Repeated measurements of per- and polyfluoroalkyl substances (PFASs) from 1979 to 2007 in males from Northern Norway: Assessing time trends, compound correlations and relations to age/birth cohort

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Abstract:

Background

Longitudinal biomonitoring studies can provide unique information on how human concentrations change over time, but have so far not been conducted for per- and polyfluoroalkyl substances (PFASs) in a background exposed population.

Objectives

Determine: i) serum PFAS time trends on an individual level; ii) relative compositions and correlations between different PFASs; and iii) assess selected PFAS concentrations with respect to periodic (calendar year), age and birth cohort (APC) effects.

Methods

Serum was sampled from the same 53 men in 1979, 1986, 1994, 2001 and 2007 in Northern Norway and analysed for 10 PFASs. APC effects were assessed by graphical and mixed effect analyses.

Results

The median concentrations of PFOS and PFOA increased five-fold from 1979 to 2001 and decreased by 26% and 23%, respectively, from 2001 to 2007. The concentrations of perfluorooctanoic acid (PFOA) and perfluorooctane sulphonic acid (PFOS) peaked during 1994-2001 and 2001, respectively, whereas perfluorohexane sulphonic acid (PFHxS) increased to 2001, but did not demonstrate a decrease between 2001 and 2007. Perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), and perfluoroundecanoic acid (PFUnDA) displayed increasing trends throughout the entire study period (1979-2007). Although PFOS comprised dominating and stable proportions of PFAS burdens during these years, the contributions from PFOA and PFHxS were considerable. The evaluation of APC effects demonstrated that calendar

year was the dominating influence on concentrations of PFOA, PFUnDA, and PFOS, although time-variant and weaker associations with age/birth cohort were indicated.

Conclusions

The concentration changes of 10 PFASs in the repeated measurements from 1979 to 2007 demonstrated divergent time trends between the different PFASs. The temporal trends of PFASs in human serum during these 30 years reflect the overall trends in historic production and use, although global transport mechanisms and bioaccumulation potential of the different PFASs together with a varying extent of consumer exposure influenced the observed trends. Sampling year was the strongest descriptor of PFOA, PFUnDA and PFOS concentrations, and the calendar-year trends were apparent for all birth year quartiles. Discrepancies between the trends in this current longitudinal study and previous cross-sectional studies were observed and presumably reflect the different study designs and population characteristics.

Key words: Age-period-cohort effects; Per- and polyfluoroalkyl substances; Repeated measurements; Serum; Time trends.

Abbreviations:

AIC, Akaike`s Information Criterion; APC, Age-period-cohort; FOSA, Perfluorooctane sulfonamide; FTSA, Fluorotelomer sulphonic acids; LOD, Limit of detection; PFASs, Poly- and perfluorinated alkyl substances; PFBA, Perfluorobutanoic acid; PFBS, Perfluorobutane sulphonic acid; PFCAs, Perfluoroalkyl carboxylic acids; PFDA, Perfluorodecanoic acid; PFDcS, Perfluorodecane sulphonic acid; PFHpA, Perfluoroheptanoic acid; PFHpS, Perfluoroheptane

sulfonic acid; PFHxA, Perfluorohexanoic acid; PFHxS, Perfluorohexane sulfonic acid; PFPeA, Perfluoropentanoic acid, PFNA, Perfluorononanoic acid; PFOA, Perfluorooctanoic acid; PFOS, Perfluorooctane sulfonic acid; PFSAs, Perfluoroalkyl sulphonic acids; PFUnDA, Perfluoroundecanoic acid; POP, Persistent organic pollutant; SRM, Standard reference material.

1. Introduction

Production and use of per- and polyfluoroalkyl substances (PFASs) started in the 1950s and increased considerably during the 1970s (Paul et al., 2009; Prevedouros et al., 2006). PFASs continue to be in high demand today due to their widespread use in industrial and consumer product applications (Buck et al., 2011). Two major PFAS groups are the perfluoroalkyl carboxylic acids (PFCAs) and the perfluoroalkyl sulphonic acids (PFSAs) (Buck et al., 2011), of which perfluorooctanoic acid (PFOA) and perfluorooctane sulphonic acid (PFOS) have received most attention in studies of humans and the environment. Increasing concern for their persistency and bioaccumulative properties has led to voluntary and regulatory efforts restricting their use, including: i) phasing out PFOS and related compounds while providing shorter chain PFSAs as replacements during 2000-2002 by 3M, the major producer (US EPA, 2002); ii) inclusion of PFOS in the Stockholm Convention in 2009 (Stockholm Convention); and iii), initiation of a PFOA stewardship program to phase out PFOA and longer chain PFCAs by 2015 (US EPA, 2006). As a consequence of these actions, the global production of PFOS and related chemicals decreased drastically after the peak between 1990 and 2000 (Paul et al., 2009), although production of PFOS has continued in China (Zhang et al., 2012) and it is likely that production of longer chain PFASs continued for some years after 2002 (Armitage et al., 2009).

