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Analysis and distribution of per- and polyfluoroalkyl substances in decidua and villi placenta explants

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ABSTRACT

Placenta mediates the transfer of nutrients, oxygen and drugs from mother to fetus. It is constituted by two cellular layers separated by the intervillous space: the outer is in direct contact with maternal blood (decidua placenta), and the inner (villi) directly in contact with the fetus. Environmental contaminants, such as per- and polyfluoroalkyl substances (PFAS) also demonstrated the ability to cross the tissue multiple layers, posing at risk the health of the fetus. The aim of the present study was to analyse the PFAS amount in decidua and villi placenta explants and to study differences in their distribution among the two side of this organ. The determination of 23 PFAS was carried out by liquid chromatography coupled to high-resolution accurate mass spectrometry (LC-HRAM). Our research included women who delivered at term between 2021 and 2022. Our data indicated that all samples contained at least one PFAS, demonstrating the ubiquitarian presence of these compounds in our population. A high occurrence of PFOS, PFOA and PFHxS, followed by PFHxA, PFBS and PFUnA was found. The fluorotelomer 6:2 FTS was also present in more than 40% of samples and this represent the first data on placenta explants. Mean and median PFAS values for decidual explants were 0.5 ng/g and 0.4 ng/g (SD 0.3), while for villi explants mean and median values were 0.6 ng/g and 0.4 ng/g (SD 0.4). A different pattern of accumulation was observed between villi and decidual explants for PFOS, PFOA and PFUnA (villi > decidua) and PFHxA, PFHxS, PFBS and 6:2 FTS (decidua > villi). Even if the mechanism of this selectively accumuation is not yet understood, molecular degree of ionization and its lipophilicity could at least in part explain this difference. This study expands the limited data describing PFAS levels in the placenta and pose attention on PFAS exposure during pregnancy.

1. Introduction

The placenta is a disc-shaped organ which provides the sole physical link between mother and fetus. It is a complex organ, constituted by two cellular layers separated by the intervillous space: the outer which is in direct contact with maternal blood (decidual placenta), and the inner side (villi) directly in contact with the fetus and that represent the vascular projections of foetal tissue. Nutrients and drugs in the maternal blood pass from the intervillous space into the foetal blood. Factors affecting drug transfer include placental metabolism, molecular weight of the drug, lipid solubility, pKa, protein binding and concentration gradient across placenta. The rate-limiting barrier for placental drug transfer is at the villi side (Griffiths and Campbell, 2015). In case of environmental contaminants, previous studies on paired maternal and cord serum samples indicated that placenta is permeable to per- and polyfluoroalkyl substances (PFAS) (Zhang et al., 2017; Zhao et al., 2020) and hypothesis that the efficiency of placental transfer is strictly correlated to the chemical structure of the molecule has been proposed

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(Beesoon et al., 2011). PFAS represent a class of anthropogenic contaminants of great concern, currently determining a geographically global exposition but with characteristics associated to the industrial activities at the site (Piva et al., 2021). Some of them (PFOA, PFOS) are defined as persistent organic pollutants (POPs) by the Stockholm Convention. Since their introduction, in the mid-1900s, PFAS have since been incorporated into numerous industrial and consumer products due to their excellent grease/water repellent properties, including: grease resistant papers (e.g., fast food containers/wrappers, microwave popcorn bags, pizza boxes, candy wrappers); stain resistance coating; water-resistant clothing and footwear, cleaning products, personal care products (e.g., shampoo, dental floss, cosmetics such as nail polish and eye makeup); paints, varnishes, ski wax and sealants. PFAS are individually characterized by carbon chain length, side group structure and their history of use. "Legacy" PFAS include compounds with a long-standing history of use and/or long biologic/environmental persistence, whereas replacement and alternative chemistry PFAS, designed to comply with more eco-sustainability government-derived standards, are generally referred to as "emerging PFAS". They are considered multi-system toxicants affecting thyroid functions, immune system, kidney reproductive system. Placenta has also been recognized as a target organ. Recently, a study stated that the highest exposure of placental PFOS was associated with lower birth weight for gestational age in infant males and higher birth weight in female infants (Hall et al., 2022). Since the complexity of this transient organ and the potentially dangerous consequence of an intoxication, it is of pivotal importance to study how PFAS behave and distribute. The aim of the present study was to determine PFAS in decidual and villi placenta samples in order to identify any disparity of distribution between the two sample sets. Currently, no study has yet clarified if a difference in the presence/concentration of PFAS exists between the maternal and foetal side and if a preferential transfer for some PFAS could be supposed. The determination of 23 PF AS, including legacy, emerging and fluorotelomers compounds in placenta was carried out by liquid chromatography coupled to high-resolution accurate mass spectrometry (LC-HRAM). This methodology represents an innovative approach for the analytical determination of PFAS in biological samples, since many methods still exploit low resolution mass spectrometry (Di Giorgi et al., 2023). However, the limited fragmentation pattern of some short-chain PFAS may hamper LC-MS/MS selectivity with a reduced number of MRM transitions. This could represent an extreme limitation in case of emerging short-chain PFAS (ADONA, C_6O_4, GenX …) which cannot be determined by enough MRM transitions and consequently specificity. To overcome this limitation high resolution high accurate mass spectrometer instruments (HRAM-MS) could be used, also in consideration of the achieved sensitivity of modern instrumentation. In this research, placenta samples have been analysed separately by considering the mother (decidual) and foetal (villi) explant. Our analyses included women who delivered between 2021 and 2022 and from whom sufficient placental tissue was available (n = 20).

