Temporal trends of perfluoroalkyl acids in plasma samples of pregnant women in Hokkaido, Japan, 2003–2011

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ABSTRACT

Perfluoroalkyl acids (PFAAs) are persistent organic pollutants that are used in a wide range of consumer products. Recent epidemiological studies have shown that prenatal exposure to toxic levels of PFAAs in the environment may adversely affect fetal growth and humoral immune response in infants and children. Here we have characterized levels of prenatal exposure to PFAA between 2003 and 2011 in Hokkaido, Japan, by measuring PFAA concentrations in plasma samples from pregnant women. The study population comprised 150 women who enrolled in a prospective birth cohort study conducted in Hokkaido. Eleven PFAAs were measured in maternal plasma samples using simultaneous analysis by ultra-performance liquid chromatography coupled to triple quadrupole tandem mass spectrometry. At the end of the study, in 2011, age- and parity-adjusted mean concentrations of perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA), perfluorotridecanoic acid (PFTrDA), perfluorohexane sulfonate (PFHxS), and perfluorooctane sulfonate (PFOS) were 1.35 ng/mL, 1.26 ng/mL, 0.66 ng/mL, 1.29 ng/mL, 0.25 ng/mL, 0.33 ng/mL, 0.28 ng/mL, and 3.86 ng/mL, respectively. Whereas PFOS and PFOA concentrations declined 8.4%/y and 3.1%/y, respectively, PFNA and PFDA levels increased 4.7%/y and 2.4%/y, respectively, between 2003 and 2011. PFUnDA, PFDoDA, and PFTrDA were detected in the vast majority of maternal samples, but no significant temporal trend was apparent. Future

studies must involve a larger population of pregnant women and their children to determine the effects of prenatal exposure to PFAA on health outcomes in infants and children.

Key words: Perfluorooctane sulfonate, perfluorooctanoic acid, perfluorononanoic acid, perfluorodecanoic acid, human maternal plasma, temporal trend

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Ethics approval: This study was conducted with written informed consent from all patients and was approved by the institutional ethics board for epidemiological studies at the Hokkaido University Graduate School of Medicine.

Abbreviations:

PFAAs, perfluoroalkyl acids;

PFCAs, perfluorinated carboxylic acids;

PFSAs, perfluoroalkane sulfonates;

PFHxA, perfluorohexanoic acid;

PFHpA, perfluoroheptanoic acid;

PFOA, perfluorooctanoic acid;

PFNA, perfluorononanoic acid;

PFDA, perfluorodecanoic acid;

PFUnDA, perfluoroundecanoic acid;

PFDoDA, perfluorododecanoic acid;

PFTrDA, perfluorotridecanoic acid;

PFTeDA, perfluorotetradecanoic acid;

PFHxS, perfluorohexane sulfonate;

PFOS, perfluorooctane sulfonate;

BEH, ethylene-bridged hybrid;

UPLC-MS/MS, ultra-performance liquid chromatography coupled to triple quadrupole tandem mass spectrometry;

MDL, method detection limit;

CI, confidence interval;

GM, geometric mean;

1. Introduction

Perfluoroalkyl acids (PFAAs) are used in a broad range of consumer products because of their surface properties, which include insulation and water resistance. These compounds are persistent and widespread organic pollutants within the environment, wildlife, and humans (Lau et al., 2007). Contamination of drinking water, foodstuffs such as seafood, leaching from food packaging and non-stick cookware, and household dust are known major routes of human exposure (Fromme et al., 2009). Potential health effects associated with PFAA exposure in humans are made worse by both bioaccumulation and persistence. In 2002, after 50 years of production, the 3M Company phased out the production and distribution of perfluorooctane sulfonate (PFOS) (Renner, 2001). PFOS has subsequently been regulated by the governments of the United States (Significant New Use Rules, United States Environmental Protection Agency, 2000), Canada (Schedule 1 of CEPA 1999, Environment Canada, 2006), and the European Union (Directive 76/769/EEC, European Commission, 2006). PFOS was also included in Annex B of the 2009 Stockholm Convention on Persistent Organic Pollutants (UNEP, 2007; Wang et al., 2009). The Environmental Protection Agency of the United States (2006) launched a 2010/2015 PFOA Stewardship Program to voluntarily reduce perfluorooctanoic acid (PFOA) emissions. Recent studies have indicated that concentrations of PFOS and PFOA are declining in the general human population (Kato et al., 2011; Olsen et al., 2012; Sundström et al., 2011; Wang et al., 2011). In contrast, concentrations of long-chain perfluorinated carboxylic acids (PFCAs) in the general human population are increasing (Wang et al., 2011).

PFOS and PFOA pass the placental barrier and are transferred to the fetus in humans (Midasch et al., 2007; Monroy et al., 2008). Previous epidemiological studies have reported a negative association between prenatal PFOS or PFOA exposure and birth weight (Andersen et al., 2010; Chen et al., 2012; Fei et al., 2007; Washino et al., 2009). Moreover, maternal PFOS levels correlate negatively with antibody concentrations in children aged 5 years (Grandjean et al., 2012). However, the effects of prenatal exposure to other PFAAs [e.g., long-chain perfluorinated carboxylic acids (PFCAs), such as perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), and perfluorododecanoic acid (PFDoDA)] remain unclear. PFCAs with chains longer than those in PFOA have high bioconcentration factors, suggesting their environmental persistence (Martin et al., 2003). It is necessary to measure, therefore, levels of exposure of pregnant women to PFOS, PFOA, and other PFAAs. It is also critical to determine whether environmental levels of these compounds are changing over time.

Here we have measured the concentration of 11 PFAAs in blood samples taken from pregnant women in Hokkaido, Japan. Analysis of samples from 2003 to 2011 allowed us to assess temporal trends associated with changes in the levels of these compounds.

2. Materials and Methods

2. 1. Study population

Study participants included 150 pregnant women, between 28 and 32 weeks of gestation, who were enrolled in a prospective birth cohort study (the Hokkaido Study on Environment and

Children's Health). This ongoing cohort study was initiated in February 2003, and details have been described (Kishi et al., 2011). Briefly, subjects were considered eligible if they were indigenous Japanese women who had antenatal care at one of 37 participating hospitals within Hokkaido during the first trimester of pregnancy. Healthcare personnel introduced the study and provided each potential participant with an invitation, which included a consent form and a baseline questionnaire. All participants provided written informed consent. Among the 20,737 women that registered between February 2003 and December 2011, only patients associated with a consent form, a baseline questionnaire, medical records at birth, and a maternal blood sample were included in this study. This represented 1,944 women selected during 2003, 2,459 women selected during 2005, 1,820 women selected during 2007, 1,274 women selected during 2009, and 1,103 women selected during 2011. From these populations, 30 women from each year were randomly selected for analysis. The protocol used in this study was approved by the institutional ethics board for epidemiological studies at the Hokkaido University Graduate School of Medicine.