Overall, biomonitoring of PFASs in human serum has demonstrated decreasing concentrations of PFOS and PFOA since the early 2000s, whereas trends for other PFASs have been variable (Calafat et al., 2007a; Glynn et al., 2012; Harada et al., 2004; Jin et al., 2007; Kannan et al., 2004; Kato et al., 2011; Olsen et al., 2005; Olsen et al., 2012; Schröter-Kermani et al., 2012; Toms et al., 2009; Wang et al., 2011; Yeung et al., 2013a, 2013b). A cross-sectional study of pooled sera from 40-50 year old men in Norway during 1976 to 2007 reported that many PFASs increased during the study period and that PFOS, PFOA and perfluoroheptane sulphonic acid (PFHpS) started declining around year 2000 (Haug et al., 2009). The observed time trends of PFOS and PFOA in human serum, to a large extent, mirror the changes in global production. However, the reasons for differing time trends for different PFAS homologues and between different studies are not well understood. Furthermore, the decline in human concentrations of PFOA and PFOS after the phase-out initiated in year 2000 was observed after a short time lag considering its relatively long human elimination half-life (Olsen et al., 2007) and the absence of consistent decreasing trends in wildlife for the same time period (Butt et al., 2010). Time trends in human biomonitoring primarily reflect a combination of the temporal changes in exposure (intensity, duration and intake rates), and elimination kinetics (Quinn and Wania, 2012; Ritter et al., 2009). With respect to exposure pathways, the body burden of PFASs is greatly influenced by dietary intake, although drinking water, inhalation of indoor air, ingestion of house dust, and direct contact with consumer/commercial products may also contribute to a varying extent (Egeghy and Lorber, 2011; Fromme et al., 2009; Haug et al., 2011; Lorber and Egeghy, 2011;Vestergren and Cousins, 2009). Consequently, temporality in human exposure depends on the response time of the major source media to changes in PFAS production. Furthermore, exposure to PFASs in human populations in Arctic regions may have a different response time to changes in production due to the time-lag of long-range transport of PFASs by air and ocean currents (Butt et al., 2010).

Local or regional differences in contamination status together with life style differences and dietary habits may therefore result in different time trends between studies.

In addition to different population exposures, observed human time trends may also be affected by the study design and demographic characteristics of the study group. Previous studies on legacy persistent organic pollutants (POPs) have demonstrated that an improved understanding of age, period and birth cohort effects is needed to correctly interpret time trends in biomonitoring studies (Nøst et al., 2013; Quinn and Wania, 2012; Ritter et al., 2009). Generally, no association (Calafat et al., 2007a, 2007b; Harada et al., 2007; Olsen et al., 2008; Yeung et al., 2006) and variable associations with age (Haug et al., 2009; Kato et al., 2011) have been reported for PFASs in cross-sectional studies. In one such study of pooled samples from Norwegian subjects, both positive and negative associations to age were reported, which varied between sampling years and the different PFASs (Haug et al., 2009).

The present study describes changes in PFAS concentrations and compositional patterns in repeated serum samples during 1979-2007 and, to the best of our knowledge, this is the first to report repeated measurements of a number of PFASs in a non-occupationally exposed population. The rare longitudinal study design allowed for an assessment of periodic time trends during nearly 30 years in addition to the age and birth cohort effects (APC effects) in concentrations of selected PFASs.

2. Subjects and methodology

2.1. Study population and subject selection

Five repeated population surveys called the Tromsø study (summarized by Jacobsen et al., 2012) took place in the municipality of Tromsø, Northern Norway in 1979, 1986-1987 (hereafter referred to as 1986), 1994-1995 (1994), 2001 and 2007-2008 (2007). Adult men (n = 60) were randomly selected from 1438 males who had participated and donated blood in all five surveys of the Tromsø study. Of these, 53 had sufficient sample volumes in ≥3 sampling years and the present analyses comprised 254 serum samples (11 samples randomly distributed across sampling years were missing). Birth year information was extracted from questionnaires, while individual dietary information was inadequate. The range in birth years was 1925-1950, and the median ages at the first and last sampling were 43 and 71. Serum samples were stored at -70 °C until analysis. The study was approved by the Regional Committees for Medical Research Ethics. Participation was voluntary and participants gave informed consents.

