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Maternal serum concentrations of per- and polyfluoroalkyl substances and their predictors in years with reduced production and use

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ABSTRACT

Determining maternal concentrations of per- and polyfluoroalkyl substances (PFASs) and the relative impact of various demographic and dietary predictors is important for assessing fetal exposure and for developing proper lifestyle advisories for pregnant women.

This study was conducted to investigate maternal PFAS concentrations and their predictors in years when the production and use of several PFASs declined, and to assess the relative importance of significant predictors.

Blood from 391 pregnant women participating in The Northern Norway Mother-and-Child Contaminant Cohort Study (MISA) was collected in the period 2007–2009 and serum analyses of 26 PFASs were conducted. Associations between PFAS concentrations, sampling date, and demographic and dietary variables were evaluated by multivariate analyses and linear models including relevant covariates.

Parity was the strongest significant predictor for all the investigated PFASs, and nulliparous women had higher concentrations compared to multiparous women (10 ng/mL versus 4.5 ng/mL in median PFOS, respectively). Serum concentrations of PFOS and PFOA of women recruited day 1–100 were 25% and 26% higher, respectively, compared to those women recruited in the last 167 days of the study (day 601–867), and the concentrations of PFNA, PFDA and PFUnDA increased with age. Dietary predictors explained 0–17% of the variation in concentrations for the different PFASs. Significantly elevated concentrations of PFOS, PFNA, PFDA and PFUnDA were found among high consumers of marine food. The concentrations of PFHxS, PFHpS and PFNA were also increased in high consumers of game and elevated concentrations of PFHpS and PFOS were detected in high consumers of white meat. Study subjects with a high intake of salty snacks and beef had significantly higher concentrations of PFOA.

The present study demonstrates that parity, sampling date and birth year are the most important predictors for maternal PFAS concentrations in years following a decrease in production and use of several PFASs. Further, dietary predictors of PFAS concentrations were identified and varied in importance according to compound.

1. Introduction

Per- and polyfluoroalkyl substances (PFASs) are fluorinated aliphatic substances, widely used in consumer products like textiles, paper products and lubricants (Lehmler, 2005). The most studied compounds to date are perfluoroalkyl carboxylic acids (PFCAs), like perfluorooctanoate (PFOA) and perfluoroalkyl sulfonic acids (PFSAs), like perfluorooctane sulfonate (PFOS) (D'eon and Mabury, 2011; Martin et al., 2010). Several PFASs are persistent substances that have been directly emitted to the environment during their production and use (Prevedouros et al., 2006). PFASs have been produced since the 1950s with increasing intensities from 1966 to the 1990s. The production remained constant

Abbreviations: ANCOVA, analysis of covariance; BMI, body mass index; FFQ, food frequency questionnaire; FOSA, perfluorooctane sulphonamide; LOD, limit of detection; MISA, The Northern Norway Mother-and-Child Contaminant Cohort Study; PFAS, per- and polyfluoroalkyl substance; PFCA, perfluoroalkyl carboxylic acid; PFDA, perfluorodecanoate; PFDoDA, perfluorododecanoate; PFHpA, perfluoroheptanoate; PFHpS, perfluoroheptane sulfonate; PFHxS, pefluorohexane sulfonate; PFNA, perfluorononanoate; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate; PFSA, perfluoroalkyl sulfonic acid; PFUnDA, perfluoroundecanoate; PLS, partial least square; POP, persistent organic pollutant; SRM, standard reference material; UHPLC-MS/MS, ultra-high pressure liquid chromatography triple–quadrupole mass-spectrometry.

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from 1990 to 2000 until a phase-out was announced in 2000, resulting in a rapid drop of PFOS related compounds from the year 2002 (Paul et al., 2009).

Concerns about the persistence of PFASs in the environment, bioaccumulation potential and risk for toxicological effects in animals and humans have classified PFOS as a persistent organic pollutant (POP) (Stockholm convention, 2009). Restricted use of PFOS was implemented in Europe from June 2008 (European Parliament, 2006) and in the US in 2001 (Paul et al., 2009). In addition, the US launched the "PFOA Stewardship Program" (US EPA, 2006) where eight of the major PFOA-producing companies committed to reduce emissions of PFOA and related chemicals by 95% by 2010. In Norway, the ban of PFOA in Norwegian consumer products by 1st of June 2014 was recently announced (Miljøverndepartementet, 2013).

Following the regulatory initiatives, a decrease in PFOS and PFOA has been observed in humans in later years (Calafat et al., 2007; Glynn et al., 2012; Haug et al., 2009; Schroter-Kermani et al., 2012). Conversely, a decreasing time trend has not been observed for longer chained PFCAs (Buck et al., 2011). Still, there are concerns about potential human health effects of PFASs such as hormonal changes, hepatotoxicity, developmental toxicity and immunotoxicity (Grandjean and Budtz-Jorgensen, 2013; Grandjean et al., 2012; Lau et al., 2007). PFASs are transferred from the mother to the fetus via the placenta during pregnancy and from mothers milk postpartum (Liu et al., 2011). Fetuses and infants are thereby exposed to these compounds at critical developmental stages.

Diet is currently suspected to be the major on-going exposure route of PFASs for humans (Fromme et al., 2009; Haug et al., 2011a; Vestergren and Cousins, 2009). In addition, these chemicals are passed to humans through air, house dust, drinking water and water based beverages (Eschauzier et al., 2013; Haug et al., 2011a, 2011b; Ullah et al., 2011). Elevated concentrations of PFASs have been associated with consumption of marine food (Berger et al., 2009; Haug et al., 2010b; Rylander et al., 2009; Vestergren et al., 2012), but also to consumption of red meat, animal fat and snacks (Halldorsson et al., 2008; Haug et al., 2010a; Noorlander et al., 2011; Ostertag et al., 2009; Vestergren et al., 2012).

Cross-sectional population studies of polychlorinated biphenyls have demonstrated increasing concentrations with age that reflect birth year dependent past exposures due to time-variant emission (Alcock et al., 2000; Moser and McLachlan, 2002; Nost et al., 2013; Ritter et al., 2009). Similar relationship of individual exposures and to historic production and use could be expected for PFAS concentrations in the general population. We hypothesize that individual maternal PFAS exposures are largely influenced by variables such as sampling date, dietary habits, birth year, parity and breastfeeding. Further, concentrations of PFASs in maternal blood during pregnancy are relevant as indicator of the exposure experienced by the fetus (Verner et al., 2009). Therefore, the aims of the study were to investigate maternal PFAS concentrations and their predictors in years when production and use of several PFASs declined, and to assess the relative importance of significant predictors.

2. Materials and methods

2.1. Study participants and collection of blood samples

The selected subjects in the present study represent the 391 women who completed The Northern Norway Mother-and-Child Contaminant Cohort Study (MISA) which consists of 515 enrolled pregnant women, recruited from June 2007 to October 2009 (recruitment period; 867 days). All participants answered a detailed questionnaire about diet and lifestyle at enrolment, and donated a blood sample at three time points (around gestational week 20, 3 days after delivery and 6 weeks after delivery). Detailed information about the study group characteristics, ethical approvals, the food frequency questionnaire (FFQ), dietary calculations and the blood collection procedures have been reported elsewhere (Hansen et al., 2010; Veyhe et al., 2012). Blood samples donated at mean gestational week 18.6 (9–36) were analyzed for a variety of PFASs. Thirteen women did not complete the food frequency questionnaire adequately, thus the total number included in the statistical analyses was 378.

2.2. Chemical analyses

A total of 26 PFASs, thirteen PFCAs (C_4-C_{14}, C_{16}, C_{18}), six PFSAs (C_4-C_8, C_{10}), three phosphonic acids (C_6, C_8, C_{10}), three fluortelomer sulfonates (4:2, 6:2, 8:2) and one perfluroalkyl sulfonamide (C_8), were initially screened for in a sub-group of 50 serum samples. PFASs detected (>LOD) in more than 20% of the samples were further quantified in the remaining serum samples (N = 391). Analytes were determined in serum samples using sonication-facilitated liquid–liquid extraction, activated ENVI-carb clean-up (Powley et al., 2005) and analyzed by ultrahigh pressure liquid chromatography triple–quadrupole mass-spectrometry (UHPLC-MS/MS). The sample preparation, treatment and extraction were performed as described by Hanssen et al. (2013) except for the volumes used; 25 μL of an 0.1 ng/μL internal standard mixture was added to 0.25 mL serum before the addition of 1 mL methanol. 20 μL of a 0.1 ng/μL branched PFDA solution was added as the recovery standard. Prior to analysis, an aliquot of 100 μL extract was transferred to a vial and mixed with an equal amount of 2 mM aqueous ammoniumacetate (NH_4OAc, ≥99%, Sigma-Aldrich, St. Louis, MO, USA). The analytical method, reagents and instrumentation are described in detail by Hanssen et al. Briefly, 10 μL was injected on a Acquity UPLC HSS T3 column (2.1 × 100 mm, 1.8 μm) (Waters Corporation, Milford, MA, USA) coupled to an Acella 1250 UHPLC pump and a TSQ Vantage (Thermo Fisher Scientific Inc., Waltham, MA, USA). Details on compounds analyzed, analytical conditions, the parent ions, monitored transitions, collision energies and S-lens settings are provided in the supplemental material Table S1. Quantification was conducted using the LCQuan software from Thermo Scientific (Thermo Fisher Scientific Inc., Waltham, MA, USA; Version 2.6).