2. 2. Standards and reagents

Acetonitrile, methanol, ultrapure water, and an HPLC-grade ammonium acetate solution (1 mol/L) were purchased from Wako Pure Chemical Inc., Osaka, Japan. Bulk ENVI-Carb sorbent was purchased from Supelco, Bellefonte, PA, USA. Acetic acid (purity: 99.7%) was purchased from Kanto Chemicals, Tokyo, Japan. Perfluorohexane sulfonate (PFHxS; >98%), PFOS (>98%), and a mixture of native PFCAs [perfluorohexanoic acid (PFHxA; >98%), perfluoroheptanoic acid

(PFHpA; >98%), PFOA (>98%), PFNA (>98%), PFDA (>98%), PFUnDA (>98%), PFDoDA (>98%), perfluorotridecanoic acid (PFTrDA; >98%), and perfluorotetradecanoic acid (PFTeDA; >98%)] were obtained from Wellington Laboratories, Inc., Guelph, Ontario, Canada. Wellington Laboratories also supplied $^{13}C_3$-labeled PFHxS (≥99%), $^{13}C_4$-labeled PFOS (≥99%), and a mixture of ^{13}C-labeled PFCAs [$^{13}C_2$-PFHxA (≥99%), $^{13}C_4$-PFOA (≥99%), $^{13}C_5$-PFNA (≥99%), $^{13}C_2$-PFDA (≥99%) and $^{13}C_2$-PFUnDA (≥99%)].

2. 3. Sample preparation

A 10-mL blood sample was taken from the maternal peripheral vein between 28 and 32 weeks of pregnancy. All samples were stored at –80 °C before analysis. An internal standard, which consisted of $^{13}C_3$-labeled PFHxS, $^{13}C_4$-labeled PFOS, and $^{13}C_4$-labeled PFCAs (2.5 ng of each), was added to each human plasma sample (0.5 mL). Samples were extracted with 2 mL acetonitrile by vortexing for 30 s. After centrifugation (3,000 × *g* for 15 min), supernatants were transferred into new tubes containing 25 mg ENVI-Carb and 50 μL acetic acid. Solutions were mixed by vortexing for 30 s. After centrifugation (3,000 × *g* for 15 min), each supernatant taken from above the ENVI-Carb was concentrated to 0.25 mL under nitrogen, and 0.25 mL methanol was added with subsequent mixing.

2. 4. Ultra-performance liquid chromatography coupled to triple quadrupole tandem mass spectrometry (UPLC-MS/MS)

Extracted solutions were analyzed using UPLC-MS/MS instrumentation. The ACQUITY UPLC system (Waters, Tokyo, Japan) was used with ethylene-bridged (BEH) C18 columns (1.7 μm, 2.1 × 50 mm). The retention gap technique was used by installing retention gap columns [BEH C18 columns (1.7 μm, 2.1 × 100 mm)], which improved PFAA sensitivity by trapping mobile-phase PFAAs (contaminants) in the retention gap column. The column temperature was 55 °C, and the column oven was maintained at 57 °C. A Micromass Quattro Premier tandem quadruple mass spectrometer (Waters) was used for MS/MS. Conditions for MS/MS were as follows: desolvation and source temperatures were set at 350 °C and 120 °C, respectively. The capillary was held at a potential of 3.5 kV relative to the counterelectrode in the negative-ion mode for all compounds. Cone and desolvation gas flow rates were 50 and 800 L/h, respectively. Cone and collision voltages, and monitored transition ions are listed in Table 1. Analytes were eluted from the column with a linear gradient involving solvent A (2 mM ammonium acetate in water) and solvent B (2 mM ammonium acetate in acetonitrile) as follows: 10% B for the initial 0.2 min, then a gradient of 10–100% B from 0.2 min to 9 min. The effluent was maintained at 100% B from 9 min to 12 min. The total UPLC cycle time was 15 min including column re-equilibration. An eluent flow rate of 0.3 mL/min was employed for all analyses. The injection volume was 5 μL.

2. 5. Quality control

Levels of PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA, PFHxS, and PFOS were measured. Calibration curves were prepared using calibration standards

that consisted of seven concentrations (between 0.1 and 10 ng/mL) prepared in 1:1 acetonitrile/methanol. Each calibration standard also contained the internal standard (5 ng/mL). Calibration curves were constructed to perform linear regressions (1/× weighting) that compared plots of peak area/internal standard area versus standard concentration/internal standard concentration. Plasma samples were quantified using calibration curves that showed good linearity and correlation coefficients (R^2) > 0.995 for all compounds. Quantification was performed using a relative-response ratio to an internal standard that most structurally matched the target analyte (Table 1).

Recoveries and relative standard deviations were evaluated using five replicate fortifications (fortified to 10 times the original concentration) of a human plasma sample with low levels of contamination (Table 1). The procedural blank levels were determined using 0.5 mL of ultrapure water. Instrumental detection limits were defined as the mass of analyte that produced a peak with a signal-to-noise ratio of 3. These values ranged from 0.1 ng/mL (PFCAs) to 0.2 ng/mL (PFSAs). The method detection limit (MDL) was defined as the mass of analyte that produced a peak with a signal-to-noise ratio of 10. These values ranged from 0.1 ng/mL (PFHxA, PFHpA, PFDA, PFUnDA, PFDoDA, PFTrDA and PFTeDA) to 0.3 ng/mL (PFNA and PFOS) (Table 1). Chromatographic resolution of branched and linear isomers in plasma samples was achieved using UPLC-MS/MS. For quantification of PFOS, the total area of the branched and linear isomer peaks was integrated.

To assess potential inter-laboratory differences, we analyzed NIST standard reference

material (SRM) 1957 (Table 1). Our methods yielded reliable data given that values for PFHpA to PFUnDA, PFHxS, and PFOS were comparable to those measured during inter-laboratory comparisons (Harada et al., 2011; Keller et al., 2010; National Institute of Standards & Technology, Certificate of Analysis, SRM 1957).

