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Poly- and perfluoroalkyl substances (PFAS) exposure through infant feeding in early life

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ABSTRACT

Background and aims: Per- and polyfluoroalkyl substances (PFAS) are non-degradable, man-made-chemicals with an elimination half-life of multiple years, causing accumulation in the environment and humans with potential harmful effects. However, longitudinal PFAS levels in human milk, daily PFAS intake and the association with infant plasma PFAS levels have never been reported. We investigated longitudinal PFOA and PFOS levels in human milk and the daily PFAS intake through infant feeding in the first 3 months of life, the most important determinants and the correlation with PFAS plasma levels at age 3 months and 2 years.

Methods: In 372 healthy term-born Dutch infants, we determined PFOA and PFOS levels in human milk given at age 1 and 3 months, in 6 infant formula brands and in infant plasma at 3 months and 2 years, using liquid-chromatography-electrospray-ionization-tandem-mass-spectrometry(LC-ESI-MS/MS). We studied the associations between daily PFAS intake and predictive characteristics by multiple regression models.

Results: PFOA and PFOS levels in human milk decreased between 1 and 3 months after delivery, regardless whether breastfeeding was given exclusively(EBF) or in combination with formula feeding. PFOA and PFOS could not be detected in any formula feeding. Daily PFAS intake(ng/kg) was highest in EBF-infants. Higher amount of human milk, older maternal age, lower parity and first-time breastfeeding were associated with higher daily intake. Daily PFAS intake in early life was strongly correlated with PFAS plasma levels at age 3 months and 2 years($R = 0.642–0.875$, $p < 0.001$).

Conclusions: Human milk contains PFOA and PFOS, in contrast to formula feeding. Daily PFOA and PFOS intake in early life is highest in exclusively breastfed infants and it is highly correlated with infant's plasma levels throughout infancy. Our findings show that breastfeeding is an important PFAS exposure pathway in the first months of life, with unknown but potential adverse effects. Knowing the important health benefits of breastfeeding, our findings warrant more research about the health outcomes in later life.

1. Introduction

Endocrine disrupting chemicals (EDC's) are non-degradable chemicals, which can accumulate in humans. They interfere with endocrine processes and can cause adverse effects on perinatal, neurodevelopmental, metabolic and reproductive outcomes. Especially when exposure occurs during susceptible periods of human development, such as early life (Kahn et al., 2020). Multiple EDC's have been thoroughly studied, such as Bisphenol A and phthalates. However, human data about another class of EDC's, per- and polyfluoroalkyl substances (PFAS), are very limited.

PFAS are a group of >3000 man-made chemicals, produced since the 1950s. They are used in a variety of consumer and industrial products. Because of their water-, dirt- and grease-repellent quality, they are used in food-packing materials and non-stick coating in pans, among other things. PFAS can easily migrate into the environment. Most PFAS are not

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degradable and can spread through the air and water. They have a tendency to accumulate in humans, because of their very long elimination half-life up to 8.5 years (Bokkers et al., 2019; Zeilmaker et al., 2018)and they have known adverse effects in adults, such as lipid and insulin dysregulation, cancer and altered thyroid function (Fenton et al., 2021). Rodent studies show concerning effects in offspring that was exposed to high levels of PFAS during pregnancy or in early life. These adverse effects consist of a wide range of developmental effects, such as growth restriction, altered behavioral patterns and endocrine disruption (Zeilmaker et al., 2018; Johansson et al., 2009). Besides for a decreased vaccination response (Grandjean et al., 2017), infant studies to confirm or refute other adverse developmental effects are scarce and have conflicting conclusions, partly because of small study populations and short or even lack of follow-up periods (Starling et al., 2019; Braun, 2017; Averina et al., 2021).

In contrast to other EDC's, PFAS have an increased affinity with proteins in the human body. PFAS have been detected in human serum and cord blood, so PFAS can migrate from mother to child during the prenatal period (Pérez et al., 2013; Yang et al., 2016). Later in life, PFAS can be taken up by inhalation of dust or by ingestion of PFAS in drinking water and food, particularly from fish, fruit and eggs (Schrenk et al., 2020). To avoid potential adverse effects, the European Food Safety Authority (EFSA) lowered the tolerable weekly intake (TWI) through food in 2020 to 4.4 ng/kg for PFOA, PFOS, PFHxS and PFNA combined (Schrenk et al., 2020). However, data on PFAS exposure in infancy through infant feeding are very scarce. In infant formula feeding, PFAS levels are reported to be low, the mean level of 15 PFAS together being 2.47 ng/mL (Macheka et al., 2021). In human milk, divergent levels have been measured, with mean PFOA and PFOS levels ranging from 0.05–0.18 ng/mL to 0.05–0.32 ng/mL, respectively (Macheka-Tendenguwo et al., 2018). PFAS are probably transferred over the mammary epithelial membrane through both protein binding and direct membrane transport mechanisms (Macheka-Tendenguwo et al., 2018). The highest PFAS levels have been measured in human milk of primiparous woman who lactate for the first time, especially in highly industrialized countries (Zheng et al., 2021; Fiedler and Sadia, 2021). The influence of other factors, such as maternal diet and macronutrient composition of the milk have not been elucidated yet. The World Health Organization (WHO) recommends to exclusively breastfeed an infant for at least the first 6 months of life (World Health O, 2001). The Agency for Toxic Substances and Disease Registry (ATSDR) stated that based on the current scientific understanding the benefits of breastfeeding outweigh potential risks of PFAS exposure through breastmilk (ATSDR, 2021). However, longitudinal PFAS levels in human milk and daily PFAS intake through infant feeding and their association with infant plasma PFAS levels have never been reported in a large cohort of healthy infants.