2.3. Quality control

Quantification of the contaminants was performed by the internal-standard addition method with isotope-labeled PFASs (Hanssen et al., 2013). Concentrations of PFASs in all samples were within the linear range of the instrument and the calibration curve. For each compound in the mass spectrometry analyses, a second mass transition served to confirm compound specificity. The quality of the analysis was assured through repetitive analysis of blank samples and reference samples. One standard reference material (SRM1957® from the National Institute of Standards and Technology, Gaithersburg, MD, USA; N = 31), one bovine serum blank and one water blank were prepared for each batch of 30 samples. Validation data (recoveries, LODs and linear regression values for the calibration curves) and analytical uncertainties for certified concentrations in SRMs are available in the supplemental material Tables S2, S3 and S4. Additionally, our laboratory participates in the Artic Monitoring and Assessment Programme ring test for POPs in human serum, an international comparison program, organized by Institut National de Santé Publique du Québec, Canada (Institut national de santé publique du Québec, 2014). Interlaboratory comparisons indicate that the uncertainties of our analysis are within ± 15–20% of the assigned values. The linear PFOS isomers was chromatographically separated from the branched isomers and quantified separately. The coelution of branched isomers (quantified as one peak) was not structurally elucidated but rather identified as eluting earlier than the linear PFOS as described elsewhere (Rylander et al., 2009). The internal standard for linear PFOS was used for quantification of the branched isomers as well (Arsenault et al., 2008). When

discussing PFOS results, it is the sum of linear and branched isomers unless otherwise is specified.

2.4. Statistical analyses

Statistical analyses were performed using SPSS statistic software, version 19 (IBM SPSS Inc. Chicago, IL, USA) with the partial least square (PLS) extension module (integration plug-in for Python). In statistical analyses, concentrations below LODs were replaced by LOD/√2 (Anda et al., 2007) and only compounds with detection frequencies above 80% were evaluated in statistical models. Different lifestyle variables (demographic, dietary and date of blood sampling) were evaluated as possible predictors of PFAS concentrations. Parity and total months of breastfeeding were highly correlated ($r = 0.92$, $p < 0.0001$) and as information about nursing was missing for 24 participants, only parity was selected in the statistical models, representing the number of child births (live born) and breastfeeding. The impact of breastfeeding on PFAS concentrations was studied in multiple linear regressions, adjusting for time passed since last breastfeeding period. Partial least square (PLS) regressions were used for data reduction and for selecting variables of specific interest, which were further studied using analysis of covariance (ANCOVA). For the latter analyses, the study group was divided into three or four groups (percentiles) according to their consumption of the selected dietary variables. Details on the statistical analyses, lifestyle variables and consumption groups are provided in the supplemental material, page 5 and Table S5. We conducted a sensitivity analysis by removing six potential outliers and applied the same statistical methods on the reduced data set; however, overall results did not change substantially, and hence all samples were included in the final models.

3. Results

Study population characteristics and dietary intake for the MISA study group are presented in the supplemental material Table S5.

3.1. PFAS concentrations in the study population

Serum concentrations of 10 PFASs with detection frequencies >16% are presented in Table 1. PFOS was the dominating compound followed by PFOA, PFNA, PFHxS, PFUnDA, PFDA and PFHpS. A total of 26 compounds were targeted in a sub-group of 50 samples, but the shortest chained PFSAs (C_4-C_5) and PFCAs (C_4-C_7), as well as the phosphonic acids and fluortelomer sulfonates, were not detected above LODs (supplemental material, Table S2) and therefore not calculated in the remaining 341 samples.

Table 1
Serum concentrations of PFASs (ng/mL) in the study group (N = 391).

Concentration (ng/mL)	Median	AM	Range	LOD	% > LOD
PFHxS	0.44	0.61	<LOD–14.8	0.06	99
PFHpS	0.10	0.12	<LOD–1.10	0.06	80
∑ PFOS	8.03	8.81	0.30–35.8	0.31	
PFOS Linear	4.66	5.10	<LOD–19.1	0.31	100
PFOS Branched	3.37	3.71	<LOD–18.2	0.14	100
% linear PFOS	59.0	59.1	36.0–80.0	N/A	
FOSA	N/A	N/A	<LOD–0.38	0.01	42
PFHpA	N/A	N/A	<LOD–0.45	0.03	16
PFOA	1.53	1.70	0.28–11.0	0.07	100
PFNA	0.56	0.67	0.15–4.36	0.04	100
PFDA	0.23	0.26	0.05–2.34	0.03	100
PFUnDA	0.26	0.30	0.03–1.46	0.02	100
PFDoDA	0.03	0.04	<LOD–0.20	0.03	50

AM, arithmetic mean; LOD, method detection limit; % > LOD, percentage of samples in which the analyte was detected; N/A, not available; PFHxS, pefluorohexane sulfonate; PFHpS, perfluoroheptane sulfonate; ΣPFOS, sum of branched and linear perfluorooctane sulfonate; % linear PFOS, percentage linear PFOS related to PFOS; FOSA, perfluorooctane sulfonamide; PFHpA, perfluoroheptanoate; PFOA, perfluorooctanoate; PFNA, perfluorononanoate; PFDA, perfluorodecanoate; PFUnDA, perfluoroundecanoate; PFDoDA, perfluorododecanoate.

3.2. Parity, breastfeeding and time related predictors

In the PLS regressions PFHxS, PFHpS, PFOS and PFOA co-varied and were associated with parity and sampling date, whereas PFNA, PFDA and PFUnDA co-varied and were associated with parity, age and body mass index (BMI). For further details on the PLS regressions, see supplemental material Figs. S1 and S2. Parity was the strongest significant predictor for all the investigated PFASs, demonstrating decreasing concentrations with increasing parity in ANCOVA models (Table 2 and Fig. 1). Additionally, investigating the association between the duration of breastfeeding and PFAS concentrations in multiple linear regressions demonstrated that total months of breastfeeding (exclusively and mixed breastfeeding) were significantly associated with serum concentrations of PFHpS, PFOS and PFOA, across parity groups. Indeed, concentrations decreased by 1.1% for PFHpS ($p = 0.006$), 0.9% for PFOS ($p = 0.005$) and 1.0% for PFOA ($p = 0.000$), per month of breastfeeding. The date of sampling was significantly associated with concentrations of PFHxS, PFHpS, PFOS, PFOA and PFNA. The mean decrease in PFOS and PFOA concentrations were 0.5 and 0.1 ng/mL per 100 days from the study start, respectively. The corresponding decreases for PFHxS, PFHpS and PFNA were 0.03, 0.003 and 0.01 ng/mL, respectively (Table 2). The investigation of the impact of recruitment date on PFAS concentrations included all women, yet conducting separate analyses for nulliparous and parous women (adjusted for time passed since last pregnancy) resulted in the same significant results (not presented). Age was significantly associated with concentrations of PFNA, PFDA and PFUnDA, and the increase for each year was 2%, 5% and 4%, respectively (Table 2). BMI was significantly associated with PFDA and PFUnDA and concentrations decreased by 1% and 4% for each unit increase in BMI, respectively (Table 2).

3.3. Dietary predictors

Based on the PLS results the following dietary variables were identified as relevant predictors of interest for PFAS: (i) A cluster of marine food and meat variables, salty snacks and berries for PFHxS, PFHpS and PFOS (supplemental material, Fig. S1); (ii) salty snacks and beef for PFOA (Fig. S1); and (iii) a cluster of marine food and meat variables, tea, berries and coffee for PFNA, PFDA and PFUnDA (supplemental material, Fig. S2). Including dietary variables while adjusting for significant demographic predictors and sampling date, in ANCOVA models (Table 3), increased the explained variation (0–17% R^2 change) for most compounds, compared to the initial models including only demographic predictors and sampling date (Table 2). The largest increase in explained variation by the models was observed for PFNA followed by PFUnDA > PFDA > PFHxS > PFHpS > PFOS and PFOA. The adjusted values demonstrated that high consumers of marine food had significantly elevated concentrations of PFOS (23% difference between the highest and the lowest intake groups, Table 3), PFNA (11%), PFDA (29%) and PFUnDA (41%). Also, high consumers of game had elevated concentrations of PFHxS (20% difference between the highest and the lowest intake groups), PFHpS (21%), and PFNA (16%), and for white meat, PFHpS (14%) and PFOS (15%). Further, high consumers of beef and salty snacks had 13% and 19% higher PFOA concentrations compared to low consumers, respectively. Although the PLS regressions indicated a significant positive association between salty snacks, vegetables, berries, tea and coffee, with several of the PFASs, the concentrations in the respective intake groups were not significantly different after adjusting for significant demographic predictors in ANCOVA models (results not presented).

