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Concentrations of perfluoroalkyl substances in donor breast milk in Southern Spain and their potential determinants

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ABSTRACT

Background: Breast milk is considered to offer the best nutrition to infants; however, it may be a source of exposure to environmental chemicals such as perfluoroalkyl compounds (PFAS) for breastfeeding infants. PFAS are a complex group of synthetic chemicals whose high stability has led to their ubiquitous contamination of the environment.

Objective: To assess the concentrations and profiles of PFAS in breast milk from donors to a human milk bank and explore factors potentially related to this exposure.

Methods: Pooled milk samples were collected from 82 donors to the Human Milk Bank of the Virgen de las Nieves University Hospital (Granada, Spain). Ultra-high performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS) was applied to determine milk concentrations of 11 PFAS, including long-chain and short-chain compounds. A questionnaire was used to collect information on donors' socio-demographic characteristics, lifestyle, diet, and use of personal care products (PCPs). Factors related to individual and total PFAS concentrations were evaluated by multivariate regression analysis.

Results: PFAS were detected in 24–100% of breast milk samples. PFHpA was detected in 100% of samples, followed by PFOA (84%), PFNA (71%), PFHxA (66%), and PFTrDA (62%). Perfluorooctane sulfonate (PFOS) was detected in only 34% of donors. The median concentrations ranged from <0.66 ng/dL (perfluorohexane sulfonic acid [PFHxS]) to 19.39 ng/L (PFHpA). The median of the sum of PFAS concentrations was 87.67 ng/L and was higher for short-chain than long-chain PFAS. Factors most frequently associated with increased PFAS concentrations included intake of creatin animal food items and use of PCPs such as skin care and makeup products.

Conclusions: Several PFAS, including short-chain compounds, are detected in pooled donor milk samples. Breast milk may be an important pathway for the PFAS exposure of breastfed infants, including preterm infants in NICUs. Despite the reduced sample size, these data suggest that various lifestyle factors influence PFAS concentrations, highlighting the use of PCPs.

Abbreviations: PFCAs, Perfluoroalkyl carboxylic acids; PFSAs, Perfluoroalkane sulfonic acids; SC PFAS, Short-chain PFAS; LC PFAS, Long-chain PFAS; NICU, Neonatal intensive care unit; PCPs, Personal care products.

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1. Introduction

Breast milk is considered the best food for infants in general and for high-risk premature infants in particular, offering proper nutrition, immunological benefits, and growth-promoting components and reducing the risk of complications (American Academy of Pediatrics [AAP], 2012). When preterm infants cannot receive breast milk from their mothers, included those admitted to a neonatal intensive care unit (NICU), the World Health Organization (WHO) and AAP recommend the administration of pasteurized human milk from a milk bank rather than artificial infant formula (AAP, 2012; WHO United Nations Children's Fund [UNICEF], 2003). Donated breast milk delivers essential nutrients and therapeutic benefits to the preterm infant but also has the potential to transmit infectious diseases and transfer toxic chemicals from exposed mothers (Carroll, 2014; Lehmann et al., 2018). Consequently, the European Human Milk Banking Association (Weaver et al., 2019) and other international milk banks have established guidelines for donor selection to ensure the safety of the milk (Clifford et al., 2020). These take account of pathogenic microorganisms and certain toxic substances (*e.g.*, tobacco, alcohol, medications, caffeine, and drugs of abuse) but do not consider occupational or environmental exposure to hazardous chemicals.

Per- and polyfluoroalkyl substances (PFAS) are a group of thousands of synthetic chemicals that are widely used in commercial and industrial products. They serve as polymerization aids in the production of fluoropolymers, as surfactants in fire-fighting foams, as anti-mist agents in chromium plating, and as water and oil repellents in textiles, leather, food contact materials, and cosmetics. PFAS are also employed in the production of semiconductors, medical devices, plant protection products, biocides, feed additives, pharmaceuticals, and paints (Glüge et al., 2020). Hydrogen atoms are entirely or partially replaced by fluorine atoms in these aliphatic substances (Buck et al., 2011) and the bond between carbon and fluorine is extremely strong and stable; hence, PFAS are highly resistant to thermal, chemical, and biological degradation and can accumulate in living organisms and biomagnify in food webs (Pérez et al., 2013). The degree of bioaccumulation generally increases with greater length of perfluoroalkyl carbon chain, and the elimination kinetics are highly species-dependent, with humans showing the longest PFAS half-lives, reaching 8.5 years for perfluorohexane sulfonic acid (PFHxS) (Olsen et al., 2009). Over the past decade, exposure to certain PFAS has been associated with lipid and insulin dysregulation (Sinisalu et al., 2020; Sun et al., 2018), infertility (Bach et al., 2016), reduced fetal growth (Kashino et al., 2020), increased miscarriage risk (Liew et al., 2020), obesity (Braun, 2017), impaired cognitive development (Vuong et al., 2019), and altered thyroid (López-Espinosa et al., 2012; Preston et al., 2020) and immune (Abraham et al., 2020; Grandjean et al., 2012) functions. These associations are supported by animal studies indicating that some PFAS are endocrine and metabolic disruptors, immunotoxic, reproductively toxic, and/or carcinogenic (ATSDR, 2018; Fenton et al., 2020; Street et al., 2018).

The most widespread PFAS in the environment are perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS), long-chain PFAS that are frequently detected in sera from populations worldwide (Bartolomé et al., 2017; Calafat et al., 2007; Kannan et al., 2004; Lewis et al., 2015; Thépaut et al., 2021). Current regulations in the European Union (EU) and elsewhere mainly address PFOS and PFOA, which are listed under the Stockholm Convention on Persistent Organic Pollutants (POPs) (Regulation (EU) 2019/1021; UNEP, 2009) and have been phased out in the EU since 2008 (European Directive, 2006/112/EC). Restrictions are also in place or planned under EU chemical legislation for other PFAS, including short-chain compounds such as PFHxS and perfluorohexanoic acid (PFHxA) (ECHA, 2019). These are less bioaccumulative than long-chain PFAS but are equally persistent in the environment and may exert similar toxicity (Nian et al., 2020).

Food, drinking water, and the indoor environment are considered to be the principal sources of human exposure to PFAS (Cornelis et al., 2012; Haug et al., 2011), which also include cosmetics and all-weather textiles, among other products made from PFAS (EFSA, 2020; Schultes et al., 2018). Seafood, meat, and dairy products may be the major sources of dietary exposure, especially to PFOA and PFOS (Domingo and Nadal, 2017; Titlemier et al., 2007). Socio-demographic factors have also been related to a greater internal PFAS burden, including occupation, male sex, higher age, and low parity (Bartolomé et al., 2017; Colles et al., 2020; Guzman et al., 2016).

Several PFAS have been detected in umbilical cord blood, placenta, breast milk, and plasma samples from breastfed infants, indicating that placental transfer and breastfeeding are both potential routes of PFAS exposure (Abraham et al., 2020; Cariou et al., 2015; Lien et al., 2013; Vela-Soria et al., 2021). It has been reported that a substantial proportion of PFAS in the mother is transferred to the infant during breastfeeding, which may contribute to reduce maternal serum and breast milk concentrations over the lactation period (Bartolomé et al., 2017; Macheka-Tendenguwo et al., 2018; Mondal et al., 2014; Thomsen et al., 2010). Identification of breastfeeding as an important pathway for the exposure to PFAS of breastfed infants (Haug et al., 2011) has been supported by findings of their wide presence in breast milk samples from mothers worldwide (Hu et al., 2021; Lee et al., 2018; Macheka--Tendenguwo et al., 2018).

Human milk banks provide milk for very premature, fragile, and sometimes medically compromised infants who are especially vulnerable to the effects of toxic chemicals. The present study is part of a wider project that aims to assess the potential adverse health impact on neonates in a NICU of exposure to endocrine-disrupting chemicals (EDCs) from their medical care, diet, and environment (Iribarne-Duran et al., 2019). The purpose of this study was to evaluate the concentrations and profiles of eleven long-and short-chain PFAS in milk samples from donors to a human milk bank and to explore factors that influence their concentrations.

2. Material and methods

2.1. Study population

Between 2015 and 2018, 82 donor mothers were recruited from the Regional Human Milk Bank of the Virgen de las Nieves University Hospital in Granada (Southern Spain). In general, donor women are registered at the milk bank after breastfeeding is well-established (*i.e.*, 2–3 weeks post-delivery). Exclusion criteria for donor milk selection include: positive serology for HIV, syphilis, or hepatitis B or C; risk factor for sexual transmitted disease (*e.g.,* unstable partner, non-utilization of condom, tattooing/piercing in previous three months, acupuncture, and blood transfusion); transplantation in previous 6 months; current smoking or drug habit; and high consumption of alcohol (>2 drinks/day or >20 g/day) or caffeine-containing drinks (>3 cups/day or >30 g/day). All local donors supplying the milk bank between 2015 and 2018 (n = 446) were invited to participate in the study and were fully informed of its nature and purpose. Donors who agreed to participate (18.4%) were asked to donate a milk sample for the analysis of environmental chemicals and to complete a structured questionnaire on socio-demographic and reproductive characteristics, lifestyle, diet, and use of personal care products (PCPs). Information on dietary habits and PCP use was available for a subsample of 77 donors. An informed consent form was signed by the donors before collecting personal information and biological samples. The research protocol was approved by the Biomedical Research Ethics Committee of Granada.

2.2. Milk sample collection

Participating donors were asked by the milk bank to collect mature milk over a minimum of 1 week and a maximum of 4 weeks by manual expression and/or breast pump and to keep them frozen (−20 °C) until delivery to the bank. On their arrival at the bank, samples were stored at

−30 °C without breaking the cold chain at any time. Before their pasteurization (done within 2 weeks), samples from each donor were thawed and pooled, obtaining an aliquot of 5–30 mL of the pooled milk. This was then stored at −20 °C until analysis at the "UNETE research unit" of the Centro de Investigación Biomédica (University of Granada). The day of pasteurization was recorded as the donation date. Hence, the interval between the start of milk collection by the mother and the donation date never exceeded 6 weeks.