2. Materials and methods

2.1. Sample collection

Pregnant women were enrolled at-term delivery at the General Hospital Arcispedale Santa Maria Nuova (ASMN) – Reggio Emilia, Italy in the period March 2021–November 2022. The study was approved by the Research Ethical Board of Area Vasta Emilia Nord (protocol number 859/2021/OSS/IRCCSRE). Women were excluded from participation if they were younger than 18 years old. Mothers' population had been selected among low-risk women under no pharmacological treatment. Alcohol abuse and illicit drug consumption were considered as excluding factors for participation and were assessed through anamnestic data collected before hospitalization. All subjects gave their written informed consent. Placenta tissue was taken in-toto at time of delivery at the Unit of Obstetrics and Gynaecology, then subdivided in maternal and foetal side on the bench in laboratory and kept separated. Material submitted to the analysis (5–10 g) was sampled with scalpels from the inner part of the organs to avoid external cross-contamination. Samples were then divided into different laboratory containers. Samples were stored in polypropylene screw vials at −20 °C until analysis.

2.2. Materials

All analytes (>98% purity) and 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 of Supplemental Information (SI). 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 0.05–0.1–0.25-1-2.5–5 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 1000 ng/ml (for details on surrogate and injection standards see Table 1 in SI). 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).

2.3. Sample preparation and LC-HRAM analysis

Placenta samples (3 g ±0.5 g) were added of 2.5 μl of mass labelled standard solution and of 10 ml of purified water and homogenized (model S, VWR International, Radnor, USA) until dissolution. The homogenate was transferred into a polypropylene 50 ml vial and added of 10 ml of acetonitrile. Mechanical agitation was performed for 30″ by vortexing (IKA, Staufen, Germany). QC samples for method validation experiments were prepared in blood (0.5 ml) at the concentration of 0.5 and 1.0 ng/ml and were processed as placenta samples. Samples were then extracted by ultrasonication for 2 h. Debris were removed by centrifugation at 4500 rpm for 5 min. Supernatant was collected and purified by weak anion exchange SPE. Column conditioning was performed by 5 ml of methanol and 5 ml of 2% formic acid in water. Column washing was carried out by 5 ml of 2% formic acid in water and 5 ml of methanol. 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 (2.1 × 100 mm, 1.9 μm), (Agilent Technologies) at 40 °C. An LC C18 column (EclipsePlus -C18, 3.0 × 50 mm 1.8 μm) was placed after pump exit to delay any perfluorinated interferents possibly originating from fluidic system. Analyses were carried out in 20 mM ammonium acetate in water (mobile phase A) and methanol (mobile phase B), both added of 0.1% formic acid (v/v) under gradient (see Table 2 in SI). 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 3500 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 100–1000 m/z at a rate of 2 spectra/sec and 3376 transients/

Table 1
Concentration range, media, median and standard deviation (SD) of PFOS, PFOA, PFHxS, PFHxA, 6:2FTS and PFBS reported for decidua and villi explants.