2. 6. Statistical analysis

Because our data did not fall into a normal distribution, PFAA concentrations were converted to a natural log scale. For participants with PFAA concentrations below the MDL, a value equal to half of the MDL was assigned for statistical analyses, except for PFHxA, PFHpA, and PFTeDA. We did not include PFHxA, PFHpA, and PFTeDA in the statistical analysis because these compounds were detected very infrequently. Given that the age of pregnant women correlates negatively with PFAA concentrations and that the concentrations measured for multiparous women are lower than for primiparous women (Fei et al., 2007; Okada et al., 2012; Washino et al., 2009), age- and parity-adjusted means were calculated using a least squares mean obtained from analysis of covariance. We used linear regression to analyze temporal trends between 2003 and 2011 and calculated change per year through the period assessed from the discrete rate of change (termed lambda). To assess the temporal trends of proportion for PFAAs, we performed the Cochran-Armitage trend test. Correlations between different PFAAs were assessed using the Spearman's rank correlation coefficient (ρ). All statistical analyses were performed using the Statistical Package for Social Science (SPSS) for Windows, version 16.0J (Japanese version; SPSS,

Inc., Chicago, IL, USA) and JMP 10 Statistical Discovery Software for Windows (S.A.S. Institute Inc., Cary, North Carolina). Differences were considered statistically significant at $p < 0.05$.

3. Results

The mean age of all pregnant women included in the study was 30.3 ± 4.8 years (Table 2). Concentrations of maternal plasma PFAAs for each year are provided in Table 3. Sum-total PFAA concentrations (ΣPFAAs) are also provided. PFOA, PFNA, PFDA, PFUnDA, and PFOS were detected in all samples. Detection rates for the other compounds were: PFHxA (13.3%), PFHpA (23.3%), PFDoDA (73.3%), PFTrDA (97.3%), PFTeDA (28.0%), and PFHxS (80.7%). Age- and parity-adjusted mean concentrations at the end of the study (in 2011) were: PFOA (1.35 ng/mL), PFNA (1.26 ng/mL), PFDA (0.66 ng/mL), PFUnDA (1.29 ng/mL), PFDoDA (0.25 ng/mL), PFTrDA (0.33 ng/mL), PFHxS (0.28 ng/mL), PFOS (3.86 ng/mL), and ΣPFAAs (10.13 ng/mL).

Scatter-plots and linear regressions of age- and parity-adjusted concentrations of PFOA, PFOS, ΣPFAAs, PFNA, and PFDA from 2003 to 2011 are shown in Fig. 1. Statistical analyses of these trends are provided in Table 3. Through the period 2003 to 2011, the rate of change per year for the concentrations of PFOA, PFOS, and ΣPFAAs exhibited a statistically significant decrease: PFOA = –3.1%, PFOS = –8.4%, and ΣPFAAs = –4.0%). In contrast, the rate of change per year for PFNA and PFDA levels increased significantly: PFNA = +4.7%; and PFDA = +2.4%.

The proportion of ΣPFAA that was accounted for by each PFAA is shown in Fig. 2. Each year of collected samples is represented separately. A comparison of the 2003 and 2011 data showed

that relative levels of PFOS (as a percentage of ΣPFAA) dropped from 57.1% to 40.7%, but this apparent temporal trend of the proportion was not statistically significant. Although the proportion of other PFAAs also did not exhibit a significant trend over time, the relative levels of PFNA (7.3% to 13.3%), PFDA (4.3% to 7.0%), and PFUnDA (9.5% to 13.6%) increased comparing 2003 with 2011.

Correlation coefficients among PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFHxS, and PFOS for all samples are listed in Table 4. The level of each PFAA correlated significantly with that of the corresponding PFAA having a different chain length. Significant correlation coefficients (ρ) were found for the following pairs: PFNA and PFDA (0.702), PFDA and PFUnDA (0.698), PFDA and PFDoDA (0.616), PFUnDA and PFTrDA (0.675), and PFDoDA and PFTrDA (0.707). In general, strong correlations were measured between PFAAs with similar chain length.

4. Discussion

Here we determined the concentration of 11 PFAAs in plasma samples of pregnant women in Hokkaido, Japan. Detectable levels of PFNA, PFDA, PFUnDA, and PFOS were found in all samples. Between 2003 and 2011, plasma concentrations of PFOS and PFOA decreased, whereas PFNA and PFDA concentrations increased. To our knowledge, this is the first report that has investigated maternal levels of 11 PFAA in Japan.

The declining levels of PFOS and PFOA in humans are consistent with previous studies (Calafat et al., 2007; Harada et al., 2011; Olsen et al., 2008, 2012). In our study, the proportion of

PFOS in ΣPFAA also decreased as compared with 2003 and 2011. This is because the 3M Company phased out their manufacture and distribution of PFOS in 2002, following 50 years of production (Renner, 2001). Given that PFOS subsequently ceased to accumulate in the environment and indeed human exposure to this compound decreased, PFOS concentrations in human plasma decreased dramatically between 2003 and 2005. Further reductions in PFOS levels by 2011 may be explained by two developments. First, PFOS was listed on Annex B of the Stockholm Convention on Persistent Organic Pollutants in 2009 (UNEP, 2007; Wang et al., 2009). Second, Japan designated PFOS a Type I Specified Chemical Substance (characterized by persistence, bioaccumulation, and long-term human toxicity) in the Act on the Evaluation of Chemical Substances and Regulation of Their Manufacture, etc., in 2010 (Ministry of Health, Labor and Welfare, Ministry of Economy, Trade and Industry and Ministry of the Environment, 2010). A similar decline in PFOA concentrations may have resulted from phasing out of the manufacture and use of PFOA in 2006 as stipulated by the U.S. Environmental Protection Agency *via* the 2010/2015 PFOA Stewardship program.

Between 2003 and 2011, concentrations of PFNA and PFDA increased by 4.7% and 2.4% per year, respectively. These results are consistent with recent reports. For example, a study of Swedish primiparous women between 1996 and 2010 revealed that PFNA and PFDA concentrations increased by 4.3% and 3.8% per year, respectively (Glynn et al., 2012). In the National Health and Nutrition Examination Survey (NHANES), which evaluated the United States population, elevated levels of PFNA were measured when samples collected from females during 1999–2000 were

compared with samples collected during 2003–2004 [geometric mean (GM) values of 0.5 ng/mL and 0.9 ng/mL, respectively] (Calafat et al., 2007). In Sendai, Japan, PFNA concentrations in samples from females increased from 1.01 ng/mL in 2003 to 1.80 ng/mL in 2008; moreover, an increase in PFDA concentration was also observed in these samples (0.52 ng/mL to 0.72 ng/mL) (Harada et al., 2011). Here we measured comparable PFNA and PFOA concentrations in samples collected during 2011. Moreover, the proportion of long-chain PFCAs generally increased over time. This result may be explained by higher environmental persistence (Martin et al., 2003) and longer half-lives of long-chain PFCAs (Ohmori et al., 2003), which generally have longer chains than PFOA. The toxicity of PFCAs has been correlated with the length of the carbon chain and the nature of the functional group (Liao et al., 2009; Wolf et al., 2008). Given the increased levels of PFNA and PFDA detected in human blood samples around the world, it is important to evaluate the potential health effects of PFCAs with chains longer than those in PFOAs.

