Assessment of per and polyfluoroalkyl substances (PFAS) in maternal blood

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ABSTRACT

Introduction: per- and polyfluoroalkyl substances (PFAS) are chemicals widely used in various industrial applications and consumer products due to their water and oil-repellent properties, since the ’50s. Their persistence in the environment and potential health impacts have raised significant concerns, particularly regarding their presence in human blood. This study focuses on the analysis of PFAS in maternal blood, given the potential implications for both maternal and fetal health.
Methods: by using liquid chromatography coupled to high resolution accurate mass spectrometry (LC-HRAMS), we quantitatively analyzed the concentrations of 23 PFAS compounds in maternal blood samples. The study cohort consisted of 16 pregnant women from different demographic backgrounds, providing a comprehensive overview of PFAS exposure.
Results: detectable levels of multiple PFAS compounds across all samples, namely PFOS, PFOA, PFHxS. PFBS, PFHxA have been identified. Total PFAS content in samples ranged between 0.18 ng/mL and 3.84 ng/mL. Among the single molecule, PFOS was the compound found at the highest concentrations (2.15 ng/mL), followed by PFHxA (1.41 ng/mL).
Discussion: the presence of PFAS in maternal blood underscores the potential for prenatal exposure, raising concerns about the developmental and long-term health effects on offspring. This research highlights the urgent need for regulatory measures to reduce PFAS exposure and calls for further studies to explore the mechanisms of PFAS transfer from mother to fetus and the subsequent health impacts.

Key words: *PFAS, maternal blood, liquid chromatography-mass spectrometry*

INTRODUCTION

The term per- and polyfluoroalkyl substances (PFAS) refers to a heterogeneous group of synthetic compounds that have undergone a process of fluorination; among them, the most studied are perfluorooctanesulfonate (PFOS), perfluorohexanesulfonic acid (PFHxS), perfluorooctanoic acid (PFOA), perfluorohexanoic acid (PFHxA), perfluorodecanoic acid (PFDA) and perfluorobutanesulfonic acid (PFBS). These compounds are defined by the presence of a variable number of carbon atoms, and at least one perfluorinated methyl group (−CF3) or a perfluorinated methylene group (−CF2−) as well as other chemical groups (1). They are a class of over 12 000 compounds that are extensively utilized in industry and in a variety of goods and procedures, such as water-repelling textiles, grease-resistant paper, nonstick packaging and cooking appliances, medical and laboratory tubing, aqueous film-forming foams, and industrial detergents. Hundreds of distinct PFAS exist, each with a carbon chain of varying length that is either fully or partially saturated with fluorine atoms;

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the rate of detoxification is, generally, inversely proportional to the number of fluorine substitutes. Altogether these molecules are highly persistent toxins, with tendency to bioaccumulation through the food chain, thus heavily contaminating the environment (2). Higher levels of these contaminants are recognized in sewage, house dust, rivers, groundwater, soils, and drinking water, especially near contaminated sites (i.e. military bases, perfluorination sites and airports) (3). Exposure to PFAS in humans occurs mainly from contaminated food and water consumption, cosmetics, cooking utensils, and a number of other commonly used products such as housekeeping trash and cleansers. An interesting review has been recently published on the analysis of PFAS in human conventional and unconventional matrices, describing the most interesting analytical techniques employed in this field (4). The evaluation of PFAS toxicity is challenging due to various factors such as exposure levels, route of exposures, and the multifaceted factors associated with individual susceptibility. Nevertheless, epidemiological and experimental evidence linking PFAS exposure and adverse health outcomes is increasing; toxicity effects have been identified in immune suppression, thyroid dysfunction, liver disease, dyslipidemia, kidney damage, reproductive and developmental issues. A systematic review highlighted the association between PFOA exposure and a slight decrease in infant birth weight. Meta-analysis indicated that even a small increase in PFOA levels correlated with reduced birth weight. Experimental studies supported these findings, concluding that there is sufficient evidence of PFOA's adverse impact on birth weight (5). A recent case-control study observed that plasma concentrations of both PFDA and PFOS were associated with higher odds of late-onset preeclampsia (6). Another recent nested case-control study highlighted that exposure to PFAS was associated with increased risk of gestational diabetes and disturbance in glucose homeostasis, especially in normal weight women, through a disruption of maternal thyroid function (7). A review also indicated the presence of mechanisms linking higher exposure to PFAS to adverse pregnancy outcomes, namely preterm birth and preeclampsia, including disrupted trophoblast migration, altered growth factor signaling, oxidative stress, and inflammatory responses (8). Furthermore, some effects may appear later in life; actually, studies also correlate *in utero* exposure to various environmental contaminants including PFAS, with childhood obesity (9). A Swedish cohort-study found an association between high PFAS exposure, dominated by PFOS and PFHxS contamination of drinking water, and an increased risk for Polycystic Ovary Syndrome (PCOS); furthermore they also found a potential increased risk for uterine leiomyoma (10).