Molecule	Range (ng/g)		Mean (ng/g)		Median (ng/g)		SD	
	villi	decidua	villi	decidua	villi	decidua	villi	decidua
PFOS	0.06–0.84	0.07–0.42	0.33	0.22	0.20	0.20	0.25	0.09
PFOA	0.01–0.48	0.01–0.21	0.13	0.08	0.08	0.06	0.14	0.06
PFHxS	0.02–0.16	0.01–0.08	0.03	0.04	0.03	0.03	0.02	0.02
PFHxA	0.02–0.50	0.01–0.40	0.03	0.05	0.03	0.04	0.02	0.04
6:2FTS	0.006–0.04	0.007–0.11	0.013	0.04	0.008	0.02	0.01	0.04
PFBS	0.005–0.03	0.007–0.07	0.012	0.017	0.006	0.01	0.01	0.01
PFUnA	0.008–0.08	0.008–0.03	0.04	0.02	0.04	0.02	0.03	0.01

spectrum. References masses were acquired throughout the run and were 112.9855 and 980.0163 m/z. Tune was set as range m/z 3200 at 10 GHz, high resolution. Data analysis was performed by the Masshunter software (version B.10.1, Agilent Technologies). Identification of compounds was by considering accurate mass (≤5 ppm) of the [M-H]- or relative adducts, isotopic pattern distribution and retention time compared to reference material. 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 asingle PFAS and as sum of all detected compounds at concentrations above the LOQ. Compounds detected but not quantified counted as zero for arithmetical average calculation. When present, branched isomers were quantified with linear isomers.

2.4. Method validation

PFAS were quantified by isotopic dilution using 13C-labelled compounds and the corresponding mass labelled standards. Linear calibration curves were prepared using standard solution consisting of a concentration series of 0.05, 0.1, 0.25, 1.0, 2.5, 5 ng/ml for each analyte. Origin was not included and a weight factor of 1/x was applied. Matrix-matched calibration was not required due to the isotope-labelled surrogate used as internal standard for each target analyte. Matrix-effect and recovery were calculated through isotope-labelled surrogate performance. In-house quality controls were repeatedly analysed (n = 5) for three consecutive days for bias and precision data. Sensitivity of the method, defined as limit of detection (LOD) and limit of quantification (LOQ), were determined for mass labelled analogues in each type of sample by using a signal-to-noise ratio of 3 and 10 (peak-to-peak). The LOD and LOQ concentrations were experimentally verified by preparing in double spiked samples. Samples displaying PFAS concentrations below LOQ were considered only for frequency calculation. Prepared laboratory blanks were processed in parallel with the samples to evaluate contamination throughout the analytical procedure.

3. Results

3.1. Method validation

Analytes and associated isotope analogues used as surrogate and injection standards respectively were selected on the base of guidelines set for environmental analysis and are reported in Table 1 of the Supplementary Information. Also, the optimized gradient is summarized in this section as table 2. Sample extraction and purification procedures were optimized on the base of the existing literature and previous authors' experience (Piva et al., 2021, 2022, 2023). Briefly, ultrasound-assisted extraction in acetonitrile was selected before solid-phase purification carried out by weak anion exchange cartridges. Good chromatographic separation was obtained for all PFAS within 12 min. Peak coelution was minimized and when present it did not limit method performances. Under the described conditions, the sensitivity, reported as LOD and LOQ were in the range 0.002–0.02 ng/g and 0.008–0.05 ng/g, respectively. When measurable, intra and interday bias (%) for all PFAS was suitable for method purposes and always below ±20%. Unfortunately, for PFOA, PFOS, PFHxS and PFBA bias values were not calculated due to endogenous presence in blood of these compounds above the spiked concentrations. Matrix-effect was in the range 54–125%, with most analytes in the range 80–118%. All validation data are summarized in Table 3 of Supplementary Information. No carryover was observed during the experiments; processed procedural blanks did not report any PFAS above LOQ except for PFBA. For this reason, PFBA values were excluded from the evaluation of the results. In future experiments containers should be tested and selected on the base of the absence of measurable amounts of this molecule.

