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Key Points:

- Per- and poly-fluoroalkyl substances and steroid hormones concentrations in human milk measured in an e-waste disassembly area
- PFOA was positively associated with DHEA and estrone concentrations in human milk
- PFOS was positively associated with A-dione in human milk

Supporting Information:

Supporting Information may be found in the online version of this article.

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Association Between Prenatal Exposure to Per- and Poly-Fluoroalkyl Substances From Electronic Waste Disassembly Areas and Steroid Hormones in Human Milk Samples

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Abstract Per- and poly-fluoroalkyl substances (PFAS), which are long-lasting environmental contaminants that are released into the environment during the e-waste disassembly process, pose a threat to human health. Human milk is a complex and dynamic mixture of endogenous and exogenous substances, including steroid hormones and PFAS. Therefore, in this study, we aimed to investigate the association between PFAS and steroid hormones in human milk from women living close to an e-waste disassembly area. In 2021, we collected milk samples from 150 mothers within 4 weeks of delivery and analyzed them via liquid chromatography-tandem mass spectrometry to determine the levels of 21 perfluorinated compounds and five steroid hormones (estrone, estriol, testosterone, progesterone, and androstenedione [A-dione]). We also performed multiple linear regression analysis to clarify the association between maternal PFAS exposure and steroid hormone concentrations. Our results indicated that PFOA and PFOS were positively associated with estrone (β, 0.23; 95% CI, 0.08–0.39) and A-dione (β, 0.186; 95% CI, 0.016–0.357) concentrations in human milk, respectively. Further, the average estimated daily intake of PFOA and PFOS were 36.5 ng/kg bw/day (range, 0.52–291.7 ng/kg bw/day) and 5.21 ng/kg bw/day (range, 0.26–32.3 ng/kg bw/day), respectively. Of concern, the PFAS intake of breastfeeding infants in the study area was higher than the recommended threshold. These findings suggested that prenatal exposure to PFAS from the e-waste disassembly process can influence steroid hormones levels in human milk. Increased efforts to mitigate mother and infant exposure to environmental pollutants are also required.

Plain Language Summary We recruited 150 mothers who provided human milk samples within 4 weeks of delivery. Using liquid chromatography-tandem mass spectrometry, we measured concentrations of 21 perfluorinated compounds and five steroid hormones (estrone, estriol, testosterone, progesterone, and androstenedione [A-dione]) in the human milk samples. Multiple linear regression analysis was performed to examine the association between maternal PFAS exposure and steroid hormone concentrations. Our findings revealed significant positive associations between specific PFAS compounds (PFOA and PFOS) and certain steroid hormone concentrations in human milk. Notably, PFOA showed positive associations with estrone concentrations, PFOS was associated with A-dione levels. These results suggest that prenatal exposure to PFAS from e-waste disassembly areas can impact the levels of steroid hormones in human milk.

1. Introduction

Per- and poly-fluoroalkyl substances (PFAS) constitute a class of man-made chemicals that are widely used in industrial and consumer applications, such as chemicals, textiles, paper and packaging, coatings, construction products, and healthcare products (S. Wang et al., 2013). Primarily, PFAS are disposed of in landfills, via incineration, or wastewater treatment (Stoiber et al., 2020). Even though the Stockholm Convention included perfluorinated compounds in the list of controlled chemicals after 2009, PFAS are still being detected in water, air, dust, and cord blood (Sunderland et al., 2019).

PFAS are widely used in the electronics and electrical industries, for example, in semiconductors, wires, cable components, and printed circuit boards (Gluge et al., 2020), and during the disassembly of e-waste containing

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PFAS, the PFAS may be released into air and dust (Tansel, 2022; Zhao et al., 2023), resulting in exposure to higher levels of PFAS for individuals living close to these e-waste disassembly areas. A previous study revealed that older adults living around e-waste disassembly areas have significantly higher serum PFAS levels than their counterparts living in non-electronic waste disassembly areas (Zhang et al., 2019). Additionally, the results of this previous study showed a negative association between fasting blood glucose levels and serum PFUnDA and PFTeDA levels in individuals living around e-waste disassembly areas. This observation suggested that exposure to PFAS in e-waste may potentially impact blood glucose regulation (Zhang et al., 2019). Another study involving mothers in Guiyu, China, revealed that maternal PFOA concentrations are negatively associated with gestational age, birth length, birth weight, and Apgar score, suggesting the existence of an association between prenatal PFOA exposure and poor neonatal outcomes (Wu et al., 2012).

Owing to their high persistence in the environment, PFAS, including PFOS and PFOA (Li et al., 2018), can remain in the body for an extended period, posing a threat to human health. Several studies have shown that PFAS exposure can cause reproductive toxicity, neurotoxicity, liver toxicity, and steroid hormone disorders in humans (Bonato et al., 2020; Johansson et al., 2008; Roth et al., 2021).

