Journal Pre-proof

Early-life exposure to per- and polyfluoroalkyl substances: Analysis of levels, health risk and binding abilities to transport proteins

Yaqi Xu, Xinyao Sui, Jinhong Li, Liyi Zhang, Pengpeng Wang, Yang Liu, Huijing Shi, Yunhui Zhang

PII: S2772-9850(24)00035-8

DOI: https://doi.org/10.1016/j.eehl.2024.04.007

Reference: EEHL 105

To appear in: *Eco-Environment & Health*

Received Date: 28 December 2023

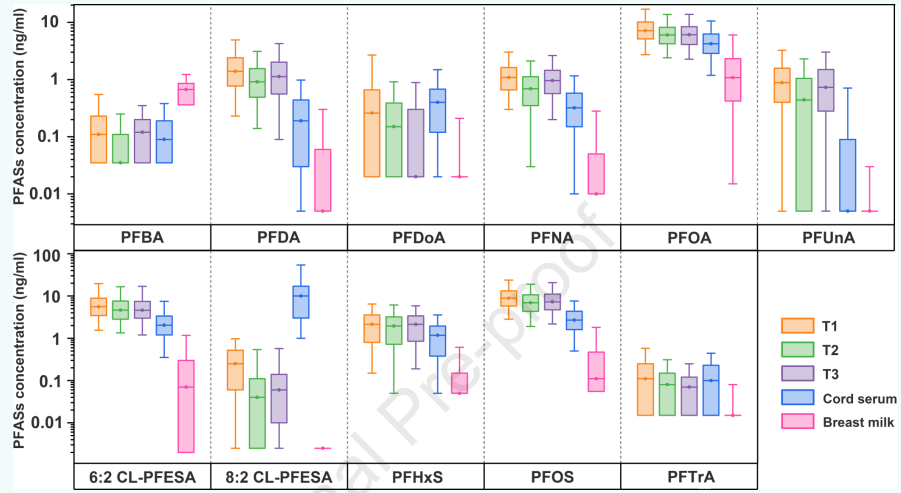
Revised Date: 5 March 2024

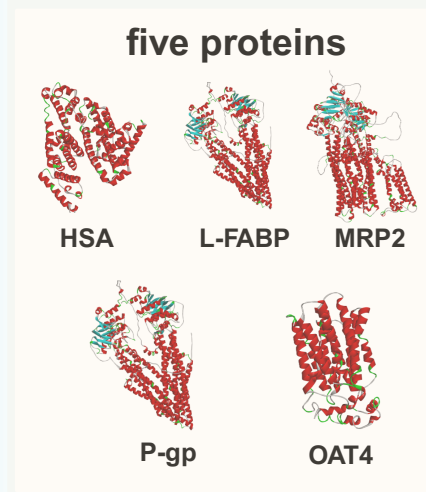
Accepted Date: 14 April 2024

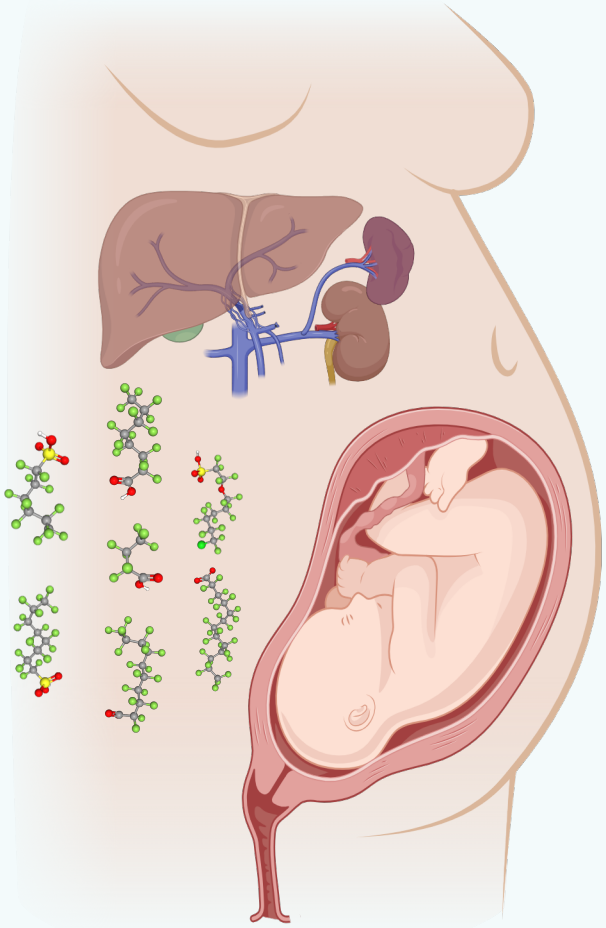
Please cite this article as: Y. Xu, X. Sui, J. Li, L. Zhang, P. Wang, Y. Liu, H. Shi, Y. Zhang, Early-life exposure to per- and polyfluoroalkyl substances: Analysis of levels, health risk and binding abilities to transport proteins, *Eco-Environment & Health*, https://doi.org/10.1016/j.eehl.2024.04.007.

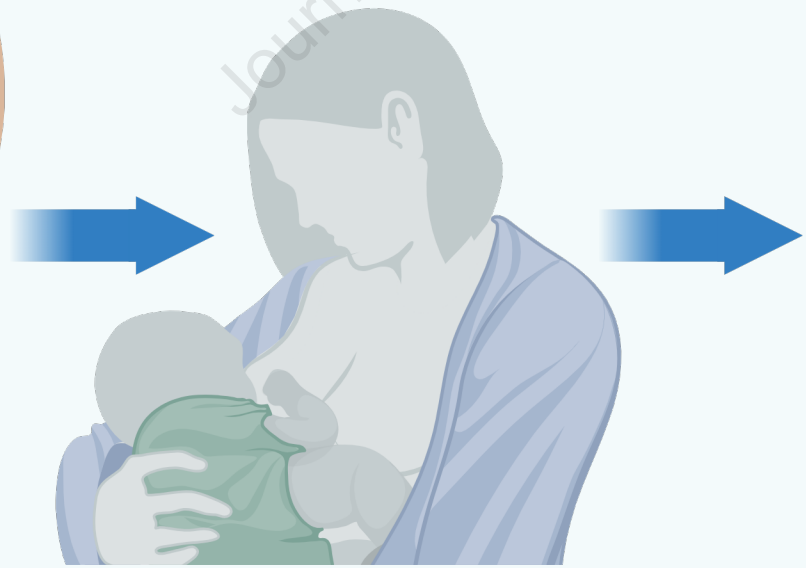
Early-Life exposure to per- and polyfluoroalkyl substances

Levels of PFASs in maternal serum , cord serum and breast milk

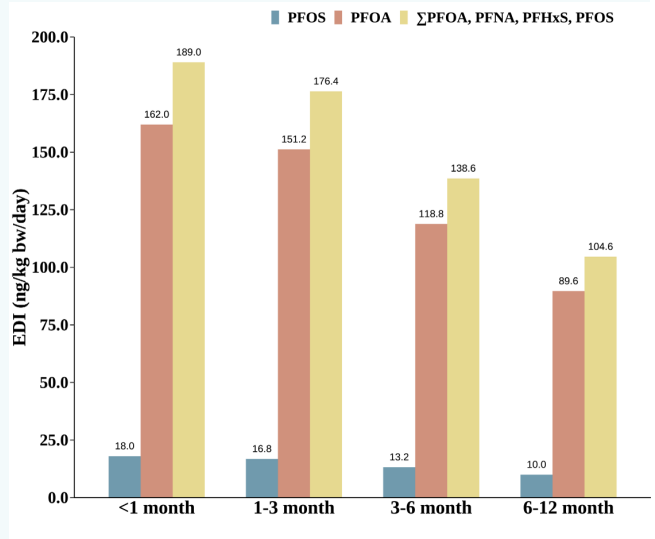








EDIs for breastfeeding infants



Early-life exposure to per- and polyfluoroalkyl substances: Analysis of levels, health risk and binding abilities to transport proteins

Yaqi Xu[a,b,1], Xinyao Sui[a,1], Jinhong Li[b,1], Liyi Zhang[b], Pengpeng Wang[b], Yang Liu[b], Huijing Shi[a,b], Yunhui Zhang [a,b,*]

[a] Key Lab of Health Technology Assessment, National Health Commission of the People's Republic of China, Fudan University, Shanghai 200032, China.

[b] Key Laboratory of Public Health Safety, Ministry of Education, School of Public Health, Fudan University, Shanghai 200032, China.

[1] *These authors contribute to this work equally.*

[*] *Corresponding author: Yunhui Zhang (yhzhang@shmu.edu.cn);*

Abstract

Per- and polyfluoroalkyl substances (PFAS) can pass through the placenta and adversely affect fetal development. However, there is a lack of comparison of legacy and emerging PFAS levels among different biosamples in pregnant women and their offspring. This study, based on the Shanghai Maternal-Child Pairs Cohort, analyzed the concentrations of 16 PFAS in the maternal serum, cord serum, and breast milk samples from 1,076 mother-child pairs. The placental and breastfeeding transfer efficiencies of PFAS were determined in maternal-cord and maternal-milk pairs, respectively. The binding affinities of PFAS to five transporters were simulated using molecular docking. The results suggested that PFAS were frequently detected in different biosamples. The median concentration of perfluorooctane sulfonate (PFOS) was the highest at 8.85 ng/mL, followed by perfluorooctanoic acid (PFOA) at 7.13 ng/mL and 6:2 chlorinated polyfluorinated ether sulfonate at 5.59 ng/mL in maternal serum. The median concentrations of PFOA were highest in cord serum (4.23 ng/mL) and breast milk (1.08 ng/mL). PFAS demonstrated higher placental than breastfeeding

transfer efficiencies. The transfer efficiencies and the binding affinities of most PFAS to proteins exhibited alkyl chain length-dependent patterns. Furthermore, we comprehensively assessed the estimated daily intakes (EDIs) of PFAS in breastfeeding infants of different age groups and used the hazard quotient (HQ) to characterize the potential health risk. EDIs decreased with infant age, and PFOS had higher HQs than PFOA. These findings highlight the significance of considering PFAS exposure, transfer mechanism, and health risks resulting from breast milk intake in early life.