3.2. PFAS in placenta samples

The developed method showed suitable for the purposes of the study and allowed for the detection of several PFAS in placenta explants. A total of 40 placenta samples collected at delivery, were analysed, subdivided in 20 decidual and 20 villi explants. Among the tested 23 PFAS, 16 were found in placenta samples. No negative sample was found; the occurrence of each PFAS is depicted in Fig. 1 and ranged between 2.6% (for PFEESA and 11Cl-PF3OUdS) up to 97% (PFOS). PFOA and PFHxS were detected in 87% and 89% of samples respectively. PFBS and PFHxA occurred in 58% and 63% of cases, respectively; while PFHpA and 6:2FTS were present in 42% of analysed placentas. The most frequently detected compounds were PFOS (97%), PFHxS (90%), PFOA (87%), PFHxA (63%), PFBS (58%). All the other PFAS were below 30% of occurrence Samples resulted simultaneously positive to more than one compound with most samples positive to more than 4 PFAS simultaneously (Fig. 2). In fact, the majority of placentas contained from 5 to 7 different molecules. One sample displayed detectable concentration of up to 12 PFAS. The most common association was PFOA + PFOS + PFHxS + PFHxA. The sum of PFAS in placenta samples ranged between 0.02 ng/g and 1.75 ng/g. PFOS was the compound which reached the highest concentrations (0.84 ng/g), followed by PFHxA (0.50 ng/g) and PFOA (0.48 ng/g). However, PFOS and PFOA also displayed the widest dispersion of concentration values in each sample group (villi/decidual) when compared to PFHxA. When considering villi and decidual material separately, mean and median PFAS sum values for decidual explants were 0.5 ng/g and 0.4 ng/g (SD 0.3), while for villi explants mean and median values were 0.6 ng/g and 0.4 ng/g (SD 0.4), respectively. Data were also analysed by considering the most frequently encountered and quantifiable PFAS (PFOS, PFOA, PFHxS, PFHxA, 6:2 FTS, PFBS, PFUnA). Range of concentration, calculate mean and median values for the most frequently detected PFAS in the two explants are reported in Table 1 and Fig. 3a and b. Mean and median values for the most detected PFAS differed in the villi group, while in decidual group they overlapped in many cases. Although T-Test analysis ($p < 0.05$) carried out on total/single amount of PFAS in the two groups did not allow to exclude the null hypothesis, a different pattern of accumulation can be observed between villi and decidual explants for the most frequently detected molecules (Fig. 3a and b). PFOS, PFOA and PFUnA presented higher concentration values in villi samples, while PFHxA, PFHxS, PFBS and 6:2 FTS displayed higher amounts in decidual samples. Placenta samples

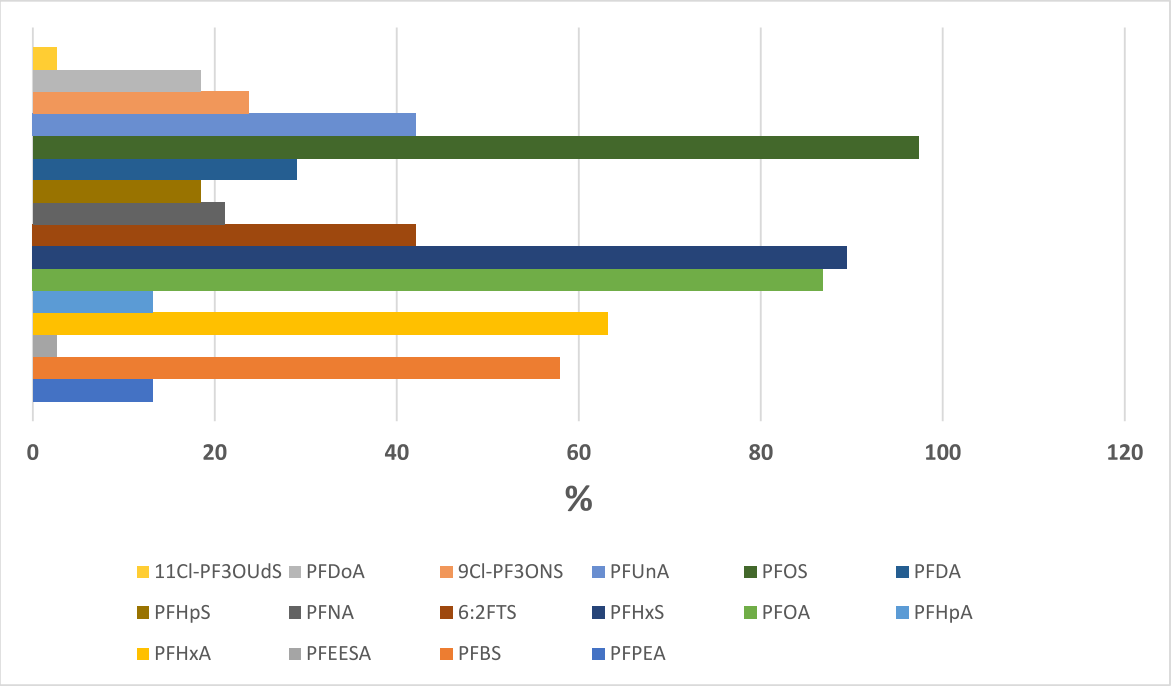

Fig. 1. PFAS occurrence in the analysed samples.

