17 days for PFOS, 11 and 16 days for PFDA, and 3.9 and 3.9 days for PFOA. These results indicated that exposure to 1.0 mg mixture of PFOS/PFDA/PFOA/kg bw has no adverse effects on the endpoints measured in juvenile chickens.

Future research directions are given at the end of this thesis. In the present project, only PFCAs and PFASs were evaluated in different environmental matrices. PFCs (C2-C3) could be detected as high as PFOS or PFOA in environmental samples, however, the recoveries were less than 10%. Effort should be made to development methods to determine these PFCs in the environmental samples. In addition, other precursors like FTOHs, FOSAs, FOSEs, and other new PFCs like PAPs are getting much attention because they are thought to be degraded to PFCAs or PFASs. Tracing these precursors can identify whether these chemicals are major sources of these PFCAs or PFASs. Biomagnifications of PFCs had been evaluated in aquatic food web, however, biomagnifications of EOF and the patterns of EOF and known PFCs should be carried out to have a broader analysis of the accumulation and transfer of fluorine compounds in the environment. The characterization of this unknown organic fluorine should be conducted.
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