Keywords: Emerging PFAS; Placental transfer; Breast milk; Health risk; Binding affinity

1 Introduction

Per- and polyfluoroalkyl substances (PFAS) constitute a category of artificial organic compounds characterized by containing a chain of two neighboring carbon atoms. One carbon atom is bonded to at least two fluorine atoms, while the other is bonded to at least one fluorine atom, with neither bound to hydrogen[1–3]. PFAS have been extensively used in the production of packaging, textiles, lubricants, and cooking utensils for their excellent hydrophobic and oleophobic properties[4]. PFAS are widely utilized and emitted, leading to their pervasive presence in the air, water, and soil. They can penetrate the human body through various pathways, resulting in significant health hazards. Furthermore, certain emerging PFAS, such as F-53B, exemplified by 6:2 chlorinated polyfluorinated ether sulfonate (6:2 Cl-PFESA) and 8:2 chlorinated polyfluorinated ether sulfonate (8:2 Cl-PFESA), are primarily employed as chromium mist inhibitors within the electroplating sector. These substances have become considerably prevalent in China in recent years[5]. Short-chain PFAS have replaced long-chain compounds in many fields, and can be widely detected in various tissues and organs of animals and humans[6]. The levels of short-chain PFAS in Chinese and European populations have also shown an upward trend, leading to public concern about the health risks of these chemicals[6]. Many studies have shown that PFAS lead to metabolic and immune system disorders, endocrine disrupting effects, neurotoxic,

reproductive and developmental toxicity, and visceral and organ toxicity[7,8]. Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) have been banned[9]. Perfluorohexanesulfonic acid (PFHxS) was also incorporated into the Stockholm Convention on June 27, 2022, and banned globally.

The initial stages of life are a pivotal period for human development[10]. Evidence has shown that both legacy and emerging PFAS in pregnant women can pass through the placental barrier[11,12]. Prenatal PFAS exposure is associated with an elevated susceptibility to infectious diseases, autism, and Attention Deficit Hyperactivity Disorder (ADHD) in children[13,14]. Recent research in Sweden[15] and Ohio, the United States[16] found a decreasing trend in PFAS levels in maternal serum, possibly due to maternal physiological changes during pregnancy, such as increased body weight and blood volume[17], or the transmission of PFAS from the mother to fetal tissues, such as fetal lung and liver[18]. Furthermore, breast milk is commonly recognized as the primary source of nutrition for infants aged 1–6 months, and it is the decisive source of exposure to environmental pollutants[19] . Infants can be exposed to PFAS through breastfeeding, leading to postnatal exposure for newborns. These findings[20,21] emphasize the need to comprehensively assess early-life PFAS exposure in mother–child pairs.

The placenta is a vital organ that can prevent the transfer of foreign substances, thus serving as a protective barrier[22]. The placental transfer properties of PFAS have become a highly concerning and urgent scientific issue that needs to be addressed within the field of environmental science[23]. The placental transfer mechanism is typically described as being characterized by passive diffusion and active transport[24]. The main method for transplacental transfer of PFAS is passive diffusion, with the levels of free PFAS in serum being a critical determinant[25]. Human serum albumin (HSA) and liver-fatty acid binding protein (L-FABP) are the main binding proteins of PFAS[26]. Additionally, the active transport mediated by the adventitial pump and transporter family can transport PFAS from either the maternal or fetal side to the opposite side of the placenta[23]. An in vitro placenta perfusion experiment demonstrated that PFOS and PFOA bind to organic anion transporter 4 (OAT4),

affecting their placental transfer[27]. PFAS are the substrate of OAT4 in the placenta. The stronger binding affinities of PFAS with OAT4 restrict the potential for transfer from the fetal side to the maternal side, consequently leading to elevated concentrations of PFAS in the placental tissue. Recent in vitro studies have exhibited that these transporters may affect the active transport of organochlorine pesticides, such as how they screen the outflow of PFAS[28]. In addition, the breast cancer resistance protein is thought to be independent of the transfer of PFOA or PFOS[27]. The current understanding of the binding modes between PFAS and these transporters is still very limited. Thus, evaluating the affinities of PFAS homologs with momentous transporters in serum and the placenta by molecular docking calculation can enhance our understanding of the placental transfer of PFAS[29].

The objective of this study was to determine the levels of legacy and emerging PFAS compounds in matched samples of maternal serum, cord serum, and breast milk samples. Additionally, molecular docking calculations were employed to investigate the interaction between PFAS and HSA, L-FABP, OAT4, P-gp, and MRP2. Furthermore, we estimated daily intakes (EDIs) of PFAS for breastfeeding infants to evaluate their potential health hazards.

2 Materials and methods

2.1 Study design and sample collection

The study utilized data from the Shanghai Maternal-Child Pairs Cohort. More detailed information has been mentioned before[30]. The study enrolled pregnant women who fulfilled the following criteria: 1) Shanghai resident; 2) age ≥ 18 years; 3) without severe chronic illnesses; and 4) able to provide a biological sample from at least one phase of the study, including serum from the first, second, or third phase of follow-up, along with cord serum and breast milk (serum samples were hemolysis free with the sample volumes being > 100 μL and the breast milk sample volume being > 1.00 mL). In total, the study encompassed 1,076 participants, and 1,039 maternal serum samples at the first follow-up (16–18 weeks), 995 maternal serum samples at the second follow-up (24–28 weeks), 887 maternal serum samples at the

third follow-up (30–34 weeks), and 988 cord serum and 551 breast milk samples were collected within 2–3 days after delivery. The serum was collected using a coagulant collecting vessel, while breast milk was collected using a 15-mL centrifuge tube and stored at -80 ℃ freezer. The samples were placed at room temperature to thaw, and each tube of sample was mixed well before measurement.

The Fudan University Institutional Review Board granted approval for the Shanghai Maternal–Child Pairs Cohort study (IRB#2016-04-0587), and informed consents were duly obtained from participants.

2.2 Determination of PFAS in serum and breast milk samples

The 16 target PFAS included 9 legacy long-chain PFAS, 5 short-chain PFAS, and 2 new substitutes [i.e., PFOS, perfluorononanoic acid (PFNA), PFOA, perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnA), perfluorolauric acid (PFDoA), perfluorotridecane acid (PFTrA), PFHxS, PFOS, perfluorooctanesulfonamide (PFOSA), perfluorobutyric acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), potassium perfluorobutane sulfonate (PFBS), 8:2 Cl-PFESA and 6:2 Cl-PFESA] were analyzed in three matrices using a high-performance liquid chromatography-triple quadrupole mass spectrometer (HPLC-QqQ-MS, Agilent 1290-6490, USA).

The instrument parameters and assay process for serum samples were described in our previous study[31] (see Methodology of PFAS Detection in the appendix). The disparities in pretreatment between breast milk and serum samples are as follows: After thawing and mixing, a small volume (100 μL for serum samples or 1.00 mL for breast milk samples) was taken and added to a centrifuge tube. Before instrumental analysis, the breast milk sample was filtered through a 0.22-μM filter membrane, while this step was not needed for the serum samples.

In this analysis process, isotope internal standards were added to each test sample to control the loss of the target substance during the pre-treatment process. Methanol was used as the substrate for the method blank (one blank control sample was added for every 28 samples) to control for the impact of human and

environmental factors. The limit of detection (LOD) and limit of quantitation for each analyte were determined as 3 and 10 times the concentrations producing a signal-to-noise ratio, respectively, ranging from 0.004 to 0.16 ng/mL and 0.03 to 0.54 ng/mL. The recoveries of substances in serum and breast milk range from 70.0% (8:2 Cl-PFESA) to 103% (PFNA) and from 69.4% (PFOSA) to 119% (6:2 Cl-PFESA), respectively. The intra-day and inter-day RSDs are both < 20.0% (Tables S1 and S2).

2.3 Docking simulations

We obtained the 3D structures of PFBA (CID: 9777), PFOA (CID: 9554), PFNA (CID: 67821), PFDA (CID: 9555), PFTrA (CID: 23084971), PFHxS (CID: 67734), PFOS (CID: 74483), 6:2 Cl-PFESA (CID: 22568738), and PFOSA (CID: 69785) from PubChem and used ChemDraw to draw the 3D structures of 8:2 Cl-PFESA, PFUnA and PFDoA. The three-dimensional crystal structures of HSA (PDB ID: 4e99) and L-FABP (PBD ID: 3stm) were obtained from the Protein Data Base. Since the crystal structures of OAT4, P-gp, and MRP2 cannot be obtained to date, the corresponding gene sequences were obtained from NCBI, and homologous modeling was performed using the SWISS-MODEL server. Online protein structure scoring was conducted using SAVES V6.0. The quality of the protein structure obtained through homologous modeling was evaluated using the Ramachandran plot (Figure S1). To characterize the active site residues of five protein receptors and target compounds and predict their binding modes, we used the Lamarckian genetic algorithm provided by AutoDock Vina software for molecular docking calculations. In the docking calculation process, the protein structure was set as a rigid structure, while the target ligand structure was a flexible structure (Table S3). Each ligand was subjected to 9 independent docking tests. In molecular docking, the binding between ligand and receptor will cause a change in Gibbs free energy (ΔG), which is determined by the thermodynamic and kinetic parameters of the interaction between ligand and receptor.

