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Time trends of perfluorinated alkyl acids in serum from Danish pregnant women 2008–2013

Christian Bjerregaard-Olesen [a], Cathrine C. Bach [b], Manhai Long [a], Mandana Ghisari [a], Rossana Bossi [c], Bodil H. Bech [d], Ellen A. Nohr [e], Tine B. Henriksen [b,f], Jørn Olsen [d], Eva C. Bonefeld-Jørgensen [a,*]

[a] Centre for Arctic Health & Unit of Cellular and Molecular Toxicology, Department of Public Health, Aarhus University, Aarhus, Denmark
[b] Perinatal Epidemiology Research Unit, Aarhus University Hospital, Skejby, Denmark
[c] Department of Environmental Science, Aarhus University, Roskilde, Denmark
[d] Section for Epidemiology, Department of Public Health, Aarhus University, Aarhus, Denmark
[e] Research Unit for Gynaecology and Obstetrics, Institute of Clinical Research, University of Southern Denmark, Odense, Denmark
[f] Department of Pediatrics, Aarhus University Hospital, Skejby, Denmark

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ABSTRACT

We aimed to estimate the levels and time trends of perfluorinated alkyl acids (PFAAs) in serum of 1533 Danish pregnant nulliparous women between 2008 and 2013. The selection criterion of only including nulliparous women was chosen to avoid confounding from parity. The serum samples were analyzed for sixteen PFAAs using solid phase extraction and liquid chromatography tandem mass spectrometry (LC-MS/MS). We investigated the time trends for seven PFAAs, which were detected in more than 50% of the samples: perfluorohexane sulfonate (PFHxS), perfluoroheptane sulfonate (PFHpS), perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), and perfluoroundecanoic acid (PFUnA). We found that the serum levels of all seven PFAAs decreased during the period from 2008 to 2013; on average PFHxS decreased with 7.0% per year, PFHpS with 14.8%, PFOS with 9.3%, PFOA with 9.1%, PFNA with 6.2%, PFDA with 6.3%, and PFUnA with 7.1% per year. Adjustment for maternal age, body mass index (BMI), educational level and gestational age at blood sampling did not change the time trends much. To our knowledge, we are the first to report decreasing trends of PFNA, PFDA and PFUnA since year 2000, thereby indicating that the phase-out of these compounds are beginning to show an effect on human serum levels.

1. Introduction

Perfluorinated alkyl acids (PFAAs) are anthropogenic compounds that have been used as surfactants since the 1940s (Schultz et al., 2003). Perfluorinated sulfonic acids (PFSAs) with six or more carbon atoms and perfluorinated carboxylic acids (PFCAs) with eight or more carbon atoms are defined as long-chain PFAAs (Buck et al., 2011). The long-chain PFAAs are very persistent and bio-accumulative. The average serum half-lives of perfluorohexane sulfonate (PFHxS), perfluorooctane sulfonate (PFOS), and perfluorooctanoic acid (PFOA) were 8.5, 5.4, and 3.8 years, respectively, in retired PFAA-production workers (Olsen et al., 2007a).

Food is the major source of exposure to PFAAs with fish, meat, and cereals being among the largest contributors (Haug et al., 2011; Haug et al., 2010; Trudel et al., 2008). Other sources include drinking water, dust and air (Trudel et al., 2008). Humans may also be exposed to PFAAs and their precursors from consumer products such as food packaging (D'Eon et al., 2009; Trier et al., 2011). The PFAAs are transferred from mother to fetus via the placenta (Kim et al., 2011; Needham et al., 2011; Ode et al., 2013) and to the newborn child via breast milk (Karrman et al., 2007; Needham et al., 2011). PFAAs have been associated with endocrine disruption (Bjerregaard-Olesen and Bonefeld-Jorgensen, 2015; Bjerregaard-Olesen et al., 2015; Kjeldsen and Bonefeld-Jorgensen, 2013; Long et al., 2013) and reproductive and developmental toxicities such as subfecundity (Bach et al., 2015b; Fei et al., 2009), miscarriages (Jensen et al., 2015), lower birth weight (Bach et al., 2014; Fei et al., 2007), and cerebral palsy (Liew et al., 2014). Furthermore, high levels of PFOS, PFOA, and perfluorooctane