We, therefore, evaluated PFAS levels in formula feeding and longitudinally in human milk and plasma in a large group of healthy infants aged 1 months, 3 months and 2 years. Secondly, we studied the intake of PFOA and PFOS per kilograms body weight per day through infant feeding and if maternal (dietary) characteristics and human milk macronutrients were associated with infant's daily PFOA and PFOS intake. Lastly, we investigated the correlation between daily PFAS intake and plasma levels during infancy. We hypothesized that PFAS levels in formula feeding would be lower compared to human milk and that the daily PFAS intake in early life is, therefore, highest in infants with exclusive breastfeeding. In addition, we hypothesized that PFAS intake through infant feeding would be correlated with infant's plasma levels at age 3 months and 2 years.

2. Material and methods

2.1. Subjects

This current study was embedded in the Sophia Pluto study, a birth cohort study in healthy infants, aiming to provide detailed data on body composition trajectories and determinants from early life to childhood (Breij et al., 2015; de Fluiter et al., 2020). Infants were recruited from several maternity wards in the Rotterdam area, The Netherlands. All participants met the following inclusion criteria: born singleton and term (≥37 weeks of gestation). Exclusion criteria were: known congenital or postnatal disease or intrauterine infection that could interfere with growth, severe asphyxia (defined as an Apgar-score below 3 after 5 min), sepsis or the need for respiratory ventilation, and any maternal disease or medication use that could interfere with fetal growth, including maternal corticosteroids and diabetes mellitus. For this sub-study, infants with blood samples at 3 months and/or at 2 years were included. The Medical Ethics Committee of Erasmus Medical Center approved the study and written informed consent was given by all parents or caregivers with parental authority.

2.2. Data collection and measurements

Study visits were scheduled at age 1, 3, 6, 9, 12, 18 months and 2 years. Birth data were taken from hospital and midwife records. Maternal characteristics were obtained from hospital and midwife records, standardized interviews and questionnaires. Maternal dietary characteristics were obtained by standardized food questionnaires. Details about diet during pregnancy were obtained during the first study visit, when the infant was 1 month old. Dietary habits were collected at the 3 month visit. Information about infant feeding type was recorded during every visit via questionnaires. Exclusive breast feeding (EBF) was defined as receiving solely breastfeeding until at least the age of 3 months. Exclusive formula feeding (EFF) was defined as starting solely infant formula before the age of 1 month. Mix feeding (mix) was defined as starting with formula feeding between 1 and 3 months of age. Living area at birth was based on zip codes.

2.3. Sample collections

Between 2013 and 2018, a total of 124 and 133 human milk samples were collected at infant's age 1 and 3 months, respectively. Ninety-nine mothers collected human milk samples on both time points. Mothers were instructed to collect hindmilk, immediately store the sample at −20 °C and transport the sample to the study location in an isolated bag containing cooling elements. During the study visits, a total of 276 and 310 infant blood samples were collected at age 3 months and 2 years, respectively. Of 267 children, blood was collected at both time points. Capillary blood was collected in EDTA microtubes (BD Microtainer®) from the infant's toe or finger. Blood was then centrifuged at 4 °C, after which plasma was separated and frozen immediately. All human milk and blood samples were stored at −80 °C until analysis.

2.4. Infant formula

PFAS analysis was also performed in standard formula for infants aged 0–6 months of the 6 most-used brands in The Netherlands; *Nutrilon, Hero, Kruidvat, Etos, Albert Heijn* and *Holle* (biologic goat's milk). Thirty mL of each brand of formula was prepared twice according to the enclosed prescription, with bottled water and tap water.

2.5. PFAS analysis

PFAS analysis took place at the Diagnostic Laboratory of Endocrinology, an ISO-15189 certified laboratory, at the Erasmus University Medical Center in Rotterdam, The Netherlands.

2.5.1. Sample preparation

The PFAS plasma levels were measured in human milk, infant formula, and EDTA-plasma. 500 μl of milk, calibrator or QC was mixed with 1000 μl of methanol including the internal standard of each PFAS and 100 μl of 100% formic acid. Or 50 μl of plasma, calibrator or QC was

mixed with 100 μl of methanol including the internal standard of each PFAS and 150 μl of 50% formic acid. Thereafter, the samples were subjected to an offline solid phase extraction using a WAX μ-elution plate (Waters™, the Netherlands) which was conditioned with methanol and water. After loading of the samples the plate was washed with 5% methanol plus 10% formic acid for milk and 50% formic acid for plasma. The PFAS were eluted with 1% of ammonia in methanol and subsequently 1:1 diluted with 10 mM ammonium acetate before analysis.