2.3. Laboratory analysis

A modification of a validated ultra-high performance liquid chromatography-with tandem mass spectrometry method (Vela-Soria et al., 2020) was used (see Supplementary material) to determine the concentrations of eleven PFAS in pooled milk samples, including: seven long-chain PFAS, *i.e.*, six perfluoroalkyl carboxylic acids (PFCAs) with >7 perfluorinated carbons (PFOA, perfluorononanoic acid [PFNA], perfluorodecanoic acid [PFDA], perfluoroundecanoic acid [PFUnDA], perfluorododecanoic acid [PFDoDA] and perfluorotridecanoic acid [PFTrDA]), and one perfluoroalkane sulfonic acid (PFSA) with ≥6 perfluorinated carbons (PFOS); and four short-chain PFAS, *i.e.*, two PFCAs (PFHxA and perfluoroheptanoic acid [PFHpA]), and two PFSAs (perfluorobutane sulfonic acid [PFBS] and PFHxS) (Buck et al., 2011).

Milk aliquots used for the determination of PFAS had not been analyzed before. Quality control (QC) procedures included the use of blanks, low and high-concentration QC materials prepared from a fortified breast milk pool, analytical standards, and reagent and matrix blanks to ensure the accuracy and precision of the data. We also performed repeated measurements of breast milk QC pools, reflecting inter- and intraday variations. Relative standard deviation (%RSD) values were calculated as a measure of the precision of the method. Table S2 summarizes the mean accuracy and %RSD values obtained. The accuracy of the method was also verified by injecting QCs of different concentrations every 20 samples. Limits of detection (LD) ranged between 0.66 and 0.86 ng/L and limits of quantification (LQ) between 2.19 and 2.87 ng/L (Table S2).

2.4. Explanatory variables

The questionnaire administered by the milk bank to prospective milk donors and an *ad hoc* questionnaire were used to gather the following socio-demographic, reproductive, and lifestyle data: age (years), parity (multiparous or primiparous), lifetime duration of breastfeeding, either exclusive or mixed (<1, 1–10, or >10 months), birth weight and length and gestational age of the most recent newborn, schooling (university education or not), current occupation (unemployed, manual worker, or non-manual worker), area of residence (urban, sub-urban, or rural), smoking habit (ever smoked in the past or not), and current body mass index (BMI, kg/m^2) categorized as underweight/normal (<25 kg/m^2) or overweight/obese (≥25 kg/m^2). Women were also asked about their weight gain during the most recent pregnancy (kg) and weight change from before pregnancy (gain, loss, or no change). The number of days post-delivery was calculated as the difference between milk donation and birth dates. Dietary information was collected on the main origin of drinking water and the average consumption frequency (servings per day or week) in the previous 12 months of seafood, fish (oily and lean fish), dairy products (yoghurt, milk, butter, cheese), meat (red meat and cold meats), pulses, eggs, bread, chocolate, cereals, rice, pasta, fruit, vegetables (raw and cooked), fried food, canned food, coffee, and alcoholic beverages (Table 2). Data were also gathered on the frequency with which the women used sun screen, lip protector, face treatments (cream, tonic, milk), body lotion, hand cream, hair mask, makeup products (foundation, lipstick, eyeliner, and eye shadow), nail polish, hair dye, shampoo, shower cream, deodorant, hairspray/mousse/gel, perfume, toothpaste, and mouth wash and received manicure and pedicure treatments in the previous 12 months (Table 3).

Table 1
General characteristics of milk donors (n = 82).

Variables	n (%)	Median	Range
Age (years)		33	19–42
Year of sample collection			
2015	25 (30.5)		
2016	27 (32.9)		
2017	23 (28.0)		
2018	7 (8.5)		
Multiparous	37 (45.1)		
Lifetime duration of breastfeeding (months)			
<1	41 (50.0)		
1–10	22 (26.8)		
>10	19 (23.2)		
Time since delivery (days)		71	20–273
Length of gestation (weeks)		39	26–41
Birth weight (g)		3130	840-4500
Birth length (cm)		50	16–56
Current BMI (kg/m^2)		22.86	17.30–36.09
Overweight/obese	26 (33.8)		
Weight gain during pregnancy (kg)		12	1–36
Weight change from before pregnancy			
Weight loss	19 (22.1)		
Weight gain	39 (49.4)		
No weight change	24 (28.6)		
Area of residence			
Rural	26 (31.2)		
Sub-urban	24 (29.9)		
Urban	32 (39.0)		
Maternal university education	51 (66.2)		
Occupation			
Unemployed	6 (6.3)		
Manual worker	22 (26.3)		
Non-manual worker	54 (67.5)		
Ex-smoker	39 (47.6)		

BMI: Body mass index.

In addition, the protein content of unpasteurized pooled milk samples (g/100 mL) was determined as a potential explanatory variable, given evidence that perfluorinated compounds are mainly transported bound to human serum albumin (Luo et al., 2012) and their lactational transfer is produced by binding to milk protein (Fromme et al., 2010). The total lipid, lactose (g/100 mL), and caloric (kcal/100 mL) contents of samples were also measured as independent variables.

2.5. Statistical analysis

The detection frequency of PFAS in milk samples and 50th, 75th, and 95th percentiles of their concentrations were calculated, including the total concentration of all PFAS (∑PFAS), the most abundant PFAS commonly found in human blood samples (∑4 PFAS = [PFOA + PFOS + PFNA + PFHxS] and ∑5 PFAS = [∑4 PFAS + PFHpA]) (Cousins et al., 2020; EFSA, 2020), long-chain PFAS (∑LC PFAS), short-chain PFAS (∑SC PFAS), PFSAs (∑PFSAs), and PFCAs (∑PFCAs). Total concentrations were calculated as the sum of molar concentrations of the compounds based on molecular weight and were expressed as PFOA (∑PFAS, ∑4 PFAS, ∑5 PFAS, ∑LC PFAS, ∑PFCAs), PFOS (∑PFSAs), or PFHpA (∑SC PFAS). When PFAS were detected in at least 70% of samples, concentrations below the LD were assigned a value of LD/$\sqrt{2}$ and were treated as continuous variables, as were the sums of the different PFAS groups. PFAS detected in less than 70% of the milk samples were categorized as detected or non-detected (binary variables). Spearman's correlation test was used to assess relationships between PFAS concentrations (Fig. 1).

Multivariate regression analyses were performed with natural-logarithm-transformed continuous (linear regression) or binary (logistic regression) PFAS concentrations as dependent variables. A forward stepwise procedure was used to enter independent variables in the models. All variables described in section 2.4, and the year of sample collection (2015, 2016, 2017, or 2018), were tested as potential

Table 2
Food intake frequency of milk donors (n = 77).

Variables	n (%)	Variables	n (%)
Coffee intake = 1 cup/day	17 (20.7)	**Pulse**	
Alcohol intake ≥ 1 drink/ month	4 (4.9)	1 sv/week	13 (16.9)
Origin of drinking water		2 sv/week	29 (37.7)
Tap water	53 (68.8)	>2 sv/week	35 (45.5)
Bottled water	24 (31.2)	**Eggs**	
Seafood		1 sv/week	16 (20.8)
<1 sv/week	11 (14.3)	2 sv/week	28 (36.4)
1 sv/week	19 (24.7)	>2 sv/week	33 (42.9)
>1 sv/week	47 (57.3)	**Bread**	
Lean fish		<1 sv/day	15 (19.5)
<1 sv/week	18 (23.4)	1 sv/day	25 (32.5)
1 sv/week	37 (48.1)	>1 sv/day	37 (48.1)
>1 sv/week	22 (28.6)	**Chocolate**	
Oily fish		Never	10 (13.0)
<1 sv/week	29 (37.7)	<1 sv/day	44 (57.1)
1 sv/week	34 (44.2)	≥1 sv/day	23 (28.0)
>1 sv/week	14 (18.2)	**Cereals**	
Yoghurt		Never	27 (35.1)
<1 sv/day	31 (40.3)	<1 sv/day	35 (45.5)
≥1 sv/day	46 (59.7)	≥1 sv/day	15 (19.5)
Milk		**Rice**	
<1 glass/day	14 (18.2)	1 sv/week	66 (85.7)
≥1 glass/day	63 (81.8)	>1 sv/week	11 (14.3)
Cheese		**Pasta**	
Never/rarely	22 (28.6)	1 sv/week	66 (85.7)
>2 sv/week	34 (44.2)	>1 sv/week	11 (14.3)
≥1 sv/day	21 (27.3)	**Fruit**	
Butter		≤2 sv/week	12 (15.6)
Never	23 (29.9)	>2 sv/week	65 (84.4)
1 sv/week	36 (46.8)	**Raw vegetables**	
>1 sv/week	18 (23.4)	≤2 sv/week	15 (19.5)
Meat		>2 sv/week	62 (80.5)
≤1 sv/week	11 (14.3)	**Cooked vegetables**	
2 sv/week	13 (16.9)	≤2 sv/week	17 (22.1)
>2 sv/week	53 (68.8)	>2 sv/week	60 (77.9)
Cold meat		**Fried food**	
<2 sv/week	43 (55.8)	<1 sv/week	37 (48.1)
2 sv/week	34 (44.2)	1 sv/week	25 (32.5)
Red meat		>1 sv/week	15 (19.5)
Never	20 (26.0)	**Canned food (ever)**	62 (80.5)
<1 sv/week	33 (42.9)		
≥1 sv/week	34 (31.2)		
sv: serving			

Table 3
Use of personal care products among milk donors (n = 77).