Further, PFAS can act as "endocrine disruptors," affecting various organ systems by disrupting hormonal balance, which in turn affects normal physiological functioning (DeWitt et al., 2009; Ivantsova et al., 2024; Ramskov Tetzlaff et al., 2021; Tsai et al., 2020; Vilhelmsson et al., 2023). For example, PFAS in the reproductive system can disrupt the metabolic processes of endogenous hormones as well as the secretion of steroid hormones, including estrone, estradiol, progesterone, and testosterone (Ding et al., 2020; Green et al., 2021; Rickard et al., 2022). Another study conducted in Hebei Province, China, showed positive correlations between cord serum PFOS levels and the levels of estrone and estriol, and a negative correlation between the PFOS levels and the level of estradiol. A positive correlation has also been observed between serum PFOA and estrone levels (H. Wang et al., 2019).

Human milk is a complex and constantly changing mixture of endogenous and exogenous substances, including steroid hormones and PFAS (Andreas et al., 2015; Y. X. Liu et al., 2021). As the primary source of nutrition for infants, human milk provides not only basic nutrients but also essential hormones, such as testosterone and insulin (Vass et al., 2023). While the association between PFAS and steroid hormones in maternal and cord blood has been examined in numerous previous studies, there are no previous reports on the relationship between PFAS and steroid hormones in human milk. Therefore, in this study, we aimed to investigate the association between PFAS and steroid hormones in human milk and explore the potential impact of maternal exposure to PFAS from an e-waste disassembly area in China on infant health. The findings of this study may provide valuable insights that can enhance understanding regarding how PFAS exposure affects infant hormonal balance through breastfeeding. Such understanding can inform healthcare professionals and policymakers with respect to decision-making to mitigate the potential risks associated with PFAS exposure and ensure the health of mothers and their nursing infants in e-waste removal areas and beyond.

2. Materials and Methods

2.1. Study Population

The study area for this study was Taizhou City, located on the southeastern coast of Zhejiang Province, China. From January to July 2021, 150 pregnant women were recruited from Taizhou Hospital, where they were admitted awaiting delivery or visited for routine prenatal care visits. We collected milk samples from these women within 4 weeks of delivery. The study protocol was approved by the Human Ethics Committee of Jiaxing University, and all the participants provided written informed consent after a detailed explanation of the study's objective and its potential implications prior to enrollment.

2.2. Questionnaire Administration and Sample Collection

Before sample collection, a structured questionnaire was administered to gather demographic and health parameter data for all the participants. The questionnaire covered various aspects, including the mother's age, body mass index (BMI), pre-pregnancy height, pre-pregnancy weight, delivery weeks, sex of offspring, employment status, education level (completed college or lower than college), alcohol consumption, smoking status, mode of delivery (vaginal or cesarean section), and primiparity.

Four weeks after delivery, all the enrolled participants were summoned to the hospital and 10 mL breast milk samples were collected from each participant by trained nurses via hand expression between 8:00 a.m. and 10:00 a.m. The collected samples were stored in foam boxes and kept chilled on ice at 4°C until transported to the laboratory, they were stored at −80°C for subsequent analysis.

2.3. Estimated Daily Intake (EDI)

PFAS intake for breastfeeding infants, aged 0–6 months, was determined using the following equation:

$$\mathrm{EDI} = \frac{C_{\mathrm{PFAS}} \times DV_{\text{breast milk}}}{BW}$$

where EDI represents the estimated daily intake normalized according to body weight (ng/kg bw/day), C_{PFAS} represents the concentration of a given PFAS in human milk (ng/mL), $DV_{\text{human milk}}$ represents the volume of human milk consumed per day (mL/day), and BW represents body weight (kg). Further, for 0–6-month-old infants with an average weight of 7.2 kg (Ministry of Environment Protection of the People's Republic of China, 2016), we considered the average milk intake per day to be 750 ml (Chinese Nutrition Society, 2014). Thus,

$$\mathrm{EDI\ (PFOA)} = 36.5\ \mathrm{ng/kg\ bw/day\ (range, 0.52–291.7\ ng/kg\ bw/day)}$$

$$\mathrm{EDI\ (PFOS)} = 5.21\ \mathrm{ng/kg\ bw/day\ (range, 0.26–32.3\ ng/kg\ bw/day).}$$

2.4. Analyses Methods

2.4.1. PFAS Analysis

2.4.1.1. Chemicals and Standards

Mass-Labeled PFCA/PFSA Extraction Standard (13) (concentration, 2 mg/mL; volume, 1.2 mL) and Native PFC/PFSA Solution/Mixture (21) (concentration, 2 mg/mL; volume, 1.2 mL) were purchased from Wellington Laboratories (Ontario, Canada). Acetonitrile and methanol (chromatography grade) were purchased from Merck (Billerica, MA, USA) and ammonium acetate (chromatography grade) was purchased from CNW Technologies (Düsseldorf, Germany).