2.5.2. LC-ESI-MS/Ms

After the sample preparation, the different PFAS were measured using liquid chromatography-electrospray-ionization tandem mass spectrometry (LC-ESI-MS/MS) (Acquity UPLC liquid chromatograph and a Xevo-TQ-S Mass Spectrometer (Waters™, the Netherlands)). An Acquity CSH Phenyl-Hexyl column was used for separation with a gradient utilizing 10 mM ammonium-acetate as solvent A and 10 mM ammonium acetate in methanol as solvent B. A 10-minute linear gradient was used with 50%A:50%B as initial condition, leading to 37% A:63%B after 10 min before re-equilibrating the column for the next injection. Mass spectrometer setting were: capillary voltage 1.00 kV in the negative mode, cone voltage 40 V, desolvation temperature 450 °C at a gasflow of 750 L/hr and cone gasflow of 200 L/hr. Argon was used as collison gas at a flowrate of 0.19 ml/min. The targeted PFAS were total and linear Perfluorooctane sulfonic acid (PFOS & LinPFOS), total and linear Perfluorooctanoic acid (PFOA & LinPFOA), total and linear Perfluorohexane sulfonic acid (PFHxS & LinPFHxS), Perfluorononanoic acid (PFNA) and Perfluorodecanoic acid (PFDA). Two mass transitions were used for each PFAS, whereby the result of samples with an ion-ratio deviating more than 10% from the mean ion-ratio were discarded. Quantification of the PFAS was performed using separate calibration curves and a ^{13}C-labeled internal standard for each PFAS and Masslynx software was used to determine the levels. Between run precision of the assay was ensured by using quality control samples in each batch of samples along with reagent blanks. Absolute- and relative matrix effects were negligible (<5%). The lower limit quantification (LLoQ) for PFOA and PFOS was set at 0.015 ng/mL in human milk and 0.15 ng/mL in infant plasma. Other PFAS could not be detected (levels < LLoQ) in (human) milk. Precision in plasma was between 1.9% and 2.9% for both tested PFAS. Precision in human milk was between 7.6% and 10.4% for both PFAS. If PFAS level was below the specific LLoQ, the plasma level was considered to be LLoQ/$\sqrt{2}$.

2.6. Human milk macronutrient analysis

Analysis of human milk macronutrients was performed in 180 samples at Erasmus University Medical Center in Rotterdam, The Netherlands (de Fluiter et al., 2021). Samples were warmed to 40 °C and homogenized using an ultrasonic processor (MIRIS, Uppsala, Sweden). Analysis of the concentrations of fat, carbohydrate, true protein and energy was performed in three-fold, using a Human Milk Analyzer (HMA, MIRIS, Uppsala, Sweden). All samples were analyzed with the same device by one person. The device was used, cleaned and calibrated according to the manufacturer's protocol. The intra-assay coefficients of variance (mean [95% confidence interval]) were 1.1[1.0–1.2]% for fat, 2.9[2.5–3.3]% for true protein, 1.7[1.1–2.3]% for carbohydrate and 1.1 [0.9–1.2]% for energy. Samples with protein values of < 0.5 g/100 ml were considered of poor quality and were excluded from further analysis. Samples were divided in tertiles based on containing 'high', 'middle' and 'low' concentrations of macronutrients.

2.7. Daily intake

For each child, PFAS intake per kilogram body weight per day (daily PFAS intake), through infant feeding was calculated as the product of the concentration of PFAS (ng/mL) and the amount of infant feeding per day (mL) divided by the weight of the child (kg). When the amount of infant feeding was unknown, the average daily amount of infant feeding was assumed (150 ml/kg/day).

2.8. Statistical analysis

SD scores for birth length and birth weight were calculated to correct for AD and sex (Villar et al., 2014) and calculated by Growth Analyser RCT software https://growthanalyser.org. Baseline characteristics are expressed as mean (SD). Independent student's *t*-test was used to determine differences in the baseline characteristics. Not normally distributed values are expressed as median [interquartile range]. Mann-Whitney *U* test was used to determine differences in concentrations between high and low macronutrient content. Correlations between PFAS levels at age 1 and 3 months and between daily PFAS intake and infant plasma level were determined with Spearman's correlation coefficient.

Multiple linear regression analysis was used to determine associated maternal, child and intake characteristics. Daily PFAS intake (ng/kg/day) at 1 and 3 months were the dependent variables. Maternal characteristics were ethnicity, education level, living area at childbirth, fertility treatment, gestational age (GA), parity, delivery mode, maternal age at birth, maternal BMI before pregnancy, history and total duration of breastfeeding in a previous child, dietary habits during pregnancy (amount of vegetables, fruit, meat, fish and eggs per week) and human milk macronutrients (fat, protein and carbohydrates). Child and intake variables were sex and amount of formula feeding at age 1 and 3 months. Using backward elimination, non-significant characteristics were removed from the model. As the sample collection date was a non-significant characteristic for daily PFAS intake, the collection date was not included in the models.

Repeated measures, ANOVA analysis and Mann-Whitney U tests were used to determine differences between infant feeding types.

All statistical tests were performed with SPSS statistical package version 25.0 (SPSS Inc. Chicago, Illinois). Tests were performed two-sided and results were regarded statistically significant if the p-value was < 0.05.

3. Results

Infant characteristics are presented in Table 1. Of all infants, 57.7 % was male and 68.8% Caucasian. Besides a small difference in AD, there were no differences in clinical characteristics between infants with exclusive breast-, formula or mixed feeding.

3.1. PFAS levels in infant feeding

Median [IQR] human milk of PFOA and PFOS are presented in Table 2. Other targeted PFAS could not be detected in human milk (levels < LLoQ). At age 1 month, the median [IQR] PFOA and PFOS levels in human milk were 0.043 [0.03–0.06] ng/mL and 0.035 [0.02–0.05] ng/mL, respectively. PFOA and PFOS levels decreased to 0.036 [0.03–0.05] ng/mL and 0.031 [0.02–0.04] ng/mL at age 3 months, respectively (all $p < 0.001$). The total level of PFOA consisted almost entirely of the linear isomer, while of the total PFOS 2/3 consisted of the linear isomers. There were no differences in PFAS levels between boys and girls (data not shown).