Variables	**n (%)**	**Variables**	**n (%)**
Sunscreen (ever)	37 (48.1)	**Eye shadow**	
Sunscreen application		Rarely/never	49 (63.6)
None	40 (51.9)	<once a day	18 (23.4)
Face	26 (33.8)	≥once a day	10 (13.0)
Entire body	11 (14.3)	**Nail polish (traditional)**	
Sunscreen protection factor		Rarely/never	65 (84.4)
None	40 (51.9)	≥once a week	12 (15.6)
<50	12 (15.6)	**Acrylic nail polish**	
50	25 (32.5)	<once a month	70 (90.9)
Lip protector (ever)	30 (39.0)	once a month	7 (9.1)
Face cream		**Manicure**	
<once a day	24 (31.2)	<once a month	67 (87.0)
once a day	31 (40.3)	once a month	10 (13.0)
>once a day	22 (28.6)	**Pedicure**	
Face tonic		<once a month	66 (85.7)
Rarely/never	60 (77.9)	once a month	11 (14.3)
≥once a week	17 (22.1)	**Hair dye**	
Face milk		Never	36 (46.8)
Rarely/never	70 (90.9)	<once a month	23 (29.9)
≥once a week	7 (9.1)	once a month	18 (23.4)
Face treatment		**Shampoo**	
Never	59 (76.6)	<3 times/week	25 (32.5)
<once a month	12 (15.6)	≥3 times/week	52 (67.5)
Once a month	6 (7.8)	**Shower cream**	
Body lotion		<once a day	6 (7.8)
Rarely/never	28 (36.4)	≥once a day	71 (92.2)
<once a day	15 (19.5)	**Hairspray/mousse/gel**	
≥once a day	34 (44.2)	Rarely/never	58 (75.3)
Hand cream		≥once a day	19 (24.7)
<once a day	43 (55.8)	**Deodorant**	
once a day	19 (24.7)	<once a day	8 (10.4)
>once a day	15 (19.5)	once a day	52 (67.5)
Hair mask		>once a day	17 (22.1)
Rarely/never	38 (49.4)	**Perfume**	
≥once a week	39 (50.6)	Rarely/never	12 (15.6)
Foundation makeup		<once a day	24 (31.2)
Rarely/never	41 (53.2)	≥once a day	41 (53.2)
<once a day	19 (24.7)	**Toothpaste**	
≥once a day	17 (22.1)	≤once a day	17 (22.1)
Lipstick		>once a day	60 (77.9)
Rarely/never	39 (50.6)	**Mouthwash**	
<once a day	24 (31.2)	Rarely/never	47 (61.0)
≥once a day	14 (18.2)	<once a day	11 (14.3)
Eyeliner		≥once a day	18 (23.4)
Rarely/never	36 (46.8)		
<once a day	21 (27.3)		
≥once a day	20 (26.0)		

explanatory variables). Given the modest sample size, the p-value threshold of 0.10 was selected to retain explanatory variables in the model. Associations were expressed as exponentiated regression coefficients (exp [β]) or odds ratios (OR) with 95% confidence intervals (CI). The overall R-squared for each model was calculated to determine the percent variability in exposure explained by explanatory variables. R version 4.0.4 (SAS Institute Inc., Cary, NC, USA) was used for data analyses.

3. Results

General characteristics of the study participants are displayed in Table 1. Donors had a median age of 33 years and 45% were multiparous (33 mothers had 1 previous birth). Most of the milk samples were collected in 2015–2017, with only 8% being collected in 2018. The lifetime breastfeeding duration was <1 month for 50% and >10 months for 23%. The median interval between delivery and milk donation was 98 days (3.3 months), ranging from 20 days (<1 month) to 273 days (9 months). In their most recent pregnancy, the birth was preterm (<37 weeks) in 23% of deliveries and the infant had low birth weight (<2500 g) in 18%. Around one-third of donors were overweight or obese; 49% gained weight from before pregnancy and 22% lost weight. More than one-third of the donors resided in the metropolitan urban area of Granada, 66% had completed university education, 26% were manual workers, and 48% were ex-smokers. Most donors drank tap water and consumed >2 servings/week of meat and >1 serving/week of seafood (44% consumed >1 serving/week of oily fish) (Table 2). More than half of donors reported the daily use of face cream, hand cream, shower cream, deodorant, and toothpaste and the frequent use of shampoo (≥3 times/week) and perfume (≥once a day) (Table 3).

The median protein content of milk samples was 1.10 g/100 mL (range = 0.20–6.80 g/100 mL), their median fat content was 3.70 g/100 mL (range = 1.16–8.30 g/100 mL), median lactose content was 7.36 g/100 mL (range = 6.54–8.00 g/100 mL), and median energy content was 68 kcal/100 mL (range = 44–110 kcal/100 mL).

Table 4 shows that detection frequencies (DF) of PFAS ranged from 24.4 to 100%, with PFHpA being detected in all samples (median concentration = 19.39 ng/L), followed by PFOA (DF = 84.1%, median = 7.17 ng/L), PFNA (DF = 70.7%, median = 2.59 ng/L), PFHxA (DF = 65.9%, median = 1.58 ng/L), and PFTrDA (DF = 62.2%, median = 1.69 ng/L). Remaining compounds were detected in less than 40% of samples. The median sum of PFAS concentrations was 87.67 ng/L (range = 7.57–1899 ng/L) and was higher for short-chain than for long-chain PFAS (median = 52.69 [range = 2.74–1168] ng/L *vs.* 20.01 [range = 3.06–571.1] ng/L, respectively) and for PFCAs than for PFSAs (median = 74.97 [range = 5.65–1399] ng/L *vs.* 2.45 [range = 0.72–223.2] ng/L,

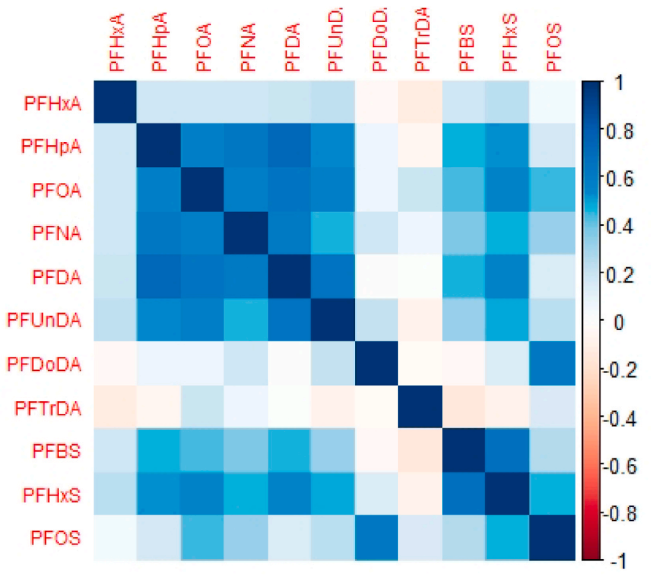

Fig. 1. Correlation heatmap for PFAS concentrations in breast milk.

respectively). At least seven PFAS compounds were detected in the breast milk of 24 donors (29%), 5–6 were detected in 38 (46%), and 2–4 in 20 (24%). Positive correlations were observed between all PFCA compounds except for PFDoDA and PFTrDA, while PFSA concentrations were positively correlated with PFHpA, PFOA, PFNA, and PFDA concentrations (Fig. 1).

Explanatory variables that were associated with PFAS concentrations are exhibited in Table 5 (linear regression models) and Tables S3–S10 (logistic regression models). The R-squared value of models ranged from 14% (∑LC PFAS) to 61% (PFDoDA). Milk samples collected in 2016 or 2017 had lower PFNA, PFDoDA, and ∑LC PFAS but higher PFHxA and ∑SC PFAS concentrations in comparisons to 2015. Multiparous donors had significantly higher concentrations of PFHpA in their milk, while lifetime duration of breastfeeding was associated with higher concentrations of PFOA, PFDA, ∑5 PFAS, ∑PFCAs, ∑LC PFAS, and ∑PFAS. Weight change from before pregnancy (gain or loss) was associated with higher PFDA, PFOS, ∑4 PFAS, and ∑5 PFAS concentrations, and residing in an urban area with higher PFHpA, ∑5 PFAS, and ∑PFAS concentrations.

With regard to food intake, red meat was associated with higher concentrations of ∑5 PFAS; oily fish, milk, and cold meat with higher PFHxA; yoghurt with higher PFDoDA and ∑PFSAs; cheese with higher PFTrDA; butter with higher PFBS; pulses with higher ∑4 PFAS; chocolate with higher PFHpA; and fried food with higher PFOS concentrations. The PCPs most frequently related to increased PFAS concentrations were hand cream, whose use was related to higher PFNA, PFDA, PFBS, PFHxS, PFOS, ∑4 PFAS, ∑SC PFAS, ∑PFCAs, and ∑PFAS; followed by face treatment, associated with higher PFHpA, PFNA, ∑5 PFAS, and ∑PFCAs; and lipstick use, associated with higher PFOA, PFUnDA and PFDoDA. Face cream and body lotion were associated with higher PFUnDA; foundation makeup with higher ∑4 PFAS; eyeliner with higher PFHxA and PFHxS; eye shadow with higher PFOA and PFDA; hair dye with higher PFOA and PFOS; shampoo with higher PFOA; hair mask with higher PFHxS; deodorant with higher ∑PFSAs; and perfume with higher ∑SC PFAS concentrations.

On the other hand, certain factors such as a higher intake of cheese, eggs, cereals, and fish, and a more frequent use of deodorants were associated with a decrease in the milk concentrations of some individual PFAS or total PFAS (Table 5 and Tables S3–S10).

4. Discussion

The concentrations of eleven PFAS were measured in milk samples from 82 donors to a human milk bank in Spain in 2015–2018. More than two-thirds of milk samples had detectable concentrations of PFHpA (100%), PFOA (84%), and PFNA (71%), and almost one-third showed the presence of at least seven PFAS; also, concentrations of short-chain PFAS were higher than of long-chain PFAS, especially in more recently collected samples. These results suggest that breast milk may be an important pathway of PFAS exposure for breastfed infants. Given that donated milk is used for premature newborns with low or very low birth weight (<1500 g) in NICUs, it appears crucial to monitor concentrations of environmental chemicals such as PFAS in human milk banks. Despite the small sample size, these findings suggest that PFAS concentrations in human milk are influenced by various lifestyle factors, such as intake of

Table 4
Concentrations (ng/L) of PFAS in donor breast milk (n = 82).