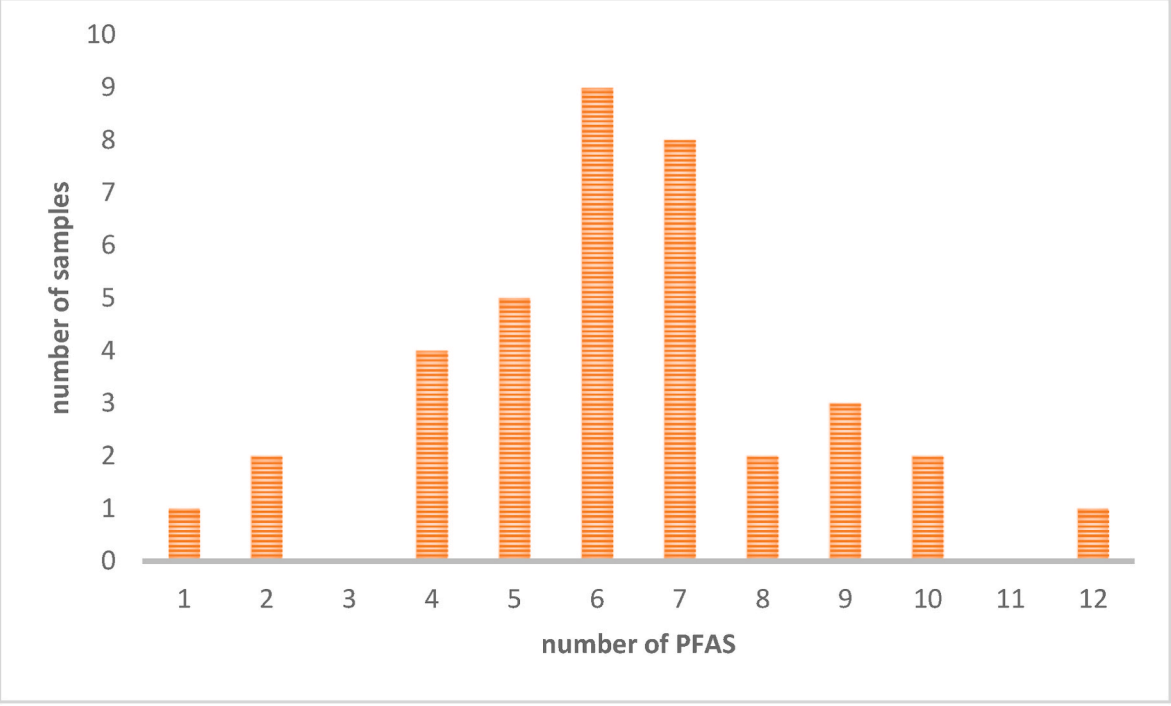

Fig. 2. Representation of the samples resulted positive to PFAS.

deriving from the same subject showed a correlation for the presence of a specific compound in most cases, but not always. In some cases, especially for concentrations near the LOQ, the compound was present only in one type of the explant. In particular, PFEESA and 11Cl-PF3OUdS were found only in decidual samples, while PFNA, PFDoA and PFDA were found more frequently in villi explants. Branched isomers of PFOA and PFOS were detectable under the described analytical conditions, although they were seldomly noticed in our set of samples. However, when present, they accounted with linear counterparts.