2.4.1.2. Sample Preparation

First, a 100-μL human milk sample was placed in a 1.5-mL centrifuge tube, and 200 μL of methanol was added. This was followed by the addition of 100 μL of the internal standard solution and vortexing 5 min to ensure thorough mixing. Thereafter, centrifugation was performed at 13,000 rpm and 4°C for 15 min using a low-temperature and high-speed centrifuge. The supernatant thus obtained (300 μL) was then transferred into another 1.5-mL centrifuge tube, freeze-dried in vacuum, and lyophilized. Next, 100 μL of methanol solution was added and centrifugation was again performed at 13,000 rpm and 4°C for 10 min using the low-temperature high-speed centrifuge. The resulting supernatant was collected and stored for further analysis.

2.4.1.3. Instrumental Analysis

The separation and quantification of PFAS (PFOA, PFOS, PFNA, PFDoA, PFHxDA, PFBA, PFPeA, PFBS, PFHxA, PFHpA, PFHxS, PFHpS, PFDA, PFUdA, PFDS, PFTrDA, PFTeDA, PFODA, PFNS, PFPeS, PFDoS) were performed using an Ultra Performance Liquid Chromatography Mass Spectrometer (UPLC-I-CLASS-XEVO® TQ-S, Waters, Milford, MA, USA) with an ACQUITY UPLC HSS T3 (2.1 × 100 mm, 1.8 μm) set at 40°C. The mobile phase, at a flow rate of 0.4 mL/min, consisted of a water/acetonitrile mixture to which 10 mM ammonium acetate was added. Further, the mass spectrometry (MS) analysis was based on electrospray ionization (ESI) with measurements performed in the negative ion mode. Detailed ion source parameters are provided in Table S2 in Supporting Information S1.

2.4.1.4. Quality Assurance and Quality Control

To prevent potential contamination by laboratory materials and instruments and ensure that target PFAS were not detected, one procedural blank and one solvent blank were analyzed each time after the analysis of 20 milk samples. The limit of detection (LOD) was set at three times the signal-to-noise ratio (S/N = 3), while the limit of quantification (LOQ) was set at 10 times the signal-to-noise ratio (S/N = 10). The standard curve association coefficient (R^2) was >0.99. Further, the accuracy of the analysis method was based on the percentage recovery rate, with the sample-spiked recovery rate ranging between 95% and 120%. Furthermore, the precision of the method was expressed as a percentage relative standard deviation (%RSD), 4%–18%, and was determined via repeated measurements using the in-house prepared QC sample. LOQ and LOD varied in the ranges 0.01–0.3 and 0.005–0.1 ng/mL, respectively.

2.4.2. Steroid Hormone Analysis

2.4.2.1. Chemicals and Standards

The steroid hormone standards used in this study included: androstenedione-d7 (Nature Standard, USA); estrone, testosterone, androstenedione, and progesterone (Dr. Ehrenstorfer, Augsburg, Germany); estriol (Cmass, UK); estrone-d4 (TRC, Canada); progesterone-d9 (CDN, Canada); estriol-d3 (Bepure, USA); testosterone-d3 (Cerilliant, Round Rock, TX, USA). Acetonitrile and methanol (chromatography grade) were purchased from Merck, and zinc sulfate and ammonium fluoride (analytical grade) were purchased from CNW Technologies GmbH (Dusseldorf, Germany).

2.4.2.2. Sample Preparation

First, 20 μL of 10% zinc sulfate solution and 20 μL of the internal standard solution were measured in a 2-mL centrifuge tube. Thereafter, 300 μL of human milk sample was added to the tube and the mixture was vortexed for 2 min after which 1,000 μL of the extractant was added and vortexing was again performed for 5 min. Next, centrifugation was then performed at 13,000 rpm and 4°C for 5 min, and 800 μL of the resulting supernatant was aspirated into a 1.5-mL centrifuge tube and lyophilized in vacuum. Next, 100 μL of the resulting mixture was placed in another centrifuge tube, vortexed for 10 min, and centrifuged at 13,000 rpm and 4°C for 10 min. Finally, the resulting supernatant was collected and analyzed. The extraction solvent was acetonitrile and the concentration of the internal standard was 5 ng/mL.

2.4.2.3. Instrumental Analysis

Steroid hormone separation and quantification were performed on an Ultra Performance Liquid Chromatography Mass Spectrometer (UPLC-I-CLASS- XEVO® TQ-S, Waters, USA) with an Agilent ZORBAX Eclipse Plus C18 2.1 × 50 mm 1.8 μm set at 40°C. For chromatography, the mobile phase consisted of an aqueous solution of ammonium fluoride (0.3 mmol/L) mixed with acetonitrile at a flow rate of 0.4 mL/min, and for MS, an ESI source with positive and negative ion switching was used. Detailed ion source parameters are briefly described in Table S3 in Supporting Information S1.

