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Longitudinal trends of per- and polyfluoroalkyl substances in children's serum

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ABSTRACT

Studies suggest negative health impacts from early life exposure to per- and polyfluoroalkyl substances (PFASs). However, information on longitudinal exposure to PFASs during childhood is scarce for background-exposed individuals. This study sought to fill this gap by investigating children's longitudinal exposure trends through measurement of PFAS serum concentrations and calculation of body burdens (μg, total in body). Blood of 54 Finnish children was sampled 2005–2015 and analyzed for 20 PFASs at 1, 6 and 10.5 years of age. The body burden was calculated by multiplying the serum concentration by the volume of distribution and the bodyweight for each individual. Associations between serum concentrations or body burdens and parameters, such as sex, breastfeeding duration, body mass index as well as indoor dust and air PFAS concentrations, were evaluated. Serum concentrations of perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA) and perfluorohexane sulfonic acid (PFHxS) decreased significantly ($p < 0.001$) with age. In contrast to serum concentrations, body burdens stayed unchanged or even increased significantly ($p < 0.05$), except for PFOA in female children. Breastfeeding duration was positively correlated ($p < 0.001$) with serum concentrations of PFHxS, PFOS, PFOA and PFNA at 1 year of age. Some associations were found at 10.5 years with sex and indoor PFAS concentrations. Observations of longitudinal decreasing trends of serum concentrations can be misleading for understanding exposure levels from external media during childhood, as the serum concentration is influenced by parallel temporal changes and growth dilution. Body burdens account for growth dilution and thus better reflect differences in early-life to adolescence exposure than serum concentrations.

1. Introduction

Per- and polyfluoroalkyl substances (PFASs) are a structurally diverse group of > 3000 anthropogenic chemicals (KEMI, 2015) that have been classified into various sub-families (Buck et al., 2011), such as the most studied group of perfluoroalkyl acids (PFAAs). PFASs have been commercially produced since the early 1950s (Okazoe, 2009) and are nowadays widely used in many industrial applications, as well as in consumer products (Buck et al., 2011; Kotthoff et al., 2015; Prevedouros et al., 2006). PFAAs are ubiquitous in the environment and present in both environmental and human matrices (Fromme et al., 2009). "Long-chain" PFAAs (i.e. ≥7 perfluorinated carbons for perfluoroalkyl carboxylic acids (PFCAs) and ≥6 perfluorinated carbons for perfluoroalkane sulfonic acids (PFSAs)) bioaccumulate in humans as a result of their slow elimination rates (Barry et al., 2013; Kennedy et al., 2004; Lau et al., 2004; Olsen et al., 2007; Stahl et al., 2011).

PFASs, especially long-chain PFAAs, have displayed an array of toxicological effects in animal studies (Kennedy et al., 2004). Epidemiological studies have shown associations between exposure to elevated concentrations of long-chain PFAAs and adverse health outcomes

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(Barry et al., 2013; Lopez-Espinosa et al., 2016). Several studies have examined associations between prenatal and childhood PFAS exposure and health outcomes. These studies have shown that early life exposure to PFASs affects development during later childhood, e.g. due to lower immune system response (Dalsager et al., 2016; Pennings et al., 2016), lower levels of sex hormones and delayed sexual maturation (Lopez-Espinosa et al., 2011, 2016) and adiposity at later childhood (Braun et al., 2016). In addition, some epidemiological studies have shown a positive association between childhood PFAS exposure and thyroid function in teenage boys (Ballesteros et al., 2017), and association between PFAS exposure and body development of fetuses and children, such as reduced birth weight and increased body mass index (BMI) (Gyllenhammar et al., 2018). These results clearly show that childhood exposure has to be investigated more closely in order to assure that the regulation of PFASs, which is currently predominantly based on data derived from adults, also protects children.

Perfluorooctane sulfonic acid (PFOS) and related substances were added to Annex B of the Stockholm Convention on Persistent Organic Pollutants (POPs) in 2009 (UNEP, 2009), and perfluorooctanoic acid (PFOA) and perfluorohexane sulfonic acid (PFHxS) are currently under consideration for addition to the convention. Recently, several long-chain PFCAs (C8-C14) as well as PFHxS were added to the Candidate List of Substances of Very High Concern (SVHC, ECHA, 2013, 2015, 2017) of the EU chemicals legislation REACH (regulation EU 1907/2006). The uses of PFOS were restricted through EU-legislation almost a decade ago (directive 2006/122/EC, and later moved to regulation EU 757/2010). More recently the EU began to regulate PFOA, its salts and related substances in a wide range of products and applications (regulation EU 2017/1000). The phase-out of perfluorooctanesulfonyl fluoride-based substances by the major manufacturer resulted in a decrease of perfluorooctane sulfonic acid (PFOS) concentrations in human serum samples after the year 2000 in America, Europe and Australia (Calafat et al., 2007; Gebbink et al., 2015; Glynn et al., 2012; Haug et al., 2009; Toms et al., 2014). At the same time, several long-chain PFCAs have increased steadily in the general population (Calafat et al., 2007; Gebbink et al., 2015) despite mitigation actions by the main producers (US EPA, 2006). There is also an increasing concern for human exposure to short-chain PFAAs and various per- and polyfluoroether acids, which are being used as replacements for long-chain PFAAs (Blum et al., 2015; Glynn et al., 2012; Ritter, 2010; Scheringer et al., 2014; Shi et al., 2016; Wang et al., 2013, 2015). Therefore, the quantitation of multiple PFASs (phased-out substances and replacements) in human serum is needed to adequately assess human PFAS exposure and associated health risks.