Abbreviations: BMI, body mass index; EPA, environmental protection agency; GM, geometric mean; LC, liquid chromatography; LOD, limit of detection; LOQ, limit of quantification; MS, mass spectrometry; PFAA, perfluorinated alkyl acid; PFBS, perfluorobutane sulfonate; PFCA, perfluorinated carboxylic acid; PFDA, perfluorodecanoate; PFDoA, perfluorododecanoate; PFDS, perfluorodecane sulfonate; PFHpA, perfluoroheptanoate; PFHpS, perfluoroheptane sulfonate; PFHxA, perfluorohexanoate; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoate; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate; PFOSA, perfluorooctane sulfonamide; PFPeA, perfluoropentanoate; PFSA, perfluorinated sulfonic acid; PFTeA, perfluorotetradecanoate; PFTrA, perfluorotridecanoate; PFUnA, perfluoroundecanoate; REACH, Registration, Evaluation, Authorisation and Restriction of Chemicals.

* Corresponding author at: Centre for Arctic Health & Unit of Cellular and Molecular Toxicology, Department of Public Health, Aarhus University, Bartholins Allé 2, Build. 1260, DK-8000 Aarhus C, Denmark.
E-mail address: ebj@ph.au.dk (E.C. Bonefeld-Jørgensen).

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sulfonamide (PFOSA) have also been associated with a higher risk of breast cancer (Bonefeld-Jorgensen et al., 2011; Bonefeld-Jorgensen et al., 2014).

In 2000, 3M, one of the largest PFAA manufacturers, announced that they would phase out their production of C6, C8, and C10 products, including PFOS and PFOA, by 2002 (Butenhoff et al., 2006; Land et al., 2015; Olsen et al., 2007b). In 2006, the US EPA invited the eight major fluoropolymer manufacturers (Arkema, Asahi, Daikin, BASF, Clariant, DuPont, 3M, and Solvay Solexis) to a global stewardship program to completely eliminate the production of PFOA, PFOA-precursors, and higher PFCA homologues by 2015 (US EPA, 2015a). Since then, all eight companies have reduced their production of these compounds, and according to a recent progress report, some of them already reached the goal in 2013 (US EPA, 2015b). In 2006, the EU restricted the marketing and use of PFOS (Schroter-Kermani et al., 2013), and in 2009, PFOS was added to Annex B of the Stockholm convention (Stockholm Convention, 2009). Other countries, including Australia, Canada, and Japan, have also made regulations on the use of PFOS (Okada et al., 2013; Toms et al., 2014). In 2012–2013, the European Union's regulation REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) included PFOA, perfluoroundecanoic acid (PFUnA), perfluorododecanoic acid (PFDoA), perfluorotridecanoic acid (PFTrA), and perfluorotetradecanoic acid (PFTeA) on a candidate list of substances of very high concern (Land et al., 2015). However, manufacturers from some countries, including China, have increased the production of long-chain PFAAs (Land et al., 2015). Furthermore, short-chain PFAAs like perfluorobutanoic sulfonate (PFBS) have been introduced as alternatives (Glynn et al., 2012). Even though the short-chain PFAAs are considered less persistent than their long-chain counterparts, short-chain PFAAs like PFBS, perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), and perfluoroheptanoic acid (PFHpA) have also been detected in human serum (Ehresman et al., 2007; Glynn et al., 2012; Haug et al., 2009; Okada et al., 2013; Schroter-Kermani et al., 2013). In a recent study, Glynn et al. reported that the levels of PFBS in 36 samples of pooled serum from 413 Swedish primiparous women on average increased with 11% per year from 1996 to 2010 (Glynn et al., 2012).

In the present study, we aimed to determine the temporal trends of PFAAs in serum of Danish pregnant nulliparous women from 2008 to 2013. To our knowledge, this is the first study to report the PFAA time trends for nulliparous pregnant women.

