Environmental Pollution 199 (2015) 166–173

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Temporal changes (1997–2012) of perfluoroalkyl acids and selected precursors (including isomers) in Swedish human serum

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ARTICLE INFO

Article history:
Received 26 October 2014
Received in revised form
19 January 2015
Accepted 21 January 2015
Available online

Keywords:
Per- and polyfluoroalkyl substances
Precursors
Human serum
Temporal trend
PAPs
Isomers

ABSTRACT

Concentrations (including isomer patterns) and temporal changes (1997–2012) of perfluoroalkyl acids (PFAAs) and selected perfluorooctane sulfonate (PFOS) and perfluoroalkyl carboxylic acid (PFCA) precursors were determined in serum samples from Swedish women. Perfluorooctane sulfonamide (FOSA) and perfluorooctane sulfonamidoacetic acid (FOSAA), as well as its N-methyl and N-ethyl derivatives (MeFOSAA and EtFOSAA) were consistently detected. Highest PFOS precursor concentrations were found for EtFOSAA (before year 2000) or MeFOSAA and FOSAA (after 2000). Disappearance half-lives for all PFOS precursors were shorter compared to PFOS. 4:2/6:2 and 6:2/6:2 polyfluoroalkyl phosphate diesters (diPAPs) were detected in <60% of the samples, whereas 6:2/8:2 and 8:2/8:2 diPAPs were detected in >60% of the samples, but showed no significant change in concentrations over time. Linear and sum-branched isomers were quantified separately for three PFAAs and three precursors. Significant changes between 1997 and 2012 in the % linear isomer were observed for PFOA and FOSA (increase) and PFOS (decrease).

1. Introduction

Per- and polyfluoroalkyl substances (PFASs) are chemicals that have been used in industrial applications and in consumer products since the 1950s, and have been identified as environmental pollutants with perfluoroalkane sulfonic acids (PFSAs) and perfluoroalkyl carboxylic acids (PFCAs) as major compound classes of concern (Buck et al., 2011). PFSAs and PFCAs are persistent in the environment and their long-chain homologues ($\geq C_6$ PFSAs and $\geq C_8$ PFCAs) have bioaccumulative properties. There are, however, also PFASs that are less persistent and that can undergo transformation. Methyl and ethyl perfluorooctane sulfonamidoethanols (Me- and EtFOSE) and perfluorooctane sulfonamides (Me- and EtFOSA) are PFASs that have been identified as precursors to perfluorooctane sulfonic acid (PFOS) (Benskin et al., 2009; Peng et al., 2014; Xie et al., 2009). The manufacturing of PFOS and its precursors resulted in a mixture of linear and branched isomers (Martin et al., 2010), and production was phased out by the main manufacturer between 2000 and 2002 in North America and Europe. Fluorotelomer-based chemicals such as polyfluoroalkyl phosphate esters (PAPs) have been identified as precursors to PFCAs (D'Eon and Mabury, 2011a). The production of fluorotelomer-based chemicals (producing only linear isomers) is still ongoing, however, industry has committed to reduce production of PFCAs with a chain length $\geq C_8$ and other fluorinated chemicals that can degrade to these PFCAs, and to eliminate emissions of these chemicals by 2015 (US EPA, 2006).

External human exposure to PFOS isomers, PFCAs and precursors has recently been estimated by Gebbink et al. (2015a). Exposure pathways such as ingestion of food, dust, and drinking water and inhalation of air were all identified as sources of PFOS, PFCAs and/or their precursors to humans. Cumulative exposure intakes of PFOS precursors were estimated to be comparable to the daily intakes of PFOS itself, while daily intakes of 6:2, 8:2, and 10:2 fluorotelomer based chemicals were higher compared to perfluorohexanoic acid (PFHxA), perfluorooctanoic acid (PFOA) and perfluorododecanoic acid (PFDA). In Sweden, diet and drinking water have been identified as exposure pathways for the general population for PFOS and its precursors (FOSA, perfluorooctane sulfonamidoacetic acid (FOSAA) and its methyl and ethyl derivatives (MeFOSAA and EtFOSAA)), while the diet was a source of

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http://dx.doi.org/10.1016/j.envpol.2015.01.024

diPAP exposure (Filipovic and Berger, 2015; Gebbink et al., 2013, 2015b; Ullah et al., 2014). In food basket samples and herring, the relative importance of precursors for PFAA exposure has recently decreased over time (Gebbink et al., 2015b; Ullah et al., 2014), however, such temporal trend studies for other exposure pathways are lacking.

With respect to human biomonitoring, PFSAs and PFCAs have been studied extensively in different populations (Glynn et al., 2012; Haug et al., 2009; Kato et al., 2011; Yeung et al., 2013a, 2013b). For most of these populations, declining temporal trends in serum were seen for PFSAs and in particular PFOS. This was linked to the phase out of PFOS and related chemicals by industry in 2002. For longer chain length PFCAs ($>C_8$), increasing trends were often observed in the studied populations. Recently, human biomonitoring studies have reported a variety of precursor compounds. FOSAA, MeFOSAA, and EtFOSAA, as well as diPAPs were detected in human serum from the US, Germany, and Hong Kong (D'Eon et al., 2009; Lee and Mabury, 2011; Loi et al., 2013; Olsen et al., 2004; Yeung et al., 2013a, 2013b). Declining temporal trends were seen in whole blood or serum for PFOS precursors, while diPAP concentrations and detection frequency were generally low. In Swedish serum samples only the PFOS precursor FOSA was analyzed so far with rapidly declining concentrations between 1996 and 2010 (Glynn et al., 2012). On the other hand, Liu et al. (2015) recently suggested that the relative importance of PFOS precursors for total internal exposure to PFOS has increased in the Swedish population (based on isomer and enantiomer pattern biomarkers). However, it is unclear which PFOS precursors (other than FOSA) and PFCA precursors the Swedish population has been and still is exposed to.

