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Probing mechanisms for bioaccumulation of perfluoroalkyl acids in carp (*Cyprinus carpio*): Impacts of protein binding affinities and elimination pathways

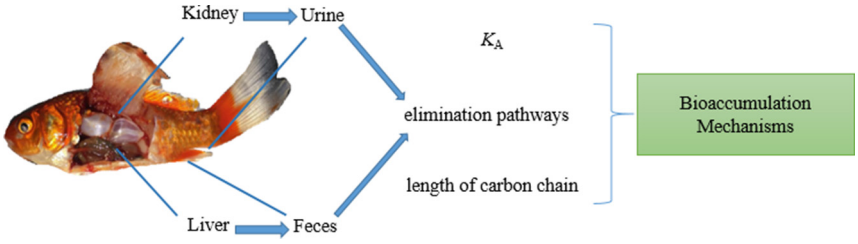
Wenjue Zhong [1], Liyuan Zhang [1], Yannan Cui, Meng Chen, Lingyan Zhu *

Tianjin Key Laboratory of Environmental Remediation and Pollution Control, Key Laboratory of Pollution Processes and Environmental Criteria of Ministry of Education, College of Environmental Science and Engineering of Nankai University, Tianjin 300350, China

HIGHLIGHTS

- Contrast to the results in mammals, PFHxS had much short half-life than PFOS in carp.
- K_A values and BCFs of PFCAs and PFSAs increased as the carbon chain length increased.
- Compared to PFCAs, PFSAs were not efficiently transported from fish blood to liver and kidney.
- PFHxS was eliminated more via the urine, feces, and gill than most of PFAAs.
- The *n*-PFOS was eliminated more via the feces but *n*-PFHxS was eliminated more via the urine.

GRAPHICAL ABSTRACT



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ABSTRACT

With regulations on the manufacture and usage of perfluorooctanoate (PFOA), perfluorooctane sulfonate (PFOS) and related compounds, short-chain perfluoroalkyl acids (PFAAs) are increasingly being used as alternatives. However, there are limited studies on their bioaccumulation mechanisms, especially for short-chain PFAAs. In this study, we examined the binding affinity of PFAAs with fish serum proteins and tissue distributions of perfluoroalkyl carboxylates (C7-C11 PFCAs) and perfluoroalkyl sulfonates (C4, C6, and C8 PFSAs) in carp (*Cyprinus carpio*), including the isomers of PFOS and perfluorohexane sulfonate (PFHxS). For both PFCAs and PFSAs, the fish serum protein binding constant (K_A) and bioconcentration factor (BCF) increased with an increase in the carbon chain length. PFHxS (C6 PFSA) had a much higher K_A but displayed a much lower BCF than those of C7-C11 PFCAs. It indicated that not only fish blood proteins, but also other proteins in the liver and kidney, mediated the accumulation of PFAAs in fish. The lowest concentration ratios of PFHxS in liver to blood and in kidney to blood suggested that it could not be effectively transported to liver and kidney by fatty acid binding proteins and organic anion transporters. PFOS and PFHxS displayed different elimination pathways, although their linear (*n*-) isomers were accumulated more in fish than the corresponding branched (*br*-) isomers. The *n*-PFOS was eliminated more via the feces but *br*-PFOS was eliminated more via the urine; while the opposite trend was observed for PFHxS isomers.

* Corresponding author.
E-mail address: zhuly@nankai.edu.cn (L. Zhu).
[1] Wenjue Zhong and Liyuan Zhang contributed equally.