2.2. Analytical methodology

Analyses were performed at the laboratories of NILU-Norwegian Institute for Air Research, Fram Centre, Tromsø, Norway. All serum samples were quantified for 10 target analytes and a subset of 43 samples were initially quantified for 21 analytes (see Supplemental Material, Table S1).

2.2.1. Extraction and clean up

Serum samples were analysed using the internal-standard method and sonication-facilitated liquid-liquid extraction in methanol, activated charcoal clean up, and analysed by ultrahigh pressure liquid chromatography triple-quadrupole mass spectrometry (Thermo Fisher Scientific Inc, Waltham, MA, USA).

Extraction was performed as per Hanssen et al. (2013) with the following changes; i) 100 µl serum was extracted in a 1.5 ml eppendorf tube; ii) the internal standards (see Supplemental Material, Table S2 for list); iii) the volume methanol (750 µl) added; and iv) amount of branched perfluorodecanoic acid (br-PFDA) recovery standard (20 µl of 0.102 ng/µl) used.

2.2.2. Instrumental analysis

The analytical specifications are described in Hanssen et al. (2013). The quantification was conducted with the LC Quan software, version 2.6.0 (Thermo Fisher Scientific Inc, Waltham, MA, USA). Of the 21 PFASs included in the analyses, 10 were detected in >20% of samples in a subset of 20 samples and the remaining samples were quantified for these 10 PFASs. The linear and branched PFOS isomers were chromatographically separated ("branched PFOS" was identified as one or several peaks eluting earlier than the linear PFOS; see Supplemental Material, Fig. S1A). The mass-labeled internal standard for linear PFOS was also used for quantification of the branched isomers. Concentrations of branched PFOS presented were calculated as the mean concentrations of two transitions in the analysis (m/z 499-80 and 499-99), since response factors have been reported to differ between transitions of different isomers of PFOS (Berger et al., 2011). Data presented as "PFOS" represent the sum of the linear and the coeluted peaks of branched isomers. Chromatographically separated branched and linear isomers could also be observed for perfluorooctane sulfonamide (FOSA) (see Supplemental Material, Fig. S1B); however, due to high variation (<80%) between isomers in parallel injections in some samples, the presented concentrations of FOSA represent the sum of isomers. For other PFASs, only the linear isomer was detected and quantified.

2.3. Quality assurance and sample integrity

2.3.1. Quality control in PFAS analyses

Blanks (n = 9) and standard reference materials (SRMs) [SRM® 1958 (n = 9) and 1957 (n = 9), both from the National Institute of Standards and Technology, Gaithersburg, MD, USA] were processed along with samples. The results for the SRM analyses were within +/-20% of reference values, except for perfluoroheptanoic acid (PFHpA), PFDA, PFUnDA for which the mean quantified concentrations were -30, -50 and -60% of reference values, respectively. The laboratory routinely participates in the Arctic Monitoring and Assessment Programme Ring Test for Persistent Organic Pollutants in Human Serum, and has performed within +/-20% of assigned values, which is considered excellent performance. [Ring test results are available from the Institut national de santé publique du Québec (2013).]

All concentrations presented were within the calibration curve and the linear range of the instrument. For each compound in the mass spectrometry analyses, a second isotopic mass transition served to confirm compound specificity. The limits of detections (LODs) were set to three times the mean concentrations determined in blank samples (Table A.1 in the Appendix). Mean recoveries (range) of internal standards were 108% (69-145), 101% (67-132), 103% (66-147), 108% (69-159), and 85% (42-142) for the 1979, 1986, 1994, 2001, 2007 samples, respectively. The recoveries in one sample preparation batch of 2007 samples were low (53% of 2007 samples), although there was no association between recoveries and concentrations (data not presented).

2.3.2. Estimation of desiccation

To correct for spuriously elevated PFAS concentrations caused by evaporation during long-term storage, serum sodium (Na^+) was measured and used to adjust plasma volumes as described in Nøst et al. (2013).