2. Materials and methods

2.1. Participants and samples

The study included women from the Aarhus Birth Cohort Biobank. The eligible candidates for the biobank were pregnant women who planned to give birth at Aarhus University Hospital, Denmark. Information about the biobank, a questionnaire, a consent form and a postage-paid envelope for response were sent to the pregnant women in early pregnancy (Mortensen et al., 2013). Of the women referred to give birth at Aarhus University Hospital, between 45 and 48% (partly depending on lab staff availability) were included in the Aarhus Birth Cohort Biobank (Mortensen et al., 2013). In the present study, only nulliparous women were included as the PFAA levels have been shown to decrease with parity (Berg et al., 2014). Out of 2853 eligible pregnant nulliparous women, who gave birth to a live born singleton neonate between 2008 and 2013, 1533 were randomly selected from the Aarhus Birth Cohort for inclusion in the present study (Bach et al., 2015a). Most participants gave a blood sample between 11 weeks and 14 weeks of gestation. The samples were processed within 2 h after blood draw and stored at −80°C (Mortensen et al., 2013). The gestational age at birth was estimated by ultrasound and used to calculate the gestational age at the time of blood draw. The Aarhus Birth Cohort also holds information on height and pre-pregnancy weight reported by the women during pregnancy as well as information about the delivery and the newborn reported by the attending midwife at delivery. All participants provided written consent to the storing of their blood samples in the biobank, and consented that the samples and information could be used for research. The study was approved by the Danish National Committee on Health Research Ethics (j.nr: M-20110054) and the Danish Data Protection Agency (j.nr: 2011-41-6014).

2.2. Chemical analysis

Sixteen PFAAs [PFBS, PFHxS, perfluoroheptane sulfonate (PFHpS), PFOS, perfluorodecane sulfonate (PFDS), PFOSA, PFPeA, PFHxA, PFHpA, PFOA, perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), PFUnA, PFDoA, PFTrA, PFTeA] were analyzed at the Department of Environmental Science, Aarhus University, using a method consisting of solid phase extraction and liquid chromatography tandem mass spectrometry (LC-MS/MS). The analytical performance specifications of the method, including limits of quantification (LOQ), are given in Table S1 in the supporting information. ^{13}C-labeled PFASs were used as internal standards. Details of the method have been described elsewhere (Bonefeld-Jorgensen et al., 2014; Bossi et al., 2005).

2.3. Statistics

Only the PFAAs that were detected in at least 50% of the samples were included in the trend analyses. For the samples that contained PFAAs at concentrations below the LOQ, the value was replaced by the LOQ/2. In addition to the single compounds, the analyses were also performed for three groups of PFAAs including all PFAAs independent of quantification frequency: 1) the sum of perfluorinated sulfonic acids ($\sum$PFSA) including PFBS, PFHxS, PFHpS, PFOS, PFDS and PFOSA, 2) the sum of perfluorinated carboxylic acids ($\sum$PFCA) including PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnA, PFDoA, PFTrA, and PFTeA, and 3) the sum of all PFAAs ($\sum$PFAA) including the PFSAs and the PFCAs.

The time trend analysis was performed by log-linear regression analysis. The time trends were analyzed for the crude data and with adjustment for maternal age (years, continuous), pre-pregnancy body mass index (BMI) (four categories), educational level (four categories), and gestational age at the time of blood draw (weeks, continuous). Moreover, we repeated the analyses with restriction to women, who had given blood no later than during gestational week 13.

The trends were used to assess the time to halve the GM serum concentrations (decrease half-times; $T_{dec1/2}$) of the PFAAs for the included 1533 pregnant women during the study period from 2008 to 2013: ln (2)/β where β is the slope obtained from the trend analysis (Noren and Meironyte, 2000).

The compositional patterns were determined as the level of each PFAA relative to the total level of the PFAAs that were detected in more than 50% of the samples. Spearman correlation analysis was performed to assess the bivariate correlation between the PFAAs including only samples with PFAA concentrations above the LOQ.

The statistical analyses and graphs were produced using STATA/IC 13 (StataCorp, College Station, TX, USA) and Microsoft Excel. The statistical significant level was set to $p < 0.05$.

3. Results and discussion

3.1. Study participants

The characteristics of the study participants are presented on a yearly basis in Table 1. The maternal age and pre-pregnancy BMI were similar across the study period. 4.4% of the blood samples were taken after gestational week 13 corresponding to 67 women in 2008 and one woman in 2012.