When $\Delta G < 0$, the interaction between the ligand and the receptor is favorable, and they tend to bind. When $\Delta G > 0$, the interaction between the ligand and the receptor is weaker and less likely to occur. Therefore, for molecular docking studies, the negative value of ΔG is usually used as a quantitative indicator to judge the stable binding

between the ligand and the receptor. There is a quantitative relationship between the dissociation constant (Kd) and the molar Gibbs free energy:

$$\Delta G = RTlnKd \quad (1)$$

In the equation, ΔG represents the standard free energy change, Kd refers to the dissociation constant, R represents the ideal gas constant, which is equal to 8.314 J/(mol·K). T signifies the temperature measured in Kelvin. The dissociation constant of the target compound and the receptor protein was calculated at human body temperature in this study.

A random forest score (RF score = pKd = −logKd) was utilized: a higher RF score indicates a smaller Kd, which translates into a higher binding ability between the small molecule and protein.

2.4 Health risk assessment

The EDIs of PFAS (expressed in ng/kg body weight [bw] per day) for infants consuming breast milk were compared with exposure guidelines. EDIs of PFAS were calculated in different age groups of breastfeeding infants using Eq. 2 adapted from Zhu et al[32]. The hazard quotients (HQ) were computed to evaluate potential health risks using Eq. 3. The calculation equations are as follows:

$$EDI = C_{milk} \times FIR \quad (2)$$

$$HQ = \frac{EDI}{RfD} \quad (3)$$

Where C_{milk} is the median concentration of each substance in breast milk, measured in ng/mL. FIR refers to the food ingestion rate, expressed as mL/(kg bw·day). The average FIR values for different age groups can be referenced from the Exposure Factors Handbook of the United States Environmental Protection Agency (EPA). For infants aged less than 1, 1–3, 3–6, and 6–12 months, the average daily intake of breast milk was 150, 140, 110, and 83.0 mL/(kg bw·day), respectively[33].

RfDs (Reference Doses) are set by the European Food Safety Agency (EFSA).

These include the following: the 2008-proposed daily tolerable intake (TDI) for PFOA, which is 1,500 ng/(kg·d); the 2008-proposed daily tolerable intake (TDI) for PFOS, which is 150 ng/(kg·d); and the 2020-proposed weekly tolerable intake (TWI) for the sum of PFOA, PFNA, PFHxS, and PFOS, which is 4.40 ng/(kg·wk). When the HQ is less than or equal to 1.00, the exposure does not exceed the adverse effect threshold; when the HQ value exceeds 1.00, the exposure level is deemed unacceptable.

2.5 Data analysis

The concentrations of PFAS congeners in serum and breast milk had skewed distributions. To describe the distribution of the target substance levels with a detection rate > 0, we utilized the geometric mean (GM), frequencies, and quartiles on a volume-based scale (ng/mL). For concentrations below the LOD, we assigned a value of LOD/2. The PFAS congeners with a detection rate > 50% were used in the statistical analyses. To assess the temporal variability of PFAS levels during pregnancy, we calculated the intraclass correlation coefficient (ICC) and its corresponding 95% confidence interval (CI). In our study, we categorized low variability as an ICC > 0.75, moderate variability as an ICC between 0.40 and 0.75, and high variability as an ICC < 0.40. The ICC was used to measure the variability of repeated measurements over time. To examine the correlations between PFAS levels in maternal serum and cord serum, we employed the Spearman correlation coefficient.

To obtain an accurate estimation of the placental transfer efficiencies (C:T3 ratio) of PFAS, we included only matched samples with detectable concentrations (>LOD) in both the third follow-up and cord serum. Similarly, when calculating the breastfeeding transfer efficiencies (M:T3 ratio), only paired samples with T3 and breast milk concentrations > LOD were used. Table S4 shows the correspondence between paired samples and compounds.

All statistical analyses were conducted using R software (version 4.0.5). The standard of statistical significance was $p < 0.05$ (two-tailed).

3 Results

3.1 Demographic characteristics

The demographic characteristics of the 1,076 pregnant women included in the study are displayed in Table 1. The average age at delivery was 29.3 ± 4.37 years, and their average BMI was 21.4 ± 2.96 kg/m^2. Among the participants, 69.5% had a BMI between 18.5 and 24.0, and 42.8% had a normal range of gestational weight gain. Additionally, 79.9% had attained at least a high school education. During pregnancy, 43.1% of mothers were exposed to passive smoking. More than half (57.1%) of pregnant women were first-time mothers. The average gestational week of pregnant women was 39.3 ± 1.23 weeks, and 3.6% of pregnant women gave birth preterm.

Table 1. Demographic characteristics of the pregnant women (*n* = 1,076) in the study

Characteristic	Mean ± SD or *n*(%)
Age at delivery (year)	29.3 ± 4.37
Pre-pregnancy BMI[a] (kg/m^2)	21.4 ± 2.96
＜ 18.5	149(13.8)
18.5–24	747(69.5)
≥ 24	180(16.7)
Gestational weight gain[b] (kg)	13.7 ± 5.23
Normal	461(42.8)
Inadequate	129(12.0)
Excessive	486(45.2)
Education	
High school and below	216(20.1)
Above high school	860(79.9)
Household income (CNY per year)	
＜ 100k	248(23.0)
100k–300k	737(68.5)
＞ 300k	91(8.50)
Passive smoking	
Yes	464(43.1)
No	612(56.9)
Parity	
Primiparity	614(57.1)
Multiparity	462(42.9)
Gestational weeks at delivery	39.3 ± 1.23
Premature delivery	39(3.6)

	Term delivery	1037(96.4)

[a] Pre-pregnancy BMI and pregnancy weight gain classification referred to Chinese standards. [b] The normal range of pregnancy weight gain of low-weight pregnant women (BMI < 18.5) is 11.0–16.0 kg, that of normal-weight pregnant women (18.5 ≤ BMI < 24.0) is 8.00–14.0 kg, that of overweight pregnant women (24.0 ≤ BMI < 28.0) is 7.00–11.0 kg, and that of obese pregnant women (BMI ≥ 28.0) is 5.00–9.00 kg.

3.2 PFAS concentrations in maternal, cord serum and breast milk

Table S5 lists the detection rates, geometric mean (GM) values, and distributions of PFAS concentrations in the 25th, 50th (median), and 75th percentiles in both serum and breast milk. The dominant analytes observed in all samples were the legacy long-chain PFOS and PFOA, and the new substitute 6:2 Cl-PFESA. PFOSA was detected in maternal and cord serum samples but not in breast milk. PFPeA, PFHpA, and PFBS were not detected in any samples. The detection rates of PFOA, PFNA, PFDA, PFHxS, PFOS, and 6:2 Cl-PFESA in maternal serum (three follow-up visits) were all > 90.0%. The detection rates of PFUnA, PFTrA, and 8:2 Cl-PFESA were all > 50.0% in three follow-up visits. The detection rates of PFBA in the first follow-up visit (T1) and the third follow-up visit (T3) were > 60.0%, but the detection rate in the second follow-up visit (T2) was 35.8%.

The highest median concentration observed during pregnancy was PFOS (8.85 ng/mL), followed by PFOA (7.13 ng/mL) and 6:2 Cl-PFESA (5.59 ng/mL) (Figure 1). The detected concentrations of PFAS varied among the three trimesters. Except for those of 6:2 Cl-PFESA and PFTrA, the median concentrations of PFAS in maternal serum followed the order of T1 > T3 > T2. The concentrations of PFTrA and 6:2 Cl-PFESA in maternal serum at different trimesters decreased with increasing trimesters (T1 > T2 > T3). The detection rates of five kinds of PFAS, namely, PFBA, PFDA, PFDoA, PFTrA, and 8:2 Cl-PFESA, were also above 50% in cord serum. The median concentrations of PFOA (4.23 ng/mL), PFOS (2.70 ng/mL), 6:2 Cl-PFESA (2.04 ng/mL), and PFHxS (1.18 ng/mL) in cord serum were notably higher than those of other PFAS. The levels of PFBA and PFTrA were low. The level of 6:2 Cl-PFESA in serum was higher than that of 8:2 Cl-PFESA.

In 551 breast milk samples, the detection rates of four PFAS were above 50.0%: PFBA (86.6%), PFOA (86.6%), 6:2 Cl-PFESA (63.0%), and PFOS (50.0%). PFOA had the highest median concentration in breast milk (1.08 ng/mL). The detection rate and concentration of 6:2 Cl-PFESA in breast milk were significantly lower than those observed in maternal and cord serum (Figure 1, Table S5).

Figure 1. Box-plots of concentrations of PFAS with > 50% detection in maternal serum across trimesters (T1-T3), cord serum or breast milk (ng/mL). The lower and upper edges of the box represent the first and third quartiles, respectively, while the line inside the box denotes the median level. The whiskers mark the 5th and 95th percentiles.

There were significant positive correlations between the concentration of PFAS in serum and breast milk samples at each time point ($p < 0.05$) (Figure S2). A detailed correlation analysis between the concentrations of various PFAS in the three matrices is shown in the Supporting Information (Figure S2).

The ICCs and 95% CIs for the concentrations of PFAS in serum during different trimesters are listed in Table S6. The ICC values ranged from 0.07 to 0.83, indicating the reproducibility of the PFAS measurements. PFHxS (ICC = 0.79) and 6:2 Cl-PFESA (ICC = 0.83) had high reproducibility across the three follow-up visits. PFOA and PFOS showed moderate reproducibility with ICC values of 0.70 and 0.81, respectively. This suggests that the measurements of these PFAS were relatively consistent but not as consistent as those of PFHxS and 6:2 Cl-PFESA. On the other hand, PFTrA had a low ICC, indicating poor reproducibility. This suggests that the measurements of PFTrA varied significantly across the three follow-up visits.