2.4. Data treatment and statistical methods

Statistical analyses were executed using the software R, ver. 3.0.0 , and a statistical significance threshold of $p < 0.05$ was used. The statistical analyses included 254 samples (N = 53, 52, 48, 49 and 52 at the five time points). All PFAS concentrations were log-normally distributed (Shapiro-Wilk tests) and therefore $\log_e$-transformed in the statistical analyses.

Summary statistics for compounds with detection frequencies >80% were calculated by conventional methods, whereas those occurring less frequently (20% - 80%) were computed for each sampling year using the Kaplan-Meier method employing the NADA package for R according to Helsel (2005).

Spearman`s ρ values were calculated for correlations. Wilcoxon signed rank test was used to assess differences in PFAS concentrations between different sampling years, and Kruskal-Wallis rank sum test between birth year quartiles in each sampling year. The non-parametric Friedman`s test of repeated measurements was employed for differences across all sampling years.

APC effects were assessed with age and birth cohort variables as quartiles. Mixed effect models (lme4 package for R) that allowed for subject-specific random variation, were used to assess periodic changes and potential age- and birth cohort-specific effects in concentrations of PFASs. The analyses were restricted to the fully detected PFOA, PFUnDA and PFOS to obtain the appropriate APC evaluation and model estimates. All models included a subject-specific random term and a random slope for sampling year, and the best fitted model was chosen based on Akaike`s Information Criterion (AIC) values (for details, see Nøst et al., 2013). Furthermore, selected graphical examinations of APC effects in concentrations of 8 PFASs were carried out.

3. Results

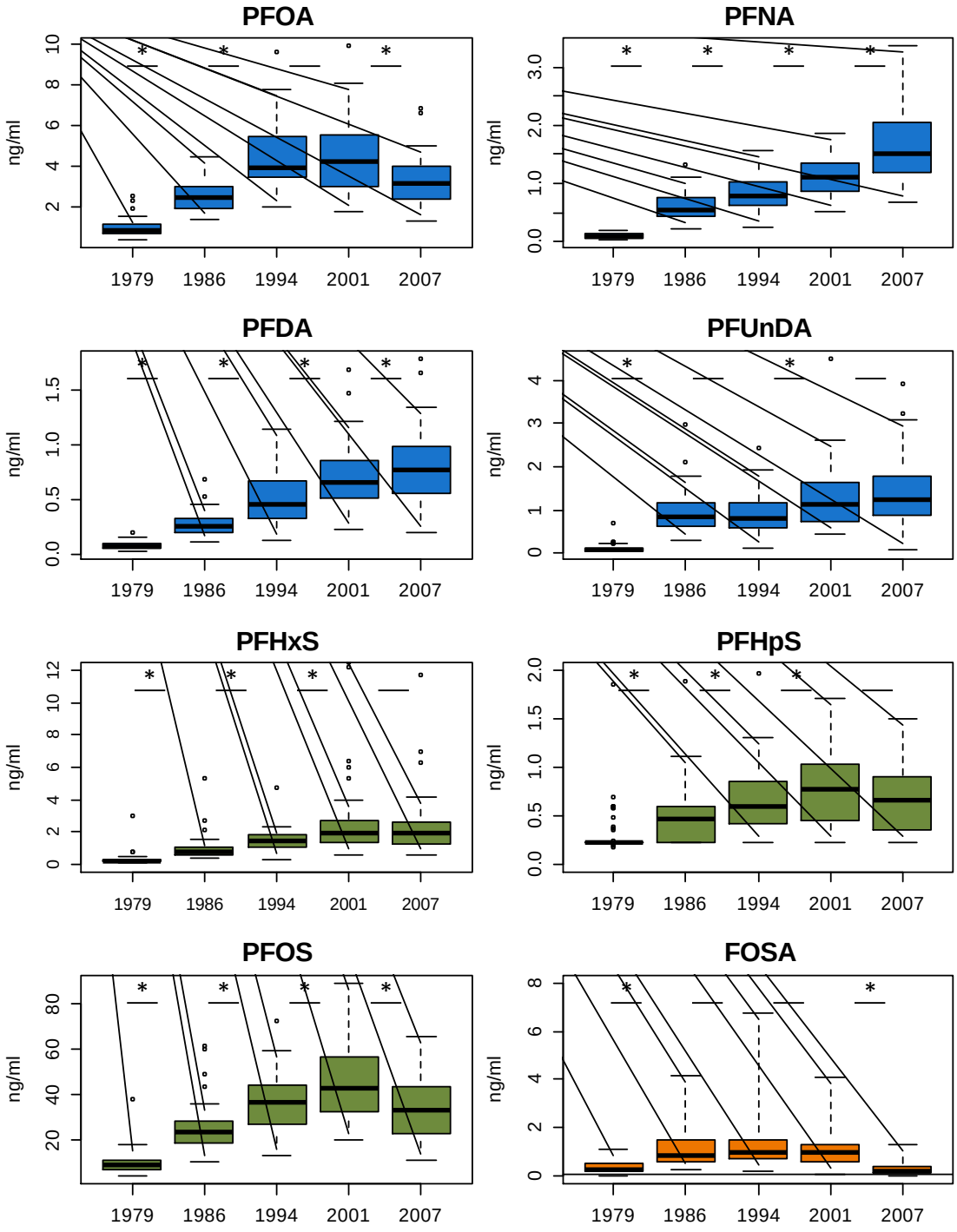
3.1. Changes in PFAS concentrations during 1979-2007