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1. Introduction

Perfluoroalkyl acids (PFAAs) are wildly used in numerous industrial and household products due to their excellent surfactant properties, thermal stability, and hydrophobic and oleophobic nature (Lau et al., 2007; Prevedouros et al., 2006; Xie et al., 2013). Perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS) are two common PFAAs which are detected most frequently in the environment (Benskin et al., 2010; Xie et al., 2013). Because of their ubiquitous environmental occurrence (Cousins, 2015; Giesy and Kannan, 2001; Houde et al., 2011; Lindstrom et al., 2011b; Shoeib et al., 2006), persistence, and potential toxicities (Shi et al., 2012; Verner et al., 2015; Wang et al., 2015), PFOS and its related products were listed in Annex B of the Stockholm Convention on Persistent Organic Pollutions in 2009, and are being regulated in many countries (Lindstrom et al., 2011a; Xie et al., 2013). Some short-chain PFASs, such as perfluorobutane sulfonate (PFBS) and perfluorohexane sulfonate (PFHxS), are therefore used as alternatives to PFOS, because of their similar properties but less toxicities (Naile et al., 2012). Consequently, concentrations of short-chain perfluoroalkyl sulfonates (PFSAs) are increasing in the environment (Chen et al., 2015c; D'Eon et al., 2006; Kang et al., 2016; Lam et al., 2016; Wang et al., 2016). However, some recent studies indicated that the short-chain PFSAs may have different toxic mechanisms from those of long-chain PFSAs (Naile et al., 2012). These prompt the necessity to investigate the environmental and toxic behaviors of short-chain PFSAs.

Many studies demonstrated that PFAAs can accumulate in organisms, and their accumulation potentials are dependent on the carbon chain length and functional groups (Conder et al., 2008; Houde et al., 2011). Previous studies generally suggested that perfluoroalkyl carboxylates (PFCAs) with less than seven perfluoroalkyl carbons (<C7), and PFSAs with < C6 displayed insignificant bioaccumulation potential (Conder et al., 2008; Martin et al., 2003a). For long-chain PFCAs with ≥C7, their bioaccumulation ability increased with an increase in perfluoroalkyl carbon chain length (Dai et al., 2013; Fang et al., 2016; Jeon et al., 2010; Martin et al., 2003a). However, it was reported that PFOS and PFHxS displayed contrary trends in the elimination rate and half-life in fish and humans, which are related to the bioaccumulation potential. For example, it was reported that PFHxS eliminated more rapidly than PFOS in rainbow trout (Martin et al., 2003a), but much slower than PFOS in rodent species, monkeys, and humans (Kowalczyk et al., 2013; Olsen et al., 2007; Sundström et al., 2012; Zhang et al., 2013). However, the underlying mechanisms accounting for these discrepant behaviors between fish and mammals are unclear and need further investigation.

Previous studies have indicated that PFAAs associate strongly with proteins such as serum albumin (SA) (Bischel et al., 2012; Chen and Guo, 2009; Hebert and MacManus-Spencer, 2010; Ng and Hungerbühler, 2013; Ng and Hungerbühler, 2014). SA is a typical in model protein that can bind with a variety of ligands, such as fatty acids (Zhang et al., 2009). Due to the similar chemical structures, it is assumed that PFAAs may compete with fatty acids for the binding sites of proteins (Bischel et al., 2012). It was reported that PFAAs could interact with bovine serum albumin (BSA) via ionic binding, Van-der Waals interaction, and hydrogen bonding (Chen et al., 2015a; Zhang et al., 2009). The binding affinity between PFAAs and SA is closely related to the perfluoroalkyl carbon chain length (Chen and Guo, 2009). The binding sites and affinities of short-chain PFAAs to proteins may differ from those of long-chain PFAAs (Hebert and MacManus-Spencer, 2010). This could affect the uptake and elimination of PFAAs and thus their bioaccumulation capacities. However, studies on the protein-binding behavior of PFAAs were limited to BSA and human serum albumin (HSA) (Bischel et al., 2011). There is sparse information on their binding affinities with fish blood protein (FBP), particularly for short-chain PFSAs.