4. Discussion

Differently from the few existing studies on PFAS presence in placenta, this research analysed separately PFAS in decidua (mother-side) and villi (fetus-side) placenta samples in order to study differences in the accumulation pattern. Our research was aimed at understanding if a different behaviour of the two side of placenta regarding PFAS transfer exists and to suppose the underlying biological mechanism. A method for the analysis of 23 PFAS among legacy, precursors and emerging

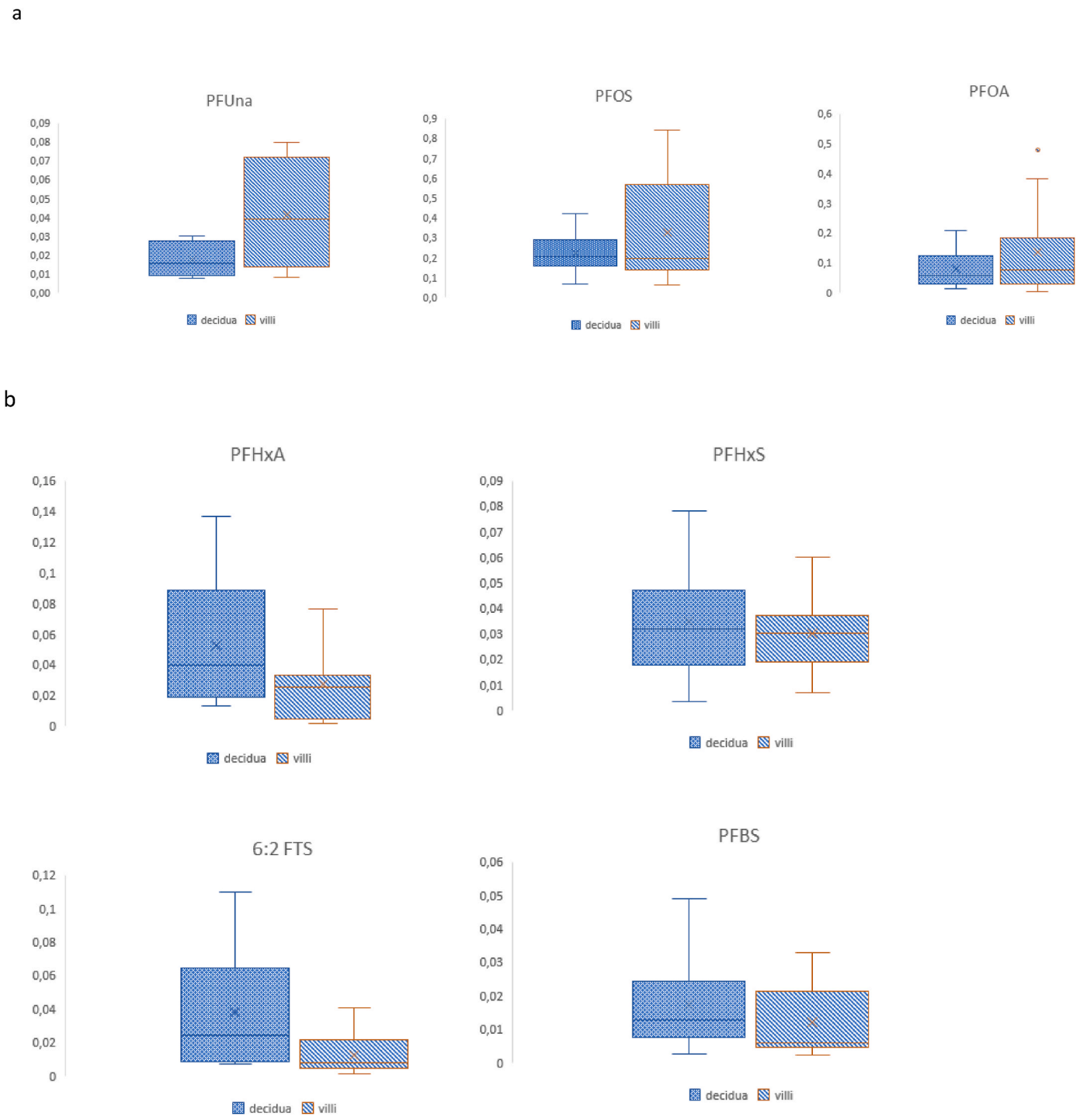

Fig. 3. aGraph of the distribution of concentration for PFAS with C length >8: PFUnA, PFOS, PFOA. Mean and median values are described by a cross and a line respectively. Standard deviation is also reported with bars. Graph of the distribution of concentration for PFAS with C length <8: PFHxA, PFHxS, 6:2 FTS, PFBS. Mean and median values are described by a cross and a line respectively. Standard deviation is also reported with bars.

