Environment International 97 (2016) 7–14

Contents lists available at ScienceDirect

Environment International

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

Full Length Article

Perfluoroalkyl and polyfluoroalkyl substances in cord blood of newborns in Shanghai, China: Implications for risk assessment

Bin Wang [a,1], Qian Chen [a,1], Lixiao Shen [a], Shasha Zhao [a], Weiyi Pang [b], Jun Zhang [a,*]

[a] MOE-Shanghai Key Laboratory of Children's Environmental Health, Xinhua Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200092, China
[b] School of Public Health, Guilin Medical University, Guilin 541004, Guangxi, China

ARTICLE INFO

Article history:
Received 8 August 2016
Received in revised form 29 September 2016
Accepted 8 October 2016
Available online xxxx

Keywords:
Perfluoroalkyl substances
Cord blood
Exposure
China
Risk assessment

ABSTRACT

Perfluoroalkyl and polyfluoroalkyl substances (PFASs) are commonly used in industrial applications and consumer products, and their potential health impacts are of concern, especially for vulnerable population like fetuses. However, *in utero* exposure to PFASs and health implications are far from fully characterized in China. To fill in the gap, we analyzed 10 PFASs in cord plasma samples (N = 687) collected in Shanghai between 2011 and 2012, one of the regions widely polluted with PFASs in China. A questionnaire survey on maternal and diet-related factors was conducted. Except for perfluoroheptanoic acid (PFHpA) and perfluorooctane sulfonamide (PFOSA), all other PFASs were detected in >90% of the samples. Perfluorooctanoic acid (PFOA) was the most predominant PFAS (median value: 6.96 ng/mL), followed by perfluorooctane sulfonate (PFOS) (2.48 ng/mL). PFOA and PFOS combined contributed to 80% of the total PFASs. The final multiple regression models showed that maternal factors including maternal age, body mass index, gestational age, economic status and educational level as well as consumption of fish and wheat were significantly related with concentrations of PFASs in cord blood. The risk assessment using the hazard quotients (HQs) approach on the basis of plasma PFAS levels indicated no potential concern for developmental toxicity in the local newborns. The results demonstrate the unique profiles of local prenatal exposure to PFASs, suggesting that PFOA has been the primary human exposure due to its widespread use and pollution. Special attention to high PFOA exposure and confirmation of potential determinants should be taken as a priority in the future plan for risk management and actions in this area.

1. Introduction

Perfluoroalkyl and polyfluoroalkyl substances (PFASs) comprise a class of synthetic fluorinated chemicals that are used in a wide range of consumer and industrial applications such as surfactants, household cleaning products, textiles, carpets, fire-fighting foams and food packaging for decades (Lehmler, 2005). PFASs, especially perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), have been found world-wide in environmental matrices, wildlife and humans (Houde et al., 2006, 2011). With the strong fluorine-carbon covalent bonds, PFASs are resistant to chemical and biological degradation under normal environmental conditions (Lau et al., 2007). Animal studies have demonstrated hepatotoxicity, developmental toxicity, immunotoxicity and hormonal changes as the main toxicity effects of PFASs (Kennedy et al., 2004; Lau et al., 2007; Andersen et al., 2008). In 2009, PFOS and its salts were added to the list of "restricted use" compounds under the Stockholm Convention on persistent organic pollutants (UNEP, 2009).

The ubiquitousness and potential adverse health effects of PFASs have raised concerns about human exposure to these compounds (Houde et al., 2006). Food and water constitute the major routes of exposure to PFASs for humans, followed by indoor air and house dust (Fromme et al., 2009; Vestergren and Cousins, 2009). Human biomonitoring has been an important tool for human exposure assessment of PFASs (Calafat et al., 2007; Zhang et al., 2010b; Wilhelm et al., 2009; Yamaguchi et al., 2013). Biomonitoring studies in subpopulations susceptible to the effects of potentially harmful environmental chemicals such as fetuses and newborns are of particular interest, because the exposure occurs during this critical window of growth may impact health later in life (Landrigan et al., 2002). Evidence shows that PFASs can be transferred from the mother to the fetus through the placenta and result in several adverse effects on fetal growth and development, including reduced head circumference and birth weight (Chen et al., 2012; Apelberg et al., 2007b).