3.3 Placental and breastfeeding transfer efficiency of PFAS

We calculated the C:T3 ratio of each substance to evaluate the placental and breastfeeding transfer efficiency of each substance. As shown in Figure 2a, the median C:T3 ratios of PFOSA, PFDoA, and PFTrA were far greater than 1.00 (1.40–2.00), and PFBA was close to 1.00. This indicates that these substances have a higher transfer efficiency and can easily cross the placental barrier, leading to their

enrichment in cord blood and fetal exposure. The median C:T3 ratio of other substances was < 1.00, indicating that the placental barrier could partially block its transfer from mother to fetus. It was observed that perfluoroalkyl carboxylates (PFCA) were more easily transferred through the placental barrier than perfluoroalkane sulfonates (PFSA) under the same chain length. For instance, PFOA demonstrated a median C:T3 ratio of 0.75, which was two fold greater than that of PFOS. A U-shaped pattern in placental transfer efficiency was observed as the molecular chain length increased for both carboxylates and sulfonates. As the chain length increased, the transfer efficiency initially decreased, then reached the lowest point and finally increased. PFUnA has the lowest transfer efficiency. These findings offer insights into the transfer efficiency of different PFAS pass through the placenta and their potential for fetal exposure.

As shown in Figure 2b, compared to the median C:T3 ratio, most PFAS had lower M:T3 values, ranging from 0.03 to 3.22. However, the median PFBA of M:T3 was 3.31, which was significantly higher than the C:T3 value. The breastfeeding transfer of carboxylic acids showed an obvious U-shaped trend with increasing chain length. The sulfonic acid decreased with increasing chain length: PFHxS (0.26) > PFOS (0.07) > 6:2 Cl-PFESA (0.04). A comparison of the two routes of transfer showed that PFAS more readily crossed the placenta into the fetal side. Moreover, the efficiencies of breastfeeding transfer surpassed those reported by Zheng et al[21,33]. As far as we are aware, this study represents the first report on the breastfeeding transfer efficiency of full-chain PFCA ranging from C4 to C13. PFBA (C4) is the PFAS with the shortest carbon chain reported in transfer efficiency studies[33–35].

Figure 2. Distributions of (a) C:T3 and (b) M:T3. The lower and upper edges of the box represent the first and third quartiles, respectively, while the line inside denotes the median level. The whiskers mark the 10th and 90th percentiles. The C:T3 represents the efficiency of placenta transfer and the M:T3 represents the efficiency of breastfeeding transfer.

3.4 The binding affinities of PFAS to proteins

The proteins in maternal serum and the placenta can be an important factor affecting their distribution and transport. The binding affinities of PFAS to five transport proteins, namely, HSA, L-FABP, OAT-4, P-gp, and MRP2, are listed in Table S7. The distinct target PFAS exhibited variable binding affinities with the same transporter, while the binding affinity of the same PFAS to different transporters also differed. This heterogeneity can be explained by the structural dissimilarities between PFAS homologs and transporters.

Overall, all target PFAS demonstrated a strong affinity to five transporters, whereas HSA had the strongest binding affinity toward the target PFAS. In the case of PFCA and PFSA, binding affinities to HSA escalated with elongating chain lengths. Likewise, the binding affinities of PFSA and Cl-PFESAs to L-FABP and OAT4 demonstrated an ascent alongside chain length augmentation. However, except for that of PFDoA, the binding affinities of PFCA to L-FABP were observed to increase with longer chain lengths, highlighting PFUnA as the pivotal point (or the turning point) for the binding affinity shift. Apart from PFOA, PFUnA, and PFDoA, the binding affinity of the remaining PFCA with OAT4 rose with increasing chain length, still showing an upward trend. Excluding that of PFDoA, the binding affinities of PFCA to P-gp and MRP2 exhibited an ascending pattern with increasing chain length, which underlined PFUnA as the turning point in their affinity trend. The binding affinities of Cl-PFESAs to P-gp and MRP2 were found to rise with longer chain lengths. However, PFOS exhibited a lower binding affinity to P-gp compared to PFHxS. The binding affinity of PFSA to MRP2 still increased as the chain length increased. The results of molecular docking indicated that the binding affinity of the PFAS homolog to five transporters was closely linked to chain length.

The visualization of the results is depicted in Figure 3 using Discovery Studio to further explore the intermolecular forces between PFUnA and five transporters. Hydrogen bonds and halogen (fluorine) bonds formed between PFUnA and the amino acid residues of the five transporters, accounting for more than 70.0% of all intermolecular forces formed. The binding energy of PFUnA to five transporters was < -8.30 kcal/mol, and the affinity between L-FABP and PFUnA was the strongest.

PFUnA was completely encapsulated in the L-FABP cavity (Figure S3).

Figure 3. The two-dimensional docking conformation of PFUnA in the substrate binding pocket of (a) HSA, (b) L-FABP, (c) MRP2, (d) OAT4, and (e) P-gp model

3.5 Risk assessment of PFAS exposure in breastfeeding infants

The study assessed the health risks of infants by calculating EDIs for PFAS ingested via breast milk, comparing them with RfDs, and calculating HQs. Table 2 summarizes the EDIs of PFAS ingested by infants of different age groups through breast milk. The median EDIs of ΣPFAS for infants in different age groups, namely, less than 1, 1−3, 3−6, and 6−12 months, were 313, 292, 230, and 173 ng/(kg bw·day), respectively. The EDIs of infants in different age groups decreased with increasing age. This trend can be attributed to the high standardized intake rate of weight in this particular age group. The change in EDIs is due to the increase in the EPA breast milk intake reference level and body weight with age. Meanwhile, the EDIs of different substances in the same age group were also different. Among all age groups, the maximum median EDI was PFOA [89.7−162 ng/(kg bw·day)], followed by PFBA [55.6−101 ng/(kg bw·day)], PFOS [9.96−18.0 ng/(kg bw·day)], and 6:2 Cl-PFESA [5.81−10.5 ng/(kg bw·day)]. The EDI of PFOA was higher than the United States EPA standard in 2016, which specified an RfD of 20.0 ng/(kg bw·day) for both PFOA and PFOS. Notably, the median EDI for infants less than one month old reached 18.0 ng/(kg bw·day), nearing the RfD (Table 2). The EDIs exceeding the RfD indicated that the exposure of infants to PFAS in Shanghai needed attention.

To assess the potential health risks of PFAS exposure to breastfeeding infants, EDIs were compared with TDI and TWI. The EDIs of PFOS and PFOA were far lower than the TDI of 1,500 and 150 ng/(kg·day) (Table 2), aligning consistently with prior research findings. The median HQs based on TDI for PFOA and PFOS for breastfeeding infants of different ages ranged from 0.06 to 0.11 and from 0.07 to 0.12, respectively. The risk caused by PFOS is higher than that caused by PFOA. The median HQs calculated with TWI for the sum of PFOA, PFOS, PFHxS, and PFNA for

different age groups of breastfeeding infants ranged from 166 to 301 (Table 3). When using the TWI for assessment, the exposure risk of breastfeeding infants was higher. These results indicated the risk of infant exposure to PFAS by ingesting breast milk in Shanghai.

Table 2. The median estimated daily intake [EDI, ng/(kg bw·day)] of PFAS by infants through breastfeeding and the tolerable exposure levels (TDI, TWI)

Analyte	age, months				TDI[a]	TWI[b]
	1	1–3	3–6	6–12		
PFBA	100	93.8	73.7	55.6	-	-
PFHxA	6.00	5.60	4.40	3.32	-	-
PFOA	162	151	119	89.6	1,500	-
PFNA	1.50	1.40	1.10	0.83	-	-
PFDA	0.75	0.70	0.55	0.42	-	-
PFUnA	0.75	0.70	0.55	0.42	-	-
PFDoA	3.00	2.80	2.20	1.66	-	-
PFTrA	2.25	2.10	1.65	1.25	-	-
PFHxS	7.50	7.00	5.50	4.15	-	-
PFOS	18.0	16.8	13.2	9.96	150	-
6:2 Cl-PFESA	10.5	9.80	7.70	5.81	-	-
8:2 Cl-PFESA	0.38	0.35	0.28	0.21	-	-
∑(PFOA, PFNA, PFHxS, PFOS)	189	176	139	105	-	4.40
ΣPFAS	313	292	230	173	-	-

[a] the 2008-proposed daily tolerable intake [ng/(kg·day)] by the EFSA;

[b] the 2020-proposed tolerable weekly intake [ng/(kg·wk)] by the EFSA.

Table 3. Calculated hazard quotients (HQs) for PFAS intake by infants through breastfeeding.

	HQ		
	PFOA	PFOS	∑(PFOA, PFNA, PFHxS, PFOS)
< 1 month	0.11	0.12	301
1–3 months	0.10	0.11	281
3–6 months	0.08	0.09	221
6–12 months	0.06	0.07	166

4 Discussion

In this study, PFAS were detectable in most serum and colostrum samples of mother-child pairs in Shanghai, China. In addition to the widely used PFOA and PFOS, we observed a high level of 6:2 Cl-PFESA in our samples. The concentration of PFAS showed a dynamic change during pregnancy. ICC values showed that PFUnA, PFTrA, and 8:2 Cl-PFESA had large time variability, suggesting that multipoint measurements should be performed to comprehensively assess the exposure levels of PFAS during pregnancy and avoid a misleading assessment of intrauterine fetal exposure. Pan et al.[20] observed a gradual decrease in the median concentration of PFAS in pregnancy serum and that the concentrations of various substances in each pregnancy were highly correlated, which was not entirely consistent with this study. The discrepancy could be attributed to differences in sample size, sampling time, dietary pattern, and region of the study population[36,37]. The sample size of this study population is relatively larger, with a total of 1,076 participants recruited from April 2016 to May 2018. Moreover, Tian et al.[38] found that the primary source of PFAS for adults in Shanghai was diet, which would affect the concentration of PFAS in serum. The high levels of PFOA and PFOS in animal-derived foods were reported in Shanghai, and the PFOA levels of aquatic products in Shanghai were apparently higher than those in other cities[39]. A Shanghai birth cohort study[38] found that higher maternal age at delivery, increased levels of education, and multiparity were associated with higher PFAS levels. Women with higher levels of education may purchase more consumer goods containing PFAS, such as seafood products and sports equipment[40,41]. Cariou et al[42] found that freshwater fish consumption was a dietary predictor of PFNA level in maternal serum during the third trimester in France. The dietary pattern of consuming fatty fish was observed in both Europe[43] and Shanghai[41]. Considering the accumulation and long half-life of PFAS, their levels in the third trimester of this study are higher than those in the second trimester, which mainly depends on the diet and consumption patterns of the study population during pregnancy.