2.4.2.4. Quality Assurance and Quality Control

To check for potential contamination by laboratory materials and instruments, one procedural blank and one solvent blank were analyzed each time after the analysis of 20 milk samples. The standard curve association coefficient (R^2) was >0.996. The precision of the analysis was based on the percentage relative standard deviation (%RSD; 3%−20%), determined via repeated measurements on the in-house prepared QC samples (n = 20).

2.5. Statistical Analyses

For the statistical analysis of milk samples with PFAS and steroid hormone concentrations lower than the LODs, we substituted these concentrations with half of the value corresponding to the test line. Data were presented as the median, interquartile range, range, mean, and standard deviation. Prior to analysis, the normality of the collected PFAS and steroid hormone concentration data were verified using the Shapiro-Wilk test, which showed non-normal distributions. Thus, the associations between PFAS and steroid hormone concentrations in human milk samples were investigated using Spearman's correlation analysis. Further, given that the PFAS

Table 1
Basic Characteristics of the Mothers (n = 150) in E-Waste Dismantling Area, China

Characteristics	Median (IQR) or *n*%
Age (years)	28.5 (26.0, 28.5)
BMI (kg/m^2)	20.8 (19.1, 23.2)
Pre-pregnancy height (cm)	159 (156, 162)
Pre-pregnancy weight (kg)	54 (48, 60)
Delivery weeks (week)	38.9 (38.1, 39.3)
Sex of the baby	
Male, *n* (%)	88 (58.7%)
Female, *n* (%)	62 (41.3%)
Career	
No, *n* (%)	64 (42.7%)
Yes, *n* (%)	86 (57.3%)
Education	
Lower than college, *n* (%)	109 (72.7%)
Completed college, *n* (%)	41 (27.3%)
Alcohol Consumption	
No, *n* (%)	147 (98.0%)
Yes, *n* (%)	3 (2.0%)
Smoking	
No, *n* (%)	144 (96.0%)
Yes, *n* (%)	6 (4.0%)
Delivery method	
Smooth delivery, *n* (%)	103 (68.7%)
Cesarean delivery, *n* (%)	47 (31.3%)
Primary production	
No, *n* (%)	36 (24.0%)
Yes, *n* (%)	114 (76.0%)

Note. BMI, body mass index; IQR, interquartile range.

concentrations were skewed to the right, all the statistical tests requiring normality were performed on log-transformed concentrations. We also performed multiple linear regression analyses to examine the relationship between maternal PFAS exposure and steroid hormone concentrations. For this analysis, we normalized the data by subtracting the mean from the original data and dividing it by the standard deviation. Thus, the adjusted variables included in the regression model were maternal age, BMI, smoking status, alcohol consumption, and primiparity (Barrett et al., 2019; S. Lee et al., 2018). All the statistical analyses were performed using SPSS software version 26.0 (SPSS Inc., Chicago, IL, USA), and statistical significance was set at $P < 0.05$.

3. Results

3.1. General Characteristics of the Included Mothers

As shown in Table 1, the median maternal age and BMI of the 150 participants were 28.5 years and 20.8 kg/m^2, respectively. Most of them had a college education (72.7%), and more than half of them were stably employed (57.3%). Further, almost all of them were non-smokers (96.0%) and non-alcoholic consumers (98.0%), and they all delivered their babies at full term. Furthermore, more than half of them chose to have a natural birth (68.7%) and the majority of them were primiparous (76.0%). Furthermore, the percentages of male and female infants among their offspring were 58.7% and 41.3%, respectively.

3.2. Human Milk PFAS Exposure

The concentrations of the PFAS (ng/ml) in the human milk samples ($n = 150$) examined in this study are shown in Table 2. Considering all the 150 collected human milk samples, the median PFAS concentration was 0.27 ng/mL, and PFOA showed the highest detection rate (97.3%), while the detection rates of the other PFAS decreased in the order: PFOS (30.7%) > PFDoA (22.0%) > PFHxDA (13.3%) > PFNA (10.0%) > PFHxS (2.0%). The remaining 15 PFAS (PFBA, PFPeA, PFBS, PFHxA, PFHpA, PFHpS, PFDA, PFUdA, PFDS, PFTrDA, PFTeDA, PFODA, PFNS, PFPeS, and PFDoS) were not detected.