Compound		LOD	DF (%)	Median	P75	P95	Max.
PFHxA	Perfluorohexanoic acid	0.73	65.9	1.58	26.15	152.3	322.4
PFHpA	Perfluoroheptanoic acid	0.79	100	19.39	55.71	232.3	743.9
PFOA	Perfluorooctanoic acid	0.86	84.1	7.17	23.86	55.12	251.8
PFNA	Perfluorononanoic acid	0.69	70.7	2.59	10.69	25.48	136.5
PFDA	Perfluorodecanoic acid	0.72	24.4	<0.72	1.57	23.01	210.3
PFUnDA	Perfluoroundecanoic acid	0.74	39.0	<0.74	1.60	3.29	14.01
PFDoDA	Perfluorododecanoic acid	0.77	35.4	<0.77	1.66	1.66	131.8
PFTrDA	Perfluorotridecanoic acid	0.78	62.2	1.69	1.69	8.84	13.34
PFBS	Perfluorobutane sulfonic acid	0.80	35.4	<0.80	1.73	66.35	195.0
PFHxS	Perfluorohexane sulfonic acid	0.66	24.4	<0.66	0.74	16.01	45.45
PFOS	Perfluorooctane sulfonic acid	0.86	34.1	<0.86	6.26	26.01	64.75
Sum of PFAS		**P5**	**P25**	**Median**	**P75**	**P95**	**Max.**
∑4 PFAS[a]	PFOA + PFOS + PFNA + PFHxS[b]	<2.04	5.51	14.66	39.69	104.7	437.8
∑5 PFAS[a]	PFOA + PFOS + PFNA + PFHxS + PFHpA[c]	2.19	5.88	53.31	103.9	280.5	1284
∑LC PFAS[a]	PFOA + PFOS + PFNA + PFDA+ PFUnDA + PFDoDA + PFTrDA[d]	3.81	7.32	20.01	47.03	155.1	571.1
∑SC PFAS[a]	PFHpA + PFHxA + PFBS + PFHxS[e]	<2.74	13.75	52.69	125.1	398.4	1168
∑PFSA[a]	PFBS + PFHxS + PFOS	<0.72	<0.72	2.45	8.09	136.6	223.2
∑PFCA[a]	PFHxA + PFHpA + PFOA + PFNA+ PFDA + PFUnDA + PFDoDA + PFTrDA	7.11	30.16	74.97	141.8	450.1	1399
∑PFAS[a]	Sum of all 11 PFAS	11.46	44.58	87.67	208.2	475.4	1899

LOD: Limit of detection; DF: Detection frequency; P75, P95: 75th and 95th percentiles.
LC: long-chain PFAS; SC: short-chain PFAS; PFSAs: Perfluoroalkyl sulfonic acids; PFCAs: Perfluoroalkyl carboxylic acids.
[a] Weighted molar sum of PFAS concentrations (sum of molar concentrations of PFAS based on molecular weight; [b]Most abundant PFAS in human serum (EFSA, 2020); [c]Most abundant PFAS in human serum including PFHpA (Cousins et al., 2020); [d]Long-chain PFAS; [e]Short-chain PFAS.

Table 5
Significant explanatory variables for breast milk concentrations of most prevalent PFAS and summed concentrations of PFAS groups (n = 77).

Predictors	PFHpA	PFOA	PFNA	∑4PFAS	∑5PFAS	∑PFSAs	∑PFCAs	∑Long-chain PFAS	∑Short-chain PFAS	∑PFAS
Year of sample collection (ref: 2015)										
2016			**0.23 (0.10–0.53)**					**0.39 (0.21–0.73)**	**2.91 (1.36–6.17)**	
2017			0.65 (0.27–1.53)					0.64 (0.34–1.18)	**2.75 (1.22–6.22)**	
2018			0.32 (0.09–1.05)					0.55 (0.22–1.38)	0.73 (0.24–2.23)	
Multiparous *vs.* primiparous	2.12 (0.96–4.72)									
Total breastfeeding (ref: <1 month)										
1–10 months		**2.75 (1.31–5.79)**			**2.99 (1.72–5.21)**		**2.27 (1.26–4.11)**	**1.80 (1.01–3.19)**		**2.61 (1.49–4.96)**
>10 months		1.11 (0.51–2.42)			1.01 (0.54–1.89)		0.90 (0.48–1.67)	1.04 (0.58–1.89)		1.31 (0.75–2.29)
BMI (kg/m^2)	0.91 (0.82–1.01)									
Weight change from pre-conception										
Weight gain				**2.22 (1.02–4.81)**	1.66 (0.89–3.09)					
Weight loss				**3.03 (1.58–5.78)**	**1.71 (1.01–2.89)**					
Area of residence (ref: rural)										
Sub-urban	1.20 (0.44–3.30)				1.32 (0.72–2.42)				**0.48 (0.23–0.99)**	0.62 (0.34–1.12)
Urban	**4.04 (1.51–10.8)**				**3.08 (1.77–5.36)**				1.68 (0.80–3.55)	**1.82 (1.06–3.11)**
Ex-smoker									**0.44 (0.22–0.87)**	
Coffee intake: 1 *vs.* <1 cup/day				**0.44 (0.21–0.90)**						
Lean fish intake (ref: <1 sv/week)										
1 sv/week									**0.29 (0.13–0.63)**	**0.46 (0.26–0.84)**
>1 sv/week									0.48 (0.21–1.12)	0.64 (0.34–1.19)
Oily fish intake (ref: <1 sv/week)										
1 sv/week										**0.56 (0.34–0.93)**
>1 sv/week										0.99 (0.52–1.56)
Yoghurt intake: ≥1 *vs.* <1 sv/day						**2.09 (1.02–4.29)**				
Cheese intake (ref: rarely/never)										
>2 sv/week	**0.30 (0.11–0.76)**				**0.46 (0.26–0.81)**	0.46 (0.20–1.08)				**0.51 (0.29–0.86)**
≥1 sv/day	**0.32 (0.10–0.99)**				**0.51 (0.27–0.95)**	0.40 (0.15–1.05)				**0.50 (0.27–0.93)**
Red meat intake (ref: ≤1 sv/week)										
2 sv/week					0.94 (0.52–1.69)					
>2 sv/week					**1.85 (1.07–3.21)**					
Pulses intake (ref: 1 sv/week)										
2 sv/week				1.33 (0.60–2.93)						
>2 sv/week				**2.53 (1.17–5.45)**						

(*continued on next page*)

Table 5 (*continued*)

Predictors	PFHpA	PFOA	PFNA	∑4PFAS	∑5PFAS	∑PFSAs	∑PFCAs	∑Long-chain PFAS	∑Short-chain PFAS	∑PFAS
Egg intake (ref: 1 sv/week)										
2 s/week	**0.30 (0.10–0.92)**									
>2 sv/week	0.46 (0.15–1.38)									
Chocolate intake (ref: never)										
<1 sv/day	3.27 (0.93–11.5)									
≥1 sv/day	2.98 (0.74–12.0)									
Cereal intake (ref: never)										
<1 sv/day				**0.38 (0.21–0.70)**						**0.53 (0.32–0.89)**
≥1 sv/day				**0.36 (0.16–0.80)**						0.65 (0.35–1.22)
Face treatment (ref: never)										
<once a month	1.99 (0.98–2.38)		2.12 (0.85–5.26)		1.65 (0.89–3.06)		1.57 (0.78–3.14)			
once a month	**3.13 (1.26–7.80)**		**4.26 (1.26–14.42)**		**4.02 (1.71–9.47)**		**3.59 (1.38–9.36)**			
Body lotion use (ref: rarely/never)										
<once a day							0.95 (0.48–1.89)			
≥once a day							**0.53 (0.29–0.98)**			
Hand cream use (ref: <once a day)										
once a day			2.09 (0.95–4.61)	**2.05 (1.07–3.92)**			1.77 (0.95–3.29)		**2.03 (1.00–4.19)**	**2.07 (1.23–3.49)**
>once a day			1.46 (0.60–3.56)	1.74 (0.86–3.52)			1.62 (0.79–3.33)		1.30 (0.58–2.91)	1.20 (0.69–2.07)
Foundation makeup use (ref: never)										
<once a day				1.55 (0.80–2.97)						
≥once a day				1.96 (0.98–3.95)						
Lip protector use: ever *vs.* never				**0.46 (0.27–0.80)**						
Lipstick use (ref: rarely/never)										
<once a day		**3.42 (1.50–7.84)**								
≥once a day		0.47 (0.18–1.25)								
Eye shadow use (ref: rarely/never)										
<once a day		0.60 (0.25–1.44)								
≥once a day		**6.39 (2.32–17.6)**								
Hair dye use (ref: never)										
<once a month		1.03 (0.46–2.32)								
once a month		**2.24 (1.01–4.98)**								
Shampoo use: ≥ *vs.* <3 times/ week		**2.01 (1.01–4.00)**								

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Table 5 (*continued*)

Predictors	PFHpA	PFOA	PFNA	∑4PFAS	∑5PFAS	∑PFSAs	∑PFCAs	∑Long-chain PFAS	∑Short-chain PFAS	∑PFAS
Shower cream use: ≥ *vs.* <once a day					**0.37 (0.16–0.89)**					
Deodorant use (ref: <once a day)										
once a day		**0.24 (0.10–0.65)**			**0.40 (0.19–0.86)**	1.17 (0.36–3.86)				
>once a day		0.85 (0.26–2.77)			0.81 (0.35–1.87)	**4.30 (1.13–16.3)**				
Perfume use (ref: rarely/never)										
<once a day									**3.35 (1.25–8.95)**	
≥once a day									1.81 (0.75–4.39)	
R^2	24%	35%	21%	26%	43%	16%	15%	14%	40%	34%

PFSAs: Perfluoroalkyl sulfonic acids; PFCAs: Perfluoroalkyl carboxylic acids.
Associations are reported as exponentiated regression coefficients (exp[β]) with 95% confidence intervals (CI).
Bold: p-value<0.05.

certain animal food items and the use of PCPs. Some of these factors were previously reported, as discussed below.

4.1. PFAS concentrations in milk

There has been increasing research into the presence of PFAS in human breast milk over the past decade (Hu et al., 2021; Macheka-Tendenguwo et al., 2018; Supplementary material, Table S11). Studies have indicated a wide variation in the geographical distribution of PFAS concentrations and profiles, revealing a global decline in the concentration of some PFAS congeners, especially in countries where their production and utilization have been restricted, such as the USA and Germany (Bjerregaard-Oelsen et al., 2016; Černá et al., 2020; Macheka-Tendenguwo et al., 2018). In this regard, the Stockholm Convention (UNEP, 2016) and EU regulations (Commission Regulations [EU] 2017/1000; 207/2011) have contributed to a gradual decline in levels of PFOS and PFOA. In the present study, PFAS concentrations in the donor milk samples collected in 2015–2018 were generally several times lower than in samples collected between 2012 and 2015 in hospitals or primary health care centers in other Spanish regions (Beser et al., 2019; Lorenzo et al., 2016; Motas Guzmán et al., 2016). In the most recent Spanish study by Beser et al. (2019), PFOA, PFOS, and PFNA concentrations in milk samples collected in 2015 were higher than in the present samples, while they did not detect any of the remaining nine PFAS analyzed (Table S11). Likewise, concentrations of PFOS, PFOA, PFNA, and PFHxS in breast milk samples gathered in Catalonia in 2007–2008 were higher in comparison to the present findings (Kärrman et al., 2010; Llorca et al., 2010), although the presence of other PFAS such as PFDA and PFUnDA was not detected (Kärrman et al., 2010). The lower concentrations of PFAS found in donor milk samples from Granada *versus* other Spanish regions may be attributable to the lower level of economic development in the South of Spain, as indicated by the results of a Spanish biomonitoring study of PFAS concentrations in serum samples from adults in 2009–2010 (Bartolomé et al., 2017).