PFUnDA, PFDoDA, and PFTrDA are frequently detected in maternal plasma. Olsen et al. (2012) found that the relative levels of long-chain PFCAs in humans could be ordered as follows: PFOA > PFNA > PFDA ≈ PFUnDA > PFHpA > PFDoDA. In our study, PFCA relative levels were similar to those reported by Olsen et al.: PFOA > PFNA > PFUnDA > PFDA > PFTrDA > PFHxA ≈ PFHpA ≈ PFDoDA. PFUnDA, PFDoDA, and PFTrDA levels were higher than seen in many countries but lower than reported for other areas of Japan (Harada et al., 2011). Total levels of long-chain PFCAs are equal to or greater than PFOA levels, and long-chain PFCA levels seem to be increasing in Japan and Korea (Harada et al., 2011). The composition of long-chain PFCAs within

human blood samples can be used like a fingerprint to identify residents of East-Asian countries, including Japan (Harada et al., 2011). PFNA, PFUnDA, and PFTrDA are manufactured primarily in Japan *via* the oxidation of a mixture of linear fluorotelomer olefins (Prevedouros et al., 2006). Industrial application of these PFCAs may have contributed to our observed increase in PFNA concentrations over time and the accumulation of PFNA in East-Asian populations. In our study, although PFUnDA and PFOA concentrations were comparable in 2011 samples, no temporal trends were observed for PFUnDA, PFDoDA, or PFTrDA. Given that the ΣPFAA concentration was generally lower than for other regions in Japan, ΣPFAA may not be increasing over time. Strong correlations between PFAAs of similar chain length were detected, particularly between long-chain PFCAs. As such, sources of exposure for different long-chain PFCAs may be quite similar. In addition, it is likely that long- and short-chain PFCA sources of exposure are different. There is little data, however, concerning long-chain PFCA concentrations in different human populations around the world (particularly PFCAs that have a longer-chain than PFDA). It is important to evaluate long-term trends associated with long-chain PFCAs in human samples and to continue to monitor levels of these compounds. Further investigation of the source of human exposure to longer-chain PFCAs is needed to evaluate in detail the effects of longer-chain PFCA levels in East-Asian populations.

The maternal PFOS and PFOA concentrations we measured were generally lower than those measured in other parts of the world. These previous epidemiological studies include the NHANES study conducted in the United States (GM concentrations were 12.29 ng/mL PFOS and 2.6 ng/mL

PFOA; Woodruff et al., 2011), the Danish National Birth Cohort study (mean concentrations were 35.3 ng/mL PFOS and 5.6 ng/mL PFOA; Fei et al., 2007), and the Family Study in Canada (mean concentrations were 18.31 ng/mL PFOS and 2.54 ng/mL PFOA; Monroy et al., 2008). Moreover, our previous study conducted in Sapporo City in Hokkaido between 2002 and 2005 also revealed low PFOS and PFOA concentrations (5.2 ng/mL and 1.3 ng/mL, respectively; Okada et al., 2012; Washino et al., 2009). All of Hokkaido, therefore, is an area where human exposure to PFAAs is relatively low. Note that the time of blood sampling during pregnancy could have affected concentrations owing to increased maternal blood volume during gestation (i.e., a dilution effect). The times of blood sampling during pregnancy in previous studies were as follows: the NHANES (first–third trimester), the Danish National Birth Cohort study (4–14 weeks of gestation), the Family Study in Canada (24–28 weeks of gestation), and the study in Sapporo City (23–35 weeks of gestation). As compared with those studies, the blood sampling time in our study was relatively late, i.e., between 28 and 32 weeks of gestation, although it was comparable to the study in Sapporo City. Also, levels of PFCAs in human breast milk in Kyoto in 2010 were reportedly higher than those in other East-Asian countries—GM concentrations were 0.083 ng/mL PFOA, 0.027 ng/mL PFNA, 0.017 ng/mL PFDA, and 0.030 ng/mL PFUnDA (Fujii et al., 2012). Levels of PFOA and PFNA in breast milk in the Sapporo City study were 0.089 ng/mL and 0.035 ng/mL, respectively, and these were similar to those of in Kyoto in 2010 (Nakata et al., 2009). However, because it was collected between 2002 and 2005, the breast milk concentrations of present Hokkaido may be lower than this. Although the levels of maternal PFOS and PFOA are low in Sapporo City, there is still a negative

association between maternal PFOS/PFOA levels and birth weight or cord-blood IgE levels (Okada et al., 2012; Washino et al., 2009). As such, it remains important to assess risks associated with prenatal exposure to PFAAs even when environmental levels are low.

A limitation of our study is that we did not analyze samples taken from individuals over time (i.e., temporal trends within individuals were not assessed). Given that the participants were selected at random, however, our temporal-trend measurements reflect the general population of Hokkaido, Japan. A strength of our study is that it represents a large-scale cohort study of the general population of Hokkaido, Japan.

In conclusion, maternal plasma samples obtained from Hokkaido, Japan, contained lower levels of PFOS and PFOA than has been measured for similar samples from other regions of Japan and around the world. Whereas concentrations of PFOS and PFOA decreased over time, levels of PFNA and PFDA, which are longer-chain PFCAs, increased between 2003 and 2011. Future studies must continue to monitor long-term human-exposure trends associated with long-chain PFCAs and assess the effects of prenatal exposure to these compounds.

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Table 1

Mass transitions, MS/MS conditions, recovery, and detection limits for each PFAA and internal standard.