Serum concentrations and detection frequencies of 10 PFASs are presented in Table A.1 of the Appendix. Concentrations of PFOA, PFNA, PFDA, PFUnDA, PFHxS, PFHpS, PFOS and FOSA in each sampling year are depicted in Fig. 1, and temporal changes between consecutive sampling years are presented in Table 1.

Table 1: Change in median concentrations (ng/ml) of the most abundant PFASs analysed in serum samples of men (N = 53, 52, 48, 49 and 52 in 1979, 1986, 1994, 2001 and 2007, respectively) in Northern Norway. Significant differences between years are indicated in Fig. 1.

	1979-1986		1986-1994		1994-2001		2001-2007	
Compound	Change in ng/ml	%	Change in ng/ml	%	Change in ng/ml	%	Change in ng/ml	%
PFOA	1.46	170	1.79	72	-0.23	-6	-0.99	-23
PFNA	0.44	500	0.20	37	0.30	38	0.45	41
PFDA	0.18	250	0.25	100	0.17	37	0.11	18
PFUnDA	0.74	850	-0.05	-6	0.26	32	0.09	8
PFHxS	0.54	260	0.61	85	0.41	27	-0.12	-6
PFHpS	0.14	62	0.11	23	0.19	32	-0.10	-13
PFOS	14.70	170	11.34	49	9.17	25	-9.39	-22
FOSA	0.63	210	-0.05	-6	-0.11	-10	-0.59	-60

