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Temporal trends (1999–2010) of perfluoroalkyl acids in commonly consumed food items

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ABSTRACT

The aim of this study was to determine how dietary exposure to PFAAs has changed over the period when major production changes occurred. Archived samples (1999–2010) of eggs, milk and farmed rainbow trout were analyzed by ultra performance liquid chromatography coupled to tandem mass spectrometry. Statistically significant decreasing trends were observed for concentrations of perfluorooctane sulfonic acid (PFOS) and perfluorohexane sulfonic acid (PFHxS) in fish ($p < 0.002$ and $p < 0.032$, respectively) and eggs ($p < 0.001$ for both compounds). Concentrations of PFOS in fish and eggs decreased by a factor of 10 and 40, respectively. In eggs there was also a statistically significant decreasing trend in concentrations of perfluorooctanoic acid (PFOA). The results of this study demonstrate that PFAA concentrations in food items from agricultural food chains and aquatic food chains close to sources respond rapidly to changes in environmental emissions. Implications for the overall understanding of human exposure are discussed.

1. Introduction

Perfluoroalkane sulfonic acids (PFSAs) and the structurally similar perfluoroalkyl carboxylic acids (PFCAs) are two classes of perfluoroalkyl acids (PFAAs) that belong to the family of per- and polyfluoroalkyl substances (PFASs). This family includes a wide range of aliphatic substances, all containing a fully fluorinated (perfluorinated) moiety ($C_nF_{2n+1}-$) linked to different functional groups (Buck et al., 2011). The PFASs make up a diverse group of commercially important chemicals that have been produced since the 1950s (Buck et al., 2011; Paul et al., 2009; Prevedouros et al., 2006). Due to their exceptional surfactant properties and resistance to degradation (Kissa, 2001), some PFASs have been widely used in industrial processes (e.g. production of fluoropolymers) and in commercial products, such as water and stain proofing agents, lubricants, paints and fire-fighting foams (Kissa, 2001; Prevedouros et al., 2006). The worldwide presence in humans and wildlife (Giesy and Kannan, 2001; Glynn et al., 2012; Houde et al., 2006; Kannan et al., 2004; Lau et al., 2007; Martin et al., 2004), as well as hazardous properties (Lau et al., 2007; Steenland et al., 2010), has been reported for certain PFCAs and PFSAs.

Between 2000 and 2002, the major global manufacturer (3M Company) ceased their production of perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorohexane sulfonic acid (PFHxS), perfluorodecane sulfonic acid (PFDS), as well as related C_6, C_8 and C_{10} chemistries (US EPA, 2000). After the 3M phase out, production of PFOA, perfluorononanoic acid (PFNA) and their precursors has continued by other companies because these compounds are still in high commercial demand (Prevedouros et al., 2006). However, voluntary and regulatory actions have been taken to reduce environmental discharges of PFOA and other long-chain PFCAs (C_8-C_{20} PFCAs, which are those with 7–19 perfluorinated carbons) (US EPA, 2006).

Since the phase-out of production and use of PFOS and PFOA by 3M, a significant decline of PFOS and PFOA levels has been reported in North American and European studies on human blood, serum and plasma (Calafat et al., 2007; Glynn et al., 2012; Haug et al., 2009; Kato et al., 2011; Olsen et al., 2008; Spliethoff et al., 2008). The concentrations of PFOA have decreased at a slower rate compared to those of PFOS despite that the intrinsic elimination

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half-life of PFOA is shorter than that of PFOS (Olsen et al., 2007). This indicates that the industrial phase out actions have led to a more efficient reduction of exposure sources to PFOS compared to PFOA. The relative importance of different human exposure pathways for PFAAs after the 3M phase-out is under debate (Renner, 2007). Several recent exposure modeling studies have estimated dietary intake to be the major current human exposure pathway for PFOS and PFOA (Egeghy and Lorber, 2011; Fromme et al., 2007; Lorber and Egeghy, 2011; Vestergren and Cousins, 2009; Vestergren et al., 2008). According to a Swedish dietary intake study (Vestergren et al., 2012a), food contributes to more than 80% of the ongoing total exposure to PFOS and PFOA for the adult Swedish population. The study by Vestergren et al. (2012a) further reported preliminary findings of a declining temporal trend of PFOS in egg and meat samples from 1999, 2005 and 2010. In order to allow for a refined assessment of the historical and ongoing human exposure to PFAAs, the current study investigated the temporal trends of a range of homologues of PFSAs and PFCAs in a few important food items before, during and following the 3M manufacturing phase out.