Compound	Precursor ion (*m*/*z*)	Product ion (*m*/*z*)	Cone (V)	Collision (eV)	Recovery (%)	RSD[a] (%)	IDL[b,c] (ng/mL)	MDL[d,e] (ng/mL)	SRM1957	
									This study[f] (ng/mL) (SE)	Reference values[g] (µg/kg)
PFCAs										
PFHxA	313	269	10	9	94.9	(9.29)	0.1	0.1	< 0.1	-
$^{13}C_2$-PFHxA	315	270	10	9	-	-	-	-	-	-
PFHpA	363	319	16	10	93.1	(9.09)	0.1	0.1	0.338 (0.022)	0.305
PFOA	413	368	17	11	94.9	(6.41)	0.1	0.2	4.76 (0.23)	5.00
$^{13}C_4$-PFOA	417	372	17	11	-	-	-	-	-	-
PFNA	463	419	15	11	92.9	(5.74)	0.1	0.3	0.924 (0.049)	0.88
$^{13}C_5$-PFNA	468	423	15	11	-	-	-	-	-	-
PFDA	513	469	15	13	94.5	(4.90)	0.1	0.1	0.267 (0.053)	0.39
$^{13}C_2$-PFDA	515	470	15	13	-	-	-	-	-	-
PFUnDA	563	519	15	13	85.8	(4.60)	0.1	0.1	0.165 (0.046)	0.174
$^{13}C_2$-PFUnDA	565	520	15	13	-	-	-	-	-	-
PFDoDA	613	569	20	13	90.1	(5.55)	0.1	0.1	0.141 (0.007)	-
PFTrDA	713	669	22	15	85.7	(5.59)	0.1	0.1	0.110 (0.009)	-
PFTeDA	663	619	15	14	100.0	(4.23)	0.1	0.1	< 0.1	-
PFSAs										
PFHxS	399	80	50	30	91.5	(6.13)	0.2	0.2	4.01 (0.27)	4.00
$^{13}C_3$-PFHxS	402	80	50	30	-	-	-	-	-	-
PFOS	499	80	45	40	75.2	(4.76)	0.2	0.3	22.3[h] (1.09)	21.1
$^{13}C_4$-PFOS	503	80	45	40	-	-	-	-	-	-

[a]RSD: Relative standard deviation

[b]IDL: Instrument detection limit

[c]5-µL injection

[d]MDL: Method detection limit

[e]0.5-mL plasma sample

[f]0.5-mL serum sample of NIST SRM

[g]Reference values in NIST, Certificate of Analysis, SRM® 1957

[h]PFOS concentration reflects integration of peak areas for both the branched and linear isomers

Table 2

The study population.

Year	n	Age (years)			Parity	
		Mean	(SD[a])	Range	Primiparous	Multiparous
2003	30	29.7	(4.8)	23–39	15	15
2005	30	29.2	(4.8)	19–37	17	13
2007	30	30.6	(3.8)	24–37	18	12
2009	30	29.6	(5.8)	19–38	16	14
2011	30	32.5	(3.9)	25–40	9	21

[a]SD: standard deviation.

Table 3

Concentration of each PFAA compound in plasma collected from pregnant women from 2003 to 2011.

Compound	Year	Detection		Concentration (ng/mL)						Adjusted concentration (ng/mL)[a]		*p* for trend[c]
		No.	(%)	Range	Mean	(SD[b])	25th	50th	75th	Mean	(95%CI)	
PFCAs												
PFHxA (C6[e])	2003	8	(26.7)	< 0.1–0.16	< 0.1	-	< 0.1	< 0.1	0.11	< 0.1	-	-
	2005	5	(16.7)	< 0.1–0.14	< 0.1	-	< 0.1	< 0.1	< 0.1	< 0.1	-	
	2007	1	(3.3)	< 0.1–0.12	< 0.1	-	< 0.1	< 0.1	< 0.1	< 0.1	-	
	2009	0	(0)	< 0.1–< 0.1	< 0.1	-	< 0.1	< 0.1	< 0.1	< 0.1	-	
	2011	6	(20)	< 0.1–0.13	< 0.1	-	< 0.1	< 0.1	< 0.1	< 0.1	-	
PFHpA (C7[e])	2003	17	(56.7)	< 0.1–0.26	< 0.1	-	< 0.1	0.11	0.16	< 0.1	-	-
	2005	1	(3.3)	< 0.1–0.11	< 0.1	-	< 0.1	< 0.1	< 0.1	< 0.1	-	
	2007	0	(0)	< 0.1–< 0.1	< 0.1	-	< 0.1	< 0.1	< 0.1	< 0.1	-	
	2009	2	(6.7)	< 0.1–0.14	< 0.1	-	< 0.1	< 0.1	< 0.1	< 0.1	-	
	2011	15	(50.0)	< 0.1–0.20	< 0.1	-	< 0.1	< 0.1	0.12	< 0.1	-	
PFOA (C8[e])	2003	30	(100)	0.71–6.88	2.05	(1.26)	1.33	1.93	2.18	1.77	1.51–2.08	0.023
	2005	30	(100)	0.70–2.35	1.25	(0.44)	0.88	1.16	1.56	1.15	0.98–1.35	
	2007	30	(100)	0.55–4.89	1.56	(0.78)	1.09	1.44	1.71	1.36	1.16–1.60	
	2009	30	(100)	0.30–5.45	1.36	(0.99)	0.72	1.19	1.68	1.09	0.93–1.27	
	2011	30	(100)	0.54–2.93	1.42	(0.63)	0.98	1.27	1.77	1.35	1.13–1.61	
PFNA (C9[e])	2003	30	(100)	0.41–3.14	1.13	(0.66)	0.72	0.92	1.14	0.98	0.84–1.13	< 0.001
	2005	30	(100)	0.49–1.52	0.81	(0.25)	0.65	0.74	0.91	0.75	0.64–0.86	
	2007	30	(100)	0.57–6.74	1.31	(1.06)	0.90	1.19	1.33	1.11	0.96–1.28	
	2009	30	(100)	0.42–3.57	1.32	(0.59)	0.98	1.24	1.45	1.20	1.04–1.39	
	2011	30	(100)	0.60-2.54	1.34	(0.57)	0.82	1.26	1.73	1.26	1.07–1.48	
PFDA (C10[e])	2003	30	(100)	0.41–1.20	0.60	(0.18)	0.49	0.56	0.68	0.58	0.52–0.65	0.016
	2005	30	(100)	0.26–0.66	0.42	(0.09)	0.36	0.40	0.48	0.41	0.36–0.46	
	2007	30	(100)	0.25–1.25	0.54	(0.20)	0.40	0.52	0.61	0.50	0.45–0.56	
	2009	30	(100)	0.28–1.24	0.57	(0.20)	0.42	0.51	0.67	0.53	0.48–0.60	
	2011	30	(100)	0.29–1.27	0.71	(0.26)	0.50	0.69	0.89	0.66	0.59–0.75	
PFUnDA (C11[e])	2003	30	(100)	0.71–2.22	1.34	(0.41)	1.09	1.26	1.64	1.27	1.10–1.47	0.876
	2005	30	(100)	0.55–1.64	1.08	(0.31)	0.79	1.13	1.30	1.03	0.89–1.19	
	2007	30	(100)	0.47–2.28	1.37	(0.52)	0.84	1.44	1.87	1.24	1.08–1.44	
	2009	30	(100)	0.42–2.90	1.19	(0.53)	0.77	1.21	1.46	1.09	0.95–1.26	
	2011	30	(100)	0.43–3.40	1.45	(0.70)	0.90	1.30	1.80	1.29	1.10–1.52	