Biomonitoring data reflecting the prenatal PFAS exposure levels have already been provided for different countries globally (Monroy et al., 2008; Cariou et al., 2015; Lee et al., 2013; Apelberg et al., 2007a; Fei et al., 2007; Fromme et al., 2010; Hanssen et al., 2010; Inoue et al.,

* Corresponding author at: MOE-Shanghai Key Laboratory of Children's Environmental Health, Xinhua Hospital, Shanghai Jiao Tong University School of Medicine, 1665 Kongjiang Road, Shanghai 200092, China.
E-mail address: junjimzhang@sina.com (J. Zhang).
[1] These authors contributed equally to this work and are considered as co-first authors.

http://dx.doi.org/10.1016/j.envint.2016.10.008
0160-4120/

2004; Lien et al., 2013), but data from China are still limited (Liu et al., 2011; Yang et al., 2016; Zhang et al., 2011). Since 3 M Company, the major manufacturer of PFOS, announced to phase out perfluorooctyl chemicals in 2000, global production volumes of PFOS and related compounds had declined significantly (Paul et al., 2009). However, PFASs are still manufactured and used in China. In fact, the manufacturing of PFOS and related compounds in China has increased since 2002 and reached the peak in 2006 with ~250 tons per year (Xie et al., 2013), which raises special concern towards increasing body burden of PFASs among Chinese. In addition, although human blood PFAS levels from several cities in China have been characterized, little is known about exposure levels from eastern areas of China, especially in Shanghai. As the most developed city in China, Shanghai had the highest concentrations of PFASs in multiple environmental media such as soils, sediments and surface water (Wang et al., 2015). Recently, a large-scale investigation covering 12 provinces of China reported that concentrations of PFASs in human milk samples from Shanghai were significantly higher than those from any other regions ($\sum$PFASs = 1252 pg/mL, followed by 381 pg/mL in pooled samples from Liaoning Province) (Liu et al., 2010). However, PFAS exposure profile to fetuses remains unknown in this area.

Risk assessment based on biomonitoring is important to understand toxicological relevance for individuals. The crucial step for risk characterization is to link PFAS levels with toxicological endpoints resulting from exposing experimental animals. For human biomonitoring studies, the Hazard Quotient (HQ) has been used to estimate the potential risk by comparing actual serum/plasma concentrations with reference serum/plasma concentrations regarded as points of departure (PoDs) to evaluate whether the exposure level is tolerable or not (Borg et al., 2013; Ludwicki et al., 2015). The PoDs are derived by modeling the dose-response relationship such as no-observable adverse-effect-level (NOAEL), lowest observed adverse effect level (LOAEL) or benchmark dose (BMD) levels for relevant toxicological endpoints (Borg et al., 2013; Ludwicki et al., 2015; US EPA, 2016a, b). Animal studies have observed series of endpoints, including liver effects, body weight changes in adults and offspring, reproductive outcomes (gestation length, fertility), developmental effects (altered puberty, survival, and developmental delays), and immune effects (Lau et al., 2007; US EPA, 2016a, b). Most available toxicological data have been collected for PFOA and PFOS, two predominant PFAS compounds in human exposure.

In the current study, a set of 10 PFASs were quantified and characterized in 687 cord blood plasma samples collected from Shanghai, China. The main objectives were to generate the exposure data of PFASs for newborns in this area; to explore potential determinants of fetal exposure; to evaluate health risk of exposure to the prominent PFASs (PFOS and PFOA) based on biomonitoring data for the vulnerable population. Since maternal-fetal transfer efficacy is congener-specific, PFAS levels measured in cord blood can reflect fetal exposure more accurately for risk assessment compared to maternal blood levels.