PFAS concentrations in the present samples are generally comparable or in the lower range of those observed in breast milk from other countries, although the majority of previous studies only measured the most abundant PFAS, *i.e.,* PFOS, PFOA, PFNA, PFDA, and PFHxS (Macheka-Tendenguwo et al., 2018; Table S11). A recent Chinese study of the same eleven PFAS as in the present study reported a higher detection frequency of PFOA, PFOS, and PFDA but a much lower detection frequency of the remaining PFAS in breast milk samples (n = 174) than in the present samples (Jin et al., 2020); for instance, PFHpA was not detected in any sample but was found in all of the present samples. In the same way, Lee et al. (2018) reported higher concentrations of PFOA, PFOS, and PFHxS but lower concentrations of the remaining PFAS in milk from 293 Korean mothers in comparison to the present donors. Overall, the present results suggest a decline in breast milk concentrations of PFOS (detected in only one out of three donors), a continued exposure to PFOA, and widespread exposure to short-chain PFAS such as PFHpA and PFHxA, whose concentrations were higher than previously reported in breast milk (Macheka-Tendenguwo et al., 2018; Table S11).

Our findings are in line with studies indicating the predominance of short-chain *versus* long-chain PFAS in breast milk (Fujii et al., 2012; Kang et al., 2016; Kim et al., 2011; Lorenzo et al., 2016). Short-chain PFAS are more soluble and have a lower molecular weight, facilitating their passage through the mammary epithelial membrane and their contamination of breast milk. In addition, the widespread and growing use of alternative short-chain PFAS over the last years would have increased human exposure (Kang et al., 2016; Lorenzo et al., 2016). It has also been suggested that the transfer of sulphonates (PFSAs) to human milk is easier than that of carboxylates (PFCAs) (Roosens et al., 2010); however, the latter were more abundant than the former in the present study.

4.2. Determinants of PFAS concentrations in breast milk

PFAS concentrations were not associated with the age of milk donors. The relationship of breast milk PFAS with age is not clear, with some studies describing higher PFAS concentrations with increasing age (Lee et al., 2018) and others showing no such association (Antignac et al., 2013; Llorca et al., 2010; Motas Guzmán et al., 2016; Nyberg et al., 2018). The BMI of donors was not related to breast milk PFAS concentrations in the present study, except for a suggestive inverse association with PFHpA. Previous reports have been contradictory, showing both positive and negative associations (Berg et al., 2014; Brantsaeter et al., 2013; Cariou et al., 2015; Jensen et al., 2015; Lee et al., 2018; Lorenzo et al., 2016). On the other hand, weight change from before pre-conception appeared to influence the increase the concentrations of PFDA, PFOS, ∑4 PFAS, and ∑5 PFAS. This finding is not easy to explain, given that weight change may be an indicator of changes in diet or lifestyle that could lead to increased PFAS exposure, as previously suggested (Lee et al., 2018).

Lifetime breastfeeding was associated with higher PFDA, PFOA, ∑PFCAs, ∑long-chain PFAS, and total PFAS concentrations, while multiparous status was associated with lower PFBS but higher PFHpA concentrations. In contrast, various studies reported lower PFAS concentrations in the milk of multiparous mothers in comparison to those who were breastfeeding for the first time (Awad et al., 2020; Barbarossa et al., 2013; Croes et al., 2012; Motas Guzmán et al., 2016; Thomsen et al., 2010), suggesting a greater transfer (either placental or via breastfeeding) of PFAS to the first newborn. Thus, Thomsen et al. (2010) reported a reduction rate of 7.7 and 3.1% per month in breast milk concentrations for PFOA and PFOS, respectively, while Mondal et al. (2014) estimated that breastfeeding was associated with monthly decrease of 1–3% in maternal serum concentrations of PFOA, PFOS, PFHxS, and PFNA and of 1–8% in breast milk concentrations of PFOA and PFOS. However, is still not well established that breast milk concentrations of PFAS decrease over the lactation period. In line with our results, a Korean study found higher PFOS, PFOA, PFNA, and total PFAS concentrations in breast milk collected at 30 *versus* 6 days after the delivery, which were attributed to changes in the dietary and lifestyle patterns of mothers throughout the lactational period (Lee et al., 2018). It has also been proposed that the interval between pregnancies may have an impact on the body burden of PFAS, with a longer interval being associated with breast milk concentrations that may be as high as observed for the first breastfeeding episode (Whitworth et al., 2012). Nevertheless, the associations with breastfeeding duration observed in this study remain poorly understood.

Some previous studies observed higher PFAS concentrations in the breast milk of women residing in urban or semi-urban *versus* rural areas (Abdallah et al., 2020; Liu et al., 2010; Tao et al., 2008a, b). In the same line, urban donors in this study showed higher concentrations of PFHpA, ∑5 PFAS, and total PFAS concentrations, while their education and occupation did not appear to influence PFAS concentrations.

Dietary intake has been identified as a substantial source of PFAS exposure (Domingo and Nadal, 2017). The intake of fish and seafood has been associated with higher internal concentrations of PFAS in several studies (Berg et al., 2014; Rylander et al., 2010; Thépaut et al., 2021; Tyrrell et al., 2013), including reports of adult serum and breast milk samples (Bartolomé et al., 2017; Motas Guzmán et al., 2016). In addition, research on the presence of PFAS in food marketed in Spain found that fish and shellfish were the most contaminated groups, showing the highest concentrations of PFOS, PFOA, PFHpA, and PFHxS (Domingo et al., 2012a, b). However, no positive relationship was found between fish/seafood intake and PFAS concentrationss in the present study, although meat consumption was associated with increased total PFAS concentrations, consistent with observations of a higher PFAS content in foods of animal *versus* non-animal origin (Tittlemier et al., 2007). The intake of other food items did not show a clear trend towards an increase in PFAS exposure. Notably, the consumption of fried food was associated with higher PFOS concentrations. This may in part be explained by a greater use of non-stick cookware or PFOS-contaminated oil for frying or by a higher intake of fried processed food contaminated with PFOS. However, no data are available to support these propositions.

Higher concentrations of several PFAS, including long- and short-chain compounds, were found in the milk from women who more frequently used various PCPs, suggesting that PCPs might be a potential source of PFAS exposure. Few data are available on the presence of PFAS in PCPs; however, nine PFAS, including PFOA and PFNA, were detected in foundation, nail polish, and sunscreen products sold in Japan (Fujii et al., 2013), and twenty-five PFAS, most frequently PFHpA and PFHxA, in foundation and cosmetic powder products sold in Sweden (Schultes et al., 2018). In the present study, the use of foundation was positively associated with the sum of PFOA, PFOS, PFNA, and PFHxS concentrations, and the use of skin care and hair products, cosmetics, perfume, and deodorant was associated with higher concentrations of long-chain and short-chain PFAS, PFCAs, and PFSAs. These results support a previous study of 264 Korean women that found the utilization of cosmetics and skin care products to be associated with breast milk concentrations of PFHpA and PFOS, respectively (Kang et al., 2016). In the same line, recent biomonitoring study of adults in Belgium and Norway reported associations between the use of cosmetics (*e.g.*, sunscreen, mouthwash, and lip balm) and serum concentrations of PFAS (Colles et al., 2020; Thépaut et al., 2021). Dermal exposure to PFAS has been considered negligible in comparison to exposure from diet, drinking water, and ingestion of house dust (Trudel et al., 2008; Vestergren et al., 2008). However, exposure assessment studies have not considered the potential contribution of PCPs to dermal uptake due to the lack of adequate dosage data. Dermal permeability studies have also shown that the skin may be a relevant route of PFAS exposure under certain conditions, underscoring the need to re-assess the potential contribution of dermal exposure (Franco et al., 2012).

4.3. Implications for newborn exposure and health

Literature reports suggest that breast milk is an important pathway for the exposure of breastfed infants to PFAS, while also acts as a route for PFAS progressive elimination from the mother's body. In general, PFAS concentrations in the present milk samples were lower than described in these studies; however, it should be taken into account that: 1) exposure may start during the fetal period via placental transfer, meaning that the infant would already have a body burden of PFAS at birth; 2) although epidemiological evidence on the effects of postnatal exposure to PFAS, particularly short-chain PFAS, remains limited, potential effects include thyroid hormone imbalances, altered postnatal growth, and a decreased antibody response to vaccines (Abraham et al., 2020; Grandjean, 2018; Jin et al., 2020; Lopez-Espinosa et al., 2012); 3) there is a lack of knowledge on the toxicological properties of many PFAS in current use and on the combined adverse effects of this complex group of synthetic chemicals; and 4) most importantly, milk donated to the human milk bank is given to highly vulnerable preterm infants in NICUs, for whom the acceptable level of risk should be zero. Interestingly, based on findings of an association between plasma PFAS concentrations and antibodies against diphtheria and tetanus in one-year-olds (Abraham et al., 2020), the EFSA estimated that critical levels in breast milk would be 60 ng/L for PFOA and PFNA, 73 ng/L for PFHxS and PFOS, and 133 ng/L for the sum of the 4 PFAS (EFSA, 2020). These values are comparable to the upper concentrations observed in the present study. Moreover, a recent study reported that the exposure of preterm infants to PFAS through human breast milk might exceed reference values for older and healthier infants (Aceti et al., 2021).