Table 2
The effect of significant predictors on the concentration of selected PFASs. Parameter estimates[a], group differences, 95 % confidence intervals (CI) and p values.

		PFHxS[c]				PFHpS[c]				PFOS[c]				PFOA[c]			
Predictor	N	Ŷ	Diff	95 % CI	p	Ŷ	Diff	95 % CI	p	Ŷ	Diff	95 % CI	p	Ŷ	Diff	95 % CI	p
Number of children (Parity)[b]																	
0	150	**0.59**	-	-	-	**0.11**	-	-	-	**10.1**	-	-	-	**2.42**	-	-	-
1	135	0.37	−0.22	(−0.27, −0.15)	**0.00**	0.09	−0.02	(−0.03, −0.05)	**0.00**	7.30	−2.84	(−3.58, −2.00)	**0.00**	1.33	−1.01	(−1.13, −0.88)	**0.00**
2	69	0.34	−0.25	(−0.31, −0.18)	**0.00**	0.08	−0.03	(−0.05, −0.02)	**0.00**	6.18	−3.92	(−4.69, −3.05)	**0.00**	1.31	−1.22	(−1.34, −1.09)	**0.00**
3–4	24	0.24	−0.35	(−0.41, −0.27)	**0.00**	0.06	−0.05	(−0.06, -0.03)	**0.00**	4.53	−5.57	(−6.39, −4.57)	**0.00**	0.86	−1.47	(−1.59, −1.29)	**0.00**
Sampling date (per 100 days)		0.56	−0.03	(−0.05, −0.01)	**0.00**	0.11	−0.003	(−0.006, −0.001)	**0.01**	9.61	−0.49	(−1.91, −0.25)	**0.00**	2.30	−0.10	(−0.13, −0.03)	**0.00**
R^2 (%)				15				11				21				44	

		PFNA[d]				PFDA[e]				PFUnDA[e]			
Predictor	N	Ŷ	Diff	95% CI	p	Ŷ	Diff	95 % CI	p	Ŷ	Diff	95% CI	p
Number of children (Parity)[b]													
0	150	**0.47**	-	-	-	**0.20**	-	-	-	**0.25**	-	-	-
1	135	0.36	−0.11	(−0.17, −0.08)	**0.00**	0.16	−0.04	(−0.06, −0.02)	**0.00**	0.19	−0.06	(−0.08, −0.03)	**0.00**
2	69	0.34	−0.13	(−0.17, −0.08)	**0.00**	0.15	−0.05	(−0.07, −0.02)	**0.00**	0.20	−0.05	(−0.08, −0.02)	**0.00**
3–4	24	0.27	−0.20	(−0.24, −0.14)	**0.00**	0.13	−0.07	(−0.09, −0.04)	**0.00**	0.14	−0.11	(−0.14, −0.07)	**0.00**
Sampling date (per 100 days)		0.46	−0.01	(−0.02, −0.003)	**0.02**								
Age (Per year)		0.48	0.01	(0.001, 0.01)	**0.03**	0.21	0.01	(0.006, 0.01)	**0.00**	0.26	0.01	(0.01, 0.02)	**0.00**
BMI (Per unit)						0.20	−0.001	(−0.005, −0.001)	**0.03**	0.24	−0.01	(−0.01, −0.003)	**0.00**
[f]R^2 (%)				12				11				15	

[a] Predicted PFAS concentrations (Ŷ) from the respective ANCOVA models. Predicted concentrations are back-transformed from log estimates and are expressed in units of ng/mL.
[b] The estimated change in PFASs in ng/mL across the number of previous born children with 0 previous births as reference group.
[c] Sampling date is included as a covariate in the model. Parity is included as a fixed factor.
[d] Sampling date and age are included as covariates in the model. Parity is included as a fixed factor.
[e] Age and BMI are included as a covariates in the model. Parity is included as a fixed factor.
[f] R^2 = The proportion of variation in the concentrations of the contaminant which is explained by the model.

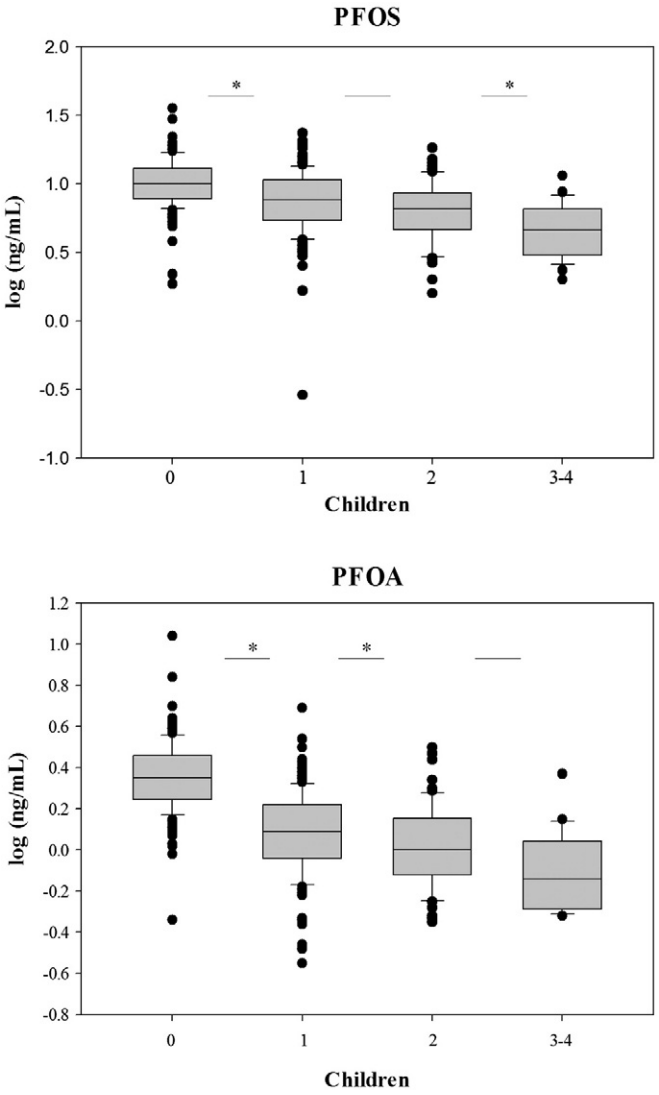

Fig. 1. Concentrations of the two most prevalent PFASs in serum of pregnant women according to parity: 0 (N = 150); 1 (N = 135); 2 (N = 69); 3–4 (N = 24). Asterisk denotes significant difference between parity groups ($p < 0.05$, pairwise comparisons: Bonferroni).

4. Discussion

This study investigates a wide range of PFASs and their lifestyle predictors in a cohort of pregnant women from Northern Norway enrolled in the period 2007–2009. Considering the decline observed in human serum since the early 2000s (Glynn et al., 2012; Haug et al., 2009; Kato et al., 2011; Okada et al., 2013; Olsen et al., 2012; Schroter-Kermani et al., 2012), the measured concentrations in the present study are low. Mean serum concentrations of PFOS, PFOA, PFNA, PFDA and PFUnDA in primiparous women in the present study were in the same range as those measured in primiparous women in Sweden in the years 2006–2008 (Glynn et al., 2012). Concentrations of PFHxS, PFOS and PFOA were lower compared to those in plasma from Norwegian pregnant women sampled in 2003–2004 (medians: 8.03 ng/mL versus 13.0 ng/mL for PFOS, respectively), whereas the PFNA concentrations were higher (Brantsaeter et al., 2013). The internal validity of the dietary information is considered good based on previous validation of the FFQ (Hjartaker et al., 2007) and intake of total energy and micronutrients are comparable to those in similar age groups in the Norwegian population (Helsedirektoratet, 2013; Veyhe et al., 2012). Overall results indicated that parity explained variation in concentrations for all PFASs, while other lifestyle variables were of varying importance according to compound.

4.1. Parity and breastfeeding

Our results underline that information on the number of child births and breastfeeding is important in the evaluation of PFAS concentrations in women. In the current study, nulliparous women had higher concentrations of all PFASs, compared to multiparous women. Similar results were observed in another study in Norway, where parity was the determinant with the largest influence on maternal PFAS concentrations (Brantsaeter et al., 2013). Serum concentrations decreased (range 1.1–2.8 ng/mL for PFOS) with increasing parity for all PFASs. In this study we could not separate the effect of breastfeeding from parity as a predictor of PFASs. Still, elimination through breast milk is believed to be greater than the transference to the fetus prenatally, based on the properties of the placenta barrier (Kim et al., 2011). Indeed, infant serum concentrations of PFHxS, PFOS and PFOA increased during breastfeeding and those of PFOA were 4.6-fold higher compared to maternal serum 6 months after birth (Fromme et al., 2010).