2. Materials and methods

2.1. Samples

The food matrices chosen for analysis were eggs, farmed fish and milk. According to Vestergren et al. (2012a) eggs and fish products each contributed with about 30–40% to the total dietary exposure of the Swedish population to PFOS in 1999. The present study used samples of rainbow trout muscle, fresh cow's milk and hen's egg yolks, which had been collected annually from 1999 to 2010 within the Swedish National Food Agency's (NFA) official food control program. A total of 36 pooled egg samples, 36 pooled milk samples and 36 individual fish samples were analyzed.

2.1.1. Eggs

Sampling of eggs was spread out over Sweden with emphasis on the largest packaging plants in the southernmost 1/3 of the country. Every year samples from 20 to 25 producers were banked for future contaminant analyses. Each banked sample consisted of a pool of 10–12 eggs from one producer. The yolks were separated from the egg whites before homogenization (using a stainless steel mixer) and freezing. Samples were stored in glass jars packed in vacuum-sealed polypropylene bags and kept at −20°C until analysis. From each year, all available egg yolk samples were divided into 3 pools, in such a manner that the samples of varying fat content (16–34%) and geographical origin were represented in all pools.

The pooled samples of egg yolks from the NFA control program comprised eggs from both conventional and organic production. Information on the number of organic eggs sampled was not available. However, the proportion of organic eggs in the pooled food control samples was probably low, since no selective sampling had been carried out. Between 2000 and 2010 the production of organic eggs increased from 2.5% to 11.5% of the total Swedish production (Lovén Persson, 2011; Jordbruksverket, 2010).

2.1.2. Milk

Fresh milk was sampled from the tanks of milk transport vehicles between 1999 and 2009 as part of the food control program. The tanks generally contained milk from 10 dairy farms. In 2010 milk samples were taken from the milk storage tanks on individual dairy farms. Sampling was conducted predominantly in the southernmost 1/3 of Sweden where most of the milk production occurs. Each year 10–25 milk samples (40 ml in volume) were collected as part of the food control program and banked for future analysis of contaminants. These samples were stored in polypropylene containers packed in vacuum-sealed polypropylene bags at −20 °C. All available samples were divided into 3 pools per year, in such a manner that samples of varying fat content (1.3–6.8%) and geographical origin were represented in all pools.

2.1.3. Fish

Farmed rainbow trout (whole fish) were collected from fish farms along the Swedish Baltic Sea coast (brackish water). Each year 1–10 samples from the food control program were banked for future analysis of contaminants. Only fish older than 12 months were sampled. Accurate information on fish age was obtained from the fish farmers, who record fish age carefully as common practice in aquaculture. Cutlets were taken between the dorsal fin and the tail fin, stored wrapped in aluminum foil and packed in vacuum-sealed polypropylene bags at −20 °C. Muscle samples were taken from each of the cutlets and analyzed individually.

2.2. Methods

Chemicals and standards used in this study as well as the sample preparation methods are described in detail in the Supplementary Data. In short, the egg yolk samples were extracted using ion pair extraction with methyl *tert*-butylether (MTBE) combined with solid phase extraction clean-up on Florisil and graphitized carbon. This method is described in detail by Vestergren et al. (2012b). The milk was extracted first with acetonitrile under acidic conditions and the residuals were subsequently back-extracted with MTBE under basic conditions, using a modified version of the method first described by Olsen et al. (2007). The extraction method used for fish samples is based on a method developed by Powley et al. (2005) with modifications for biota samples described by Berger et al. (2009). In short, samples were extracted using acetonitrile and the extracts were subjected to dispersive clean-up on graphitized carbon and glacial acetic acid.