3.3. Human Milk Steroid Hormone Concentrations

The detection rates and concentrations of the steroid hormones (pg/mL) in the collected human milk samples ($n = 150$) are shown in Table 3. Specifically, five steroid hormones, estrone, estriol, testosterone, A-dione, and progesterone were detected, and their mean concentrations were 298.32, 153.94, 13.97, 445.78, and 238.81 pg/mL, respectively. Among these steroid hormones, estrone, A-dione, and progesterone were detected in 100% of the samples, while the detection rates for the remaining hormones were as follows: estriol, 80.7%; and testosterone, 46.7%.

3.4. Human Milk PFAS Exposure and Steroid Hormones

The associations between the concentrations of the individual PFAS and steroid hormones are presented in Table 4. Spearman's correlation analysis showed that PFOA concentration was significantly associated with estrone (β, 0.23; 95% CI, 0.08–0.39). We also observed significant correlations between L-PFOS concentration and A-dione (β, 0.21; 95% CI, 0.06–0.36) and between PFDoA concentration and testosterone (β, 0.27; 95% CI, 0.07–0.39). The results obtained using our multiple regression model, which included data on maternal age, BMI, smoking, alcohol consumption, and primary delivery, and was based on log-transformed PFAS concentrations, are shown in Table 5. Consistent with the Spearman correlation analysis results, it was evident that the level of PFOA exposure of the mothers was positively associated with the levels of estrone in their milk samples (estrone:

Table 2
Distribution of Concentrations of PFASs (ng/ml) in Human Milk (n = 150) in E-Waste Dismantling Area, China

Compounds	Detection frequencies (%)	LOD	Min	Max	Median	Mean
PFASs						
PFOA	97.3	0.01	<LOD	2.80	0.27	0.35
PFOS	30.7	0.05	<LOD	0.31	<LOD	0.05
PFNA	10.0	0.01	<LOD	0.16	<LOD	0.01
PFDoA	22.0	0.005	<LOD	0.02	<LOD	0.004
PFHxDA	13.3	0.02	<LOD	0.09	<LOD	0.02
PFBA	0	0.1	<LOD	<LOD	<LOD	<LOD
PFPeA	0	0.01	<LOD	<LOD	<LOD	<LOD
PFBS	0	0.05	<LOD	<LOD	<LOD	<LOD
PFHxA	0	0.05	<LOD	<LOD	<LOD	<LOD
PFHpA	0	0.01	<LOD	<LOD	<LOD	<LOD
PFHxS	2.0	0.05	<LOD	0.1121	<LOD	0.027
PFHpS	0	0.1	<LOD	<LOD	<LOD	<LOD
PFDA	0	0.05	<LOD	<LOD	<LOD	<LOD
PFUdA	0	0.05	<LOD	<LOD	<LOD	<LOD
PFDS	0	0.05	<LOD	<LOD	<LOD	<LOD
PFTrDA	0	0.05	<LOD	<LOD	<LOD	<LOD
PFTeDA	0	0.05	<LOD	<LOD	<LOD	<LOD
PFODA	0	0.05	<LOD	<LOD	<LOD	<LOD
PFNS	0	0.05	<LOD	<LOD	<LOD	<LOD
PFPeS	0	0.05	<LOD	<LOD	<LOD	<LOD
PFDoS	0	0.05	<LOD	<LOD	<LOD	<LOD

Note. LOD, limit of detection.

β, 0.572; 95% CI, 0.188–0.956). PFOS exposure was also found to be positively associated with A-dione concentration (β, 0.186; 95% CI, 0.016–0.357). Furthermore, PFDoA exposure showed a positive association with testosterone concentration (β, 0.673; 95% CI, 0.136–1.210).

3.5. Human Milk PFAS Levels Around the World

The concentrations of PFOA (ng/ml) in human milk samples obtained from women in different countries in America, Europe, Asia, etc., are shown in Table S1 in Supporting Information S1. In this study, the concentration of PFOA in the collected samples ranged from below the LOD to 2.8 ng/mL, and its median value was 0.27 ng/ml,

Table 3
Distribution of Concentrations of Steroid Hormones (pg/mL) in Human Milk (n = 150) in E-Waste Dismantling Area, China

Compounds	Detection frequencies (%)	LOD	Min	Max	Median	Mean
Steroids						
Estrone	100.0	2	2.15	4662.60	89.25	298.32
Estriol	80.7	5	<LOD	2155.03	52.81	153.94
Testosterone	46.7	1	<LOD	318.57	<LOD	13.97
A-dione	100	20	50	5298.17	347.64	445.78
Progesterone	100	10	15.6	1576.90	153.76	238.81

Note. A-dione, Androstenedione; DHEA, Dehydroepiandrosterone; LOD, limit of detection.

