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Protein-specific distribution patterns of perfluoroalkyl acids in egg yolk and albumen samples around a fluorochemical facility

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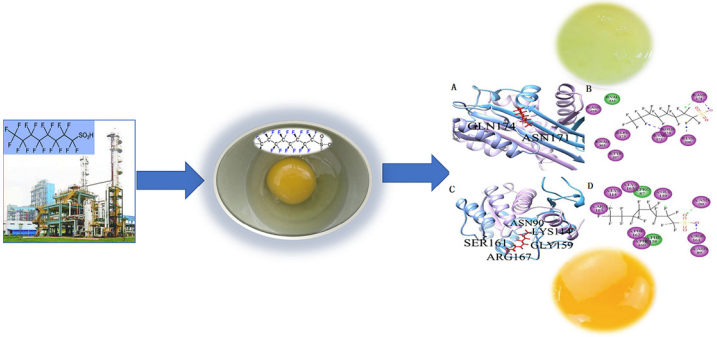
HIGHLIGHTS

- PFAAs and main isomers were detected in chicken eggs around a fluorochemical plant.
- Higher levels of PFAAs and typical isomers were in egg yolk than in albumen.
- Different binding patterns of PFAAs to protein from yolk and albumen were modeled.
- PFAAs dietary exposure around a fluorochemical plant should be of a high concern.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, eggs from free-range and barn chickens in farms around a fluorochemical facility were collected to assess the distribution profiles of perfluoroalkyl acids (PFAAs), including isomers of perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), and perfluorohexane sulfonate (PFHxS), in egg yolk and albumen. The results revealed that the concentrations of PFAAs in yolks were significantly higher than those in albumen. All 17 PFAAs examined could be detected in yolks, showing decreasing concentrations with increasing distance from the fluorochemical facility. The three predominant compounds in yolks were perfluorobutanoic acid (PFBA, mean concentration 81.4 ng/g ww), PFOS (28.0 ng/g ww), and PFOA (4.83 ng/g ww), and this result is consistent with the product structure of the facility. Moreover, *n*-PFOA, *n*-PFOS, and *n*-PFHxS were the dominant contaminants in yolk, with mean concentrations of 4.75, 25.7, and 4.29 ng/g ww, respectively. In albumen, PFBA was still the predominant PFAA congener (mean concentration = 3.93 ng/g ww), followed by PFOA. Docking analysis indicated that the PFAAs presented higher binding abilities with the low density lipoprotein, high density lipoprotein, and vitellin proteins in yolk than that with ovalbumin albumen proteins, which might be the main factor influencing the possible difference in distributions of PFAAs in yolk and albumen.

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1. Introduction

Perfluoroalkyl acids (PFAAs) constitute a group of synthetic surfactants characterized by fully fluorinated carbon chains with a hydrophilic functional group. These compounds have been widely used in consumer and industrial products such as food packaging, water and stain proofing agents, and fire-fighting foams. The ubiquitous presence of these PFAAs in various environments and their adverse effects on environment, biota (Gebbink et al., 2016), and human health (Gao et al., 2015) make it necessary to understand their sources, transport, and ultimate fate in the environment. Owing to their environmental persistence, bioaccumulation, and toxicity, perfluorooctane sulfonate (PFOS), its salts, and perfluorooctane sulfonyl fluoride (POSF) were listed as severe organic pollutants in Annex B of the Stockholm Convention in 2009. Moreover, perfluorooctanoic acid (PFOA) and perfluorohexanesulfonate (PFHxS) were listed as persistent organic pollutants in the Stockholm Convention in 2015 and 2017, respectively (Wang and Sun, 2016; Stockholm Convention, 2017).

PFAAs are manufactured by electrochemical fluorination (ECF) and telomerization processes (Lehmler, 2005). ECF process produces a mixture of linear (70%) and branched (30%) PFAA isomers, while telomerization process produces only linear isomers (Benskin et al., 2010). Differences in the structures of these linear and branched isomers result in different properties (Beesoon and Martin, 2015). According to literature, linear and branched PFHxS, PFOS, and PFOA isomers in the maternal serum can be transported across the placenta to cord serum and the transport rates of branched PFOS isomers decrease as the branching point moves away from the sulfonate moiety (Chen et al., 2017).

Unlike other lipophilic organic pollutants such as polyaromatic hydrocarbons and dioxins, PFAAs tend to bind to proteins. Studies evidenced that PFAAs bind to protein with appropriate binding sites, either as a ligand or using hydrogen-bonding as driving force to form protein–ligand complexes in the PFAAs-protein system (Bischel et al., 2011; Zhang et al., 2013). Thus, these compounds are often found in protein-rich tissues such as eggs (Lopez-Antia et al., 2017) and serum (Gao et al., 2015).

Eggs are one of the most important dietary sources in China. In this study, eggs from free-range and barn chickens in farms around a PFAA manufacturing facility in China were collected to study the distributions and congener profiles of PFAAs in egg yolk and albumen. Furthermore, the binding process of the PFAA isomers to specific proteins in the egg yolk and albumen were also modeled. The objectives of this study were as follows: 1) to examine the contents and composition profiles of PFAAs in eggs; 2) to investigate the levels and composition profiles of the PFOS, PFOA, and PFHxS isomers in eggs; and 3) to understand difference in distribution mechanisms of PFAAs and their isomers in egg albumen and yolks.