2. Material and methods

2.1. Study population

From 2011 and 2012, we recruited a total of 688 singleton pregnant women who came to two large hospitals in Shanghai for delivery with a written informed consent. All participants were native Chinese and residents of Shanghai city. After delivery, one newborn diagnosed of suspicious anomalies was excluded. Thus, 687 infant-mother pairs were finally included in our study. At enrollment, a face-to-face questionnaire survey was administered by trained interviewers. The structured questionnaire was developed to gather information on demographic characteristics and dietary and lifestyle-related factors during pregnancy. To assess the influence of dietary patterns, an average frequency of commonly consumed food items was collected including fish/shellfish intake. Several lifestyle factors such as alcohol intake, smoking status, source of drinking water and pesticides use during pregnancy were also included. Medical information, including maternal age, pre-pregnancy body mass index (BMI), parity, gestational age, pregnancy complications and infant's sex was obtained from participant medical records. The study protocol was approved by the Ethics Committees of all involved research institutions and hospitals.

2.2. Sample collection and analysis

A total of 10 mL umbilical cord blood specimens were collected at delivery. The blood specimens were centrifuged at 4000 rpm for 10 min immediately after collection in hospital. The plasma was then separated and stored at −80 °C until shipping on dry ice to the laboratory at Xinhua Hospital affiliated to Shanghai Jiao Tong University School of Medicine for analysis.

The samples were analyzed for 10 PFASs: perfluorododecanoic acid (PFDoA), perfluoroundecanoic acid (PFUA), perfluorodecanoic acid (PFDA), perfluorononanoic acid (PFNA), perfluorooctane sulfonamide (PFOSA), PFOS, PFOA, perfluoroheptanoic acid (PFHpA), perfluorohexane sulfonic acid (PFHxS), perfluorobutane sulfonic acid (PFBS). The PFASs and internal standard solution ($^{13}C_4$-PFOS and $^{13}C_4$-PFOA) were purchased from Wellington Laboratories (Guelph, Ontario, Canada) and Sigma-Aldrich (St. Louis, MO, USA). Purities of all standards were ≥95%.

The plasma samples were extracted using the protein precipitation extraction method developed by Lien et al. with some modifications (Lien et al. 2011). Briefly, 10 μL of 50 ng/mL internal standard solution was spiked into 100 μL cord plasma sample in a polypropylene centrifuge tube and vortexed for 30s. Then, 150 μL acetonitrile containing 1% formic acid and 150 μL methanol were added to each sample before the second vortex. The mixture was sonicated for 10 min and centrifuged at 12,000 rpm for 10 min. The supernatant was collected (about 100 μL) and then filtered through 0.22-μm Nylon syringe filter into a 1.5 mL auto-sampler vial for analysis. Calibration standards were prepared by spiking blank fetal bovine serum with the standard mixture of 10 analytes and isotope labeled internal standards.

PFASs were separated and quantified on liquid chromatography system coupled with tandem mass spectrometry (HPLC-MS/MS, Agilent 1290–6490, Agilent Technologies Inc., USA). A 2-μL aliquot of the sample extract was injected into a ZORBAX Eclipse Plus C18 column (2.1 × 100 mm, 1.8 μm; Agilent, USA) maintained at 35 °C and equipped with a C18 pre-column (2.1 × 5 mm, 1.8 μm). The flow rate was 0.3 mL/min. The mobile phase consisted of water contained 10 mM ammonium acetate (A) and methanol (B). The gradient elution started at 60% methanol and increased to 80% after 5 min, then increased to 90% at 10 min; it was held at 90% for 1 min, and then returned back to 40% methanol at 12 min. The instrument was operated in the electrospray ionization (ESI) negative mode with multiple reaction monitoring (MRM). The precursor and product ions for each target analyte, together with the applied collision energy and retention time are summarized in Supplementary Table S1.

2.3. Quality assurance and quality control

Quantification was performed using the internal-standard method. Procedural blank analysis was conducted using newborn fetal bovine plasma for each batch of samples. Trace levels of PFNA were detected in procedural blanks. Thus the average blank signals were subtracted from the calculated concentrations in the samples. For each analyte, a six point calibration curve with a concentration ranging from 0.5 ng/mL to 100 ng/mL was used for quantification. Calibration curves exhibited good linearity with correlation coefficients ≥0.99 for all compounds. The accuracy (% mean recovery) and precision were estimated by replicating analysis of samples at a low (1.6 ng/mL) and a high concentration (80 ng/mL). The recoveries of PFASs ranged from 80% to 110%, and intra- and inter-day calibration biases were <10% for all the analytes. The limits of detection (LOD), defined as a signal-to-noise ratio of 3, ranged from