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Effects of age, gender and region on serum concentrations of perfluorinated compounds in general population of Henan, China

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HIGHLIGHTS

- 133 Serum samples were collected from inland China aged 0–88 years for analysis.
- Compared with coastal region, the levels and composition of PFCs were different.
- Significant increases in PFOA, PFNA, and PFOS concentrations over age were found.
- Median concentrations of 4 PFCs were higher in males than in females.
- Higher PFOA concentrations were found in urban populations.

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ABSTRACT

133 Serum samples collected from Henan donors aged from 0 to 88 years were analyzed for 12 perfluorinated compounds (PFCs). Perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) accounted for 69.19% of the total PFCs in serum samples, with a median concentration of 1.43 and 1.47 ng mL^{-1}, respectively. Other PFCs were detected at much lower concentrations, with median concentrations ranging from 0.03 to 0.37 ng mL^{-1}. PFOA and PFOS were positively correlated ($r = 0.219$) in serum samples, indicating that they may have common exposure pathways. For all donors (0–88 years), significant increases in PFOA ($r = 0.239$, $p < 0.01$), perfluorononanoic acid (PFNA) ($r = 0.185$, $p < 0.05$) and PFOS ($r = 0.175$, $p < 0.05$) concentrations over age were found. Median concentrations of PFOA, PFNA, perfluorodecanoic acid (PFDA), and PFOS were higher in males than in females. Higher PFOA concentrations were found in urban populations than in rural populations. Since PFCs exposure in general population is prevalent, further studies are needed to explore its possible impacts on epidemiological factors.

1. Introduction

Perfluorinated compounds (PFCs) are a group of manmade chemicals with surface-active properties, which have been widely used as surfactants and surface protectors in lubricants, paints, polishes, fire-fighting foams, food packaging and many other applications for over 50 years (Lau et al., 2007). In recent years, PFCs have drawn great concerns internationally due to their global occurrences in environment and biota (Giesy and Kannan, 2001; Meesters and Schroder, 2004), as well as their toxicity (Lau et al., 2007) and bioaccumulation (Martin et al., 2004). PFCs have also been found in food (Tittlemier et al., 2006), drinking water (Skutlarek et al., 2006), indoor air and dust (Shoeib et al., 2005), which are potential human exposure pathways and raise concerns over human health.

PFCs have been found in human blood or serum samples in general population from many countries (Kannan et al., 2004; Fromme et al., 2009), including China (So et al., 2006; Pan et al., 2010). In the meantime, relationships between age, gender and PFCs concentrations have been discussed in a few studies. However, previous studies are controversial about the age and gender effects on PFCs concentrations in general population (Karrman et al., 2006; Zhang et al., 2010b). Furthermore, while most of the previous studies focused on coastal and industrialized areas, where lifestyles are very different with inland areas, studies focusing on inland and agricultural areas are needed.

Henan, with a population of over 94 million, is a major agricultural province located in the central part of China. Henan is the 5th largest provincial economy of China and the largest among inland

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provinces. However, as a semi-industrialized economy, per capita GDP of Henan is low compared to eastern provinces, and Henan is considered to be one of the most backward areas of China. Agriculture has traditionally been a pillar of its economy, with the nation's highest wheat output and second highest rice output, earning its reputation as the breadbasket of China. Unlike the coastal region where diet is high in fish, the diet in this region relies mainly on grain, vegetable, meat, poultry and egg. In the present study, the concentrations of 12 PFCs in 133 serum samples derived from a general population of Henan were analyzed. The aim of this study is to evaluate the PFCs concentrations in general populations in inland and agricultural area, as well as effects of age, gender and region on PFCs concentrations.

2. Materials and methods

2.1. Sampling

During October–November 2011, 133 serum samples derived from individuals living in Yuanyang County (Henan, China) were obtained from the Yuanyang Red Cross Hospital. The participants aged from 0 to 88 years old were divided into five age groups (0–15 years, 16–30 years, 31–45 years, 45–60 years, 61 years and older), two gender groups (79 males and 54 females) and two region groups (33 from urban area and 100 from rural areas), separately. Whole blood of approximate 5 mL was withdrawn from each donor, and the serum was immediately separated. Serum samples were stored at −20 °C until analysis.

Information on lifestyle and demographic factors was collected through interviews. In the present study, body mass index (BMI), per capita net income, consumptions of meat, egg, fish and seafood were examined as potential determinants on serum PFCs concentrations.