4.4. Strengths and limitations

The main limitation of this study is the small sample size, which reduced the capacity to detect possible determinants of PFAS exposure,

particularly for compounds with a low detection frequency and therefore modeled as binary variables. Nevertheless, similar or even smaller sample sizes were used by most published studies on PFAS in breast milk (Macheka-Tendenguwo et al., 2018; Table S11). In addition, extrapolation of the study findings to lactating women in general is limited, because milk donors tend to be more educated and have higher incomes in comparison to non-donor lactating women (Osbaldiston and Mingle, 2007). Indeed, most donors in this study had a university education and were non-manual workers. Moreover, data were not available to establish whether the socio-demographic profile differed between participating and non-participating donors. However, neither education nor occupation was associated with milk PFAS concentrations. A further limitation was the use of a questionnaire not specifically designed for an exhaustive investigation of sources of PFAS exposure, and the lack of more detailed data on dietary patterns, occupations, and other potential sources of exposure prevented the identification of additional exposure pathways. Further, the considerable number of explanatory factors assessed may have led to some spurious statistically significant associations. It is also possible that bias may have resulted from the misreporting of dietary intakes and other factors. Nevertheless, misclassification is unlikely to be driven by exposure levels. Finally, the wide time frame for sample collection (ranging from 20 days to 9 months since delivery) may hamper comparisons with other studies on PFAS breast milk concentrations, given that internal PFAS exposure may vary over the lactation period due to toxicokinetics and/or lifestyle changes. In fact, our research group is currently investigating time-dependent variations in breast milk PFAS concentrations over the lactation period.

The main strength of this study is the assessment of pooled milk samples (over a maximum of 4 weeks) rather than spot samples. It is well established that lactational transfer of PFAS occurs by binding to milk protein. Protein levels decrease linearly in human milk over the first year of lactation, particularly over the first 6 weeks post-partum (Ballard and Morrow, 2013). Hence, PFAS assessments in spot breast milk samples may increase the risk of exposure misclassification in comparison to the assessment of pooled samples. Moreover, some of the eleven PFAS measured (*e.g.,* PFUnDA, PFDoDA, and PFTrDA) have been less well studied in breast milk samples and human biomonitoring studies. To our knowledge, this is the first report on the presence of PFAS in breast milk samples supplied by donor mothers to a human milk bank. The results suggest that requirements for donor selection may not be sufficient to minimize the exposure of breastfed infants to environmental chemicals.

5. Conclusions

This study of the concentrations of eleven PFAS in donor breast milk demonstrated the wide presence of these compounds in milk samples, especially short-chain PFAS such as PFHpA and PFHxA. PFOA and PFNA showed lower concentrations than observed in previous studies but were still detected in a large proportion of samples, whereas the findings for PFOS suggest a decrease in exposure levels. The data also suggest that certain lifestyle patterns, such as the use of PCPs, may have an influence on the presence of PFAS in breast milk; however, these data should be interpreted with caution given the limited sample size. Further studies are required to elucidate the main factors contributing to the increase in PFAS concentrations in breast milk and to determine changes in exposure levels over the lactation period This issue is especially urgent in relation to the supply of human milk to preterm infants and the need to limit their exposure to harmful chemicals.

Declaration of competing interest

The authors declare no actual or potential competing financial interests.

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Appendix A. Supplementary data

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References

Abdallah, M.A., Wemken, N., Drage, D.S., Tlustos, C., Cellarius, C., Cleere, K., Morrison, J.J., Daly, S., Coggins, M.A., Harrad, S., 2020. Concentrations of perfluoroalkyl substances in human milk from Ireland: implications for adult and nursing infant exposure. Chemosphere 246, 125724.

Abraham, K., Mielke, H., Fromme, H., Völkel, W., Menzel, J., Peiser, M., Zepp, F., Willich, S.N., Weikert, C., 2020. Internal exposure to perfluoroalkyl substances (PFASs) and biological markers in 101 healthy 1-year-old children: associations between levels of perfluorooctanoic acid (PFOA) and vaccine response. Arch. Toxicol. 94 (6), 2131–2147.

Aceti, A., Barbarossa, A., Gazzotti, T., Zironi, E., Pagliuca, G., Vitali, F., Beghetti, I., Corvaglia, L., 2021. Exposure to perfluoroalkyl substances through human milk in preterm infants. Eur. J. Pediatr. https://doi.org/10.1007/s00431-021-04073-4. Apr 10.

Agency for Toxic Substances and Disease Registry (ATSDR), 2018. Toxicological Profile for Perfluoroalkyls. (Draft for Public Comment). U.S. Department of Health and Human Services, Public Health Service, Atlanta, GA.

American Academy of Pediatrics (AAP), 2012. Breastfeeding and the use of human milk. Pediatrics 129 (3), e827–e841.

Antignac, J.P., Veyrand, B., Kadar, H., Marchand, P., Oleko, A., Le Bizec, B., Vandentorren, S., 2013. Occurrence of perfluorinated alkylated substances in breast milk of French women and relation with socio-demographical and clinical parameters: results of the ELFE pilot study. Chemosphere 91 (6), 802–808.

Awad, R., Zhou, Y., Nyberg, E., Namazkar, S., Yongning, W., Xiao, Q., Sun, Y., Zhu, Z., Bergman, Å., Benskin, J.P., 2020. Emerging per- and polyfluoroalkyl substances (PFAS) in human milk from Sweden and China. Environ. Sci. Process Impacts 22 (10), 2023–2030.

Bach, C.C., Vested, A., Jorgensen, K., Bonde, J.P., Henriksen, T.B., Toft, G., 2016. Perfluoroalkyl and polyfluoroalkyl substances and measures of human fertility: a systematic review. Crit. Rev. Toxicol. 46 (9), 735–755.

Ballard, O., Morrow, A.L., 2013. Human milk composition: nutrients and bioactive factors. Pediatr. Clin. 60 (1), 49–74.

Barbarossa, A., Masetti, R., Gazzotti, T., Zama, D., Astolfi, A., Veyrand, B., Pession, A., Pagliuca, G., 2013. Perfluoroalkyl substances in human milk: a first survey in Italy. Environ. Int. 51, 27–30.

Bartolome, M., Gallego-Pico, A., Cutanda, F., Huetos, O., Esteban, M., Perez- Gomez, B., Bioambient, es, Castano, A., 2017. Perfluorinated alkyl substances in Spanish adults: geographical distribution and determinants of exposure. Sci. Total Environ. 603–604, 352–360.

Berg, V., Nost, T.H., Huber, S., Rylander, C., Hansen, S., Veyhe, A.S., Fuskevag, O.M., Odland, J.O., Sandanger, T.M., 2014. Maternal serum concentrations of per- and polyfluoroalkyl substances and their predictors in years with reduced production and use. Environ. Int. 69, 58–66.

Beser, M.I., Pardo, O., Beltrán, J., Yusà, V., 2019. Determination of 21 perfluoroalkyl substances and organophosphorus compounds in breast milk by liquid