Table 4
Association of PFAS Levels and Steroid Hormones in Human Milk (n = 150)

Compounds	PFOA β (95% CI)	*P* value	PFOS β (95% CI)	*P* value	PFNA β (95% CI)	*P* value	PFDoA β (95% CI)	*P* value	PFHxDA β (95% CI)	*P* value
Estrone	**0.23 (0.08–0.39)**	**<0.01**	0.16 (−0.01–0.32)	0.05	0.11 (−0.04–0.27)	0.17	0.11 (−0.31–0.28)	0.17	0.022 (−0.14–0.18)	0.79
Estriol	0.11 (−0.06–0.27)	0.17	−0.004 (−0.17–0.16)	0.96	0.13 (−0.03–0.27)	0.11	0.04 (−0.11–0.19)	0.59	0.06 (−0.09–0.21)	0.48
Testosterone	0.16 (0.008–0.31)	0.05	0.09 (−0.07–0.24)	0.25	0.09 (−0.08–0.24)	0.29	**0.27 (0.07–0.39)**	**<0.01**	−0.01 (−0.16–0.14)	0.89
A-dione	0.08 (−0.08–0.24)	0.32	**0.21 (0.06–0.36)**	**<0.01**	0.02 (−0.15–0.19)	0.78	0.20 (−0.16–0.19)	0.81	0.04 (−0.10–0.18)	0.61
Progesterone	0.06 (−0.10–0.21)	0.50	0.08 (−0.07–0.24)	0.32	0.08 (−0.06–0.21)	0.36	−0.003 (−0.16–0.13)	0.97	0.06 (−0.08–0.20)	0.48

Note. CI, confidence interval; β, standardized coefficient. Bold font indicates statistically significant.

which was found to be much lower than that reported in a study conducted in South Korea (1.46 ng/mL) from 2008 to 2009 (Kim et al., 2011), but higher than those reported for other countries and the rest of China.

4. Discussion

The potential health effects of PFAS on pregnant women and their infants, particularly in regions where PFAS-containing e-waste is disassembled, have been an issue of concern in recent years. Human milk has become an increasingly valuable resource for monitoring PFAS exposure in humans owing to its non-invasive sampling method (Cariou et al., 2015; Černáet al., 2020). In this study, we investigated the association between prenatal PFAS exposure and steroid hormone concentrations in human milk from women living around an e-waste disassembly area in China. Thus, we observed that changes in maternal steroid hormone levels were associated with PFAS exposure.

Specifically, among all the PFAS analyzed in this study, PFOA showed the highest concentration (range, 0.005–2.80 ng/mL; median, 0.27 ng/mL) consistent with its detection rate being the highest (Starling et al., 2014). Further, this median PFOA concentration (0.27 ng/ml) was higher than those reported in studies conducted in other countries in Africa, Europe, North America, and Asia (except for South Korea; Table S1 in Supporting Information S1: Abdallah et al., 2020; Al-sheyab et al., 2015; Antignac et al., 2013; Awad et al., 2020; Barbarossa et al., 2013; Beser et al., 2019; Cariou et al., 2015; Černá et al., 2020; Criswell et al., 2023; Forns et al., 2015; Fromme et al., 2022; Fujii et al., 2012; Hassan et al., 2023; Iszatt et al., 2019; Jin et al., 2020; Kadar et al., 2011; Kang et al., 2016; Kärrman et al., 2007, 2010; Kim et al., 2011; Lankova et al., 2013; S. Lee et al., 2018; Y. M. Lee et al., 2020; J. Liu et al., 2010, 2011; Llorca et al., 2010; Macheka-Tendenguwo et al., 2022; Motas Guzmàn et al., 2016; Raab et al., 2013; Rawn et al., 2022; Roosens et al., 2010; Serrano et al., 2021; So et al., 2006; Tao, Kannan, et al., 2008; Tao, Ma, et al., 2008; Völkel et al., 2008; H. Wang et al., 2019; G. Zheng et al., 2021; P. Zheng et al., 2022). Possibly, these differences can be attributed to differences in the characteristics of the sampling areas, for example, for South Korea, the samples were collected in Seoul, the capital city, which is characterized by a high population density and a well-developed electronics industry, implying higher electronics

Table 5
Association of PFAS and Steroid Hormones in Human Milk Using Multiple Linear Analysis (n = 150)

	PFOA		PFOS		PFNA		PFDoA		PFHxDA	
Compounds	β	95% CI	β	95% CI	β	95% CI	β	95% CI	β	95% CI
Estrone	**0.572**	**0.188–0.956**	0.309	−0.061–0.679	0.384	−0.042–0.809	0.262	−0.202–0.726	0.022	−0.464–0.508
Estradiol	−0.071	−0.737–0.595	−0.253	−0.878–0.373	−0.164	−0.884–0.557	0.078	−0.703–0.859	0.216	−0.598–1.030
Testosterone	0.444	−0.014–0.903	0.053	−0.387–0.493	0.158	−0.348–0.664	**0.673**	**0.136–1.210**	−0.036	−0.608–0.536
A-dione	0.004	−0.178–0.186	**0.186**	**0.016–0.357**	0.020	−0.179–0.219	−0.029	−0.245–0.186	0.042	−0.183–0.266
Progesterone	0.105	−0.100–0.310	0.115	−0.081–0.310	0.095	−0.131–0.321	−0.049	−0.294–0.196	0.018	−0.238–0.274