Measurements in blood serum represent the “gold standard” for monitoring PFASs in humans in epidemiological studies. However, serum concentrations in children cannot easily be related to external exposure due to growth dilution effects resulting from the increasing blood volume and body weight during childhood. Calculation of the body burden of a chemical (defined here as the total amount in μg in the body) is one way to remove the confounding effect of growth dilution. The body burden is a measure of the total accumulated amount of a substance in the body at a given time point, which allows for the detection of changes in external exposure over time.

To date, there are very few published studies that have measured PFASs at several time points during childhood and the reported longitudinal trends in these studies display differences in the range of the PFAS concentrations and time of the peak concentrations (Fromme et al., 2010; Gyllenhammar et al., 2016; Mogensen et al., 2015). Reasons for the reported variable longitudinal trends may include methodological differences (e.g. sampling time points), but may also reflect geographical differences in exposure pathways and sources for infants, toddlers and children. The objectives of this study were to investigate a) longitudinal exposure trends of background-exposed children for PFASs during childhood through measurement of serum concentrations following the same individuals from 1 to 10.5 years of age, b) children's body burden trends of PFASs during childhood, and c) causes for the different exposure trends for PFASs during childhood. To examine causes for or associations with the serum concentrations and body burdens, breastfeeding duration, body mass index as well as PFAS dust concentrations (Winkens et al., 2018) and PFAS air concentrations (Winkens et al., 2017a) of the children's bedrooms were tested for correlation.

2. Materials and methods

2.1. Sampling

The study population consisted of a subset of individuals from a birth cohort study (LUKAS2) in Eastern Finland, for which the mothers of the study subjects were recruited at Kuopio University Hospital (Karvonen et al., 2009). The children were born between May 2004 and May 2005, and the blood samples from 54 children (26 male, 28 female) were collected from the same individuals at the ages of 1 year (range 0.97–1.06) in 2005/2006, 6 years (5.71–6.32) in 2010/2011 and 10.5 years (9.90–10.95) in 2014/2015 (5 mL serum tube, Vacutainer (Becton Dickinson, BD®, Plymouth, UK)). The blood samples were centrifuged for 10 min at 800 $\times g$ to separate the serum fractions, which were stored frozen at −20 °C until analysis. All chemical analyses were performed in 2016 to reduce analytical and methodological variations. Although some of the samples were stored frozen for several years, the chemical stability of PFASs implicates that archiving the samples under such conditions would have negligible effects on the quality of the data (Houde et al., 2006). Written informed consent was obtained from all parents of the participants and the study was approved by the Research Ethics Committee, Hospital District of Northern Savo, Kuopio, Finland (case number 48/2004 and amendments).

2.2. Sample treatment and chemical analysis

At the beginning of the serum analysis, 2.5 ng of mass labelled internal standard for quantitation of PFASs was added to the 0.2 mL serum sample (for a substance list see Table S1). Each sample was extracted with 0.3 mL of 20 mM ammonium acetate in methanol. Prior to instrumental analyses the extracts were diluted with Milli-Q water (1:2 parts, water:extract), and the samples were filtered with 0.2 μm syringe filters (Pall Life Sciences, Ann Arbor, MI). Twenty PFASs (perfluorohexanoic acid, PFHxA; -heptanoic acid, PFHpA; -octanoic acid, PFOA; -nonanoic acid, PFNA; -decanoic acid, PFDA; -undecanoic acid, PFUnDA; -dodecanoic acid, PFDoDA; -tridecanoic acid, PFTrDA; -tetradecanoic acid, PFTeDA; -hexane sulfonic acid, PFHxS; -heptane sulfonic acid, PFHpS; -octane sulfonic acid, PFOS; -decane sulfonic acid, PFDS; *N*-methyl-perfluorooctane sulfonamidoacetic acid, MeFOSAA; *N*-ethyl-perfluorooctane sulfonamidoacetic acid, EtFOSAA; perfluorooctane sulfonamide, FOSA; *N*-methyl-perfluorooctane sulfonamide, MeFOSA; *N*-ethyl-perfluorooctane sulfonamide, EtFOSA; 6:2 polyfluoroalkyl phosphoric acid diesters, 6:2 diPAP; 8:2 polyfluoroalkyl phosphoric acid diesters, 8:2 diPAP) were analyzed using liquid chromatography negative ion electrospray tandem mass spectrometry (LC-ESI-MS/MS). The details of LC-MS/MS parameters have been published earlier (Koponen et al., 2013). A seven point matrix-matched standard curve with concentrations ranging from 0.075 to 50 ng/mL ($r^2 > 0.992$ for each compound) was used for quantitation. The lowest concentration point with acceptable signal to noise ratio and chromatographic peak area was used as the limit of quantitation (LOQ) (details in Koponen et al., 2013), see Table S2 for a LOQ list of the different analytes. Chromatographic peak integration was undertaken with the help of the Xcalibur 2.0.7 software, and the final serum concentrations were calculated in Microsoft Excel.

For quality control, a blank sample and an in-house control serum sample were analyzed in the same way as the real serum samples with each serum sample batch. PFAS levels in the blank samples (n = 3)