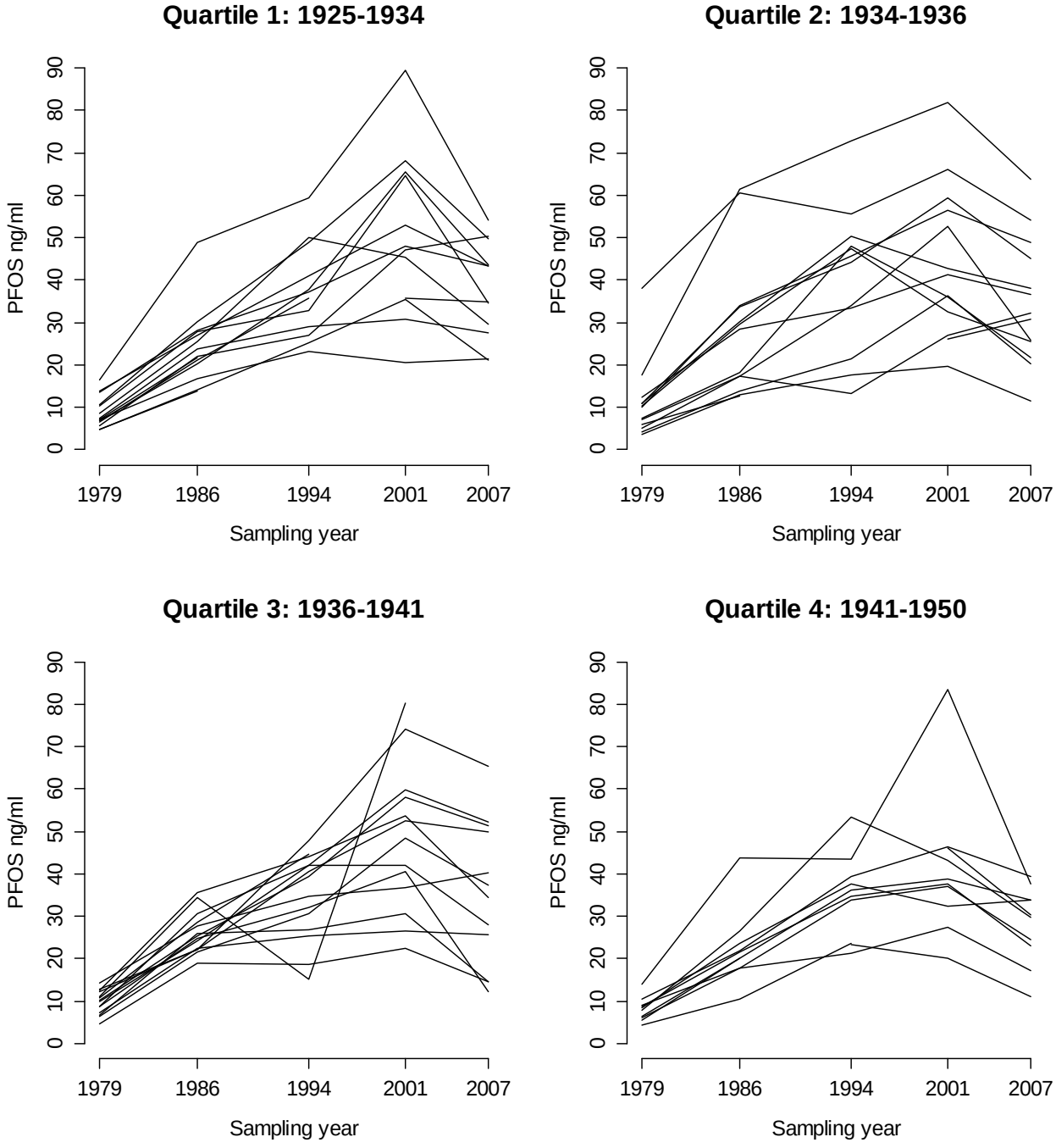
Fig. 1: Concentrations (ng/ml wet weight) of the most abundant PFASs analysed in repeated serum samples of men from Northern Norway (N =53, 52, 48, 49 and 52 in 1979, 1986, 1994, 2001 and 2007, respectively). The asterisks denote significant differences in consecutive sampling years ($p<0.001$, Wilcoxon signed rank test). The boxplots for FOSA are censored box plots with the horizontal line indicating the LOD. One outlier for FOSA (13 ng/ml) in 2001 is not shown.



All quantified PFASs except perfluorohexanoic acid (PFHxA) increased from the initial concentrations in 1979. The median concentrations of PFOA and PFOS increased five-fold from 1979 to 2001 and decreased by 26% and 23%, respectively, from 2001 to 2007. Concentrations peaked in 1994 for PFHpA; in 1994 and 2001 for PFOA; and in 2001 for PFHxS and PFOS (not significant for PFHxS). Concentrations of FOSA reached a plateau from 1986 to 2001 and decreased to 2007. Continuously increasing concentrations across the study period were observed for PFNA, PFDA, PFUnDA, although not statistically significant between all years; and the rate of increase varied between the different homologues and years. Individual trend curves are presented for PFOS and PFOA in Fig. 2 and Supplemental Material, Fig. S2, respectively; they display generally consistent trends among individuals with the largest concentration ranges in 2001 and 2007.

Perfluorobutanoic acid (PFBA) and perfluoropentanoic acid (PFPeA) were detected in the samples, but the quantified concentrations are not presented due to the lack of a confirmatory ion transition in the instrumental analysis. However, reanalysis of three samples from different years by Acquity UPLC -MS-MS (ES^-, MRM, Waters Tandem Quadrupol Detector) and HR-MS (ES^-, Full Scan, LTQ Orbitrap, Thermo Scientific) qualitatively confirmed their presence.

Fig. 2: Individual trend lines for PFOS concentrations in repeated measurements in 1979, 1986, 1994, 2001 and 2007 in serum samples of men from Northern Norway. Trend lines are displayed according to birth year quartiles.



3.2.PFAS correlations between subsequent measurements

Correlations between two subsequent measurements of a PFAS varied during the sample period (Table 2), and were the strongest between the measurements in 2001 and 2007 for most PFASs. However, those for PFOS and PFOA were robust (Spearman`s ρ >0.6) between all subsequent measurements.