As several PFASs declined rapidly from the year 2002 (Glynn et al., 2012), women giving birth before the year 2002 would transfer more PFAS to their child in the gestational period and through lactation, compared to women giving birth in e.g. 2007. Harmoniously, the decrease in maternal serum PFOA concentrations per month of breastfeeding in the present study was 1%, while a corresponding decrease of 2.5% was reported in a different population of pregnant women from Norway sampled in 2003–2004 (Brantsaeter et al., 2013). These observations are in accordance with studies on the time trend of the internal exposure in general populations, which show a decrease in PFOS and PFOA blood concentrations after 2003 (Harada et al., 2004, 2005; Inoue et al., 2004; Olsen et al., 2003, 2005). This means that the relative importance of parity as predictor likely differs in pre- and post-ban periods, which is in line with observations done by Ode et al. (2013) where parity was not identified as a predictor of PFOS and PFNA concentrations in the time period 1978–2001.

4.2. Date of sampling

The date of sampling was a significant predictor of PFHxS, PFHpS, PFOS, PFOA and PFNA concentrations in the present study, where concentrations declined throughout a recruitment period of 867 days. For PFOS and PFOA, the concentrations of women recruited day 1–100 were 25% and 26% higher, respectively, compared to those women recruited in the last days of the study (601–867 days from study start). Corresponding decrease in PFHxS, PFHpS and PFNA concentrations were less pronounced. The decrease in concentrations during the study period are in line with reported temporal trends of the subsequent compounds (Haug et al., 2009; Kato et al., 2011; Schroter-Kermani et al., 2012), while for PFNA, concentrations have been demonstrated to increase in studies from the same time period (Glynn et al., 2012; Okada et al., 2013; Olsen et al., 2012). Further, association with sampling date was not observed for PFDA and PFUnDA and may reflect different historical production and use, environmental pathways and longer half-lives of these PFASs (Glynn et al., 2012; Kato et al., 2011). Considering the short study period and inconsistent decline in PFNA concentrations between the 100 days interval groups, caution has to be made in deducing PFNA temporal trends. Still, the present observations underline the importance of considering extended recruitment periods when investigating predictors of PFAS concentrations.

4.3. Birth year

The range in birth years for the participating women were 1964–1990, thus they were all born during the period of large-scale PFAS productions and before the phase-out of PFOS related compounds.

Table 3
Parameter estimates[a], group differences, 95 % confidence intervals (CI) and p values of significant dietary predictors in the best fitted ANCOVA models of selected PFASs.

		PFHxS[c]				PFHpS[d]				PFOS[e]			
Predictor	N	Ŷ	Diff	95 % CI	p	Ŷ	Diff	95 % CI	p	Ŷ	Diff	95 % CI	p
Intake of marine food[b]													
0–24 g/day	94	**0.55**	-	-	-					**8.71**	-	-	-
24.1–37.8 g/day	95	0.57	0.02	(−0.08, 0.14)	0.74					9.46	1.36	(−0.43, 2.1)	0.22
37.9–59 g/day	95	0.66	0.11	(−0.003, 0.25)	0.07					11.0	2.33	(0.97, 3.88)	**0.00**
59.1–184 g/day	94	0.67	0.12	(−0.002, 0.26)	0.06					11.4	2.64	(1.22, 4.26)	**0.00**
Intake of game[b]													
0 g/day	188	**0.58**	-	-	-	**0.11**	-	-	-				
0–3 g/day	62	0.53	−0.05	(−0.15, 0.007)	0.33	0.11	0.004	(−0.01, 0.03)	0.69				
3.1–82 g/day	128	0.73	0.15	(0.04, 0.27)	**0.01**	0.14	0.03	(0.009, 0.05)	**0.00**				
Intake of white meat[b]													
0–18 g/day	106					**0.12**	-	-	-	**8.87**	-	-	-
18.1–28 g/day	95					0.12	0.001	(−0.02, 0.03)	0.92	9.95	1.08	(−0.2, 2.6)	0.11
28.1–33 g/day	82					0.15	0.03	(0.005, 0.06)	**0.02**	10.3	1.43	(0.15, 2.9)	**0.03**
33.1–79 g/day	95					0.14	0.02	(−0.002, 0.04)	0.07	10.5	1.58	(0.29, 3.02)	**0.01**
Intake of salty snacks[b]													
0–4.7 g/day	92												
4.8–9.4 g/day	98												
9.5–13 g/day	79												
13.1–55 g/day	109												
Intake of beef[b]													
0 g/day	125												
0–4 g/day	173												
4.1–37.2 g/day	80												
Intake of chocolate[b]													
0–4.7 g/day	88												
4.8–9.7 g/day	99												
9.8–16.5 g/day	90												
16.6–138 g/day	101												
[i]R^2 (%)				25				17				25	

[a] Predicted PFAS concentrations (Ŷ) from the respective ANCOVA models. Predicted concentrations are back-transformed from log estimates and are expressed in units of ng/mL.
[b] The estimated change in PFASs in ng/mL across intake groups with the low intake group as reference.
[c] Parity and sampling date are included as covariates in the model. Intake of game and marine food are included as fixed factors.
[d] Parity and sampling date are included as covariates in the model. Intake of game and white meat are included as fixed factors.
[e] Parity and sampling date are included as covariates in the model. Intake of marine food and white meat are included as fixed factors.
[f] Parity and sampling date are included as covariates in the model. Intake of salty snacks and beef is included as a fixed factor.
[g] Parity, sampling date and age are included as covariates in the model. Intake of game and marine food are included as fixed factors.
[h] Parity, age and BMI are included as covariates in the model. Intake of marine food and chocolate are included as fixed factors.
[i] R^2 = The proportion of variation in concentrations of the contaminant which is explained by the model.

Table 3 (*continued*)

	PFOA[f]				PFNA[g]				PFDA[h]				PFUnDA[h]			
Predictor	Ŷ	Diff	95 % CI	p	Ŷ	Diff	95 % CI	p	Ŷ	Diff	95 % CI	p	Ŷ	Diff	95 % CI	p
Predictor	Ŷ	Diff	95 % CI	p	Ŷ	Diff	95 % CI	p	Ŷ	Diff	95 % CI	p	Ŷ	Diff	95 % CI	p
Intake of marine food[b]																
0–24 g/day					**0.47**	-	-	-	**0.12**	-	-	-	**0.10**	-	-	-
24.1–37.8 g/day					0.47	−0.003	(−0.06, 0.05)	0.89	0.15	0.03	(0.008, 0.04)	**0.01**	0.13	0.03	(0.008, 0.005)	**0.01**
37.9–59 g/day					0.53	0.06	(0.001, 0.13)	**0.05**	0.17	0.05	(0.03, 0.07)	**0.00**	0.14	0.04	(0.02, 0.07)	**0.00**
59.1–184 g/day					0.53	0.06	(0.004, 0.13)	**0.04**	0.17	0.05	(0.03, 0.07)	**0.00**	0.17	0.07	(0.05, 0.11)	**0.00**
Intake of game[b]																
0 g/day					**0.43**	-	-	-								
0–3 g/day					0.43	−0.001	(−0.05, 0.06)	0.86								
3.1–82 g/day					0.51	0.080	(0.03, 0.14)	**0.00**								
Intake of white meat[b]																
0–18 g/day																
18.1–28 g/day																
28.1–33 g/day																
33.1–79 g/day																
Intake of salty snacks[b]																
0–4.7 g/day	**2.04**	-	-	-												
4.8–9.4 g/day	2.25	0.21	(−0.06, 0.51)	0.13												
9.5–13 g/day	2.34	0.30	(0.03, 0.60)	**0.03**												
13.1–55 g/day	2.54	0.50	(0.20, 0.83)	**0.00**												
Intake of beef[b]																
0 g/day	**2.04**	-	-	-												
0–4 g/day	2.13	0.10	(−0.11, 0.33)	0.44												
4.1–37.2 g/day	2.33	0.30	(0.001, 0.60)	**0.05**												
Intake of chocolate[b]																
0–4.7 g/day									**0.13**	-	-	-	**0.10**	-	-	-
4.8–9.7 g/day									0.12	−0.01	(−0.03, 0.005)	0.25	0.09	−0.01	(−0.02, 0.01)	0.81
9.8–16.5 g/day									0.11	−0.02	(−0.04, −0.008)	**0.01**	0.09	−0.01	(−0.02, 0.01)	0.68
16.6–138 g/day									0.10	−0.03	(−0.04, −0.01)	**0.00**	0.08	−0.02	(−0.03, −0.01)	**0.00**
[i]R^2 (%)			44				29				20				31	

The exposure period after the phase out is expected to be similar for all women. No association to age was observed for PFHxS, PFHpS, PFOS and PFOA concentrations, while age was positively associated to PFNA, PFDA and PFUnDA concentrations. These observations are in accordance with compound differences in half-lives, bioaccumulation potentials and continued production for some years after 2002 for PFNA, PFDA and PFUnDA (Armitage et al., 2009; Zhang et al., 2013). Our results are further supported by another Norwegian study, where increasing concentrations of longer-chained PFCAs with increasing age were described in pooled samples from 2007 (Haug et al., 2009).