At age 1 and 3 months, PFOA and PFOS levels in human milk were strongly correlated, $R = 0.872$ and $R = 0.849$, respectively (both, $p < 0.001$). Furthermore, the PFAS levels in human milk were not different whether breastfeeding was given exclusively or in combination with formula feeding (mixed feeding) (Fig. 1A).

We also analyzed PFAS levels in the 6 most commonly used infant formula brands, but all PFAS levels were below the LLoQ of 0.015 ng/mL, so PFOA and PFOS could not be measured in any of the infant formula brands. There was no difference in PFAS levels when the milk was prepared with bottled or tap water (data not shown).

Table 1
Clinical characteristics.

	EBF	Mix	EFF	p-value
N	150	125	97	
Maternal characteristics				
Ethnicity N(%)				0.854
Caucasian	101 (67.3%)	82 (65.6%)	72 (74.2%)	
Black	10 (6.7)	11 (8.8%)	6 (6.2%)	
Asian	1 (0.7%)	1 (0.8%)	1 (1.0%)	
Latin-American	1 (0.7%)	0	1 (1.0%)	
Other & mixed	33 (22.0%)	27 (21.6%)	13 (13.4%)	
Missing	4 (2.7%)	4 (3.2%)	4 (4.1%)	
Delivery mode N(%)				0.726
Vaginal	111 (74.0%)	84 (67.2%)	65 (67.0%)	
Caesarian section	38 (25.3%)	40 (32.0%)	31 (32.0%)	
Missing	1 (0.7%)	1 (0.8%)	1 (1.0%)	
Primiparous N(%)				0.052
Yes	83 (55.3%)	84 (67.2%)	51 (52.6%)	
No	67 (44.7%)	41 (32.8%)	46 (47.4%)	
First-time breastfeeding N (%)				0.074
Yes	83 (55.3%)	84 (67.2%)	57 (58.8%)	
No	62 (41.3%)	37 (29.6%)	26 (26.8)	
Missing	5 (3.3%)	4 (3.2%)	14 (14.4%)	
Child characteristics				
GA (weeks)	39.87 (1.16)	39.60 (1.23)	39.40 (1.30)	**0.011**
Sex N(%)				0.729
Boys	83 (55.3%)	74 (59.2%)	58 (59.8%)	
Girls	67 (44.7%)	51 (40.8%)	39 (40.2%)	
Birth weight SDS	0.26 (1.08)	0.16 (1.17)	0.32 (1.15)	0.569
Birth length SDS *	0.78 (1.10)	0.51 (1.31)	0.54 (1.23)	0.111

Data expressed as mean (SD). *Available in 92 EBF-, 62 mix- and 56 EFF-infants. Abbreviations: GA = gestational age, EBF = exclusive breastfeeding until age 3 months, EFF = exclusive formula feeding until age 3 months, Mix: mixed feeding until age 3 months, N = number of subjects, SDS = standard deviation score.

In plasma, median [IQR] PFAS levels at age 3 months and 2 years are presented in Table 2. Since PFOA and PFOS and other PFAS could not be measured in formula feeding, only analyses with plasma levels of EBF- and mixed fed infants are presented from this point onwards. Median [IQR] plasma levels of PFOA and PFOS at age 3 months were 3.080 [1.97–4.44] ng/mL and 1.829 [1.26–2.89] ng/mL, respectively. PFOA and PFOS levels decreased to 2.360 [1.57–3.28] ng/mL and 1.667 [1.04–2.34] ng/mL at age 2 years (all $p < 0.001$). There were no differences in PFAS plasma levels between boys and girls.

3.2. Human milk macronutrients

Human milk samples with protein content in the highest tertile, had higher PFOA levels compared to samples with protein content in the lowest tertile ($p < 0.01$) (Fig. 2). PFOA and PFOS levels were not significantly different between samples containing low and high fat or carbohydrate concentrations.

3.3. Daily PFAS intake

Intake per kg per day (daily intake) of PFOA and PFOS was highest in EBF-infants (Fig. 1B). At age 1 month, estimated marginal mean daily intake of PFOA and PFOS was, 7.56 ng/kg and 5.72 ng/kg, respectively, which declined to 6.08 ng/kg and 5.21 ng/kg, respectively, at age 3 months.

In formula feeding, PFOA and PFOS could not be detected, so daily PFAS intake through formula was negligible.

In infants with mixed feeding, the mean (SD) daily formula intake increased from 36.36 (57.6) ml/kg at age 1 month, to 109.8 (56.0) ml/kg at age 3 months. As a result of the relatively higher intake of formula compared to human milk, the estimated marginal mean daily PFOA and PFOS intake in mixed fed infants decreased, from 6.54 ng/kg and 4.49 ng/kg, respectively, at age 1 month to 1.86 ng/kg and 1.33 ng/kg, respectively, at age 3 months.

Table 2
PFAS levels in human milk and infant plasma during infancy.