Note. The models were adjusted for mother's age, BMI, alcohol consumption, smoking, primary production. A-dione, Androstenedione; DHEA, Dehydroepiandrosterone; CI, confidence interval; β: standardized coefficient. Bold font indicates statistically significant. All the raw values of the PFAS and steroid hormones were initially transformed into natural logarithms to assure the normality of residuals.

usage and disassembly rates. Additionally, the proximity of Seoul to Lake Asan, along with the presence of a nearby sewage treatment plant, raise concerns regarding the potential entry of disintegrated pollutants into the lake, their subsequent accumulation in the bodies of lactating mothers, and increased PFOA concentration in human milk consumed by infants (Y. M. Lee et al., 2020). Moreover, the PFOA concentrations observed in this study were generally higher than those reported for other regions in China, such as Hangzhou (0.031 ng/mL; Jin et al., 2020), Sichuan (0.096 ng/mL; P. Zheng et al., 2022), and Zhoushan (0.11 ng/mL; So et al., 2006). Notably, the median PFOA concentration obtained in this study was as much as three and ninefold higher than those reported for Sichuan (0.096 ng/ml) and Hangzhou (0.031 ng/mL), respectively. This may be since Taizhou is an area with frequent e-waste disposal activities and is close to the seashore, which is more contaminated by e-waste, resulting in higher levels of PFOA in the water (Yang et al., 2024). These findings suggested that the risk of maternal prenatal PFOA exposure is significantly higher in e-waste disassembly areas than in other areas.

Studies have indicated that PFAS concentrations in human milk samples are lower than those in blood (plasma and serum). Even though the mechanism of PFAS transfer from blood to milk remains unknown, several theories have been proposed in this regard. Reportedly, given that fatty acids make up a large proportion of human milk and PFAS has structural features similar to those of fatty acids, PFAS can pass through the mammary epithelial membrane via binding to albumin, similar to fatty acids (Macheka-Tendenguwo et al., 2018). Normally, the concentration of steroid hormones in human milk is 1%–5% of that in plasma (Sjövall, 1970). By analyzing the levels of estrogens in human milk and blood samples from healthy lactating women 3–5 days postpartum, McGarrigle and Lachelin (1983) observed significantly higher estrone and estriol glucuronide concentrations in serum than in human milk. In addition, Hines compared the concentrations of endogenous compounds in serum and human milk samples from lactating women at 2–7 weeks and 3–4 months postpartum and observed higher estradiol concentrations in serum than in human milk (Hines et al., 2007). Conversely, Boss et al. (2018) observed that progesterone levels in human milk were similar to those in serum. Furthermore, this previous study showed that maternal protein and meat intake could affect progesterone concentration in human milk. The differences in hormone concentrations between the two sample types suggested the existence of a progesterone transfer mechanism between human milk and blood. Further, Sahlberg and Axelson (1986) reported that different classes of steroid hormones have different transport systems. Particularly, estrogen may be transformed via conjugated estrogens, which in the gut of the mother, are hydrolyzed by intestinal bacteria to a free state and subsequently absorbed into the blood together as conjugated estrogens through the portal vein.

In this study, we observed that PFOA was positively associated with estrone concentrations, PFOS was positively associated with A-dione concentration, and PFDoA was positively associated with testosterone concentration. Previous studies have also reported similar associations between PFAS and steroid hormones. For example, in a cohort study conducted in Hebei (China), maternal blood PFOS levels were found to be positively associated with estrone and estriol concentrations (H. Wang et al., 2019). On the other hand, the results of the present study showed no association between PFAS and progesterone. However, a previous study revealed a negative association between PFOS level and the levels of progesterone in the serum of women of reproductive age of 25–35 years. A related study on the association between maternal blood PFAS levels and sex hormone levels in cord blood during pregnancy, considering the influence of genetic factors (Kobayashi et al., 2021), indicated that the interaction between PFOS level and the infant CYP 17 A1 (rs743572) genotype is significantly associated with A-dione and testosterone levels in the infants. These findings suggest that PFAS may alter maternal steroid hormone secretion by affecting the activity of steroid hormone synthase; however, the specific mode of this effect (stimulation or inhibition) requires further confirmation in future studies. These diverse findings highlight the complex and potentially contrasting effects of PFAS on hormone levels. Thus, further research to fully clarify the implications and mechanisms involved is required. Human milk is an important source of nutrition for infants aged 0–6 months, and studies have shown that infants are more exposed to PFAS through breastfeeding within the first month after birth than through cumulative exposure during gestation via placental transfer (Mosca & Gianni, 2017; Yi & Kim, 2021). Therefore, by monitoring the concentration of PFAS in human milk, the potential health risks associated with e-waste disassembly areas can be assessed. This method is suitable for breastfeeding mothers and infants. Further, in the present study, the EDIs of the different PFAS were calculated to determine their potential health risks to breastfeeding infants. The average EDIs of PFOA and PFOS were 36.5 ng/kg bw/day (range, 0.52–291.7 ng/kg bw/day) and 5.21 ng/kg bw/day (range, 0.26–32.3 ng/kg bw/day), respectively. Furthermore, the mean EDI (36.5 ng/kg bw/day) of PFOA obtained in this study was generally higher than that reported for Ireland (18 ng/kg bw/day; Abdallah et al., 2020) and Spain (7.15 ng/kg bw/day; Guzmàn