4.4. Understanding the importance of diet for PFAS concentrations

Associations with one or several PFASs for food items from all major food groups indicate an overall exposure to PFASs from the diet. In agreement with several studies (Berger et al., 2009; Rylander et al., 2009; Vestergren et al., 2012), marine food was indicated as the main predictor of dietary exposure to PFOS, PFNA, PFDA and PFUnDA. Furthermore, white meat was a predictor of PFHpS and PFOS concentrations, while beef was significantly associated with PFOA and these associations are in accordance with other studies (Halldorsson et al., 2008; Haug et al., 2010a; Noorlander et al., 2011; Vestergren et al., 2012). A significant association with game (reindeer, moose and grouse) was detected for PFHxS, PFHpS and PFNA and is in agreement with findings in Ostertag et al. (2009), although their study explored different type of game, corresponding to regional differences and hunting patterns in different parts of the world.

As the overall PFASs exposure has been and is decreasing, the relative importance of diet is likely increasing caused by the gradual elimination of direct exposure (intentionally produced compounds) as exposure pathway, and continued food web accumulation of PFASs currently residing in environmental compartments like water and air. In the present study, the relative importance of diet seems more apparent for the longer chained PFASs, as the largest increase in explained variance was observed for these compounds when including the diet in the statistical models as compared to the shorter chained PFASs. These observations are in harmony with the PFNA > PFOA pattern in biota and increasing biomagnification with PFCA chain length (Vestergren and Cousins, 2009). Exposure studies like the present investigate which dietary items explain the relative differences in PFAS concentrations within the population, as opposed to food basket studies describing the contribution of absolute intake of individual food items to the PFASs body burden. Therefore, the dietary predictors identified in this study are food items with high concentrations and/or food items with a large difference in intake between individuals. Indeed, the associations observed in the present study are in line with studies on PFAS concentrations in different food groups which demonstrate that marine food and meat generally have the highest PFAS concentrations as well as a large variation in intake (Cornelis et al., 2012; Domingo et al., 2012; Haug et al., 2010a; Herzke et al., 2013; Hlouskova et al., 2013; Noorlander et al., 2011; Tittlemier et al., 2007; Trudel et al., 2008; Vestergren et al., 2012). Conversely, no association between PFAS concentrations and high consumption food categories (vegetables, cereal products and dairy products) were observed in the present study. These food groups have been reported to contribute considerably to the total daily intake of PFASs (Haug et al., 2010a; Noorlander et al., 2011); however, the small differences in intake in the population and/or their relative low concentrations of PFASs might explain the discrepancies in observations (Vestergren et al., 2012). Evidently, the identified dietary sources of PFAS exposure will vary not only according to the concentration in food and intake rates but also according to study design. Consistently, consumption of fish and shellfish was a major determinant of serum PFAS concentrations in a subpopulation with a high intake of seafood (Haug et al., 2010b) whereas red meat and animal fat were predictors of PFOS and PFOA concentrations in a population with high intake of meat (Halldorsson et al., 2008).

5. Conclusions

The present study demonstrates that parity, sampling date and birth year are the strongest predictors for maternal PFAS concentrations in years following a decrease in production and use of several PFASs. Further, dietary predictors of PFAS concentrations were identified and varied in importance according to compound. The identification of dietary predictors of PFASs depends on variations in intake among the participants, concentrations in food of the specific compounds and study design.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.envint.2014.04.010.

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SUPPLEMENTAL MATERIAL

Maternal serum concentrations of per- and polyfluoroalkyl substances and their predictors in years with reduced production and use

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Contents:

1. Supplemental material: Materials and methods

1.1 Chemical analyses

Table S1. Overview of analysed compounds, parent ions, monitored transitions, S-lens conditions and collision energies. Transition 1 is the quantifier ion and transition 2 the qualifier ion

				Triple–quadrupole mass-spectrometry (UHPLC-MS/MS).				
Compound	**Acronym**	**Group acronym**	**Isotope labeled standard for quantification**	**Parent ion (m/z)**	**Transition 1 (m/z)**	**Transition 2 (m/z)**	**Collision energy (V)**	**S-lens (V)**
Perfluorobutane sulfonate	PFBS		$^{13}C_3$ PFHxS	298.9	80.0	99.0	44	85
Perfluoropentane sulfonate	PFPeS		$^{13}C_3$ PFHxS	349.0	80.0	99.0	44	85
Perfluorohexane sulfonate	PFHxS	Perfluoroalkane sulfonic acids (PFSAs)	$^{13}C_3$ PFHxS	399.0	80.0	99.0	45	86
Perfluoroheptane sulfonate	PFHpS		$^{13}C_3$ PFHxS	449.0	80.0	99.0	48	95
Perfluorooctane sulfonate	PFOS		$^{13}C_4$ PFOS	499.0	80.0	99.0	50	103
Perfluorodecane sulfonate	PFDS		$^{13}C_4$ PFOS	599.0	80.0	99.0	59	120
Perfluorobutanoate	PFBA		$^{13}C_4$ PFBA	213.0	169.0		11	39
Perfluoropentanoate	PFPeA		$^{13}C_5$ PFPeA	268.0	218.9		7	43
Perfluorohexanoate	PFHxA		$^{13}C_5$ PFHxA	263.0	119.1	269.1	25	43
Perfluoroheptanoate	PFHpA		13C_4 PFHpA	363.0	169.0	319.1	18	43
Perfluorooctanoate	PFOA		$^{13}C_4$ PFOA	413.1	169.1	369.1	18	55
Perfluorononanoate	PFNA	Perfluoroalkyl carboxylic acids (PFCAs)	$^{13}C_5$ PFNA	463.0	219.1	418.8	18	68
Perfluorodecanoate	PFDA		$^{13}C_6$ PFDA	513.0	269.0	469.0	19	68
Perfluoroundecanoate	PFUnDA		$^{13}C_7$ PFUnDA	563.1	268.9	518.8	18	78
Perfluorododecanoate	PFDoDA		$^{13}C_2$ PFDoDA	613.1	169.1	569.0	25	73
Perfluorotridecanoate	PFTrDA		$^{13}C_2$ PFDoDA	663.1	169.0	619.1	28	85
Perfluorotetradecanoate	PFTeDA		$^{13}C_2$ PFDoDA	713.0	168.9	669.1	30	85
Perfluorohexadecanoate	PFHxDA		$^{13}C_2$ PFDoDA	813.1	168.8	769.1	31	106
Perfluorooctadecanoate	PFODA		$^{13}C_2$ PFDoDA	913.1	219.0	869.1	29	116
Perfluorooctane sulfonamide	FOSA	Perfluoroalkane sulfonamides (FOSAs)	$^{13}C_8$ FOSA	498.0	78.0	498.0	78	124
4:2 fluorotelomer sulfonate	4:2 FTS		$^{13}C_3$ PFHxS	327.0	80.0	307.1	25	86
6:2 fluorotelomer sulfonate	6:2 FTS	Fluortelomer sulfonates (FTSs)	$^{13}C_4$ PFOS	427.0	80.0	407.1	45	105
8:2 fluorotelomer sulfonate	8:2 FTS		$^{13}C_4$ PFOS	527.0	80.0	507.0	47	118
Perfluorohexyl phosphonic acid	PFHxPA		Cl-PFHxPA	399.0	78.9		58	71
Perfluorooctyl phosphonic acid	PFOPA	Perfluoroalkyl phosphonic acids (PFPAs)	Cl-PFHxPA	498.9	78.9		19	93
Perfluorodecyl phosphonic acid	PFDPA		Cl-PFHxPA	599.0	79.0		28	147
13C labeled internal standards	$^{13}C_3$ PFHxS			401.9	80.0	99.0	45	86
	$^{13}C_4$ PFOS			502.9	80.0	99.0	50	119
	$^{13}C_4$ PFBA			217.0	171.8		11	39
	$^{13}C_5$ PFPeA			268.0	223.0		11	43
	$^{13}C_5$ PFHxA			318.0	119.1	273.0	25	43
	$^{13}C_4$ PFHpA			367.0	169.2	322.0	18	43
	$^{13}C_4$ PFOA			417.0	169.1	372.0	18	45
	$^{13}C_5$ PFNA			467.9	219.1	423.1	6	68
	$^{13}C_6$ PFDA			519.0	269.0	474.0	19	68
	$^{13}C_7$ PFUnDA			570.0	268.9	525.0	18	78
	$^{13}C_2$ PFDoDA			615.1	169.0	570.0	25	73
	$^{13}C_8$ FOSA			506.0	78.0	506.0	43	124
1-Chloro-perfluorohexyl phosphonic acid	Cl-PFHxPA			414.9	78.9	414.9	45	113
Recovery standard	3,7- Branched PFDA			469.0	269.0		23	117