Currently, PFAS with high detection rates in domestic and foreign studies

include mainly PFOA, PFNA, PFDA, PFHxS, and PFOS[16,44–63] (Figure S4), with relevant references available for further details. The concentrations of PFOA in both serum and breast milk samples were found to be higher in the studied population compared to populations in other countries or regions. Conversely, when compared to investigations carried out in Seoul, Warsaw, Denmark, Avon, and Ohio, the PFOS level in maternal serum demonstrated notably reduced levels. Moreover, PFOS exposure levels in cord serum were significantly lower than those in Denmark, Russia, and Korea and below reported levels in other domestic regions, such as Wuhan and Guangzhou. Nonetheless, the PFOS exposure level in breast milk surpassed the levels observed in Beijing, Jiangsu, and Hangzhou. There are currently fewer studies of F-53B in breast milk (Figure S5), with relevant references available for further details. The concentration of 8:2 Cl-PFESA in this population was relatively low. However, it is important to highlight that the level of 6:2 Cl-PFESA in maternal serum was notably higher in our study compared to other regions, except for Tianjin and Nanjing. Similarly, the level of 6:2 Cl-PFESA in cord serum was also higher than that observed in other regions in China.

In most studies, maternal blood samples were collected from pregnant women before delivery[21,63]. However, the third-trimester serum was used to calculate the transfer efficiency in the current study based on the following two considerations: Firstly, with the average gestational week at 39.3 weeks, the third-trimester serum samples we used were collected at 30−34 weeks, which is relatively close to childbirth. Secondly, using third-trimester serum for calculating placental transfer efficiency may provide an accurate reflection of fetal exposure, as the fetus is more likely to have been exposed to PFAS from the mother during this stage, potentially offering a representation of prenatal exposure. Additionally, we need to consider the uncertainty associated with using serum from this period to calculate transfer efficiency. Our results showed that transfer efficiency is influenced by both perfluorocarbon chain length[20,63–65] and functional groups[42,66,67]. Most studies[20,62,65] have demonstrated a U-shaped relationship between the C:T3 of PFAS and the fluorinated alkyl chain length, consistent with the findings of our study. This may be

affected by the different binding affinity between PFAS and proteins such as HSA and L-FABP. However, the decreasing and irregular[68] trend of PFAS transfer efficiency with the increase of carbon chain length has been previously reported in countries such as South Korea[11] and South Africa[66]. When analyzing functional groups, comparisons between PFSA and PFCA with identical fluorinated chain lengths reveal higher C:T3 for PFCA, which is consistent with previous findings[62]. Due to the limited compounds analyzed, the relationship between C:T3 and other functional groups and isomers of PFAS and its potential mechanism has not been clarified. Previous evidence[66,69,70] has suggested that most branched isomers have higher C:T3 ratios compared to linear isomers. The distinctive structure of F-53B, characterized by features like ester bonds and chlorine atoms, may promote placental metastasis[20]. Therefore, F-53B might not necessarily make them "safer" than PFOS in terms of transplacental transmissibility.

The binding affinities of PFAS to transport proteins could play a crucial part in the transplacental transfer of PFAS. Cao et al.[71] reported that the substitute of PFAS, 6:2 Cl-PFESA, may have a higher affinity for endogenous proteins. Recently, the biological process of PFAS transfer was studied on protein binding by using laboratory experiments and computational models (including molecular docking, molecular dynamics simulation, and QSAR modeling[71]) to calculate the binding constants of different PFAS. The presence of transporters on placental syncytiotrophoblasts adds a layer of complexity to the transfer of maternal-fetal ectopic substances. Molecular docking calculations reveal that the main driving forces are halogen and hydrogen bond interactions, with the binding geometry being contingent upon the size and strength of these interactions[23]. In alignment with earlier research, our study similarly found that PFAS have greater binding affinities to HSA than to OAT4, possibly attributed to the distinct structure of proteins. In this study, the placental transfer efficiency of PFCA decreased first and then increased, in which PFUnA was the lowest point. On the contrary, the affinity of PFCA (ranging from C4 to C13) to L-FABP and P-gp increased first and then decreased, and PFUnA was the turning point. This may be due to P-gp functioning as a pump, facilitating the transfer

of PFAS from the placenta to the maternal bloodstream[22]. The affinity of PFSA to protein is greater than that of PFCA, under the same carbon chain length. These findings support that PFAS with varying functional groups and chain lengths may exhibit different binding affinity to transporters. It is worth mentioning that those longer chains, such as PFUnA, may have more conformations and lower global molecular energy. This may bring uncertainty to the docking results. Nevertheless, the binding mode of PFAS to proteins can explain its distribution in the body and its potential toxicity to organisms.

Breast milk serves as the primary source of nutrition for the majority of newborns under six months old. The EDIs of PFAS in breastfeeding infants surpass those reported for adult dietary intake [0.58 ng/(kg bw·day)][72] by over one order of magnitude, underscoring breastfeeding as a significant exposure route for infants. The highest EDIs were identified in infants aged less than one month old. This implies a potential heightened susceptibility to adverse health outcomes linked to PFAS exposure within this specific age range. In this study, the EDIs of PFOA and PFDoA were relatively high, but the EDIs of PFNA, PFDA, PFUnA, PFOS, and 6:2 Cl-PFESA were relatively lower than those in Zheng's study[21]. The discrepancies in EDIs observed across various studies may be attributable to variations in exposure levels resulting from diverse sources of exposure in different regions, consumption patterns, individual metabolic differences, and the inconsistency of EDI estimation methods. In addition, with the growth of infants, complementary foods may also be an additional source of exposure. Given that infants are more vulnerable to external chemicals than adults, it is necessary to enhance monitoring efforts concerning PFAS exposure and health hazards, especially the effects on lactating infants.

5. Conclusion

Based on the Shanghai Maternal-Child Pairs Cohort, the exposure levels of legacy and emerging PFAS in maternal serum, cord serum, and breast milk were monitored in paired samples to comprehensively assess the exposure levels and risks of PFAS in early life. PFAS were detectable in most of the serum and colostrum

samples in mother-child pairs, with the highest level of PFOS in maternal serum and the highest level of PFOA detected in cord serum and breast milk. The placental and breastfeeding transfer efficiencies of PFAS are influenced by carbon chain length. Infants can be exposed to PFAS through breastfeeding, particularly increasing the health risks of PFOS and PFOA, which necessitates further attention. Furthermore, the study investigated the binding of PFAS to transporters to explore the mechanism of placental transport using molecular docking. However, placental transporters may play a role in the transport process of PFAS, and further experimental studies are necessary to elucidate their specific mechanisms in the metabolism and transfer of PFAS.

CRediT authorship contribution statement

Y.Q.X.: writing–original draft, visualization, formal analysis, methodology, software, data curation; X.Y.S., J.H.L., L.Y.Z.: methodology, visualization, investigation, software; P.P.W., Y.L.: investigation, resources; H.J.S.: methodology, supervision; Y.H.Z.: methodology, conceptualization, data Curation, resources, project administration, supervision, funding acquisition.

Declaration of competing interest

The authors declare no competing interests.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Grant No. 82273585) and the National Key Research and Development Program of China (Grant No. 2022YFC2705004, 2019YFE0114500). We are very grateful for the contributions of all investigators and quality controllers who participated in the Shanghai MCPC study.

Reference:

1. Hammel, E., Webster, T.F., Gurney, R., and Heiger-Bernays, W. (2022). Implications of PFAS

559 definitions using fluorinated pharmaceuticals. iScience *25*, 104020. 10.1016/j.isci.2022.104020.
560 2. Domingo, J.L. (2012). Health risks of dietary exposure to perfluorinated compounds. Environ
561 Int *40*, 187–195. 10.1016/j.envint.2011.08.001.
562 3. Ritscher, A., Wang, Z., Scheringer, M., Boucher, J.M., Ahrens, L., Berger, U., et al. (2018).
563 Zürich Statement on Future Actions on Per- and Polyfluoroalkyl Substances (PFASs). Environ
564 Health Perspect *126*, 84502. 10.1289/EHP4158.
565 4. Sajid, M., and Ilyas, M. (2017). PTFE-coated non-stick cookware and toxicity concerns: a
566 perspective. Environ Sci Pollut Res Int *24*, 23436–23440. 10.1007/s11356-017-0095-y.
567 5. He, Y., Lv, D., Li, C., Liu, X., Liu, W., and Han, W. (2022). Human exposure to F-53B in
568 China and the evaluation of its potential toxicity: An overview. Environ Int *161*, 107108.
569 10.1016/j.envint.2022.107108.
570 6. Nyberg, E., Awad, R., Bignert, A., Ek, C., Sallsten, G., and Benskin, J.P. (2018). Inter-
571 individual, inter-city, and temporal trends of per- and polyfluoroalkyl substances in human milk
572 from Swedish mothers between 1972 and 2016. Environ Sci Process Impacts *20*, 1136–1147.
573 10.1039/c8em00174j.
574 7. Zhao, J., Hinton, P., Chen, J., and Jiang, J. (2020). Causal inference for the effect of
575 environmental chemicals on chronic kidney disease. Comput Struct Biotechnol J *18*, 93–99.
576 10.1016/j.csbj.2019.12.001.
577 8. Wang, Z., DeWitt, J.C., Higgins, C.P., and Cousins, I.T. (2017). A Never-Ending Story of Per-
578 and Polyfluoroalkyl Substances (PFASs)? Environ Sci Technol *51*, 2508–2518.
579 10.1021/acs.est.6b04806.
580 9. Convention, S. UN experts recommend elimination of additional hazardous chemicals to
581 protect human health and the environment. BRSMeas.
582 https://chm.pops.int/Implementation/PublicAwareness/PressReleases/POPRC15expertsrecommen
583 deliminationofPFHxS/tabid/8153/Default.aspx.
584 10. Grippo, A., Zhu, K., Yeung, E.H., Bell, E.M., Bonner, M.R., Tian, L., et al. (2023). Indoor air
585 pollution exposure and early childhood development in the Upstate KIDS Study. Environ Res *234*,
586 116528. 10.1016/j.envres.2023.116528.
587 11. Lee, Y.J., Kim, M.-K., Bae, J., and Yang, J.-H. (2013). Concentrations of perfluoroalkyl
588 compounds in maternal and umbilical cord sera and birth outcomes in Korea. Chemosphere *90*,
589 1603–1609. 10.1016/j.chemosphere.2012.08.035.
590 12. Manzano-Salgado, C.B., Casas, M., Lopez-Espinosa, M.-J., Ballester, F., Basterrechea, M.,
591 Grimalt, J.O., et al. (2015). Transfer of perfluoroalkyl substances from mother to fetus in a
592 Spanish birth cohort. Environ Res *142*, 471–478. 10.1016/j.envres.2015.07.020.
593 13. Kim, S., Choi, K., Ji, K., Seo, J., Kho, Y., Park, J., et al. (2011). Trans-placental transfer of
594 thirteen perfluorinated compounds and relations with fetal thyroid hormones. Environ Sci Technol
595 *45*, 7465–7472. 10.1021/es202408a.
596 14. Fei, C., McLaughlin, J.K., Tarone, R.E., and Olsen, J. (2007). Perfluorinated chemicals and
597 fetal growth: a study within the Danish National Birth Cohort. Environ Health Perspect *115*,
598 1677–1682. 10.1289/ehp.10506.
599 15. Perfluorinated Alkyl Acids in Blood Serum from Primiparous Women in Sweden: Serial
600 Sampling during Pregnancy and Nursing, And Temporal Trends 1996–2010 | Environmental
601 Science & Technology https://pubs.acs.org/doi/10.1021/es301168c.
602 16. Kato, K., Wong, L.-Y., Chen, A., Dunbar, C., Webster, G.M., Lanphear, B.P., et al. (2014).

Changes in serum concentrations of maternal poly- and perfluoroalkyl substances over the course of pregnancy and predictors of exposure in a multiethnic cohort of Cincinnati, Ohio pregnant women during 2003-2006. Environ Sci Technol *48*, 9600–9608. 10.1021/es501811k.

17. Ouzounian, J.G., and Elkayam, U. (2012). Physiologic changes during normal pregnancy and delivery. Cardiol Clin *30*, 317–329. 10.1016/j.ccl.2012.05.004.

18. Borg, D., Bogdanska, J., Sundström, M., Nobel, S., Håkansson, H., Bergman, Å., et al. (2010). Tissue distribution of (35)S-labelled perfluorooctane sulfonate (PFOS) in C57Bl/6 mice following late gestational exposure. Reprod Toxicol *30*, 558–565. 10.1016/j.reprotox.2010.07.004.

19. Kosarac, I., Kubwabo, C., and Foster, W.G. (2016). Quantitative determination of nine urinary metabolites of organophosphate flame retardants using solid phase extraction and ultra performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS). J Chromatogr B Analyt Technol Biomed Life Sci *1014*, 24–30. 10.1016/j.jchromb.2016.01.035.

20. Pan, Y., Zhu, Y., Zheng, T., Cui, Q., Buka, S.L., Zhang, B., et al. (2017). Novel Chlorinated Polyfluorinated Ether Sulfonates and Legacy Per-/Polyfluoroalkyl Substances: Placental Transfer and Relationship with Serum Albumin and Glomerular Filtration Rate. Environ Sci Technol *51*, 634–644. 10.1021/acs.est.6b04590.

21. Zheng, P., Liu, Y., An, Q., Yang, X., Yin, S., Ma, L.Q., et al. (2022). Prenatal and postnatal exposure to emerging and legacy per-/polyfluoroalkyl substances: Levels and transfer in maternal serum, cord serum, and breast milk. Sci Total Environ *812*, 152446. 10.1016/j.scitotenv.2021.152446.

22. Li, J., Ma, D., Qian, C., Guo, B., Guan, R., Liu, C., et al. (2024). Assessment of Fetal Exposure and Elimination of Perfluoroalkyl and Polyfluoroalkyl Substances: New Evidence from Paired Serum, Placenta, and Meconium Samples. Environ Sci Technol *58*, 2260–2270. 10.1021/acs.est.3c08498.

23. Zhao, L., Teng, M., Zhao, X., Li, Y., Sun, J., Zhao, W., et al. (2023). Insight into the binding model of per- and polyfluoroalkyl substances to proteins and membranes. Environment International *175*, 107951. 10.1016/j.envint.2023.107951.

24. Fedorenko, M., Alesio, J., Fedorenko, A., Slitt, A., and Bothun, G.D. (2021). Dominant entropic binding of perfluoroalkyl substances (PFASs) to albumin protein revealed by 19F NMR. Chemosphere *263*, 128083. 10.1016/j.chemosphere.2020.128083.

25. Maso, L., Trande, M., Liberi, S., Moro, G., Daems, E., Linciano, S., et al. (2021). Unveiling the binding mode of perfluorooctanoic acid to human serum albumin. Protein Sci *30*, 830–841. 10.1002/pro.4036.

26. Zhang, L., Ren, X.-M., and Guo, L.-H. (2013). Structure-Based Investigation on the Interaction of Perfluorinated Compounds with Human Liver Fatty Acid Binding Protein. Environ. Sci. Technol. *47*, 11293–11301. 10.1021/es4026722.

27. Kummu, M., Sieppi, E., Koponen, J., Laatio, L., Vähäkangas, K., Kiviranta, H., et al. (2015). Organic anion transporter 4 (OAT 4) modifies placental transfer of perfluorinated alkyl acids PFOS and PFOA in human placental ex vivo perfusion system. Placenta *36*, 1185–1191. 10.1016/j.placenta.2015.07.119.

28. Walker, N., Filis, P., Soffientini, U., Bellingham, M., O'Shaughnessy, P.J., and Fowler, P.A. (2017). Placental transporter localization and expression in the Human: the importance of species, sex, and gestational age differences†. Biology of Reproduction *96*, 733–742. 10.1093/biolre/iox012.

29. Ma, D., Lu, Y., Liang, Y., Ruan, T., Li, J., Zhao, C., et al. (2022). A Critical Review on Transplacental Transfer of Per- and Polyfluoroalkyl Substances: Prenatal Exposure Levels, Characteristics, and Mechanisms. Environ. Sci. Technol. *56*, 6014–6026. 10.1021/acs.est.1c01057.
30. Zhao, Y., Wang, P., Zhou, Y., Xia, B., Zhu, Q., Ge, W., et al. (2021). Prenatal fine particulate matter exposure, placental DNA methylation changes, and fetal growth. Environ Int *147*, 106313. 10.1016/j.envint.2020.106313.
31. Li, J., Li, Q., Li, J., and Zhang, Y. (2022). Simultaneous determination of poly- and perfluoroalkyl substances and organophosphorus flame retardants in serum by ultra-performance liquid chromatography/tandem mass spectrometry. Rapid Commun Mass Spectrom *36*, e9312. 10.1002/rcm.9312.
32. Zhu, H., and Kannan, K. (2019). Occurrence of Melamine and Its Derivatives in Breast Milk from the United States and Its Implications for Exposure in Infants. Environ. Sci. Technol. *53*, 7859–7865. 10.1021/acs.est.9b02040.
33. Zheng, G., Schreder, E., Dempsey, J.C., Uding, N., Chu, V., Andres, G., et al. (2021). Per- and Polyfluoroalkyl Substances (PFAS) in Breast Milk: Concerning Trends for Current-Use PFAS. Environ Sci Technol *55*, 7510–7520. 10.1021/acs.est.0c06978.
34. Liu, L., Yan, P., Liu, X., Zhao, J., Tian, M., Huang, Q., et al. (2024). Profiles and transplacental transfer of per- and polyfluoroalkyl substances in maternal and umbilical cord blood: A birth cohort study in Zhoushan, Zhejiang Province, China. Journal of Hazardous Materials *466*, 133501. 10.1016/j.jhazmat.2024.133501.
35. Huang, S., Li, X., Deng, L., Xie, J., Huang, G., Zeng, C., et al. (2023). Exposure to per- and polyfluoroalkyl substances in women with twin pregnancies: Patterns and variability, transplacental transfer, and predictors. Journal of Hazardous Materials *460*, 132432. 10.1016/j.jhazmat.2023.132432.
36. Ji, D., Pan, Y., Qiu, X., Gong, J., Li, X., Niu, C., et al. (2023). Unveiling Distribution of Per- and Polyfluoroalkyl Substances in Matched Placenta-Serum Tetrads: Novel Implications for Birth Outcome Mediated by Placental Vascular Disruption. Environ Sci Technol *57*, 5782–5793. 10.1021/acs.est.2c09184.
37. Wang, J., Pan, Y., Wei, X., and Dai, J. (2020). Temporal Trends in Prenatal Exposure (1998-2018) to Emerging and Legacy Per- and Polyfluoroalkyl Substances (PFASs) in Cord Plasma from the Beijing Cord Blood Bank, China. Environ Sci Technol *54*, 12850–12859. 10.1021/acs.est.0c01877.
38. Huo, X., Liang, W., Tang, W., Ao, Y., Tian, Y., Zhang, Q., et al. (2023). Dietary and maternal sociodemographic determinants of perfluoroalkyl and polyfluoroalkyl substance levels in pregnant women. Chemosphere *332*, 138863. 10.1016/j.chemosphere.2023.138863.
39. Gu, Q., Wen, Y., Wu, H., and Cui, X. (2023). Uptake and translocation of both legacy and emerging per- and polyfluorinated alkyl substances in hydroponic vegetables. Sci Total Environ *862*, 160684. 10.1016/j.scitotenv.2022.160684.
40. Brantsæter, A.L., Whitworth, K.W., Ydersbond, T.A., Haug, L.S., Haugen, M., Knutsen, H.K., et al. (2013). Determinants of plasma concentrations of perfluoroalkyl substances in pregnant Norwegian women. Environ Int *54*, 74–84. 10.1016/j.envint.2012.12.014.
41. Tian, Y., Zhou, Y., Miao, M., Wang, Z., Yuan, W., Liu, X., et al. (2018). Determinants of plasma concentrations of perfluoroalkyl and polyfluoroalkyl substances in pregnant women from a birth cohort in Shanghai, China. Environ Int *119*, 165–173. 10.1016/j.envint.2018.06.015.