On this basis, we conducted a preliminary study on the presence of these contaminants in maternal blood at the end of the pregnancy. The aim of the present study was to assess the presence of 23 PFAS in maternal blood and to check an association between PFAS amounts and lifestyles and health during pregnancy. In addition to PFAS, emerging and precursors (fluorotelomers) compounds have also been included in the study; the analyses were carried out by liquid chromatography coupled to high accuracy mass spectrometry (LC-HRMS) by exploiting a previously developed and validated analytical method to which the reader is referred for details (11).

METHODS

Materials

All analytes (>98% purity), mass labelled standards used as surrogate and injection standards were from Wellington Laboratories (Guelph, Ontario, Canada). Chemical names and information about isotope-labelled analogues are reported in Table 1. Isotopically labelled compounds, added to the sample before extraction in known amount (surrogate) were used. Mass-labelled PFOA, PFBA and PFOS (chemical purities >98% and isotopic purities of >99%) were added to the purified extracts before injection and used as injection standards. Reference solution for accurate mass measurement was from Agilent Technologies (Santa Clara, USA). Target perfluoroalkyl analytes were prepared at serial dilution of 0.05; 0.1; 0.25; 1.0; 2.5; 5.0 ng/mL in water/methanol (90/10, v/v) for calibration; surrogate mass-labelled compounds were used at the concentration of 500 ng/mL; injection standards were used at the concentration of 1 000 ng/mL (for details on surrogate and injection standards see Table 1). Methanol, acetonitrile and formic acid 98-100% (all LC–MS grade) were supplied by Merck (Darmstadt, Germany). Water for mobile phase was produced by Sartorius Arium mini apparatus (Sartorius, Goettingen, Germany). Ammonium acetate was provided from Sigma-Aldrich (S.Louis, MO, USA). WAX polymer (150 mg, 6 mL) cartridges were also from Agilent Technologies (Santa Clara, US).

Study population

The participants were recruited on a voluntary basis in the Obstetric ward of S. Orsola Hospital-University of Bologna in the period 2022-2023. Inclusion criteria were: age over 18, valid informed consensus, and at term pregnancy; exclusion criteria was substance use disorder such as drugs, pharmaceutics, and alcohol.

Sample collection

All participants were asked to answer a questionnaire about their dietary habits such as 7-days consumption of fish, tap and bottled water and organic products. They were also investigated about their medical, gynecological and obstetrical history. Blood samples were collected the day after delivery in a tube containing anticoagulants and stored in freezers (-20 °C) until analysis.

Table 1
List of the used analytes, surrogate and associated injection standards

Analyte	Isotope dilution analogue	Injection standard
PFBA	13C4-PFBA	13C3-PFBA
PFPeA	13C5-PFPeA	13C3-PFBA
PFMBA	13C5-PFPeA	13C3-PFBA
PFBS	13C3-PFBS	13C4-PFOS
PFEESA	13C3-PFBS	13C4-PFOS
PFHxA	13C5-PFHxA	13C2-PFOA
4:2FTS	13C2-4:2FTS	13C4-PFOS
6:2FTS	13C2-6:2FTS	13C4-PFOS
8:2 FTS	13C2-8:2FTS	13C4-PFOS
PFPeS	13C3-PFHxS	13C4-PFOS
PFHxS	13C3-PFHxS	13C4-PFOS
HFPO-DA	13C3-HFPO-DA	13C2-PFOA
PFHpA	13C4-PFHpA	13C2-PFOA
ADONA	13C4-PFHpA	13C2-PFOA
PFOA	13C8-PFOA	13C2-PFOA
PFHpS	13C8-PFOS	13C4-PFOS
PFOS	13C8-PFOS	13C4-PFOS
9Cl-PF3ONS	13C8-PFOS	13C4-PFOS
11Cl-PF3OUdS	13C8-PFOS	13C4-PFOS
PFNA	13C9-PFNA	13C2-PFOA
PFDA	13C6-PFDA	13C2-PFOA
PFUnA	13C7-PFUnA	13C2-PFOA
PFDoA	13C2-PFDoA	13C2-PFOA