1.2 Quality control

Table S2. Limit of detections (LODs) and detection frequences of the compounds

Compound	LOD ng/mL	% > LOD
PFBS	0.004	<20
PFPeS	0.05	<20
PFHxS	0.06	99
PFHpS	0.06	80
∑PFOS	0.31	100
PFDS	0.001	<20
PFBA	0.20	<20
PFPeA	0.06	<20
PFHxA	0.05	<20
PFHpA	0.03	<20
PFOA	0.07	100
PFNA	0.04	100
PFDA	0.03	100
PFUnDA	0.02	100
PFDoDA	0.03	50
PFTrDA	0.02	<20
PFTeDA	0.03	<20
PFHxDA	0.04	<20
PFODA	0.02	<20
FOSA	0.01	42
4:2 FTS	0.01	<20
6:2 FTS	0.01	<20
8:2 FTS	0.01	<20
PFHxPA	0.001	<20
PFOPA	0.003	<20
PFDPA	0.004	<20

Table S3: Calculated recoveries of the internal standard for the compounds and linear regrssion (r^2) values for the calibration curves

	Recovery (%)	
Internal standard	Mean ± stdev	r^2
$^{13}C_3$ PFHxS	74 ±12	≥0.99
$^{13}C_4$ PFOS	74 ±9	≥0.99
$^{13}C_4$ PFBA	83 ±8	≥0.99
$^{13}C_5$ PFPeA	84 ±4	≥0.99
$^{13}C_5$ PFHxA	84 ±7	≥0.99
$^{13}C_4$ PFHpA	86 ±7	≥0.99
$^{13}C_4$ PFOA	76 ±10	≥0.99
$^{13}C_5$ PFNA	72 ±12	≥0.99
$^{13}C_6$ PFDA	82 ±9	≥0.99
$^{13}C_7$ PFUnDA	85 ±15	≥0.99
$^{13}C_2$ PFDoDA	82 ±15	≥0.99
$^{13}C_8$ FOSA	79 ±13	≥0.99
Cl-PFHxPA	67 ±8	≥0.99

Table S4. Measured analyte in SRM 1957 in % of specified concentration

	PFHpA	PFHxS	PFOA	PFOS	PFNA	PFDA	PFUnDA
Mean ± stdev	72 ±12	84 ± 14	90 ±7	110 ± 10	92 ±14	51 ±10	60 ±23

1.3 Statistical analyses

PLS regression was used as an exploratory model for evaluating the impact of the demographic and dietary variables simultaneously on serum concentrations of PFASs. To increase the model predictive ability, variables with variable importance to projection (VIP) values > 0.6 were included in the final models. To investigate the impact of the dietary variables with large influence on contaminants concentration (identified from the PLS regression) while adjusting for demographic variables, analysis of covariance (ANCOVA) was applied. For the latter analyses, the study group was divided into three or four groups (percentiles) according to their consumption of: Total marine food (shellfish, fish spread, roe, liver, crab, fatty fish, lean fish, whale and seal), game (reindeer meat, reindeer products, moose and grouse), white meat (chicken and pork), beef, salty snacks, berries, vegetables, tea, coffee and chocolate. For the PLS regression, the contaminant data and the dietary variables were log transformed and diagnostic plots of the residuals were evaluated to ensure that model assumptions were met. For the ANCOVAs; the contaminant data was log transformed while the variables were normally distributed within groups. Assumptions for performing ANCOVA were fulfilled: i) a linear relationship between the covariates and the dependent variable in the respective models was observed; ii) the variance of the dependent variable was homogeneous across all the intake groups; iii) there were homogenous regression slopes for all the intake groups; and iv) no interactions were observed.

Table S5. Study population characteristics and dietary intake for the MISA study group (N = 378)

	Characteristics		
Variables	**Median**	**Mean**	**Range**
Age (years)	32	31	18-43
Children/Parity	1	0.9	0-4
BMI (pre-pregnancy)	23	24	18-44
Sampling date (Days from study start)	328	349	0-867
Duration of breastfeeding (Months per child)			
Exclusive	4	4	0-8
Mixed	8	11	0-36
	Dietary intake g/day		
Foodstuff	**Median**	**Mean**	**Range**
Milk and yoghurt	168	236	0-821
Coffee	60	137	0-1128
Tea	170	249	0-3060
Water	675	721	0-1200
Lemonade and soda	193	232	0-1500
Bread and cereals	182	172	0-452
Jam	6	8	0-80
Meat on bread	10	16	0-57
Mayo salad	0	4	0-75
Cheese	20	26	0-130
Fish spread for bread	5	9	0-62
Rice	21	25	0-64
Pasta	26	33	0-77
Porridge made of rice	12	19	0-50
Other porridge	8	17	0-245
Soup	34	37	0-215
Fruit	190	220	0-759
Vegetables	144	159	10-506
Potatos	50	57	0-189
Fish roe	0.6	0.6	0-5
Fish liver	0.1	0.1	0-4
Processed fish products[a]	31	37	0-119
Whale and seal meat	0	0.5	0-5
Crab meat	0	0.1	0-7
Shellfish	1	1	0-4
Seagull eggs	0	0.1	0-1
Fat and sauce for fish dinners	11	14	0-82
Roast	2	2	0-20
Beef	4	5	0-37
White meat[b]	28	27	0-78
Game[c]	1.2	3.9	0-82
Other kind of meat[d]	9.4	15.7	0-81
Processed meat products[e]	74	74	0-202
Sauce for meat	14	17	0-97
Eggs	17	18	0-59
Icecream	6	8	0-36
Pastries	38	41	0-314
Berries	2	3	0-46
Desert	0	5	0-46
Chocolate	10	14	0-138
Salty snacks	9	10	0-55
Other kind of fish[f]	0	1	0-41
Lean fish[g]	16	20	0-136
Fat fish[h]	9	12	0-66
Fat on bread	16	17	0-69
	Frequency (Y/N)	**Precentage**	
Use of fish oil	103/275	27/73	
Use of fish capsules	208/170	55/45	
Smoking before pregnancy	84/294	22/78	
Smoking during pregnancy	28/350	7/93	

[a]Include fish cake, fish au gratin and deep fried fish

[b]Include chicken and cutlets

[c]Include reindeer meat, reindeer products, grouse and moose

[d]Include meat other than steak, beef, white meat, game or processed meat

[e]Include rissoles, sausages, pizza, lobscouse and bacon/ham

[f]Include fish other than lean fish or fatty fish

[g]Include boiled cod, fried cod, tuna and fresh water fish

[h]Include catfish, salmon, mackerel, herring and halibut

2. Supplemental material: Results

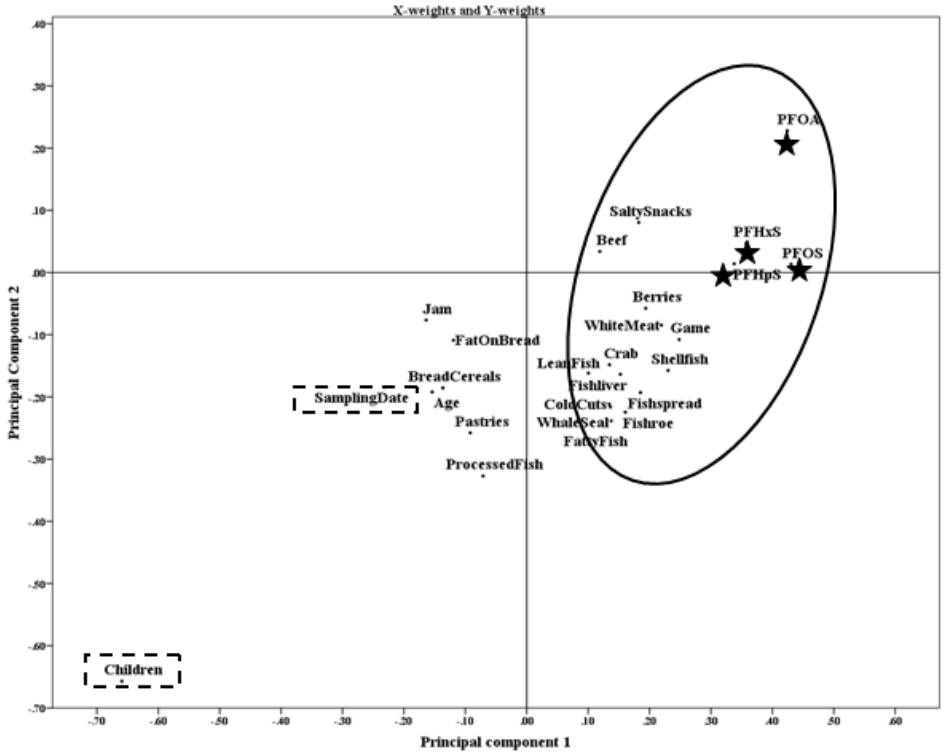

Figure S1. Partial least square loading plot for PFHxS, PFHpS, PFOS and PFOA concentrations. The plot describes the linear relationship between the independent variables (self-reported demographic and dietary variables) and the dependent variables (PFASs marked with stars) and how the variables load onto the principal components. Variables with variable importance to projection values > 0.6 are included in the PLS. Variables circled with a solid line were significantly positive associated with the investigated compounds, whereas variables boxed with a dashed line were significantly negatively associated with the same compounds (bivariate correlations with significant Pearson correlation coefficients, $p<0.05$).