PFDoDA (C12[e])	2003	30	(100)	0.17–0.35	0.24	(0.05)	0.21	0.23	0.27	0.24	0.21–0.29	0.913
	2005	14	(46.7)	< 0.1–0.15	0.08	(0.04)	< 0.1	< 0.1	0.13	< 0.1	-	
	2007	19	(63.3)	< 0.1–0.30	0.12	(0.06)	< 0.1	0.11	0.16	< 0.1	< 0.1–0.12	
	2009	18	(60.0)	< 0.1–0.21	0.11	(0.06)	< 0.1	0.12	0.15	< 0.1	< 0.1–0.11	
	2011	29	(96.7)	< 0.1–0.51	0.25	(0.08)	0.22	0.24	0.30	0.25	0.21–0.30	
PFTrDA (C13[e])	2003	30	(100)	0.25–0.81	0.41	(0.12)	0.32	0.40	0.47	0.40	0.35–0.47	0.055
	2005	29	(96.7)	< 0.1–0.40	0.24	(0.08)	0.18	0.24	0.29	0.23	0.20–0.27	
	2007	29	(96.7)	< 0.1–0.80	0.27	(0.14)	0.18	0.25	0.34	0.25	0.21–0.29	
	2009	28	(93.3)	< 0.1–0.48	0.25	(0.11)	0.17	0.25	0.34	0.23	0.20–0.27	
	2011	29	(96.7)	< 0.1–0.78	0.36	(0.14)	0.28	0.33	0.44	0.33	0.28–0.39	
PFTeDA (C14[e])	2003	16	(53.3)	< 0.1–0.16	< 0.1	-	< 0.1	0.11	0.12	< 0.1	-	-
	2005	0	(0)	< 0.1–< 0.1	< 0.1	-	< 0.1	< 0.1	< 0.1	< 0.1	-	
	2007	0	(0)	< 0.1–< 0.1	< 0.1	-	< 0.1	< 0.1	< 0.1	< 0.1	-	
	2009	0	(0)	< 0.1–< 0.1	< 0.1	-	< 0.1	< 0.1	< 0.1	< 0.1	-	
	2011	4	(13.3)	< 0.1–0.15	0.11	(0.03)	0.11	0.12	0.13	< 0.1	-	
PFSAs												
PFHxS (C6[e])	2003	29	(96.7)	< 0.2–0.60	0.40	(0.11)	0.34	0.40	0.49	0.37	0.30–0.45	0.106
	2005	22	(73.3)	< 0.2–0.53	0.27	(0.13)	< 0.2	0.26	0.35	0.22	0.18–0.27	
	2007	24	(80)	< 0.2–0.61	0.28	(0.13)	0.22	0.27	0.35	0.24	0.20–0.29	
	2009	23	(76.7)	< 0.2–0.77	0.30	(0.16)	< 0.2	0.29	0.39	0.25	0.21–0.30	
	2011	23	(76.7)	< 0.2–0.78	0.33	(0.18)	< 0.2	0.33	0.46	0.28	0.22–0.35	
PFOS (C8[e])[d]	2003	30	(100)	3.53–14.1	7.76	(2.46)	5.72	7.66	9.50	7.66	6.92–8.39	< 0.001
	2005	30	(100)	3.17–13.1	6.20	(2.24)	4.84	5.37	7.37	5.99	5.25–6.74	
	2007	30	(100)	3.11–10.8	6.23	(2.05)	4.37	6.30	7.59	6.08	5.34–6.81	
	2009	30	(100)	2.43–9.69	4.54	(1.48)	3.31	4.48	5.30	4.43	3.70–5.16	
	2011	30	(100)	1.31–8.46	3.90	(1.87)	2.49	3.52	5.05	3.86	3.04–4.68	
ΣPFAAs												
	2003	-	-	7.51–24.33	14.19	(3.90)	11.37	13.97	16.19	14.05	12.90–15.20	< 0.001
	2005	-	-	6.40–18.01	10.51	(2.73)	8.78	9.65	12.24	10.18	9.01–11.35	
	2007	-	-	6.15–23.30	11.83	(3.29)	9.60	11.76	13.41	11.52	10.36–12.67	
	2009	-	-	5.23–18.83	9.80	(2.92)	8.03	9.21	11.11	9.64	8.50–10.78	
	2011	-	-	4.42–18.22	10.02	(3.57)	7.37	9.07	12.70	10.13	8.85–11.42	

[a]Adjusted concentrations were evaluated with respect to the average maternal age of 30.32 years and parity by analysis of covariance.

[b]SD: standard deviation.

[c]Linear regressions to detect temporal trends from 2003 to 2011.

[d]PFOS concentration reflects integration of peak areas for both the branched and linear isomers

[e]C: carbon chain length.

Table 4

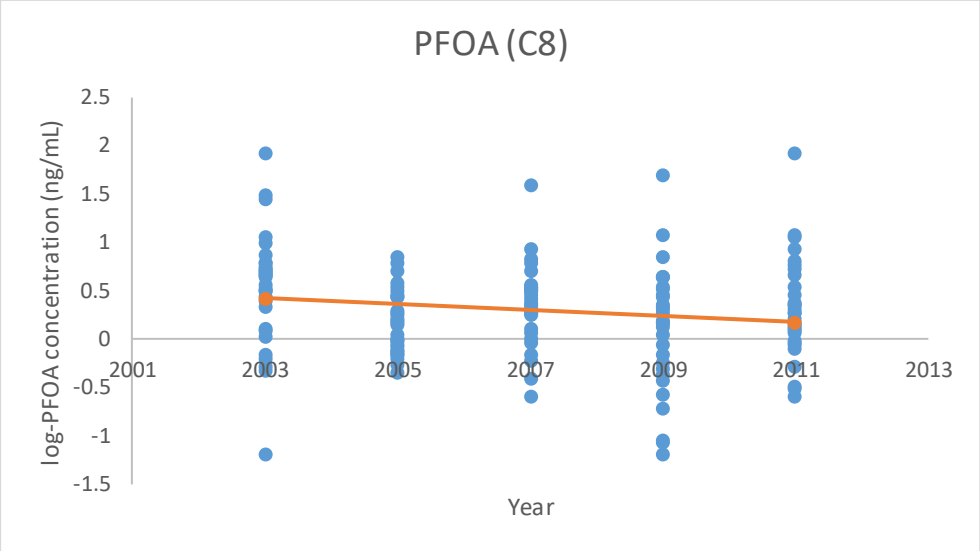
Correlations between levels of PFAA compounds with different chain lengths.