Sample extraction and analysis

Blood (0.5 mL) were added of 2.5 µL of mass labelled standard solution and of 10 mL of purified water vortexed for 1 minute. The material was transferred into a polypropylene 50 mL vial and added of 10 mL of acetonitrile. Mechanical agitation was performed for 30 seconds by vortexing (IKA, Staufen, Germany). Samples were then extracted by ultrasonication for 2 hours. Debris were removed by centrifugation at 4 500 rpm for 5 minutes. Supernatant was collected and purified by weak anion exchange solid phase extraction (SPE). Briefly, column conditioning was performed by 3 mL of methanol and 3 mL of 2% formic acid in water. Column washing was carried out by 2% formic acid in water and methanol, 3 mL each. Elution was performed in two steps, each composed of 2.5 mL of methanol/NH4OH (90/10, v/v). Extracts were taken to dryness under a stream of nitrogen at 40°C, then reconstituted in 500 µL of water/methanol (90/10, v/v) to which 2.5 µL of injection internal standard solution was added. Analyses were carried out in a 1290 Infinity II LC coupled to 6546 quadrupole-time-of-flight mass spectrometer (Q-TOF) (Agilent Technologies, Santa Clara, USA). Chromatographic separations were performed in a Poroshell EC-C18 column (2.1x100 mm, 1.9 µm), (Agilent Technologies) at 40°C. The mobile phases were constituted by 20 mM ammonium acetate in water (mobile phase A) and methanol (mobile phase B), both added of 0.1% formic acid (v/v). Flow rate was 0.250 mL/min. The injected volume was 5 µL. The Q-TOF instrument was set in negative acquisition mode and source parameters were as follow: capillary 3 500 V, gas temperature 320°C, sheat gas temperature 350 °C, drying gas 8 L/min, nebulizer 35 psi, sheat gas flow 12 L/min. All source parameters were optimized under LC conditions. Mass scan was in the range 100-1 000 m/z. Quantification was achieved by using isotope dilution technique to consider for matrix effects and adjust for any losses during sample preparation. PFAS concentrations were reported both as concentration for single PFAS and as sum of all detected compounds at concentrations above the Limit of Quantification (LOQ). Compounds detected but not quantified counted as zero for arithmetical average calculation. When present, branched isomers were quantified with linear isomers. All numbers were rounded to the first decimal digit, except for concentrations that were rounded to the second decimal digit. Under the described conditions the LOQ for the compounds were in the range 0.03-0.25 ng/mL. Linearity was tested from LOQ up to 5 ng/mL.

RESULTS

Questionnaire results

Sixteen women, between 30 and 44 years-old, were enrolled in this study. They were interviewed about their lifestyle, personal habits, heath, and pregnancy. Regarding to the place of residence, ten of them lived in urban areas, three in the suburbs and three in the countryside. With regards of their everyday diet, 87.5% of them were used to consume organic products. In addition, another 87.5% declared weekly consumption of fish and among those 64.3% were used to eating fish once a week, 14.3% two times a week, 14.3% between two-three times a week and 8.1% between three and four times a week. 62.5% of participants drank water from plastic bottles while the remaining (37.5%) of them drank tap water. Among medical, gynecological and obstetrical history, 68.75% of participants had a positive anamnesis for a disease. 27.3% of these subjects suffered from gestational hypothyroidism while 9.1% had gestational diabetes. The other pathologies were hypothyroidism (9.1%), obesity (9.1%), anemia (9.1%), myoma (9.1%), placenta previa (9.1%) and accreta (9.1%), and fetal abnormalities (9.1%).

PFAS in maternal blood

A total of 16 maternal blood samples collected the day after delivery were analysed. Among the tested 23 PFAS (as reported in Table 1), 8 were found in blood samples; only 2 blood samples were negative for all the tested compounds. Between positive samples, the occurrence of each PFAS is reported in Figure 1 and ranged between 7.1% (for PFUnA and PFPeA) up to 100% (PFOS). PFHxS were detected in 71.4% of blood samples. PFHxA and PFOA were equally detected in 57.1% of blood samples. PFBS occurred in 42.9% of blood samples, while PFNA in 14.3% of blood samples. Detection frequency in samples ranged between one compound in 28.6% of samples, while the remaining 71.4% samples resulted simultaneously positive to more than one compound with most samples positive to more than 4 PFAS simultaneously. The most common association, being found in three samples, was PFOA/PFOS/PFHxS /PFHxA. Regarding the concentration, the total of PFAS in blood samples ranged between 0.18 ng/mL and 3.84 ng/mL. Among the single molecule, PFOS was the compound which reached the highest concentrations (2.15 ng/mL), followed by PFHxA (1.41 ng/mL). However, PFOS and PFOA also displayed the widest dispersion of concentration values when compared to PFHxA. Data were also analysed by considering the most frequently encountered and quantifiable PFAS (PFOS, PFHxS, PFOA, PFHxA, PFBS). For each of these, the range of concentration, mean and median values are reported in Table 2. The observed values are lower compared to those reported in the literature (12-14). The main confounding factor concerns the recruitment of women from the general population who are not specifically exposed to these substances.