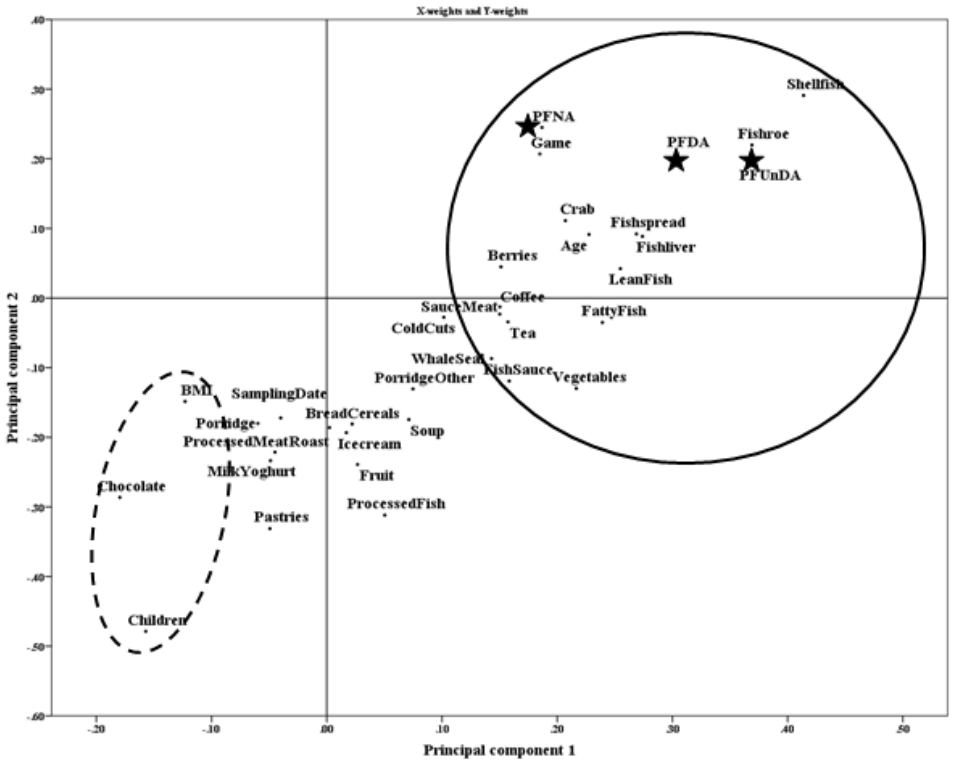

Figure S2. Partial least square loading plot for: PFNA, PFDA and PFUnDA concentrations. The plot describes the linear relationship between the independent variables (self-reported demographic and dietary variables) and the dependent variables (PFASs marked with stars) and how the variables load onto the principal components. Variables with variable importance to projection values > 0.6 are included in the PLS. Variables circled with a solid line were significantly positive associated with the investigated compounds, whereas variables circled with a dashed line were significantly negatively associated with the same compounds (bivariate correlations with significant Pearson correlation coefficients, $p<0.05$).

Table S6: Unadjusted serum PFAS concentrations in mothers with different parity, sampling date, age and BMI

Number of cildren	**0 Children (N = 150)**			**1 Child (N = 135)**			**2 Children (N = 69)**			**3-4 Children (N = 24)**		
	Median	**Mean**	**Range**	**Median**	**Mean**	**Range**	**Median**	**Mean**	**Range**	**Median**	**Mean**	**Range**
PFHxS (ng/mL)	0.56	0.81	0.08-8.34	0.39	0.44	0.04-1.40	0.36	0.64	0.04-14.8	0.22	0.48	0.04-3.22
PFHpS (ng/mL)	0.13	0.16	0.04-0.66	0.10	0.11	0.04-0.36	0.08	0.11	0.04-1.07	0.04	0.08	0.04-0.22
PFOS (ng/mL)	9.97	10.9	1.85-35.8	7.70	8.27	0.30-23.7	6.46	6.95	1.60-18.1	4.60	5.10	2.00-11.5
PFOA (ng/mL)	2.2	2.43	0.46-10.9	1.24	1.37	0.28-4.92	1.00	1.13	0.44-3.15	0.73	0.86	0.48-2.34
PFNA (ng/mL)	0.63	0.78	0.25-3.62	0.53	0.57	0.15-1.26	0.53	0.65	0.19-3.12	0.39	0.59	0.22-4.36
PFDA (ng/mL)	0.25	0.29	0.09-2.34	0.21	0.24	0.07-0.61	0.23	0.26	0.05-0.74	0.19	0.22	0.07-0.96
PFUnDA (ng/mL)	0.27	0.32	0.03-1.46	0.24	0.28	0.05-0.96	0.28	0.33	0.04-0.90	0.19	0.25	0.05-0.93
Sampling date	**0-180 days (N = 92)**			**181-328 days (N = 97)**			**329-519 days (N = 95)**			**520-867 days (N = 94)**		
	Median	**Mean**	**Range**	**Median**	**Mean**	**Range**	**Median**	**Mean**	**Range**	**Median**	**Mean**	**Range**
PFHxS (ng/mL)	0.50	0.79	0.07-17.8	0.44	0.62	0.04-7.97	0.43	0.62	0.04-8.37	0.38	0.46	0.04-1.70
PFHpS (ng/mL)	0.11	0.15	0.04-1.07	0.11	0.13	0.04-0.53	0.11	0.13	0.04-0.40	0.11	0.13	0.04-0.40
PFOS (ng/mL)	9.07	9.85	2.35-35.8	8.01	8.56	0.29-19.2	8.09	9.26	2.00-29.4	7.03	7.76	1.59-21.9
PFOA (ng/mL)	1.65	1.87	0.50-4.52	1.51	1.63	0.33-4.21	1.72	1.87	0.44-10.9	1.20	1.49	0.28-4.13
PFNA (ng/mL)	0.59	0.73	0.20-3.62	0.56	0.64	0.15-2.94	0.58	0.70	0.15-3.10	0.51	0.62	0.19-4.36
PFDA (ng/mL)	0.23	0.26	0.07-0.79	0.22	0.26	0.07-0.59	0.24	0.29	0.09-2.34	0.22	0.25	0.05-0.96
PFUnDA (ng/mL)	0.26	0.29	0.03-0.87	0.26	0.31	0.04-0.91	0.28	0.33	0.05-1.46	0.23	0.26	0.04-0.96
Age	**18-28 years (N = 90)**			**29-32 years (N = 99)**			**33-35 years (N = 93)**			**36-43 years (N = 96)**		
	Median	**Mean**	**Range**	**Median**	**Mean**	**Range**	**Median**	**Mean**	**Range**	**Median**	**Mean**	**Range**
PFHxS (ng/mL)	0.44	0.70	0.07-8.37	0.44	0.55	0.04-2.50	0.48	0.58	0.04-3.22	0.42	0.67	0.09-14.8
PFHpS (ng/mL)	0.11	0.13	0.04-0.53	0.10	0.12	0.04-0.66	0.10	0.13	0.04-0.56	0.10	0.13	0.04-1.07
PFOS (ng/mL)	8.56	9.04	2.35-19.7	7.76	8.55	1.67-29.4	8.12	0.98	0.29-35.8	7.77	8.86	1.59-26.7
PFOA (ng/mL)	1.82	1.94	0.50-4.38	1.45	1.64	0.35-4.15	1.42	1.83	0.33-10.9	1.28	1.45	0.28-3.63
PFNA (ng/mL)	0.53	0.61	0.22-3.10	0.54	0.65	0.15-3.62	0.60	0.72	0.40-3.94	0.56	0.69	0.25-4.36
PFDA (ng/mL)	0.21	0.22	0.07-0.56	0.20	0.24	0.07-0.80	0.26	0.30	0.07-2.34	0.24	0.28	0.05-0.96
PFUnDA (ng/mL)	0.18	0.22	0.03-0.91	0.24	0.28	0.04-1.46	0.30	0.33	0.05-0.96	0.31	0.36	0.04-0.90
Body mass index (BMI)	**17-21.5 (N = 92)**			**21.6-23.5 (N = 93)**			**23.6-25.6 (N = 94)**			**25.7-44.5 (N = 92)**		
	Median	**Mean**	**Range**	**Median**	**Mean**	**Range**	**Median**	**Mean**	**Range**	**Median**	**Mean**	**Range**
PFHxS (ng/mL)	0.44	0.68	0.08-8.37	0.45	0.53	0.08-2.50	0.41	0.72	0.04-14.8	0.45	0.56	0.04-3.22
PFHpS (ng/mL)	0.10	0.12	0.04-0.49	0.09	0.12	0.04-0.66	0.10	0.13	0.04-1.07	0.11	0.13	0.04-0.56
PFOS (ng/mL)	8.90	9.36	1.67-19.7	7.78	8.24	1.59-23.7	7.50	8.63	0.30-21.9	8.47	9.31	2.35-35.8
PFOA (ng/mL)	1.74	1.84	0.35-4.21	1.45	1.70	0.48-10.9	1.36	1.58	0.28-6.92	1.56	1.70	0.44-5.02
PFNA (ng/mL)	0.57	0.65	0.22-2.37	0.57	0.71	0.24-3.62	0.55	0.67	0.15-4.36	0.55	0.66	0.20-2.94
PFDA (ng/mL)	0.25	0.27	0.09-0.72	0.23	0.29	0.05-2.34	0.21	0.24	0.07-0.96	0.22	0.24	0.07-0.80
PFUnDA (ng/mL)	0.29	0.33	0.05-0.91	0.30	0.33	0.04-0.96	0.26	0.29	0.03-0.93	0.22	0.26	0.06-1.46