The instrumental analysis and quantification is described in detail elsewhere (Vestergren et al., 2012b) and in the Supplementary Data. In short, the purified sample extracts were analyzed using an Acquity ultra performance liquid chromatography system coupled to a Xevo TQS tandem mass spectrometer with an electrospray ionization interface operated in the negative ion mode (all Waters Corp.). Concentrations of two PFSAs ($C_nF_{2n+1}SO_3^-$, $n = 6, 8$) and nine PFCAs ($C_nF_{2n+1}COO^-$, $n = 5–13$) were quantified. Quantification was performed using the internal standard method (isotopic dilution) for all target analytes. For perfluoroheptanoic acid (PFHpA), perfluorotridecanoic acid (PFTrDA) and perfluorotetradecanoic acid (PFTeDA), which did not have corresponding isotope labeled standards, $^{13}C_2$-perfluorohexanoic acid ($^{13}C_2$-PFHxA, for PFHpA) and $^{13}C_2$-perfluorododecanoic acid ($^{13}C_2$-PFDoDA) were used for quantification, respectively. PFOS was quantified using the transition to *m/z* 99. In the presence of branched PFOS isomers (detected in egg samples only) the sum of branched isomers was quantified relative to a branched PFOS standard. A linear relationship ($R^2 > 0.99$) between signal and concentration was observed for all homologues in a five point external standard calibration curve with concentrations ranging from 0.2 to 4.98 pg/μl. All quantified concentrations given in this study are on a sample fresh weight (fw) basis and were not blank corrected.

2.3. Analytical quality assurance

A series of 3–5 procedural blanks were analyzed along with every batch of samples for determination of the method detection limits (MDLs) and the method quantification limits (MQLs) of the different target analytes. If the procedural blank chromatograms contained detectable signals of a certain PFAA, its MDL was defined as the arithmetic mean plus three times the standard deviation of the analyte signal in the procedural blanks. Analogously, the MQL was defined as the arithmetic mean plus ten times the standard deviation of the analyte signal. In cases where the procedural blank extracts did not contain a detectable signal, the MDLs were set equal to the lowest quantified concentration in a sample with an analyte signal displaying a signal-to-noise ratio of at least 3. Analogously, the MQLs were defined at a signal-to-noise ratio of 10. PFHxS, PFOS, PFNA, perfluoroundecanoic acid (PFUnDA), PFDoDA, PFTrDA and PFTeDA were typically non-detectable in the procedural blank chromatograms for all methods, which was reflected in lower MDLs compared to the other analytes (PFHxA, PFHpA, PFOA, perfluorodecanoic acid (PFDA)).

$^{13}C_8$-PFOA and $^{13}C_8$-PFOS were used as volumetric standards to calculate recoveries of the internal standards in all samples analyzed. The mean recoveries of the different internal standards were in the ranges 34–64%, 43–70% and 51–112% for the methods for egg yolk, fish and milk, respectively (Table S1 in the Supplementary Data). Due to the use of authentic mass-labeled internal standards for all analytes, the variability of absolute recoveries between matrices did not affect the precision and accuracy of quantification (see next paragraph).

The precision and accuracy of the analytical methods were evaluated for each sample matrix by controlled low-level laboratory "spiking" experiments with the target analytes as described in detail in the Supplementary Data, including Tables S2–S4. Precision, expressed as coefficient of variation in triplicate analysis, was in the range 7–17% for egg yolk, 1–10% for milk and 2–14% for fish, with the exception of few higher values for PFAAs that were not included in temporal trend analyses (Tables S2–S4). The good precision of all analytical methods allowed for reliable temporal trend analysis. Accuracy is expressed as relative deviation of the quantified concentration in the spiked sample matrix from the theoretical spike level (Tables S2–S4). This deviation was ≤33% for all PFAAs for which statistical temporal trend analysis was performed in the different sample matrices (see below).

2.4. Statistics

Statistical analyses of temporal trends were performed when levels of a given PFAA were above the MDL in more than 40% of the samples. Values below the MDL (non-detects) were replaced by MDL/√2 prior to statistical treatment (Loftis et al., 1989). Other methods have been suggested that are an improvement on the common practice of recalculating non-detects as MDL/√2, and which would increase the statistical power (Helsel, 2005). However, these alternative methods require several values to be above MDL in any particular year and therefore could not be applied in the present study. For samples with a PFAA concentration between MDL and MQL