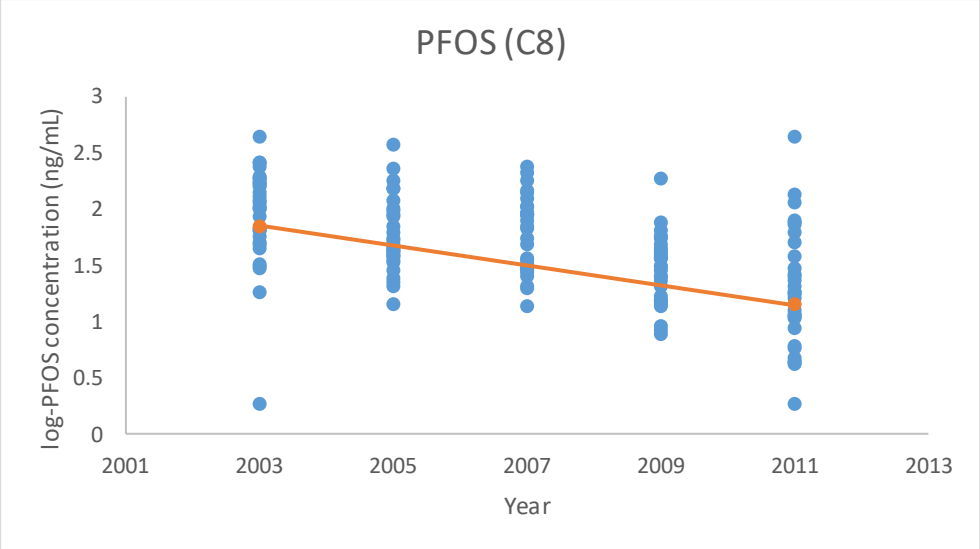
	PFOA (C8[a])		PFNA (C9[a])		PFDA (C10[a])		PFUnDA (C11[a])		PFDoDA (C12[a])		PFTrDA (C13[a])		PFHxS (C6[a])		PFOS (C8[a])
	ρ		ρ		ρ		ρ		ρ		ρ		ρ		ρ
PFCAs															
PFOA (C8[a])	1.00														
PFNA (C9[a])	0.492	***	1.00												
PFDA (C10[a])	0.480	***	0.702	***	1.00										
PFUnDA (C11[a])	0.201	*	0.482	***	0.698	***	1.00								
PFDoDA (C12[a])	0.288	***	0.271	***	0.616	***	0.459	***	1.00						
PFTrDA (C13[a])	0.128		0.227	**	0.548	***	0.675	***	0.707	***	1.00				
PFSAs															
PFHxS (C6[a])	0.268	***	0.202	*	0.239	**	0.291	***	0.264	**	0.329	***	1.00		
PFOS (C8[a])	0.404	***	0.128		0.166	*	0.273	***	0.043		0.202	*	0.408	***	1.00

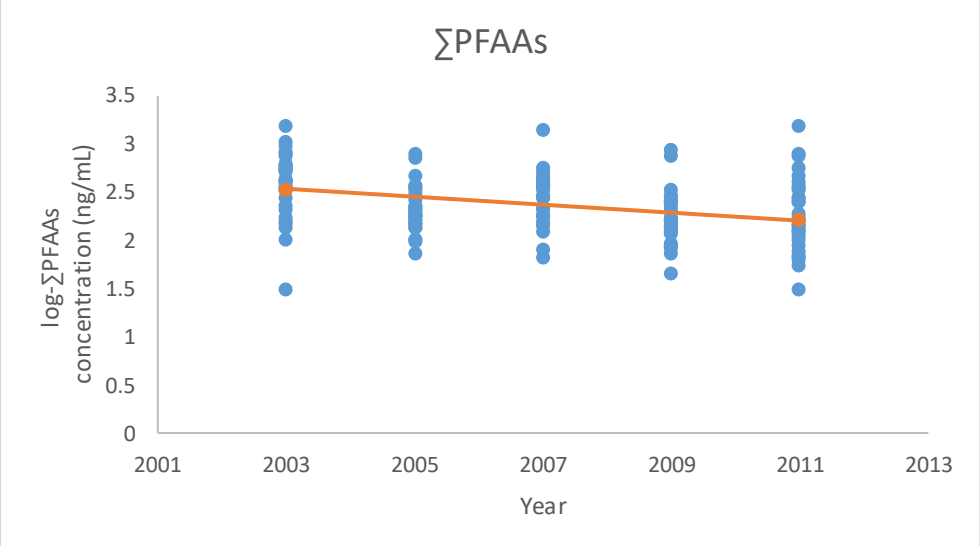
[a]C: carbon chain length.

ρ: Spearman's rank correlation coefficient.

*p < 0.05, **p < 0.01, ***p < 0.001


PFOA (C8)
log-PFOA concentration (ng/mL)
2.5
2
1.5
1
0.5
0
-0.5
-1
-1.5
2001
2003
2005
2007
2009
2011
2013
Year


PFOS (C8)
log-PFOS concentration (ng/mL)
3
2.5
2
1.5
1
0.5
0
2001
2003
2005
2007
2009
2011
2013
Year


∑PFAAs
log-∑PFAAs concentration (ng/mL)
3.5
3
2.5
2
1.5
1
0.5
0
2001
2003
2005
2007
2009
2011
2013
Year