Table S7: Unadjusted serum PFAS concentrations in different food intake groups. Grey boxes denote the groups that were significant difference (p<0.05) compared to the low intake group in the ANCOVA models (Table 3)

Intake of total marine food	**0-24 g/day (N = 94)**			**24.1-37.8 (N = 95)**			**37.9-59 (N = 95)**			**59-184 g/day (N = 94)**		
	Median	**Mean**	**Range**	**Median**	**Mean**	**Range**	**Median**	**Mean**	**Range**	**Median**	**Mean**	**Range**
PFHxS (ng/mL)	0.38	0.55	0.07-8.37	0.43	0.70	0.04-14.8	0.46	0.58	0.10-1.86	0.50	0.66	0.04-7.97
PFHpS (ng/mL)	0.09	0.11	0.04-0.53	0.09	0.13	0.04-1.07	0.12	0.13	0.04-0.56	0.11	0.13	0.04-0.49
PFOS (ng/mL)	7.24	7.87	1.60-23.7	7.74	8.30	0.29-18.8	8.73	9.72	1.85-35.8	8.44	9.51	2.51-29.4
PFOA (ng/mL)	1.52	1.72	0.35-4.92	1.47	1.59	0.33-3.86	1.56	1.92	0.28-10.9	1.61	1.61	0.44-3.63
PFNA (ng/mL)	0.53	0.59	0.19-3.11	0.52	0.66	0.15-3.62	0.60	0.75	0.25-4.36	0.60	0.67	0.15-1.89
PFDA (ng/mL)	0.19	0.20	0.05-0.74	0.21	0.25	0.07-0.79	0.26	0.31	0.09-2.34	0.25	0.28	0.09-0.80
PFUnDA (ng/mL)	0.19	0.20	0.03-0.56	0.21	0.29	0.05-0.88	0.30	0.32	0.08-0.87	0.33	0.39	0.06-1.46
Intake of white meat	**0-18 g/day (N = 106)**			**18.1-28 g/day (N= 95)**			**28.1-33 g/day (N = 82)**			**33.1-79 g/day (N=95)**		
	Median	**Mean**	**Range**	**Median**	**Mean**	**Range**	**Median**	**Mean**	**Range**	**Median**	**Mean**	**Range**
PFHxS (ng/mL)	0.43	0.54	0.04-2.52	0.38	0.62	0.04-7.97	0.43	0.62	0.04-8.37	0.49	0.71	0.08-14.8
PFHpS (ng/mL)	0.10	0.12	0.04-0.66	0.09	0.10	0.04-0.36	0.11	0.14	0.04-0.40	0.11	0.14	0.04-1.10
PFOS (ng/mL)	7.46	7.96	0.29-19.5	7.76	8.54	2.00-29.4	8.57	9.17	2.75-20.4	8.73	9.61	1.85-35.8
PFOA (ng/mL)	1.47	1.61	0.33-4.38	1.28	1.53	0.28-4.92	1.61	1.76	0.53-6.92	1.65	1.91	0.46-10.9
PFNA (ng/mL)	0.53	0.73	0.15-4.36	0.55	0.61	0.19-3.10	0.55	0.61	0.20-1.62	0.60	0.71	0.15-2.81
PFDA (ng/mL)	0.22	0.26	0.05-0.96	0.23	0.25	0.07-0.80	0.23	0.26	0.10-0.60	0.23	0.27	0.10-2.34
PFUnDA (ng/mL)	0.25	0.29	0.04-0.91	0.26	0.30	0.04-1.46	0.28	0.32	0.03-0.93	0.25	0.29	0.06-0.96
Intake of chocolate	**0-4.7 g/day (N = 88)**			**4.8-9.7 g/day (N = 99)**			**9.5-13 g/day (N = 90)**			**13.1-55 g/day (N = 101)**		
	Median	**Mean**	**Range**	**Median**	**Mean**	**Range**	**Median**	**Mean**	**Range**	**Median**	**Mean**	**Range**
PFDA (ng/mL)	0.24	0.31	0.05-2.34	0.24	0.27	0.07-0.80	0.22	0.24	0.09-0.48	0.20	0.23	0.07-0.60
PFUnDA (ng/mL)	0.30	0.33	0.03-0.91	0.28	0.34	0.05-1.46	0.27	0.3	0.05-0.73	0.20	0.24	0.06-0.88
Intake of salty snacks	**0-4.7 g/day (N = 92)**			**4.8-9.4 g/day (N = 98)**			**9.8-16.5 g/day (N = 79)**			**16.6-138 g/day (N = 109)**		
	Median	**Mean**	**Range**	**Median**	**Mean**	**Range**	**Median**	**Mean**	**Range**	**Median**	**Mean**	**Range**
PFHxS (ng/mL)	0.42	0.51	0.04-2.56	0.43	0.81	0.04-0.37	0.45	0.56	0.11-1.48	0.48	0.66	0.08-14.8
PFHpS (ng/mL)	0.09	0.11	0.04-0.66	0.10	0.13	0.04-0.43	0.10	0.13	0.04-0.53	0.12	0.14	0.04-1.07
PFOS (ng/mL)	7.42	8.10	0.29-23.7	7.78	8.80	2.82-19.7	8.55	9.48	1.59-29.4	8.45	9.16	1.98-35.8
PFOA (ng/mL)	1.39	1.55	0.33-6.92	1.56	1.74	0.28-4.92	1.65	1.76	0.44-4.15	1.60	1.84	0.48-10.9
Intake of game	**0 g/day (N = 188)**			**0-3 g/day (N = 62)**			**3.1-82 g/day (N = 128)**					
	Median	**Mean**	**Range**	**Median**	**Mean**	**Range**	**Median**	**Mean**	**Range**			
PFHxS (ng/mL)	0.43	0.52	0.04-4.04	0.39	0.47	0.04-1.86	0.49	0.85	0.04-14.8			
PFHpS (ng/mL)	0.09	0.11	0.04-0.53	0.10	0.12	0.04-0.56	0.12	0.15	0.04-1.07			
PFOS (ng/mL)	7.67	8.36	0.29-19.6	7.99	8.89	1.67-35.8	8.62	9.56	1.85-29.4			
PFOA (ng/mL)	1.46	1.68	0.28-10.9	1.53	1.69	0.35-6.92	1.64	1.78	0.44-4.15			
PFNA (ng/mL)	0.55	0.60	0.15-3.10	0.53	0.63	0.19-2.59	0.59	0.79	0.24-4.36			
PFDA (ng/mL)	0.22	0.25	0.05-2.34	0.22	0.24	0.07-0.60	0.25	0.28	0.09-0.96			
PFUnDA (ng/mL)	0.26	0.30	0.04-0.90	0.23	0.25	0.03-0.58	0.27	0.33	0.06-1.46			
Intake of beef	**0 g/day (N=125)**			**0-4 g/day (N = 173)**			**4.1-37.2 g/day (N = 80)**					
	Median	**Mean**	**Range**	**Median**	**Mean**	**Range**	**Median**	**Mean**	**Range**			
PFOA (ng/mL)	1.33	1.53	0.28-6.92	1.61	1.75	0.46-10.9	1.81	1.92	0.44-4.92			