compounds was developed and validated in blood samples. For method development and validation, blood was selected over plasma/serum as the most similar matrix to placenta extracts because recent studies showed a different partition between blood/serum i.e., ratios different from 1:1, influenced by the length of C–F chain (Jin et al., 2016). In analogy with PFAS environmental and food analysis (Piva et al., 2022, 2023), the isotope dilution method was applied for sample quantification. This approach showed particularly appropriate for to the absence of real negative samples (Mørck et al., 2015) to be used for matrix-matched calibration at very low levels of concentration. In fact, even if a set of blood was screened for PFAS presence before validation and the blood with the lowest amounts was selected, the intraday and interday bias calculation was affected by endogenous presence of PFOS, PFOA, PFHxS and PFHpS. Cross-contamination from laboratory material also represents an issue in PFAS analysis. In this case PFBA was excluded from the method application due to the unavoidable contamination from placenta containers. Overall, the validation parameters were satisfying, and the method proved suitable for the purposes of the study, revealing the presence of 16 type of PFAS in our set of samples. A comparison with the most recent research on the topic revealed this

study as one of the most widen conducted on placenta samples so far, in fact we included in our method also emerging (ADONA, HFPO-DA) and precursor of PFAS (4:2 FTS, 6:2 FTS, 8:2 FTS) for a total of 23 compounds. Our sample set included placentas from women not considered at high risk of exposure and enrolled in a limited time range (2021–2022) and represents the first attempt to study PFAS burden in placentas in Italy. The results presented a high occurrence of PFOS, PFOA and PFHxS, followed by PFHxA, PFBS and PFUnA. The fluorotelomer 6:2 FTS was also found in more than 40% of samples and this represent the first data on placenta explants. Quantitative data were lower than concentrations usually found in human blood/plasma/serum samples of at least one order of magnitude, and this is in line with the current literature. Moreover, our data displayed comparable PFAS occurrence for the most studied PFOS and PFOA, but at lower amounts, when compared to other studies present in literature and this may reflect the different time and geographical enrolment. In fact, only considering the most recent research, Mamsen et al. (2019) submitted a total of 78 placenta samples from Swedish women collected in 2014–2015 to the analysis of six PFAS, namely PFOS, PFOA, PFNA, PFDA, PFUnA and PFHxS. The most frequently detected compounds were PFOS (93%), PFOA (82%) and PFNA (83%). PFOS amounts were in the range 0.45–3.87 ng/g; PFOA amounts were in the range 0.15–0.99 ng/g, while PFNA, PFDA and PFUnA were below 0.73 ng/g. The authors also observed an increased accumulation of PFAS burden (i.e., the sum of all PFAS) in relation to gestational age, with PFOS and PFOA as the most represented. They also hypothesised a different transfer efficiency between mother and fetus in relation to the length of the C–F chain. A recent study took in consideration 11 PFAS in placenta samples from United States collected in the period 2010–2011 (Hall et al., 2022). Detection frequency for PFOS, PFOA, PFNA and PFDA were all around 96–100%, and no sample was found negative to PFAS. Median values for the most detected compounds were in the range 0.06 (PFDA)- 0.95 (PFOS) ng/g. The authors noted that longer-chain PFAS was abundantly detected in placenta and shorter-chain PFAS were detected much less frequently. A high-risk group of women were enrolled in 2015–2018 by Bangma et al. (2020) for the analysis of PFAS. The authors assessed the presence of PFOS at the maximum levels (median 0.48 ng/g) and PFHxS, PFHpS, PFOS and PFUnA occurrence over 50% of the analysed placenta samples. All these data confirm that at least PFOS and PFOA are ubiquitarians among the human population and that almost every placenta develops under the influence of one of these toxicants. The presence of other PFAS congeners may be more strictly connected to geographical distribution or time of collection. According to all these studies, placenta has been recognized as a target organ for PFAS accumulation and represent the gateway for transmission into foetal blood. However, placenta is a complex organ and is constituted of external tissue of maternal origin (decidua) separated by an interspace from foetal tissue (villi). The rate-limiting barrier for placental drug transfer is represented by the layer of cells covering the villi (Griffiths and Campbell, 2015) and for this reason the two groups of explants have been separately analysed in our research. Data from literature affirm that both long ($C \geq 8$) and short ($C < 8$) chain molecules can pass from maternal circulation to foetal blood, but with different efficiencies (i.e. long chain > short chain) (Mamsen et al., 2019). At the moment, no hypothesis of the biological mechanism exists. We tentatively studied the differential distribution of 23 PFAS in decidua and villi placenta samples, but unfortunately, only 7 of the 16 detected PFAS reached a satisfactory occurrence frequency above LOQ, namely PFOS, PFOA, PFHxS, PFHxA, 6:2 FTS, PFBS and PFUnA and further evaluations are based only for these compounds. Our data indicated a different accumulation pattern between PFOS/PFOA/PFUnA and PFHxS/PFHxA/6:2 FTS/PFBS: the former group was more concentrated in the villi explants (Fig. 3a), while the latter was more concentrated into decidua explants (Fig. 3b). A differential accumulation between the two sides of the placenta was observed also for the class of brominated flame retardants (Ruis et al., 2019). This differential accumulation confirms that placenta tissues mediate PFAS transfer to foetal blood not equally, showing in one case an exceeding transfer and in the other an incomplete transfer. The rules of drug transfer from maternal to fetus blood are diverse, including simple and facilitated diffusion along the concentration gradient. Though, other mechanisms such as pinocytosis and active transport cannot be fully excluded on the base of the current observational data. Some authors justified the different placental transfer capability on the base of the C-chain length (Mamsen et al., 2019), however also other molecular characteristics such as molecular weight, lipid solubility and degree of ionization should be considered for transfer efficiency. In fact, the evaluation of transfer efficiency on the base of solely C-chain length do not explain completely our data. If it was the only parameter to be considered, the distribution pattern of 6:2 FTS (C8) could not be fully explained, and it would be in contrast with other C8 (i.e. PFOS/PFOA) (Table 4 of Supplementary Information). Neither low molecular weight PFAS do not seem to be favourited during transfer, being PFOS and PFUnA >500 g/mol. Degree of ionization and lipophilicity, estimated respectively by pKa and LogP could at least in part explain our data. Molecules with higher pKa (PFOS, PFOA, PFUna) and higher LogP (PFOS, PFOA) could pass more easily through the layer of cells covering the villi. Unfortunately, for many PFAS all these physical-chemical data are lacking, and any general consideration would be hampered.