	PFOA	*LinPFOA*	PFOS	*LinPFOS*
Age 1 month				
Human Milk				
N	123	121	118	97
% Detected	99.2%	97.6%	95.2%	78.2%
Level (ng/mL)	0.043 [0.03–0.06]	0.041 [0.03–0.06]	0.035 [0.02–0.05]	0.023 [0.02–0.03]
Daily intake (ng/kg)	6.150 [4.67–9.15]	5.850 [4.35–8.81]	4.875 [3.24–6.90]	3.300 [1.59–4.50]
Age 3 months				
Human Milk				
N	129	127	123	101
% Detected	97.0%	95.5%	92.5%	75.9%
Level (ng/mL)	0.036 [0.03–0.05]	0.034 [0.03–0.05]	0.031 [0.02–0.04]	0.021 [0.02–0.03]
Daily intake (ng/kg)	4.950 [3.28–6.90]	4.650 [3.15–6.60]	4.053 [2.70–5.78]	2.550 [1.59–4.05]
Infant plasma*				
N	205	205	205	205
% Detected	100%	100%	100%	100%
Level (ng/mL)	3.080 [1.97–4.44]	3.020 [1.93–4.37]	1.829 [1.26–2.89]	0.931 [0.63–1.45]
Age 2 years				
Infant plasma*				
N	224	224	224	224
% Detected	100%	100%	100%	100%
Level (ng/mL)	2.360 [1.57–3.28]	2.306 [1.53–3.23]	1.667 [1.04–2.34]	0.769 [0.50–1.17]

Data are presented as median [IQR]. N = number of measurements above the lower limit of detection. % detected = amount of samples with PFAS level above the lower limit of detection. Abbreviations: PFOA = Total Perfluorooctanoic acid, LinPFOA = linear Perfluorooctanoic acid, PFOS = Total Perfluorooctane sulfonic acid, LinPFOS = linear Perfluorooctane sulfonic acid. * for detailed data on infant plasma levels (van Beijsterveldt et al., 2022).

3.4. Associations with daily intake

The results of multiple linear regression analysis are shown in Table 3. At age 1 month, the model explained 54.5% – 56.2% of variance in the daily PFAS intake. As formula did not contain PFAS, daily PFAS intake was inversely associated with the daily amount of formula, so when the infant received 100 ml of formula feeding in combination with breastfeeding, the daily PFOA and PFOS intake was respectively 0.9 and 0.7 ng/kg lower than in EBF-infants. This shows indirectly that a higher intake of breastfeeding is associated with a higher daily PFOA and PFOS intake. Daily PFOA and PFOS intake was highest in infants of primiparous mothers. With each previously born child, the daily PFOA and PFOS intake via human milk was respectively 1.005 and 0.577 ng/kg lower. For PFOS, daily intake increased also with increasing maternal age, when maternal age increased with 1 year, the daily PFOS intake of the infant increased with 0.086 ng/kg.

At age 3 months, the model explained 55.8%–62.5% of variance in the daily PFAS intake. Daily PFOA and PFOS intake was inversely associated with the daily amount of formula feeding. When the infant received 100 ml formula, the daily PFOA and PFOS intake was respectively 0.7 and 0.5 ng/kg lower compared to EBF-infants. Daily PFOA intake was higher in infants of mothers who had never breastfed a previous child. Also, infants born in the east of Rotterdam had a higher daily PFOA intake compared to infants born in the west of Rotterdam and daily PFOS intake was higher with increasing maternal age.

We found no associations between PFAS intake at age 1 and 3 months and maternal dietary habits, including consumption of fish and eggs.