et al., 2016). Even though there is no specific reference dose for infants, the Tolerable Daily Intake is based on the most stringent Health-Based Guideline Value established by the European Food Safety Authority in 2020 (PFOA: 0.857 ng/kg bw/day, PFOS: 1.857 ng/kg bw/day; EFSA et al., 2020). Potential reasons for the differences in EDI may be as follows. First, different methods of estimating the EDI were used in different studies, such as differences in infant weight and daily human milk intake, which may have impacted on the calculation of the EDI. Differences in mothers' dietary preferences may also have affected the calculation of EDI (Abdallah et al., 2020), despite being located in coastal areas. In addition, the sampling area for this study was located in an e-waste disassembly area, which may account for the significantly higher EDI. Therefore, human milk consumption, owing to PFAS exposure, poses a potential health risk for breastfeeding infants in e-waste disassembly areas in China. Generally, breastfeeding infants in e-waste disassembly areas have a relatively higher PFAS intake than their counterparts in other areas (Zhao et al., 2023). A cohort study conducted in the Netherlands showed a strong association between daily PFAS intake in early infancy and plasma PFAS levels at age 2 (van Beijsterveldt et al., 2022). Excessive PFAS intake in early infancy may result in several negative health effects, including obesity and changes in behavioral patterns (Anderko et al., 2019; Starling et al., 2019). Therefore, changes in infant PFAS intake have significant implications for later development. Considering that human milk is the sole food for infants and is beneficial for immune-system maturation, organ development, and protection against infections, it is essential to continuously monitor the potential risk of neonatal exposure to PFAS in human milk. Previous studies have shown that changes in maternal and placental hormone levels during pregnancy are associated with pregnancy complications, including pre-eclampsia, non-epithelial ovarian cancer (Chen et al., 2011; Kumar et al., 2018), and poor birth outcomes, including low birth weight (Gilles et al., 2018). In addition, epidemiological studies have shown that prenatal PFAS exposure is associated with adverse fetal birth outcomes and development (Tillaut et al., 2023; Wikström et al., 2020; Zhou et al., 2023). The associations between PFAS and steroid hormones found in this study support the hypothesis that PFAS act as endocrine disruptors, suggesting that prenatal PFAS exposure may act as a pathway to influence fetal growth trajectories. To this end, future individualized follow-up cohort studies will include an assessment of the association between prenatal maternal PFAS exposure and infant growth and development to extend our current results.

The results of this study provide evidence for the existence of an association between prenatal PFAS exposure and changes in steroid hormone levels in human milk. However, our study had some limitations. First, we did not conduct individualized follow-up cohort studies to confirm the association between maternal prenatal PFAS exposure and infant growth and development. Therefore, further studies in this regard are necessary. Second, even within a narrow window of lactation, individual differences in the levels of steroid hormones can be substantial. Such significant changes in hormonal levels possibly reduced the statistical power of our analyses. Third, our study did not consider the possible influence of the dietary habits of the 150 mothers on PFAS exposure, which is a limitation of our study design. We hope to improve our study in the future.

5. Conclusions

In this study, we investigated infant exposure to PFAS via human milk consumption by examining milk samples from 150 lactating mothers living close to an e-waste disassembly area. Our results showed a positive association between PFAS and steroid hormone levels in human milk. Thus, human milk, an essential excretion pathway for mothers and an important source of nutrition in early infancy can be used as a suitable sample for assessing the association between prenatal PFAS exposure and steroid hormone levels. Of concern, the PFAS intake of breastfeeding infants in the study area was higher than the recommended threshold. Regardless, this result should be interpreted with caution considering rapid variations in infant weight and human milk ingestion with growth. Therefore, long-term monitoring of the effects of PFAS exposure on infant growth and development is required. Increased efforts to mitigate mother and infant exposure to environmental pollutants are also required.

Conflict of Interest

The authors declare no conflicts of interest relevant to this study.

Data Availability Statement

Data is available at Sun (2024).

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