Though the study provides important new data about the prevalence of different PFAS in placenta and their potential transfer from mother to fetus, some weakness could be discussed. The absence of blank material for method development and validation hampers the capability of the method for the accurate determination of these compounds and limits any evaluation of the extraction recovery. Blood was selected for method validation for the great availability also in consideration of the needed amounts, compared to placenta samples availability, but surely this is an approximation. The selected population can be considered representative of individuals not highly exposed but an evaluation of the total PFAS burden by evaluating lifestyles or food intake for each individual was not possible at this stage of the research. Moreover, though confounding factors such as pharmacological drugs consumption or alcohol/illicit drugs exposure have been excluded in proximity of the sampling, these cannot be totally excluded for the past. Finally, as future perspectives, in the hypothesis of an altered anatomy and physiology of the organ induced by PFAS exposure during the early stages of development, histological data on the sampled placentas will be studied in function of PFAS analytical data.

As concluding remarks of the presented data, it becomes certainly important to investigate more deeply the aspects that rule the transfer of this class of molecules through placenta and to study the consequences of a chronic exposure to these contaminants in terms of altered placental development. In this view, it is totally missing any information regarding a dose-effect relationship and if effects are in relation to permanent or transient alterations at developmental stage of foetal growth. All this data could be lately used to propose pharmacological intervention to high-risk women to avoid detrimental consequences to pregnancy.

5. Conclusions

This study confirms the great permeability of placenta for most PFAS and represents the first evidence of a different grade of exposure to PFAS in this peculiar organ critical for pregnancy outcome. Also in consideration of the limited number of samples available, this study expands the limited data describing PFAS levels in the placenta. The need for PFAS biomonitoring during pregnancy among women of child-bearing age should not be underrated for the dangerous effects on the fetus health.

Author statement

JPP, EP: Conceptualization, methodology; **PF**: funding acquisition, resources; **JPP, EP**: Data curation, Writing- Original draft preparation;

MPB, CM, AS: investigation, resources; **JPP, PF**: formal analysis, writing-reviewing and editing.

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Ethical approval

The study was approved by the Research Ethical Board of Area Vasta Emilia Nord (protocol number 859/2021/OSS/IRCCSRE), Italy.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2023.115955.

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