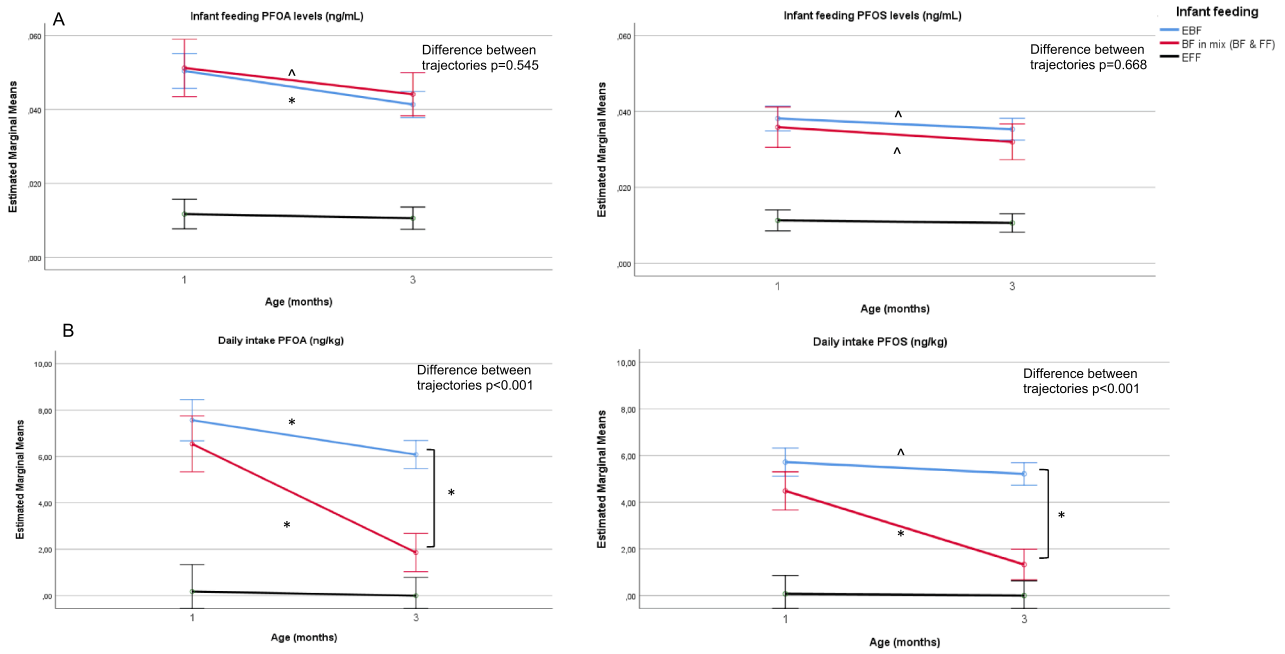

Fig. 1. PFAS in early life. A. Estimated marginal means with 95% confidence interval of human milk level of at age 1 and 3 months categorized by infant feeding type. B. Estimated marginal means with 95% confidence interval of daily PFOS and PFOA intake categorized by infant feeding type. Differences between infant feeding types at 1, 3 and Δ 1–3 months; * $p < 0.001$, ^ $p < 0.05$. Difference between trajectories is the differences between PFAS trajectories in infants with EBF and BF in mix between age 1–3 months. Abbreviations: PFOS = Total Perfluorooctane sulfonic acid and PFOA = Total Perfluorooctanoic acid, EBF = exclusive breastfeeding until age 3 months, EFF = exclusive formula feeding until age 3 months, EBF in mix: breast- and formula feeding until age 3 months.

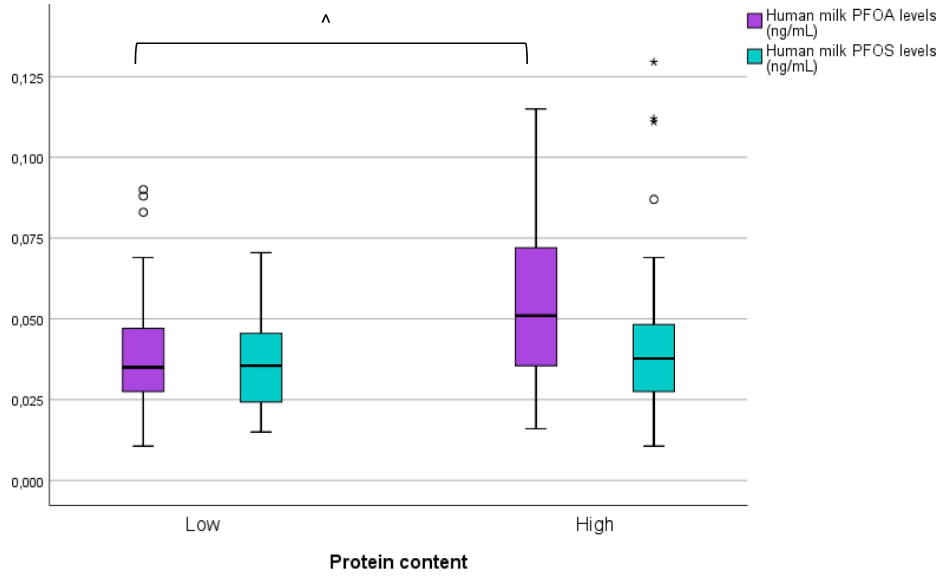

Fig. 2. Differences in PFAS levels in human milk samples with low and high protein content. ^= p-value < 0.01. PFOA = Total Perfluorooctanoic acid, PFOS = Total Perfluorooctane sulfonic acid.

3.5. Correlation with infant plasma levels

At age 3 months, daily PFOA and PFOS intake through breastfeeding was highly correlated with infant's plasma levels, with correlation coefficients of 0.836 and 0.875 (p-value < 0.001), respectively. Furthermore, daily PFOA and PFOS intake at age 3 months was also highly correlated with infant's plasma levels at age 2 years, R = 0.642 and 0.692 (p-value < 0.001), respectively.

4. Discussion

We present longitudinal daily PFAS intake through human milk and formula feeding in a large group of healthy Dutch infants. In human milk, both PFOA and PFOS levels decreased between 1 and 3 months after delivery and PFOA levels were higher in human milk with a higher protein content. The PFAS levels in human milk were not different whether breastfeeding was given exclusively or in combination with formula feeding (mixed feeding). As PFAS could not be detected in formula feeding, the daily PFAS intake (ng/kg) was significantly higher in infants with exclusive breastfeeding compared to infants with mixed

Table 3
Multiple linear regression analysis for daily PFAS intake by human milk at age 1 and 3 months.

	PFOA			*LinPFOA*			PFOS			*LinPFOS*		
	B ± SE	β	p-value	B ± SE	β	p-value	B ± SE	β	p-value	B ± SE	β	p-value
Age 1 month												
Amount of formula/day	−0.009 ± 0.00	−0.73	<0.001	−0.009 ± 0.00	−0.72	<0.001	−0.007 ± 0.00	−0.73	<0.001	−0.005 ± 0.00	−0.72	<0.001
Parity	−1.005 ± 0.26	−0.18	<0.001	−0.984 ± 0.26	−0.18	<0.001	−0.577 ± 0.20	−0.14	0.024	−0.349 ± 0.14	−0.13	0.040
Maternal age							0.086 ± 0.04	0.11	0.013	0.054 ± 0.03	0.10	0.012
Constant	9.057 ± 0.50			8.728 ± 0.50			3.663 ± 1.25			2.467 ± 0.86		
Adjusted R^2 (p-value)	0.562 (<0.001)			0.546 (<0.001)			0.566 (<0.001)			0.545 (<0.001)		
Age 3 months												
Amount of formula/day	−0.007 ± 0.00	−0.83	<0.001	−0.006 ± 0.00	−0.83	<0.001	−0.005 ± 0.00	−0.74	<0.001	−0.004 ± 0.00	0.77	<0.001
Living area	0.696 ± 0.33	0.09	0.037	0.674 ± 0.32	0.10	0.039						
Breastfeeding previous child	−1.316 ± 0.36	−0.17	<0.001	−1.337 ± 0.35	−0.18	<0.001						
Maternal age							0.088 ± 0.03	0.12	0.007	0.066 ± 0.02	0.13	0.005
Constant	5.561 ± 0.56			5.297 ± 0.55			2.293 ± 1.08			1.308 ± 0.77		
Adjusted R^2 (p-value)	0.625 (<0.001)			0.619 (<0.001)			0.586 (<0.001)			0.558 (<0.001)		