PFAAs synthesized by electrochemical fluorination (ECF) are generally a mixture of linear and branched isomers (Bischel et al., 2012). Previous investigations demonstrated that the bioaccumulation of PFOS and PFOA was isomer specific in aquatic organisms, and usually linear (*n*-) isomers were preferentially enriched (Fang et al., 2014a; Fang et al., 2014b; Houde et al., 2008). However, there have been few studies on the isomeric accumulation of short-chain PFSAs, such as PFHxS, in aquatic organisms.

The objectives of this study are as follows: 1) to investigate the uptake and elimination kinetics, as well as bioconcentration factors (BCF) of PFAAs in carps with different carbon chain lengths and functional groups; 2) to explore the mechanisms accounting for the accumulation of PFAAs, including the binding affinities to FBP and elimination pathways via the feces and urine; and 3) to unveil the isomer-specific bioaccumulation behaviors of PFOS and PFHxS, including the major accumulation tissues and elimination pathways.

2. Materials and methods

2.1. Chemicals and reagents

PFOS (98% purity) was obtained from Tokyo Kasei Kogyo. PFHxS (98%) and PFBS (98%) were purchased from Sigma Chemical Company, St. Louis, U.S.A. PFOA (95%) and perfluorononanoic acid (PFNA; 97%) were purchased from Shanghai Adam Beta Reagent, China. Perfluorodecanoic acid (PFDA; 96%) and perfluorododecanoic acid (PFDoA; 97%) were obtained from Geel, Belgium. Perfluoroundecanoic acid (PFUnA; 96%) was bought from FluoroChem Ltd., Derbyshire, United Kingdom. A mixture of mass-labelled perfluoroalkyl carboxylic acids and mass-labelled perfluoroalkylsulfonates (MPFAC-MXA) were purchased from Wellington Laboratories, Boston, U.S.A. Methanol of high performance liquid chromatography (HPLC) grade, formic acid (HPLC grade) and ammonium acetate (99%) were purchased from Dikma Technology, CA, U.S.A. Ammonium hydroxide solution (NH_4OH; 25%) and sodium hydroxide (99%) were obtained from Guangfu Fine Chemical Research Institute, Tianjin, China. Methyl tert-butyl ether (MTBE; HPLC grade) was purchased from Kangkede Technology Company, Tianjin, China. Tetrabutyl ammonium hydrogen sulfate (TBAH; 99%) was purchased from Meryer Chemical Technology Company, Shanghai, China. Sodium carbonate (99.8%) was purchased from Jinke Fine Chemical Research Institute, Shanghai, China. Phosphate buffered solution (PBS, 0.01 M, pH 7.1–7.3) was obtained from Dingguo Company, Tianjin, China. MilliQ water was used throughout the study.

2.2. Isomer nomenclature

The nomenclature for the specific PFOS isomers was adopted from Benskin et al. (2007). The prefix "*n*-" typically refers to a linear chain. The prefix "*m*-" refers to mono-methyl branched isomers with one perfluorinated methyl branched chain, and the number preceding "*m*-" indicates the position of the branch in the carbon chain, while "*iso*-" means that the branch is located at the end of the carbon chain. The prefix "*m2*-" indicates two methyl groups in a dimethyl branched isomer.

2.3. Fish exposure tests

Carp were purchased from a local farmer's market in Tianjin and acclimatized for one month in the laboratory before exposure. Three rectangular fish aquaria of 60 L volume were used to conduct the experiments. They were made of polypropylene, which prevents the adsorption of PFAAs to the tank. Each tank contained 40 fish. One of the tanks was used for the control test, in which 40 L of filtered dechlorinated water without any PFAAs was applied. The other two tanks were used for exposure tests in parallel, in which the water was spiked with PFAAs, including PFBS, PFHxS, PFOS, PFOA, PFNA, PFDA, PFUnA, and PFDoA, each at 2 nmol/L. Filtered dechlorinated water with a hardness of 95.0 ± 3.0 mg/L $CaCO_3$, pH of 7.6 ± 0.5, and dissolved oxygen concentration of 7.0 ± 0.4 mg/L, was maintained at 20 ± 3 °C