chromatography coupled to orbitrap high-resolution mass spectrometry. Anal. Chim. Acta 1049, 123–132.
Bjerregaard-Olesen, C., Bach, C.C., Long, M., Ghisari, M., Bech, B.H., Nohr, E.A., Henriksen, T.B., Olsen, J., Bonefeld-Jørgensen, E.C., 2016. Determinants of serum levels of perfluorinated alkyl acids in Danish pregnant women. Int. J. Hyg Environ. Health 219 (8), 867–875.
Brantsaeter, A.L., Whitworth, K.W., Ydersbond, T.A., Haug, L.S., Haugen, M., Knutsen, H. K., Thomsen, C., Meltzer, H.M., Becher, G., Sabaredzovic, A., Hoppin, J.A., Eggesbo, M., Longnecker, M.P., 2013. Determinants of plasma concentrations of perfluoroalkyl substances in pregnant Norwegian women. Environ. Int. 54, 74–84.
Braun, J., 2017. Early-life exposure to EDCs: role in childhood obesity and neurodevelopment. Nat. Rev. Endocrinol. 13 (3), 161–173.
Buck, R.C., Franklin, J., Berger, U., Conder, J.M., Cousins, I.T., de Voogt, P., Jensen, A.A., Kannan, K., Mabury, S.A., van Leeuwen, S.P.J., 2011. Perfluoroalkyl and polyfluoroalkyl substances in the environment: terminology, classification, and origins. Integrated Environ. Assess. Manag. 7 (4), 513–541.
Calafat, A.M., Wong, L.Y., Kuklenyik, Z., Reidy, J.A., Needham, L.L., 2007. Polyfluoroalkyl chemicals in the U.S. Population: data from the national health and nutrition examination survey (NHANES) 2003-2004 and comparisons with NHANES 1999-2000. Environ. Health Perspect. 115, 1596–1602.
Cariou, R., Veyrand, B., Yamada, A., Berrebi, A., Zalko, D., Durand, S., Pollono, C., Marchand, P., Leblanc, J.C., Antignac, J.P., Le Bizec, B., 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.
Carroll, C., 2014. Body dirt or liquid gold? How the 'safety' of donated breastmilk is constructed for use in neonatal intensive care. Soc. Stud. Sci. 44 (3), 466–485.
Černá, M., Grafnetterová, A.P., Dvořáková, D., Pulkrabová, J., Malý, M., Janoš, T., Vodrážková, N., Tupá, Z., Puklová, V., 2020. Biomonitoring of PFOA, PFOS and PFNA in human milk from Czech Republic, time trends and estimation of infant's daily intake. Environ. Res. 188, 109763.
Clifford, V., Sulfaro, C., Lee, J., Pink, J., Hoad, V., 2020. Development and evaluation of formal guidelines for donor selection for human milk banks. J. Paediatr. Child Health 56 (8), 1242–1248.
Colles, A., Bruckers, L., Den Hond, E., Govarts, E., Morrens, B., Schettgen, T., Buekers, J., Coertjens, D., Nawrot, T., Loots, I., Nelen, V., De Henauw, S., Schoeters, G., Baeyens, W., van Larebeke, N., 2020. Perfluorinated substances in the Flemish population (Belgium): levels and determinants of variability in exposure. Chemosphere 242, 125250.
Commission Regulation (EU) 2017/1000 Commission Regulation (EU) 2017/1000 of 13 june 2017 amending annex XVII to regulation (EC) No 1907/2006 of the European parliament and of the council concerning the registration, evaluation, authorisation and restriction of chemicals (REACH) as regards perfluorooctanoic acid (PFOA), its salts and PFOA-related substances. Available online: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32017R1000&from=EN (accessed on 18 February 2021).
Commission Regulation (EU) No 207/2011, Commission Regulation (EU) No 207/2011 of 2 march 2011 amending regulation (EC) No 1907/2006 of the European parliament and of the council on the registration, evaluation, authorisation and restriction of chemicals (REACH) as regards annex XVII (diphenylether, pentabromo derivative and PFOS). Available online: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32011R0207 (accessed on 18 February 2021).
Cornelis, C., Hollander, W.D., Roosens, L., Covaci, A., Smolders, R., Van Den Heuvel, R., Govarts, E., Van Campenhout, K., Reynders, H., Bervoets, L., 2012. First assessment of population exposure to perfluorinated compounds in Flanders, Belgium. Chemosphere 86 (3), 308–314.
Cousins, I.T., DeWitt, J.C., Glüge, J., Goldenman, G., Herzke, D., Lohmann, R., Miller, M., Ng, C.A., Scheringer, M., Vierke, L., Wang, Z., 2020. Strategies for grouping per- and polyfluoroalkyl substances (PFAS) to protect human and environmental health. Environ. Sci. Process Impacts. 22 (7), 1444–1460.
Croes, K., Colles, A., Koppen, G., Govarts, E., Bruckers, L., Van de Mieroop, E., Nelen, V., Covaci, A., Dirtu, A.C., Thomsen, C., Haug, L.S., Becher, G., Mampaey, M., Schoeters, G., Van Larebeke, N., Baeyens, W., 2012. Persistent organic pollutants (POPs) in human milk: a biomonitoring study in rural areas of Flanders (Belgium). Chemosphere 89 (8), 988–994.
Directive 2006/122/EC of the European parliament and of the council of 12 december 2006 amending for the 30th time council directive 76/769/EEC on the approximation of the laws, regulations and administrative provisions of the member states relating to restrictions on the marketing and use of certain dangerous substances and preparations (perfluorooctane sulfonates) (text with EEA relevance), CELEX1. Available online: http://op.europa.eu/en/publication-detail/-/publication/612a4b88-51dd-4a86-a3d0-22c33323a493/language-en (accessed on 28 December 2020).
Domingo, J.L., Ericson-Jogsten, I., Perelló, G., Nadal, M., Van Bavel, B., Kärrman, A., 2012a. Human exposure to perfluorinated compounds in Catalonia, Spain: contribution of drinking water and fish and shellfish. J. Agric. Food Chem. 60 (17), 4408–4415.
Domingo, J.L., Jogsten, I.E., Eriksson, U., Martorell, I., Perelló, G., Nadal, M., Bavel, B.V., 2012b. Human dietary exposure to perfluoroalkyl substances in Catalonia, Spain. Temporal trend. Food Chem. 135 (3), 1575–1582.
Domingo, J.L., Nadal, M., 2017. Per- and polyfluoroalkyl substances (PFASs) in food and human dietary intake: a review of the recent scientific literature. J. Agric. Food Chem. 65 (3), 533–543.
ECHA (European Chemicals Agency), 2019. Candidate list of substances of very high concern for authorisation. Available online: https://echa.europa.eu/candidate-list-table, 28 December 2020.
EFSA European Food Safety Authority, 2020. Risk to human health related to the presence of perfluoroalkyl substances in food. Scientific opinion of the panel on contaminants in the food chain. EFSA J 18 (9), 6223.
Fenton, S.E., Ducatman, A., Boobis, A., DeWitt, J.C., Lau, C., Ng, C., Smith, J.S., Roberts, S.M., 2020. Per- and polyfluoroalkyl substance toxicity and human health review: current state of knowledge and strategies for informing future research. Environ. Toxicol. Chem. 40 (3), 606–630.
Franco, J., Meade, B.J., Frasch, H.F., Barbero, M., Anderson, S.E., 2012. Dermal penetration potential of perfluorooctanoic acid (PFOA) in human and mouse skin. J. Toxicol. Health, Part A. 75 (1), 50–62.
Fromme, H., Tittlemier, S.A., Völkel, W., Wilhelm, M., Twardella, D., 2010. Perfluorinated comounds-exposure assessment for the general population in western countries. Int. J. Hyg Environ. Health 212, 239–270.
Fujii, Y., Yan, J., Harada, K.H., Hitomi, T., Yang, H., Wang, P., Koizumi, A., 2012. Levels and profiles of long-chain perfluorinated carboxylic acids in human breast milk and infant formulas in East Asia. Chemosphere 86 (3), 315–321.
Fujii, Y., Harada, K.H., Koizumi, A., 2013. Occurrence of perfluorinated carboxylic acids (PFCAs) in personal care products and compounding agents. Chemosphere 93, 538–544.
Glüge, J., Scheringer, M., Cousins, I.T., DeWitt, J.C., Goldenman, G., Herzke, D., Lohmann, R., Ng, C.A., Trier, X., Wang, Z., 2020. An overview of the uses of per- and polyfluoroalkyl substances (PFAS). Environ. Sci. Process Impacts 22 (12), 2345–2373.
Grandjean, P., 2018. Delayed discovery, dissemination, and decisions on intervention in environmental health: a case study on immunotoxicity of perfluorinated alkylate substances. Environ. Health 17 (1), 62.
Haug, L.S., Huber, S., Becher, G., Thomsen, C., 2011. Characterisation of human exposure pathways to perfluorinated compounds–comparing exposure estimates with biomarkers of exposure. Environ. Int. 37 (4), 687–693.
Hu, L., Luo, D., Wang, L., Yu, M., Zhao, S., Wang, Y., Mei, S., Zhang, G., 2021. Levels and profiles of persistent organic pollutants in breast milk in China and their potential health risks to breastfed infants: a review. Sci. Total Environ. 753, 142028.
Iribarne-Duran, L.M., Artacho-Cordon, F., Peña-Caballero, M., Molina-Molina, J.M., Jiménez-Díaz, I., Vela-Soria, F., Serrano, L., Hurtado, J.A., Fernández, M.F., Freire, C., Olea, N., 2019. Presence of bisphenol A and parabens in a Neonatal Intensive Care Unit: an exploratory study of potential sources of exposure. Environ. Health Perspect. 127 (11), 117004.
Jensen, T.K., Andersen, L.B., Kyhl, H.B., Nielsen, F., Christesen, H.T., Grandjean, P., 2015. Association between perfluorinated compound exposure and miscarriage in Danish pregnant women. PloS One 10, e0123496.
Jin, H., Mao, L., Xie, J., Zhao, M., Bai, X., Wen, J., Shen, T., Wu, P., 2020. Poly- and perfluoroalkyl substance concentrations in human breast milk and their associations with postnatal infant growth. Sci. Total Environ. 713, 136417.
Kang, H., Choi, K., Lee, H.S., Kim, D.H., Park, N.Y., Kim, S., Kho, Y., 2016. Elevated levels of short carbon-chain PFCAs in breast milk among Korean women: current status and potential challenges. Environ. Res. 148, 351–359.
Kannan, K., Corsolini, S., Fillmann, G., Kumar, K.S., Loganathan, B.G., Mohd, M.A., Olivero, J., Van Wouwe, N., Yang, J.H., Aldous, K.M., 2004. Perfluorooctane sulfonate and related fluorochemicals in human blood from several countries. Environ. Sci. Technol. 38, 4489–4495.
Kärrman, A., Domingo, J.L., Llebaria, X., Nadal, M., Bigas, E., van Bavel, B., Lindström, G., 2010. Biomonitoring perfluorinated compounds in Catalonia, Spain: concentrations and trends in human liver and milk samples. Environ. Sci. Pollut. Res. Int. 17, 750–758.
Kashino, I., Sasaki, S., Okada, E., Matsuura, H., Goudarzi, H., Miyashita, C., Okada, E., Ito, Y.M., Araki, A., Kishi, R., 2020. Prenatal exposure to 11 perfluoroalkyl substances and fetal growth: a large-scale, prospective birth cohort study. Environ. Int. 136, 105355.
Kim, S.-K., Lee, K.T., Kang, C.S., Tao, L., Kannan, K., Kim, K.-R., Kim, C.-K., Lee, J.S., Park, P.S., Yoo, W.Y., Ha, J.Y., Shin, Y.-S., Lee, J.-H., 2011. Distribution of perfluorochemicals between sera andmilk from the same mothers and implications for prenatal and postnatal exposures. Environ. Pollut. 159, 169–174.
Lee, S., Kim, S., Park, J., Kim, H.J., Choi, G., Choi, S., Kim, S., Kim, S.Y., Kim, S., Choi, K., Moon, H.B., 2018. Perfluoroalkyl substances (PFASs) in breast milk from Korea: time-course trends, influencing factors, and infant exposure. Sci. Total Environ. 612, 286–292.
Lehmann, G.M., LaKind, J.S., Davis, M.H., Hines, E.P., Marchitti, S.A., Alcala, C., Lorber, M., 2018. Environmental chemicals in breast milk and formula: exposure and risk assessment implications. Environ. Health Perspect. 126 (9), 96001.
Lewis, R.C., Johns, L.E., Meeker, J.D., 2015. Serum biomarkers of exposure to perfluoroalkyl substances in relation to serum testosterone and measures of thyroid function among adults and adolescents from NHANES 2011-2012. Int. J. Environ. Res. Publ. Health 12 (6), 6098–6114.
Lien, G.W., Huang, C.C., Wu, K.Y., Chen, M.H., Lin, C.Y., Chen, C.Y., Hsieh, W.S., Chen, P.C., 2013. Neonatal-maternal factors and perfluoroalkyl substances in cord blood. Chemosphere 92, 843–850.
Liew, Z., Luo, J., Nohr, E.A., Bech, B.H., Bossi, R., Arah, O.A., Olsen, J., 2020. Maternal plasma perfluoroalkyl substances and miscarriage: a nested case-control study in the Danish National Birth Cohort. Environ. Health Perspect. 128 (4), 47007.
Liu, J., Li, J., Zhao, Y., Wang, Y., Zhang, Y., Lei, Z., Wu, Y., 2010. The occurrence of perfluorinated alkyl compounds in human milk from different regions of China. Environ. Int. 36, 433–438.
Llorca, M., Farré, M., Picó, Y., Teijón, M.L., Alvarez, J.C., Barceló, D., 2010. Infant exposure of perfluorinated compounds: levels in breast milk and commercial baby food. Environ. Int. 36, 584–592.