Results of multivariate linear regression. B ± SE = Unstandardized coefficient B (=the amount of change in daily PFAS intake due to a change of 1 unit of the independent variable) and Standard Error. β = standardized coefficient Beta (=the higher Beta, the stronger the effect of the independent variable on the daily PFAS intake). Amount of formula / day = amount of formula in ml per 24 h, breastfeeding of previous child: 0 = no, 1 = yes. Maternal age in years, weeks, living area: 1 = West Rotterdam, 2 = East Rotterdam, PFOA = Total Perfluorooctanoic acid, PFOS = Total Perfluorooctane sulfonic acid.

feeding or exclusive formula feeding at age 3 months. Older maternal age, lower parity, first-time breastfeeding and lower amount of formula feeding were associated with higher daily PFOA and PFOS intake. Daily PFAS intake in early life was strongly correlated with PFAS plasma levels at age 3 months and 2 years.

To our knowledge, we are the first to present longitudinal data of PFAS levels in human milk and their associations with longitudinal plasma levels in infancy. A few European studies reported cross-sectional data about PFAS levels in human milk, mostly using <50 pooled samples (Macheka-Tendenguwo et al., 2018). They reported divergent results in human milk with mean PFOA and PFOS levels ranging from 0.05–0.18 ng/mL to 0.05–0.32 ng/mL, respectively, which are higher compared to the levels we found. These study populations were, however, smaller compared to ours and consisted predominantly of samples collected between 1996 and 2010. Both PFOA and PFOS have been enlisted as Persistent Organic Pollutants (POPs) by the Stockholm convention in 2009 and, therefore, guidelines to phase out the production of PFOA and PFOS from industrial production lines in Europe have been set up (Schrenk et al., 2020). We have collected samples between 2013 and 2018, which could explain why we measured lower PFAS levels in human milk.

PFOA and PFOS could not be detected in 6 most commonly used Dutch brands of infant formula, indicating that levels were below 0.015 ng/mL. In The Netherlands, PFAS has never been measured in infant formula. Globally, only 2 research groups measured PFAS in infant formula and reported low levels (Macheka et al., 2021; Lankova et al., 2013), which is in line with our results. Our findings show that PFOA and PFOS exposure in infancy through infant formula, prepared with both tap and bottled water in The Netherlands, is negligible. Of course, this could be different when PFAS contaminated water is used for the preparation of infant formula.

Median daily PFOA and PFOS intake decreased between 1 and 3 months of age. EBF-infants had about 6–8 times higher daily intake compared to EFF-infants. Besides, we found that a higher amount of formula feeding, and thus a lower human milk intake, was associated with a lower daily PFOA and PFOS intake. This is in line with our previous finding that EBF-infants have 2–3 fold higher serum PFAS levels compared to EFF-infants up until the age of 2 years (van Beijsterveldt et al., 2022). We have now evidence that breastfeeding is a major contributor to PFAS exposure in early life. In 2020, EFSA revised their safety thresholds for PFAS intake by food for children and adults, to avoid potential adverse effects (Schrenk et al., 2020). By setting this threshold, the scarce data on pre- and postnatal transmission from mother to her child were taken into account and it was chosen to prevent a lower vaccination response in children (Grandjean et al., 2017; Abraham et al., 2020). Altogether, the tolerable intake for PFOA, PFOS, PFHxS and PFNA combined was assumed to be 4.4 ng/kg per week (Schrenk et al., 2020). Our findings show that the PFOA and PFOS intake via human milk is is >38.5 ng/kg per week, which is more than 7 times the tolerable weekly intake (TWI), as advised by the EFSA. This is concerning, because the first months of life are known to be a critical window for adiposity programming and a vulnerable period for the programming of growth and neurodevelopment. Our findings might, therefore, indicate that especially infants with exclusive breastfeeding in the first months of life are potentially more prone to not only decreased vaccination response (Grandjean et al., 2017; Kramer and Kakuma, 2012), but also other potential adverse developmental effects of PFAS in early life, such as growth restriction, increased adiposity, altered behavioral patterns and endocrine disruption as has been shown in rodent studies (Zeilmaker et al., 2018; Johansson et al., 2009). Such adverse effects would reduce the health benefits of breastfeeding, in terms of growth outcomes, protection against obesity, infections and allergies (World Health O, 2001; Kramer and Kakuma, 2012; Victora et al., 2016), but have not been sufficiently studied in infants and young children. Based on the recommendation of the World Health Organization to give exclusive breastfeeding for the first 6 months of life (World Health O, 2001), the current statement of the Agency for Toxic Substances and Disease Registry (ATSDR), that the benefits of breastfeeding most likely outweigh potential risks of PFAS exposure through breastmilk (ATSDR, 2021)and our present findings, it is crucial to study if early life PFAS exposure will result in adverse effects later in childhood.