The first aim of this study was to investigate the presence, concentrations, and temporal changes of PFAAs and selected precursors in serum samples donated by nursing primiparous women from Uppsala, Sweden, between 1997 and 2012. Using a more sensitive analytical method compared to Glynn et al. (2012), quantitative data for a larger range of PFAA homologues were obtained. The second aim was to investigate whether temporal changes in the relative contribution of branched and linear PFAA and precursor isomers in serum could shed light on the importance of precursors for total human exposure to PFAAs and therewith confirm the findings for PFOS by Liu et al. (2015). The serum samples were analyzed for the following PFASs: $C_{4,6,8,10}$ PFSAs, $C_{4\text{-}14}$ PFCAs, FOSA and FOSAA and their N-methyl and N-ethyl derivatives, as well as 4 monoPAPs and 11 diPAPs.

2. Material and methods

2.1. Chemicals and reagents

Native and labeled standards of PFSAs, PFCAs, FOSAs, FOSAAs, and mono- and diPAPs used in this study are listed in Table S1 in the Supporting Information. All solvents and reagents were of the highest commercial purity and employed as received.

2.2. Serum samples

Between 1996 and 2012 primiparous women living in Uppsala County, Sweden, donated blood samples within the fourth week after delivery for a study on temporal trends of persistent halogenated organic compounds in pregnant and nursing women, the POPUP study (Persistent Organic Pollutants in Uppsala Primiparas) (Glynn et al., 2012; Lignell et al., 2009). In the present study, per year, 30 individual serum samples were pooled into 3 pools (9 or 10 individual samples per pool). Serum pools from the following years were included in this study: 1997 (each of the three pools contained two individual serum samples collected in 1996), 1998, 2000, 2002, 2004, 2006, 2008, 2010, and 2012 (Table S2). For each year, two aliquots of all three serum pools were analyzed. The study was approved by the regional ethical vetting board in Uppsala, Sweden, and the participating women gave informed consent before donating the blood samples.

2.3. Sample preparation

The extraction and clean-up of the samples is based on published methods (Gebbink et al., 2013, 2015b). Briefly, serum samples (1 g) were spiked with labeled internal standards (500 pg each, see Table S1 for all internal standards used) and 3 mL acetonitrile were added. The samples were mixed by vortex and placed in a sonication bath for 15 min, after which the samples were centrifuged for 5 min at 3000 rpm. The organic phase was transferred to a separate tube and the extraction procedure was repeated. The combined extracts were concentrated to ~1 mL under a stream of nitrogen. SPE WAX cartridges (150 mg, 6 mL, Waters) were conditioned with 6 mL methanol and 6 mL water. The sample extracts were loaded onto the WAX columns and the columns were washed with 1 mL 2% aqueous formic acid and then with 2 mL water. The columns were dried by applying a vacuum and by centrifugation before neutral compounds were eluted with 3 mL methanol (fraction 1). The ionic compounds were subsequently eluted with 4 mL of a solution of 1% ammonium hydroxide in methanol (fraction 2). Both fractions were dried under a stream of nitrogen and the residuals were redissolved in 150 μL of methanol. The extracts were filtered using centrifugal filters (modified nylon 0.2 μm, 500 μL, VWR International) and $^{13}C_8$-PFOA and $^{13}C_8$-PFOS (500 pg each) were added as recovery internal standards prior to ultra-performance liquid chromatography-tandem mass spectrometry (UPLC/MS/MS) analysis.

2.4. Instrumental analysis and quantification

Fraction 1 was analyzed for FOSAs, while fraction 2 was analyzed twice, first for PFSAs, PFCAs, and FASAAs and then for mono- and diPAPs. For all instrumental analyses, separation was carried out on an Acquity UPLC system (Waters) equipped with a BEH C18 (50 × 2.1 mm, 1.7 μm particle size, Waters) analytical column. Mobile phases were (A) 95% water and 5% methanol and (B) 75% methanol, 20% acetonitrile, and 5% water. Both mobile phases contained 2 mM ammonium acetate and 5 mM 1-methyl piperidine. The column temperature was set at 40 °C, and the injection volume was 5 μL. Mobile phases, the gradient programs and flow rates for the different UPLC methods can be found in Tables S3 and S4. Connected to the UPLC system was a Xevo TQ-S triple quadrupole mass spectrometer (Waters) operated in negative ion electrospray ionization (ESI−) mode. The capillary voltage was set at 3.0 kV, and the source and desolvation temperatures were 150 °C and 350 °C, respectively. The desolvation and cone gas flows (nitrogen) were set at 650 L/h and 150 L/h, respectively. Compound-specifically optimized cone voltages and collision energies are listed in Table S1.

Quantification was performed using an internal standard approach. Analytes lacking an analogous labeled standard were quantified using the internal standard with the closest retention time (Table S1). Quantification was performed using the precursor − product ion multiple reaction monitoring (MRM) transitions reported in Table S1. For all precursor compounds an additional product ion was monitored for confirmation. For diPAPs for which no authentic standards were available, a technical mixture was used to optimize MRM channels and for confirmation of retention times (Gebbink et al., 2013). Quantification of these diPAPs was