2. Materials and methods

2.1. Nomenclature and acronyms

The nomenclature and acronyms of PFAA isomers were adapted from the study by Benskin et al. (2007). For PFOS, linear PFOS was represented as *n*-PFOS, 1-perfluoromethyl as 1*m*-PFOS, sum of 3-perfluoromethyl and 5-perfluoromethyl as (3 + 5)*m*-PFOS, 4-perfluoromethyl as 4*m*-PFOS, perfluoroisopropyl as iso-PFOS, and total dimethyl perfluoromethyl isomers as *m2*−PFOS. For PFOA, linear PFOA was denoted as *n*-PFOA, 3-perfluoromethyl as 3*m*-PFOA, 4-perfluoromethyl as 4*m*-PFOA, 5-perfluoromethyl as 5*m*-PFOA, perfluoroisopropyl as iso-PFOA, and *t*-perfluorobutyl as *tb*-PFOA. For PFHxS, linear PFHxS was denoted as *n*-PFHxS and its branched isomer as *br*-PFHxS.

2.2. Chemicals and reagents

All the standards, including the mixture of 17 PFAAs standards (PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA, PFHxDA, PFODA and PFBS, PFHxS, PFOS, and PFDS), the mixture of 12 mass labeled internal standards ($^{13}C_4$PFBA, $^{13}C_4$PFPeA, $^{13}C_2$PFHxA, $^{13}C_2$PFHpA, $^{13}C_4$PFOA, $^{13}C_5$PFNA, $^{13}C_2$PFDA, $^{13}C_2$PFUnDA, $^{13}C_2$PFDoDA, $^{13}C_2$PFBS, $^{18}O_2$PFHxS, and $^{13}C_4$PFOS), and the isomer standards of *n*-,1*m*-, 3*m*-, 4*m*-, 5*m*-, *iso*-, (4,4)m_2-, (4,5)m_2-, and (5,5)m_2-PFOS; *n*-, 3*m*-, 4*m*-, 5*m*-, *iso*-, and *tb*-PFOA; *n*- and *br*-PFHxS mixture were purchased from Wellington Laboratories (Canada).

Methanol (LC/MS-grade) was purchased from Fisher (USA). Ultrapure water was prepared using a Milli-Q Advantage A10 system (Millipore, USA). Tetrabutylammonium Hydrogen Sulfate (TBA), formic acid, and ammonium hydroxide were purchased from Alfa Aesar (USA). OASIS WAX (6 cc, 150 mg) cartridges were purchased from Waters Co. (Ireland).

2.3. Sample collection and pretreatment

The PFAA manufacturing facility is located in the Hubei province, China. The chicken egg sampling sites are located at 500 to 3650 m away from the facility. Nineteen eggs were collected from two chicken farms, while the free-range chicken eggs were collected from 18 sampling sites around the facility. The details of sampling sites are presented in Fig. 1. A total of 109 eggs from free-range chickens were collected.

The egg albumen and yolk were separated in the laboratory. We opened a hole in the eggshell and the egg albumen flowed into a clean polypropylene tube. Then, the vitelline membrane was pierced, and the egg yolk was allowed to flow into another clean polypropylene tube. Thus, we collected the "pure" yolk. The PFAAs were extracted by an ion-pair method based on our previous study (Gao et al., 2015). Sample (0.5 g) was weighed in a 15–mL polypropylene tube and spiked with internal standard (5 ng) (details in supporting information). Next, TBA (1 mL, 0.5 M) and sodium carbonate buffer (2 mL, 0.25 M) were added and mixed thoroughly. Methyl tert-butylether (MTBE, 5 mL) was then added for extraction. The mixture was shaken vigorously at 270 rpm for 30 min and subsequently centrifuged at 3500 rpm for 10 min. Threefold extraction was performed per sample and the resultant extracts were combined. The solutions were concentrated to 0.5–1.0 mL under a gentle stream of nitrogen (N_2). Methanol (1 mL) was added and the extract was further concentrated to 0.5 mL. Finally, the sample was diluted with water (13 mL).

Solid phase extraction (SPE) was carried out for the sample cleanup. The WAX cartridges (6 cc, 150 mg) were preconditioned with 4 mL each of 0.1% ammonium hydroxide in methanol, methanol, and water in succession. The samples were then loaded onto the cartridges and ammonium acetate (4 mL, pH = 4) was added to remove the impurities. Finally, the target compounds were eluted with 4 mL each of methanol and 0.1% ammonium hydroxide in methanol. The elutes were concentrated to 1.0 mL under a gentle stream of N_2 and subsequently transferred to an injection vial for instrumental analysis.

2.4. Instrumental analysis and quantification

The purified PFAAs were analyzed by the methods reported in our previous study (Gao et al., 2015) with slight modification. The HPLC-ESI-MS/MS system comprised an UltiMate™ 3000 DGLC high-performance liquid chromatograph (Thermo Fisher Scientific, USA) and a TSQ Quantiva triple quadrupole mass spectrometer (Thermo Fisher Scientific, USA). The sample (10 μL) was injected into an Acclaim 120 C_{18} column (4.6 × 150 mm, 5 μm, Thermo Fisher Scientific, USA). Ammonium acetate (10 mM, pH 4, A) and acetonitrile (B) were used as the mobile phases. The flow rate was set at 1 mL/min. The dual mobile-phase gradient started at 10% B; held constant for 1.5 min; changed to 95% B within 4 min, remained constant until 8 min; returned to the initial condition within 8.5 min; and then balanced for 1.5 min, i.e., total 10 min per injection. The mass spectrometer was operated in the negative ion ESI-MS/MS mode using the multiple reaction monitoring mode. Aiming to achieve satisfactory qualitative and quantitative