The range and distribution of concentrations of the other molecules are reported in Figure 2.

Most of our subjects had organic food in their diet (14 out 16), however PFAS was highly detected in their blood (85.7%). Ten subjects declared fish consumption less than once a week and displayed an average total PFAS level of 1.78 ng/mL (median: 1.17 ng/mL); the remaining declared higher fish consumption and their average blood PFAS levels were 1.96 ng/mL (median 2.57 ng/mL).

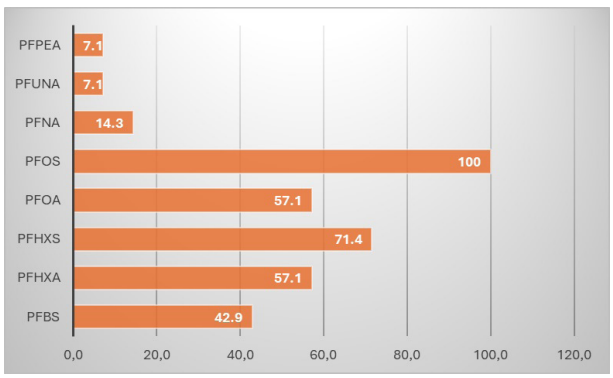

Figure 1
Percentage of the distribution of single PFAS in maternal blood samples.

Table 2
List of the most frequently encountered PFAS in the maternal blood.

Compound	Range of concentration (ng/mL)	Mean Value (ng/mL)	Median Value (ng/mL)
PFOS	0.18-2.22	0.64	0.51
PFHxS	0.08-0.14	0.11	0.11
PFOA	0.09-0.44	0.27	0.26
PFHxA	0.22-1.41	0.56	0.42
PFBS	0.03-0.07	0.05	0.05

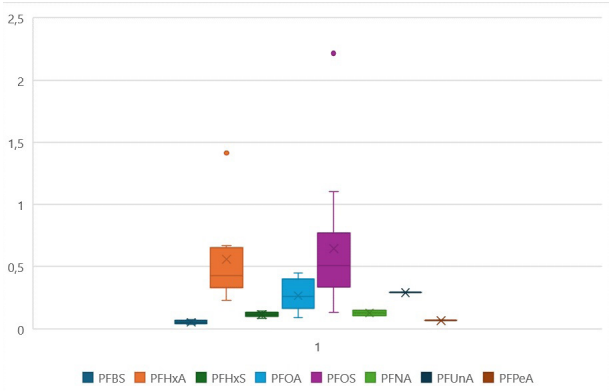

Figure 2
Box plots distribution of PFAS molecules found in maternal blood samples. Median (x) and mean (line) are included in the boxes.

While for water consumption, PFAS molecules were found in the 100% of blood samples of the subjects used to drinking tap water, while PFAS molecules were found in the 81,9% of blood samples of the subjects used to drinking water from plastic bottles.

We investigated some of the pathologies mostly associated to endocrine disruptors reported in the literature (5,10). We have found that endocrinological diseases (hypothyroidism and diabetes mellitus) were the most frequent (31.3% of all subjects) with PFAS concentration values ranging from 0.43 ng/mL up to 4.86 ng/mL (mean: 2.11 ng/mL; median: 1.12 ng/mL). For other pathologies, PFAS concentration values ranged from 0.44 ng/mL up to 3.74 ng/mL (mean: 1.35 ng/mL; median: 0.6 ng/mL). While in the blood samples of subjects without pathologies PFAS concentration ranged from 1.16 ng/mL up to 2.75 ng/mL (mean: 2.22 ng/mL; median: 2.11 ng/mL). For all sixteen cases the PFAS concentration mean was 1.79 ng/mL and the median was 1.17 ng/mL.

CONCLUSIONS

This is one of the first studies in Italy to investigate PFAS detection in a non-exposed population. It is also one of the first studies to examine the habits of subjects. Our results indicated as the most frequently detected compounds in maternal blood PFOS/PFHxS/PFOA/ PFHxA/PFBS, while the less detected were PFNA/ PFUnA/PFPeA. We confirmed the ubiquity of these

substances and their widespread distribution in pregnant women. However, the small sample size does not allow for inferences to be drawn, indicating that further research in this field is needed, especially considering the connection with obstetric conditions. Assessing PFAS levels in biological human samples represent the first step in establishing guidelines to guarantee safety thresholds for human health.

CONFLICT OF INTEREST

None

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