Our results show that daily PFOA and PFOS intake in early life does not only correlate with infant's plasma levels at age 3 months, but also at age 2 years. This can be explained by the very long elimination half-life in humans, up to 3.8 years for PFOA and up to 5.4 years for PFOS (2, 3). Our findings show that not only breastfeeding is an important PFAS exposure pathway, but also that PFAS exposure in early life has lasting effect on plasma levels throughout infancy. This could have potentially

negative consequences throughout life, but further research to the effects of PFAS exposure in early life is needed.

Our findings show that PFOA, and not PFOS, levels are higher in milk with higher protein content. PFAS are known to bind to proteins, but, the exact mechanism of PFAS secretion in and excretion through breastfeeding is not well elucidated yet (10, 11). It has been postulated that transmission occurs via transfer over the mammary epithelial membrane through binding on protein, mainly albumin or via direct membrane transport mechanisms, because the chemical structure mimics fatty acids (Macheka-Tendenguwo et al., 2018). Our findings suggest that only PFOA and not PFOS excretion is associated with protein binding.

In contrast to PFOA, the linear PFOS isomer accounted for about 2/3 of the total PFOS levels. The only other research group who reported the level of linear PFOS in human milk also described a 2/3 contribution of the linear isomer to the total PFOS level (Lankova et al., 2013). This is in line with adult plasma, in which the contribution of linear to total PFOS levels is described to be around 59–68% (Kärrman et al., 2007)and the total PFOA plasma levels predominately consist of linear isomer (Lin et al., 2021). Since the ratio of linear to total PFOA and PFOS levels in both human milk and plasma in present study are similar compared to literature, our findings suggest that both the linear and branched isomers can be equally transported in and excreted by the mammary glands.

We found parity to be associated with infant PFOA and PFOS intake, with the highest daily intake through human milk of primiparous mothers and daily PFOA intake was higher when mothers had not breastfed a previous child. This is in line with literature, since it was described that primiparous mothers, who lactate for the first time have higher PFAS levels in their milk (Zheng et al., 2021; Fiedler and Sadia, 2021). Lastly, daily PFOS intake increased with increasing maternal age. Our findings suggest that older primiparous mothers have higher PFAS plasma levels and that these could lead to higher excretion through the mammary glands and subsequently to higher levels in human milk and thus higher daily infant PFAS intake.

Daily PFOA intake of infants born in the eastern area of Rotterdam was higher compared to infants born in the western area. Rotterdam is a large city, located near a large industrial and harbor region in The Netherlands. In Dordrecht, about 20 km south-east from Rotterdam, adult residents have higher median plasma levels of PFOA (10.2 ng/mL), compared to those in other regions (3.4 ng/mL) (van Poll et al., 2017), because of the local PFAS-producing chemical company, which produced PFOA until 2012. In a radius of 50 km around this company, PFOA remained present in higher levels in soil and groundwater compared to 150 other areas in The Netherlands (Wintersen et al., 2020). Our findings are likely the result of more PFAS accumulation in mothers living closer to the chemical company resulting in higher PFAS levels in their breastmilk.

Maternal dietary habits were not associated with daily PFAS intake of the infants. PFAS can be ingested by the consumption of several products, especially by fish, fruit, vegetables and eggs (Schrenk et al., 2020). Because PFOA and PFOS have a very long elimination half-life (Bokkers et al., 2019; Zeilmaker et al., 2018) and have different affinity to accumulate in organs and tissues (Pérez et al., 2013), dietary habits could possibly have long-term effects on PFAS levels in human milk. We did not find any association with maternal dietary habits, which might be explained by the fact that we could only determine dietary habits during pregnancy and the first months after delivery and lacked information about dietary habits before pregnancy, which could potentially influence PFAS levels in human milk.

The strengths of our study are the longitudinal PFAS analyses in both human milk and infant plasma in a large prospective cohort of healthy infants. PFAS analysis were performed using a high quality measuring method. We acknowledge some limitations. As the exact amount of human milk intake cannot be measured, we used an average human milk intake per day of 150 ml/kg as an estimation. Besides, we did not collect maternal blood samples, which could have given more detailed insight in the plasma to human milk transmission of PFAS, which could be of interest in future research.

In conclusion, human milk contains measurable PFOA and PFOS levels, but PFAS could not be detected in formula feeding. Daily PFAS intake was highest in EBF-infants of older primiparous women. Furthermore, daily PFAS intake in early life was highly correlated with infant's PFAS plasma levels throughout infancy. Our findings show that breastfeeding is an important exposure pathway in the first months of life. Knowing the important health benefits of breastfeeding, our findings warrant more research, particularly about the health outcomes in later life.

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Author contributions

AHK designed the study. IvB, KdF and AHK were in charge of the cohort, design, and collecting of the data and samples. BvZ and SvdB designed the laboratory method. BvZ conducted all PFAS analysis. KdF conducted all human milk macronutrient analysis. IvB performed the statistical analysis. Drafting the manuscript was primarily done by IvB under supervision of AHK. All authors were involved in writing the manuscript and had final approval of the submitted version.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: The Sophia Pluto study is an investigator-initiated cohort study, for which Prof. Dr. A.C.S. Hokken-Koelega received an independent research grant by Danone Nutricia Research. The sponsor had no role in the study design, collection, analysis or interpretation of the data, the writing of the manuscript or the decision to submit it for publication.

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