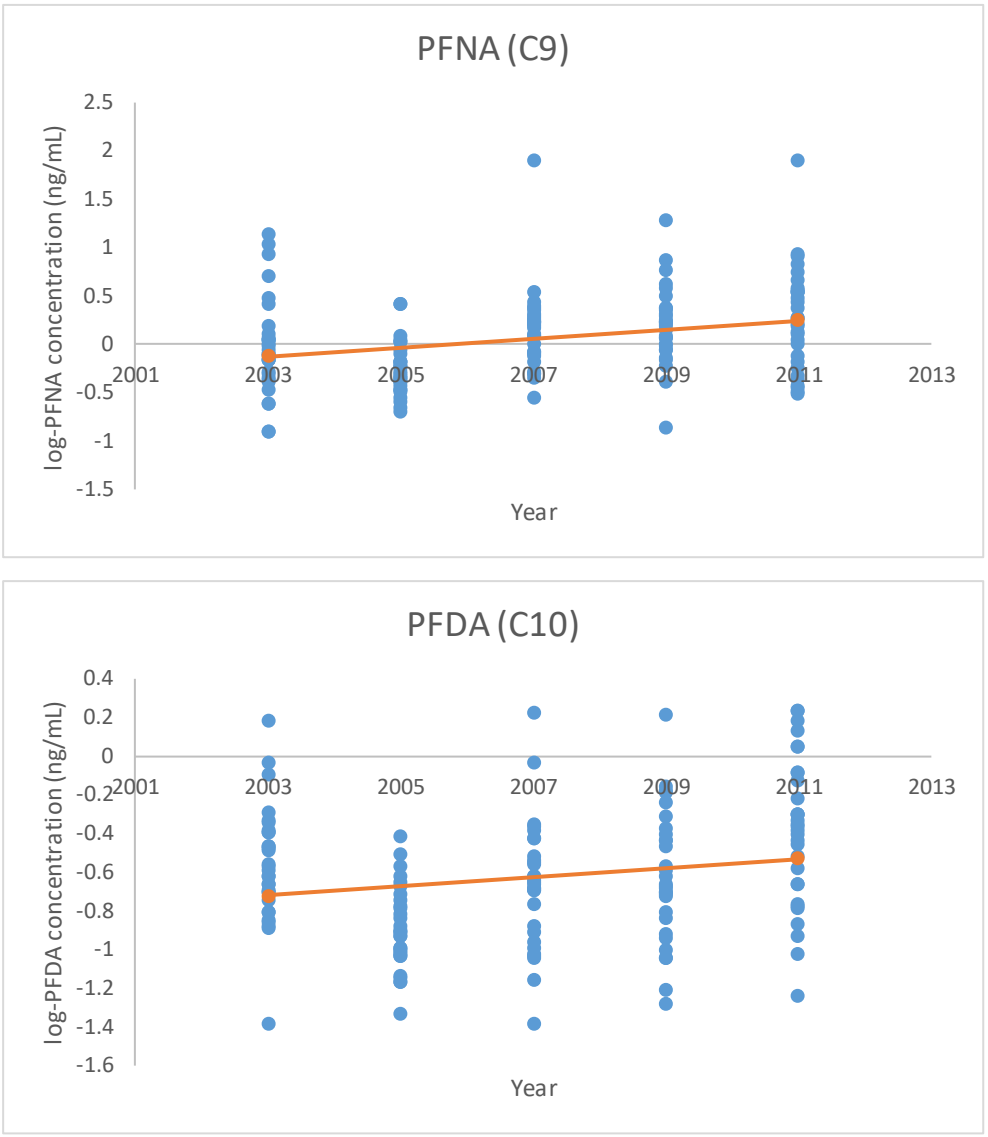


Fig. 1. Temporal trends associated with changes in PFOA, PFOS, ΣPFAA, PFNA, and PFDA, levels in maternal plasma adjusted for age and parity between 2003 and 2011. Solid lines denote the predicted fit from linear regression adjusted for age and parity (log-PFOA = –0.031 year + 0.447; log-PFOS = –0.088 year + 1.943; log-ΣPFAAs = –0.040 year + 2.574; log-PFNA = 0.046 year - 0.171; and log-PFDA = 0.024 year - 0.747). The letter 'C' indicates the carbon chain length of each compound.

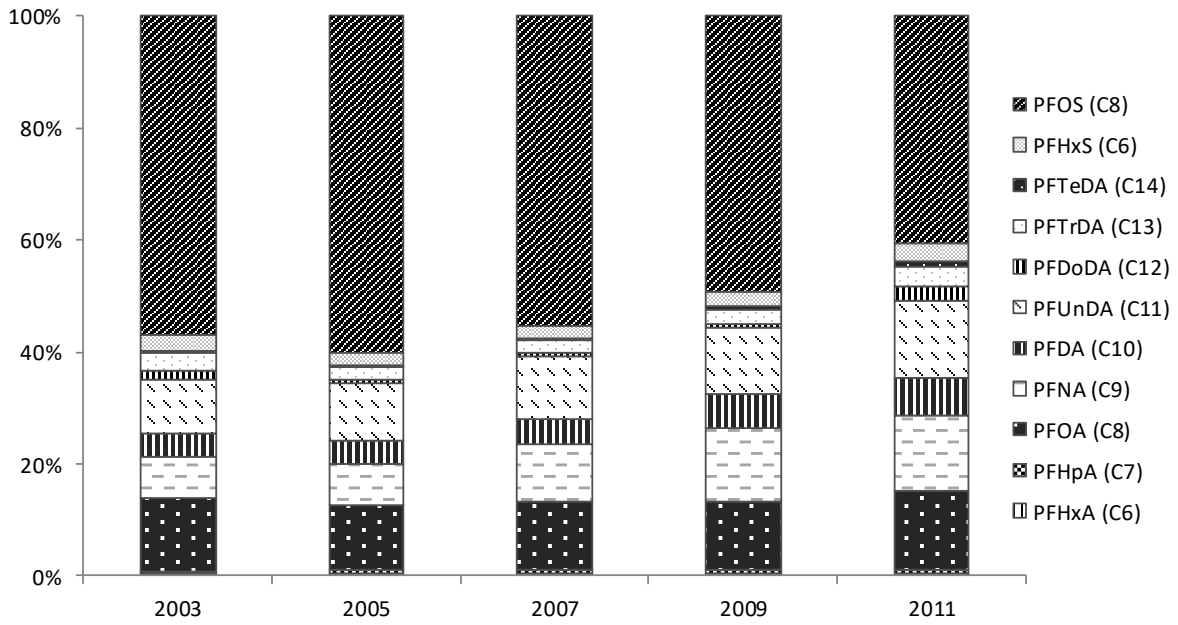


Fig. 2. The composition of total PFAA levels between 2003 and 2011. Relative levels of each PFAA (with ΣPFAA concentration set to 100%) were determined every other year between 2003 and 2011. The letter 'C' indicates the carbon chain length of each compound.