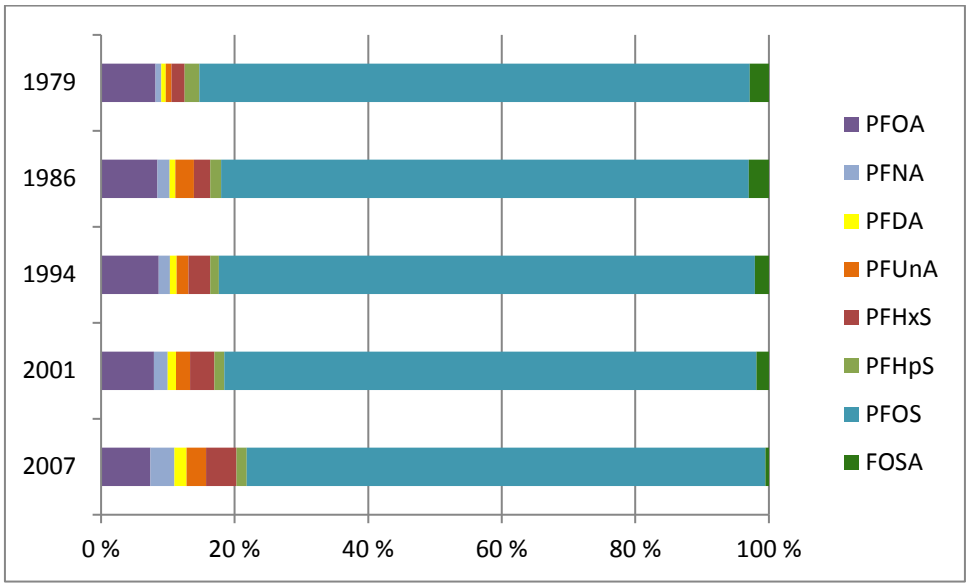
Table 2: Spearman`s ρ for significant correlations ($p<0.05$) between subsequent measurements.

Compound	1979-1986	1986-1994	1994-2001	2001-2007
PFHpA		0.35		0.55
PFOA	0.65	0.66	0.60	0.75
PFNA	0.44	0.65	0.63	0.60
PFDA	0.50	0.42	0.59	0.71
PFUnDA	0.35	0.56	0.61	0.79
PFHxS	0.59	0.63	0.46	0.81
PFHpS	0.43	0.48	0.36	0.66
PFOS	0.84	0.65	0.62	0.81
FOSA		0.39		

3.3.Compositional patterns and correlations between PFASs

The most abundant PFASs in all years were PFOS (78-82% of summed median PFAS concentrations) >PFOA (7-9%) >PFHxS (2-5%) >PFUnDA (1-3%); compositional patterns of PFASs for each sampling year are indicated in Fig. 3. Median PFOS concentrations were 9-10 times higher than those of PFOA in all sampling years. Decreasing ratios across sampling years were observed for other pairs: 9, 5, 5, 4, 2 for PFOA/PFNA, 12, 10, 9, 7, 4 for PFOA/PFDA and 9, 3, 5, 4, and 3 for PFOA/PFUnDA in 1979, 1986, 1994, 2001 and 2007, respectively.

Fig. 3: Relative contributions of the different PFASs to their sum (in %) are presented for five repeated serum measurements in men from Northern Norway (N = 53, 52, 48, 49 and 52 in 1979, 1986, 1994, 2001 and 2007, respectively).



The median percentages of linear isomers to summed PFOS concentrations were quite stable (68% - 72%; Table A.1 in the Appendix), but the percentages of linear PFOS in 1994 and 2001 were significantly different (Wilcoxon paired rank sum, $p<0.0001$). Some individual variation in temporal trends for percentage of linear isomer was observed (Supplemental Material, Fig. S3).