42. Cariou, R., Veyrand, B., Yamada, A., Berrebi, A., Zalko, D., Durand, S., et al. (2015). Perfluoroalkyl acid (PFAA) levels and profiles in breast milk, maternal and cord serum of French women and their newborns. Environ Int *84*, 71–81. 10.1016/j.envint.2015.07.014.

43. Papadopoulou, E., Haug, L.S., Sakhi, A.K., Andrusaityte, S., Basagaña, X., Brantsaeter, A.L., et al. (2019). Diet as a Source of Exposure to Environmental Contaminants for Pregnant Women and Children from Six European Countries. Environ Health Perspect *127*, 107005. 10.1289/EHP5324.

44. Leter, G., Consales, C., Eleuteri, P., Uccelli, R., Specht, I.O., Toft, G., et al. (2014). Exposure to perfluoroalkyl substances and sperm DNA global methylation in Arctic and European populations. Environ Mol Mutagen *55*, 591–600. 10.1002/em.21874.

45. Specht, I.O., Hougaard, K.S., Spanò, M., Bizzaro, D., Manicardi, G.C., Lindh, C.H., et al. (2012). Sperm DNA integrity in relation to exposure to environmental perfluoroalkyl substances - a study of spouses of pregnant women in three geographical regions. Reprod Toxicol *33*, 577–583. 10.1016/j.reprotox.2012.02.008.

46. Timmermann, C.A.G., Andersen, M.S., Budtz-Jørgensen, E., Boye, H., Nielsen, F., Jensen, R.C., et al. (2022). Pregnancy Exposure to Perfluoroalkyl Substances and Associations With Prolactin Concentrations and Breastfeeding in the Odense Child Cohort. J Clin Endocrinol Metab *107*, e631–e642. 2022011805253747200.

47. Valvi, D., Højlund, K., Coull, B.A., Nielsen, F., Weihe, P., and Grandjean, P. (2021). Life-course Exposure to Perfluoroalkyl Substances in Relation to Markers of Glucose Homeostasis in Early Adulthood. J Clin Endocrinol Metab *106*, 2495–2504. 2021122204331533100.

48. McGlinchey, A., Sinioja, T., Lamichhane, S., Sen, P., Bodin, J., Siljander, H., et al. (2020). Prenatal exposure to perfluoroalkyl substances modulates neonatal serum phospholipids, increasing risk of type 1 diabetes. Environ Int *143*, 105935. 10.1016/j.envint.2020.105935.

49. Seo, S.-H., Son, M.-H., Choi, S.-D., Lee, D.-H., and Chang, Y.-S. (2018). Influence of exposure to perfluoroalkyl substances (PFASs) on the Korean general population: 10-year trend and health effects. Environ Int *113*, 149–161. 10.1016/j.envint.2018.01.025.

50. Caron-Beaudoin, É., Ayotte, P., Laouan Sidi, E.A., Community of Lac Simon, Community of Winneway – Long Point First Nation, CSSS Tshukuminu Kanani of Nutashkuan, et al. (2019). Exposure to perfluoroalkyl substances (PFAS) and associations with thyroid parameters in First Nation children and youth from Quebec. Environ Int *128*, 13–23. 10.1016/j.envint.2019.04.029.

51. Eick, S.M., Hom Thepaksorn, E.K., Izano, M.A., Cushing, L.J., Wang, Y., Smith, S.C., et al. (2020). Associations between prenatal maternal exposure to per- and polyfluoroalkyl substances (PFAS) and polybrominated diphenyl ethers (PBDEs) and birth outcomes among pregnant women in San Francisco. Environ Health *19*, 100. 10.1186/s12940-020-00654-2.

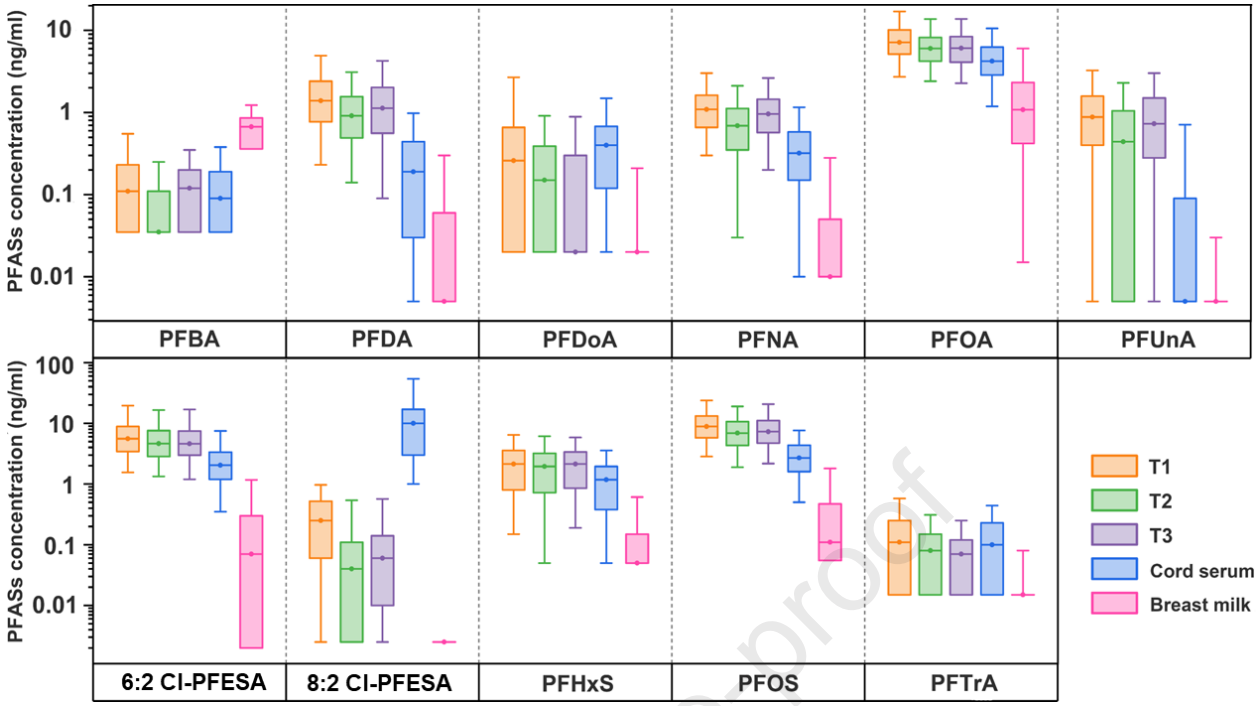
52. Vuong, A.M., Yolton, K., Xie, C., Dietrich, K.N., Braun, J.M., Webster, G.M., et al. (2019). Prenatal and childhood exposure to poly- and perfluoroalkyl substances (PFAS) and cognitive development in children at age 8 years. Environ Res *172*, 242–248. 10.1016/j.envres.2019.02.025.

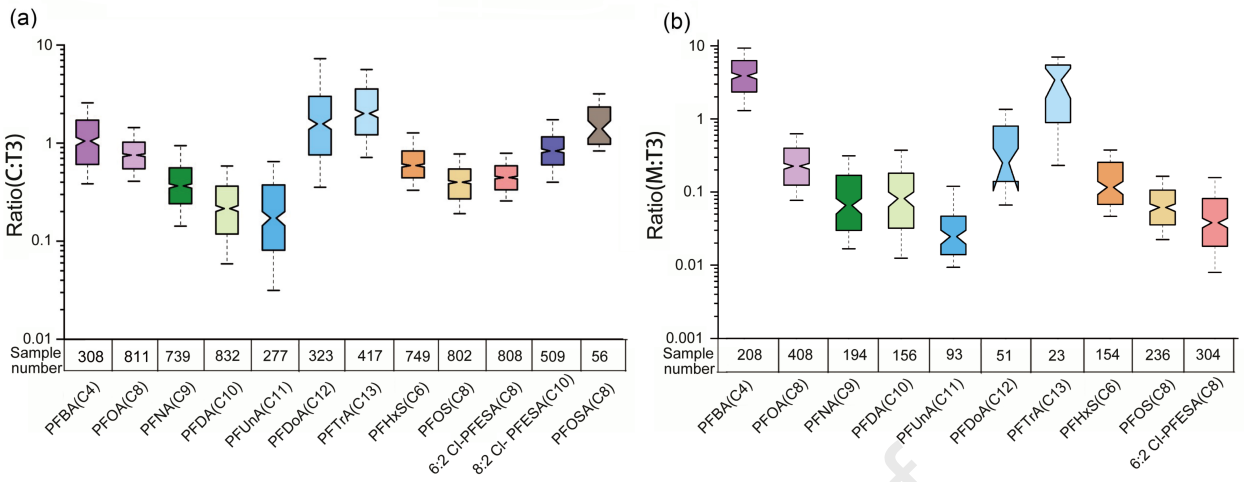
53. Prenatal and childhood exposure to perfluoroalkyl substances (PFAS) and measures of attention, impulse control, and visual spatial abilities - PubMed https://pubmed.ncbi.nlm.nih.gov/30015313/.