2.2. PFCs analyses

Serum samples were analyzed for 12 PFCs, including perfluorobutane sulfonate (PFBS), perfluorohexane sulfonate (PFHxS), perfluorooctane sulfonate (PFOS), perfluorobutyric acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUdA) and perfluorododecanoic acid (PFDoA). The external standard used for all matrix spikes was a mixture of 12 PFCs (>98%) purchased from Wellington Laboratories (Guelph, Ontario, Canada). The mixture of 9 isotope-labeled internal standards ($^{18}O_2$-labeled PFHxS, $^{13}C_4$-labeled PFOS, $^{13}C_4$-labeled PFBA, $^{13}C_2$-labeled PFHxA, $^{13}C_4$-labeled PFOA, $^{13}C_5$-labeled PFNA, $^{13}C_2$-labeled PFDA, $^{13}C_2$-labeled PFUdA and $^{13}C_2$-labeled PFDoA) was also obtained from Wellington Laboratories.

Serum samples were prepared according to the methods outlined in Hansen et al., 2001, with minor modifications. Briefly, 0.5 mL of serum, 100 μL labeled internal standards (10 ng mL^{-1}), 2 mL of sodium carbonate (Na_2CO_3) (0.25 mol L^{-1}), and 1 mL of tetrabutylammonium hydrogen sulfate (TBAHS) (0.5 mol L^{-1}) were added to a 15 mL clean polypropylene (PP) tube for extraction and mixed well. 5 mL methyl tert-butyl ether (MTBE) was then added to the solution, and the mixture was shaken for 20 min. The organic and aqueous layers were separated by centrifugation at 2160×*g* for 15 min, and the organic layer was transferred to a second polypropylene tube. The aqueous layer was then extracted twice with MTBE, and the organic layer was separated and combined with that from the first extraction. The combined MTBE extracts were evaporated to dryness under high purity nitrogen, and then the residue was reconstituted in 1 mL of a mixture of methanol and 10 mmol L^{-1} ammonium acetate (2:3, v/v). Finally, the sample was filtered through a 0.22 μm nylon filter.

PFCs were analyzed as outlined in Bao et al. (2011) using high performance liquid chromatography-tandem mass spectrometry (HPLC–MS/MS). Chromatography was performed by an Agilent 1290 HPLC system (Palo Alto, CA, USA). A 10 μL aliquot of extract was injected onto a 2.1 × 100 mm (3.5 μm) Agilent Eclipse Plus C18 column (Palo Alto, CA, USA) with 10 mmol L^{-1} ammonium acetate and acetonitrile as mobile phases starting with 30% acetonitrile at a flow rate of 0.3 mL min^{-1} and column temperature of 40 °C. The gradient was increased to 90% acetonitrile at 9 min and then held for 2 min. In addition, an 8 min re-equilibration interval was run before each following sample. The HPLC system was interfaced to an Agilent 6460 Triple Quadrupole (QQQ) mass spectrometer (Santa Clara, CA, USA) operated with electro spray ionization (ESA) in negative mode. The gas temperature and ion spray voltage were maintained at 350 °C and 4000 V. Ions were monitored with a multiple reaction monitoring (MRM) mode. The MS/MS transition for each target analyte, the applied collision energy, and the fragmentation voltage are summarized in Supplementary Table S1.

2.3. Quality assurance and quality control

Procedural blanks were prepared at an interval of every ten samples to check if contamination occurred during the extraction of serum samples. Solvent blanks containing acetonitrile and Milli-Q water (2:3, v/v) were prepared to run after every twenty samples to monitor for background contamination. Duplicate injections and calibration check standards were run after every twenty samples to assure the precision and accuracy of each run. The concentrations of serum extracts were quantified via nine-point matrix-matched calibration curves ranging from 0.01 to 100 ng mL^{-1} which were performed by adding mixed PFC standard solution into blank newborn bovine serum. The regression coefficients (r^2) of calibration curves for all the target analytes were higher than 0.99. The limit of detection (LOD) was defined as three-fold larger than the signal-to-noise ratio, and the limit of quantification (LOQ) was defined as ten-fold larger than the signal-to-noise ratio. The recovery and reproducibility of the serum sample extraction were determined on six replicate analyses of 0.5 mL of newborn bovine serum containing 2 ng of each PFC standard. No PFC contamination was found above the LOD in newborn bovine serum. The LOD, LOQ and matrix spike recoveries for all the target chemicals are summarized in Supplementary Table S1.

2.4. Statistical analysis

Statistical analyses were conducted using SPSS Statistics v19.0. Only PFCs that were detected in more than 75% of the samples were used for statistical analyses. Non-detects was included in the calculation, as a proxy value of an LOD/2. The Spearman rank correlation analyses were employed to examine the relationship between age and PFCs concentrations, and among various PFCs in the samples. Mann–Whitney *U*-tests were used to assess the effects of gender and region on PFCs concentrations in serum. Linear regression analyses were used to evaluate the effects of possible determinants on serum PFCs concentrations. PFCs concentrations were natural log transformed to satisfy the criteria of normality.

3. Results and discussion

3.1. PFCs in human serum

Serum concentrations of 12 PFCs, stratified by the characteristics of the study population are given in Table 1. PFBA (78.20%),