Lopez-Espinosa, M.J., Mondal, D., Armstrong, B., Bloom, M.S., Fletcher, T., 2012. Thyroid function and perfluoroalkyl acids in children living near a chemical plant. Environ. Health Perspect. 120, 1036–1041.
Lorenzo, M., Farré, M., Blasco, C., Onghena, M., Picó, Y., Barceló, D., 2016. Perfluoroalkyl substances in breast milk, infant formula and baby food from Valencian community (Spain). Environ. Nanotechnol. Monit. Manage. 6, 108–115.
Luo, Z., Shi, X., Hu, Q., Zhao, B., Huang, M., 2012. Structural evidence of perfluorooctane sulfonate transport by human serum albumin. Chem. Res. Toxicol. 25 (5), 990–992.
Macheka-Tendenguwo, L.R., Olowoyo, J.O., Mugivhisa, L.L., Abafe, O.A., 2018. Per- and polyfluoroalkyl substances in human breast milk and current analytical methods. Environ. Sci. Pollut. Res. 25, 36064–36086.
Mondal, D., Hernandez Weldon, R., Armstrong, B.G., Gibson, L.J., Lopez-Espinosa, M.J., Shin, H.M., Fletcher, T., 2014. Breast feeding: a potential excretion route for mothers and implications for infant exposure to perfloroalkyl acids. Environ. Health Perspect. 122 (2), 187–192.
Motas Guzmán, M., Clementini, C., Pérez-Cárceles, M.D., Rejón, S.J., Cascone, A., Martellini, T., Guerranti, C., Cincinelli, A., 2016. Perfluorinated carboxylic acids in human breast milk from Spain and estimation of infant's daily intake. Sci. Total Environ. 544, 595–600.
Nian, M., Luo, K., Luo, F., Aimuzi, R., Huo, X., Chen, Q., Tian, Y., Zhang, J., 2020. Association between prenatal exposure to PFAS and fetal sex hormones: are the short-chain PFAS safer? Environ. Sci. Technol. 54 (13), 8291–8299.
Nyberg, E., Awad, R., Bignert, A., Ek, C., Sallsten, G., Benskin, J.P., 2018. Inter-individual, inter-city, and temporal trends of per- and polyfluoroalkyl substances in human milk from Swedish mothers between 1972 and 2016. Environ. Sci. Process Impacts 20 (8), 1136–1147.
Olsen, G.W., Chang, S.-C., Noker, P.E., Gorman, G.S., Ehresman, D.J., Lieder, P.H., Butenhoff, J.L., 2009. A comparison of the pharmacokinetics of perfluorobutanesulfonate (PFBS) in rats, monkeys, and humans. Toxicology 256, 65–74.
Osbaldiston, R., Mingle, L.A., 2007. Characterization of human milk donors. J. Hum. Lactation 23 (4), 350–357.
Perez, F., Nadal, M., Navarro-Ortega, A., Fabrega, F., Domingo, J.L., Barcelo, D., Farre, M., 2013. Accumulation of perfluoroalkyl substances in human tissues. Environ. Int. 59, 354–362.
Preston, E.V., Webster, T.F., Claus Henn, B., McClean, M.D., Gennings, C., Oken, E., Rifas-Shiman, S.L., Pearce, E.N., Calafat, A.M., Fleisch, A.F., Sagiv, S.K., 2020. Prenatal exposure to per- and polyfluoroalkyl substances and maternal and neonatal thyroid function in the Project Viva Cohort: a mixtures approach. Environ. Int. 139, 105728.
Regulation (EU) 2019/1021 of the European Parliament and of the Council of 20 June 2019 on Persistent Organic Pollutants..
Roosens, L., D'Hollander, W., Bervoets, L., Reynders, H., van Campenhout, K., Cornelis, C., van Den Heuvel, R., Koppen, G., Covaci, A., 2010. Brominated flame retardants and perfluorinated chemicals, two groups of persistent contaminants in Belgian human blood andmilk. Environ. Pollut. 158, 2546–2552.
Rylander, C., Sandanger, T.M., Froyland, L., Lund, E., 2010. Dietary patterns and plasma concentrations of perfluorinated compounds in 315 Norwegian women: the NOWAC Postgenome Study. Environ. Sci. Technol. 44, 5225–5232.
Schultes, L., Vestergren, R., Volkova, K., Westberg, E., Jacobson, T., Benskin, J.P., 2018. Per- and polyfluoroalkyl substances and fluorine mass balance in cosmetic products from the Swedish market: implications for environmental emissions and human exposure. Environ. Sci. Process Impacts 20 (12), 1680–1690.
Sinisalu, L., Sen, P., Salihović, S., Virtanen, S.M., Hyöty, H., Ilonen, J., Toppari, J., Veijola, R., Oresič, M., Knip, M., Hyötyläinen, T., 2020. Early-life exposure to perfluorinated alkyl substances modulates lipid metabolism in progression to celiac disease. Environ. Res. 188, 109864.
Street, M.E., Angelini, S., Bernasconi, S., Burgio, E., Cassio, A., Catellani, C., Cirillo, F., Deodati, A., Fabbrizi, E., Fanos, V., Gargano, G., Grossi, E., Iughetti, L., Lazzeroni, P., Mantovani, A., Migliore, L., Palanza, P., Panzica, G., Papini, A.M., Parmigiani, S., Predieri, B., Sartori, C., Tridenti, G., Amarri, S., 2018. Current knowledge on endocrine disrupting chemicals (EDCs) from animal biology to humans, from pregnancy to adulthood: highlights from a national Italian meeting. Int. J. Mol. Sci. 19 (6), 1647.
Sun, Q., Zong, G., Valvi, D., Nielsen, F., Coull, B., Grandjean, P., 2018. Plasma concentrations of perfluoroalkyl substances and risk of type 2 diabetes: a prospective investigation among U.S. women. Environ. Health Perspect. 126 (3), 037001.
Tao, L., Kannan, K., Wong, C.M., Arcaro, A.F., Butenhoff, J.L., 2008a. Perfluorinated compounds in human milk from Massachusetts. U.S.A. Environ. Sci. Tech. 42, 3096–3101.
Tao, L., Ma, J., Kunisue, T., Libelo, E.L., Tanabe, S., Kannan, K., 2008b. Perfluorinated compounds in human breast milk from several Asian countries, and in infant formula and dairy milk from the United States. Environ. Sci. Technol. 42, 8597–8602.
Thépaut, E., Dirven, H.A.A.M., Haug, L.S., Lindeman, B., Poothong, S., Andreassen, M., Hjertholm, H., Husøy, T., 2021. Per- and polyfluoroalkyl substances in serum and associations with food consumption and use of personal care products in the Norwegian biomonitoring study from the EU project EuroMix. Environ. Res. 195, 110795.
Thomsen, C., Haug, L.S., Stigum, H., Frøshaug, M., Broadwell, S.L., Becher, G., 2010. Changes in concentrations of perfluorinated compounds, polybrominated diphenyl ethers, and polychlorinated biphenyls in Norwegian breast-milk during twelve months of lactation. Environ. Sci. Technol. 44, 9550–9556.
Tittlemier, S.A., Pepper, K., Seymour, C., Moisey, J., Bronson, R., Cao, X.L., Dabeka, R. W., 2007. Dietary exposure of Canadians to perfluorinated carboxylates and perfluorooctane sulfonate via consumption of meat, fish, fast foods, and food items prepared in their packaging. J. Agric. Food Chem. 55 (8), 3203–3210.
Trudel, D., Horowitz, L., Wormuth, M., Scheringer, M., Cousins, I.T., Hungerbühler, K., 2008. Estimating consumer exposure to PFOS and PFOA. Risk Anal. 28 (2), 251–269.
Tyrrell, J., Melzer, D., Henley, W., Galloway, T.S., Osborne, N.J., 2013. Associations between socioeconomic status and environmental toxicant concentrations in adults in the USA: NHANES 2001-2010. Environ. Int. 59, 328–335.
UNEP Stockholm Convention, 2009. SC-4/17: Listing of Perfluorooctane Sulfonic Acid, its Salts and Perfluorooctane Sulfonyl Fluoride.
Vela-Soria, F., García-Villanova, J., Mustieles, V., de Haro, T., Antignac, J.P., Fernandez, M.F., 2021. Assessment of perfluoroalkyl substances in placenta by coupling salt assisted liquid-liquid extraction with dispersive liquid-liquid microextraction prior to liquid chromatography-tandem mass spectrometry. Talanta 221, 121577.
Vela-Soria, F., Serrano-López, L., García-Villanova, J., de Haro, T., Olea, N., Freire, C., 2020. HPLC-MS/MS method for the determination of perfluoroalkyl substances in breast milk by combining salt-assisted and dispersive liquid-liquid microextraction. Anal. Bioanal. Chem. 412 (28), 7913–7923.
Vestergren, R., Cousins, I.T., Trudel, D., Wormuth, M., Scheringer, M., 2008. Estimating the contribution of precursor compounds in consumer exposure to PFOS and PFOA. Chemosphere 73 (10), 1617–1624.
Vuong, A.M., Yolton, K., Xie, C., Dietrich, K.N., Braun, J.M., Webster, G.M., Calafat, A. M., Lanphear, B.P., Chen, A., 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.
Weaver, G., Bertino, E., Gebauer, C., Grovslien, A., Mileusnic-Milenovic, R., Arslanoglu, S., Barnett, D., Boquien, C.Y., Buffin, R., Gaya, A., Moro, G.E., Wesolowska, A., Picaud, J.C., 2019. Recommendations for the establishment and operation of human milk banks in Europe: a consensus statement from the European Milk Bank Association (EMBA). Front. Pediatr. 7, 53.
Whitworth, K.W., Haug, L.S., Baird, D.D., Becher, G., Hoppin, J.A., Skjaerven, R., Thomsen, C., Eggesbo, M., Travlos, G., Wilson, R., Longnecker, M.P., 2012. Perfluorinated compounds and subfecundity in pregnant women. Epidemiology 23 (2), 257–263.
World Health Organization (WHO) United Nations Children's Fund (UNICEF), 2003. Global strategy for infant and young child feeding. Geneva. Available online: http://whqlibdoc.who.int/publications/2003/9241562218.pdf. (Accessed 15 January 2021).