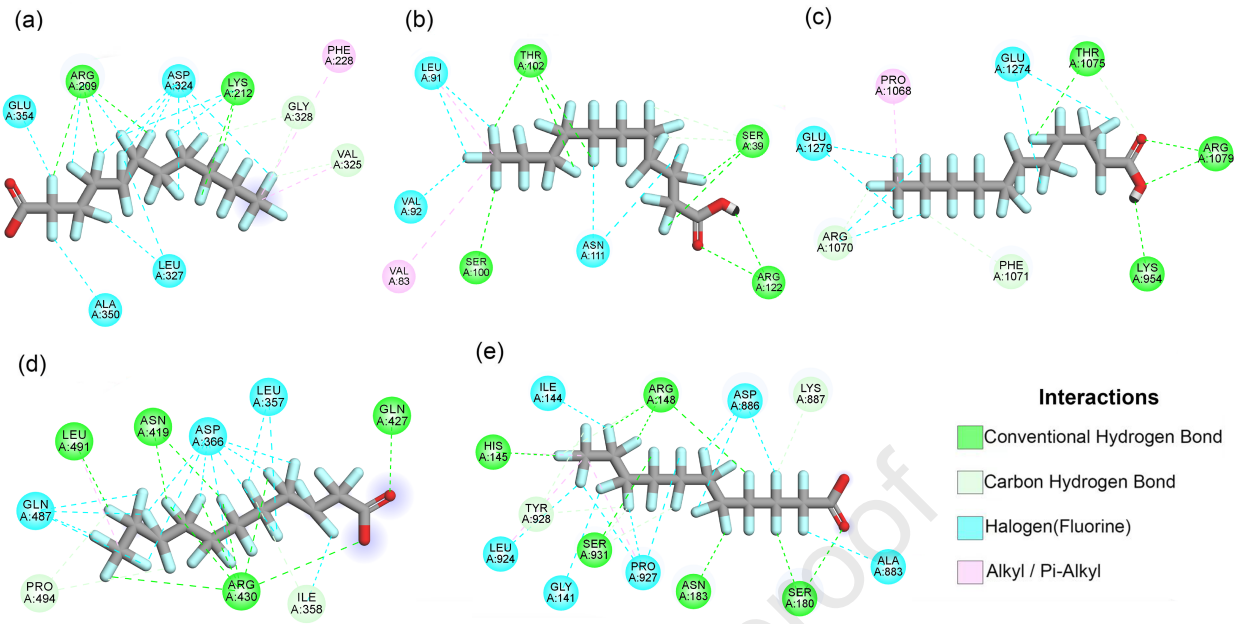
54. Vuong, A.M., Yolton, K., Xie, C., Dietrich, K.N., Braun, J.M., Webster, G.M., et al. (2021). Childhood exposure to per- and polyfluoroalkyl substances (PFAS) and neurobehavioral domains in children at age 8 years. Neurotoxicol Teratol *88*, 107022. 10.1016/j.ntt.2021.107022.

55. Prenatal Exposure to Perfluoroalkyl Substances: Infant Birth Weight and Early Life Growth - PubMed https://pubmed.ncbi.nlm.nih.gov/30272047/.
56. Prenatal exposure to per- and polyfluoroalkyl substances (PFAS) and neurobehavior in US children through 8 years of age: The HOME study - PubMed https://pubmed.ncbi.nlm.nih.gov/33545124/.
57. Buck, C.O., Eliot, M.N., Kelsey, K.T., Calafat, A.M., Chen, A., Ehrlich, S., et al. (2018). Prenatal exposure to perfluoroalkyl substances and adipocytokines: the HOME Study. Pediatr Res *84*, 854–860. 10.1038/s41390-018-0170-1.
58. Exposure to Perfluoroalkyl Substances in a Cohort of Women Firefighters and Office Workers in San Francisco - PubMed https://pubmed.ncbi.nlm.nih.gov/32100527/.
59. Chang, C.-J., Barr, D.B., Zhang, Q., Dunlop, A.L., Smarr, M.M., Kannan, K., et al. (2021). Associations of single and multiple per- and polyfluoroalkyl substance (PFAS) exposure with vitamin D biomarkers in African American women during pregnancy. Environ Res *202*, 111713. 10.1016/j.envres.2021.111713.
60. Exposure of Norwegian toddlers to perfluoroalkyl substances (PFAS): The association with breastfeeding and maternal PFAS concentrations - PubMed https://pubmed.ncbi.nlm.nih.gov/27453094/.
61. Richterová, D., Fábelová, L., Patayová, H., Pulkrabová, J., Lanková, D., Rausová, K., et al. (2018). Determinants of prenatal exposure to perfluoroalkyl substances in the Slovak birth cohort. Environ Int *121*, 1304–1310. 10.1016/j.envint.2018.10.051.
62. Hartman, T.J., Calafat, A.M., Holmes, A.K., Marcus, M., Northstone, K., Flanders, W.D., et al. (2017). Prenatal Exposure to Perfluoroalkyl Substances and Body Fatness in Girls. Child Obes *13*, 222–230. 10.1089/chi.2016.0126.
63. Gao, K., Zhuang, T., Liu, X., Fu, J., Zhang, J., Fu, J., et al. (2019). Prenatal Exposure to Per- and Polyfluoroalkyl Substances (PFASs) and Association between the Placental Transfer Efficiencies and Dissociation Constant of Serum Proteins-PFAS Complexes. Environ Sci Technol *53*, 6529–6538. 10.1021/acs.est.9b00715.
64. Zhang, T., Sun, H., Lin, Y., Qin, X., Zhang, Y., Geng, X., et al. (2013). Distribution of poly- and perfluoroalkyl substances in matched samples from pregnant women and carbon chain length related maternal transfer. Environ Sci Technol *47*, 7974–7981. 10.1021/es400937y.
65. Monroy, R., Morrison, K., Teo, K., Atkinson, S., Kubwabo, C., Stewart, B., et al. (2008). Serum levels of perfluoroalkyl compounds in human maternal and umbilical cord blood samples. Environ Res *108*, 56–62. 10.1016/j.envres.2008.06.001.
66. Hanssen, L., Röllin, H., Odland, J.Ø., Moe, M.K., and Sandanger, T.M. (2010). Perfluorinated compounds in maternal serum and cord blood from selected areas of South Africa: results of a pilot study. J Environ Monit *12*, 1355–1361. 10.1039/b924420d.
67. Radosavljevic, V., and Belojevic, G. (2009). A new model of bioterrorism risk assessment. Biosecur Bioterror *7*, 443–451. 10.1089/bsp.2009.0016.
68. Gützkow, K.B., Haug, L.S., Thomsen, C., Sabaredzovic, A., Becher, G., and Brunborg, G. (2012). Placental transfer of perfluorinated compounds is selective--a Norwegian Mother and Child sub-cohort study. Int J Hyg Environ Health *215*, 216–219. 10.1016/j.ijheh.2011.08.011.
69. Beesoon, S., Webster, G.M., Shoeib, M., Harner, T., Benskin, J.P., and Martin, J.W. (2011). Isomer profiles of perfluorochemicals in matched maternal, cord, and house dust samples: manufacturing sources and transplacental transfer. Environ Health Perspect *119*, 1659–1664.

779 10.1289/ehp.1003265.
780 70. Wu, Y., Bao, J., Liu, Y., Wang, X., and Qu, W. (2023). A Review on Per- and Polyfluoroalkyl
781 Substances in Pregnant Women: Maternal Exposure, Placental Transfer, and Relevant Model
782 Simulation. Toxics *11*, 430. 10.3390/toxics11050430.
783 71. Cao, H., Zhou, Z., Wang, L., Liu, G., Sun, Y., Wang, Y., et al. (2019). Screening of Potential
784 PFOS Alternatives To Decrease Liver Bioaccumulation: Experimental and Computational
785 Approaches. Environ. Sci. Technol. *53*, 2811–2819. 10.1021/acs.est.8b05564.
786 72. Poothong, S., Papadopoulou, E., Padilla-Sánchez, J.A., Thomsen, C., and Haug, L.S. (2020).
787 Multiple pathways of human exposure to poly- and perfluoroalkyl substances (PFASs): From
788 external exposure to human blood. Environment International *134*, 105244.
789 10.1016/j.envint.2019.105244.
790







Highlights:

• 6:2 Cl-PFESA exhibited a high detection rate and concentration in maternal serum, cord serum, and breast milk samples.

• PFAS were more easily transferred through the placenta than breastfeeding.

• With increasing carbon chain length, the placental and breastfeeding transfer efficiencies of PFAS showed a structure-dependent pattern.

• The EDIs decreased with breastfed infant age and the HQs of PFOS were higher than that of PFOA.