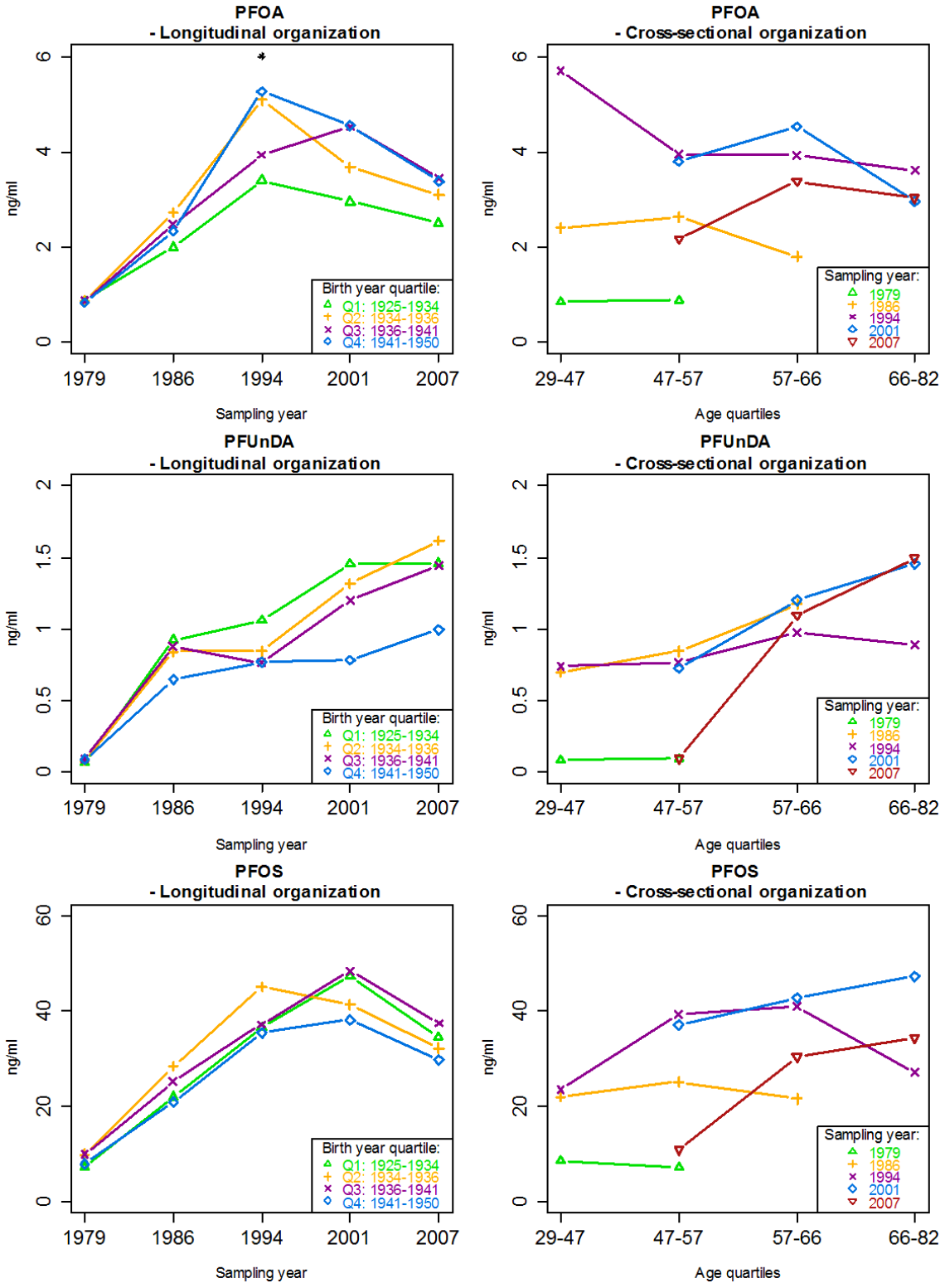
The correlations between PFASs in each sampling year are presented in Supplemental Material, Table S3. Those for PFOS and PFOA were quite stable (Spearman`s ρ: 0.3-0.4) during the study period, while they generally increased for PFNA, PFDA and PFUnDA (especially between PFDA and PFUnDA, with Spearman`s ρ= 0.4, 0.6, 0.7, 0.8, 0.9 in 1979, 1986, 1994, 2001 and 2007, respectively). Furthermore PFNA and PFOS correlated well (Spearman`s ρ >0.6) throughout the period, and the associations of PFDA and PFUnDA with PFOS strengthened.

However, those between PFHxS and PFOS decreased slightly and the correlation between FOSA and PFOS increased from 1979 to 1994 and declined thereafter.

3.4. Age-period-cohort effects in concentrations of PFOA, PFUnDA and PFOS

Estimates from mixed effect models for PFOA, PFUnDA and PFOS are presented in Supplemental Material, Tables S4-6. The best fitted model for PFOS and PFOA included only sampling year as a fixed predictor, whereas for PFUnDA it also included birth cohort as a fixed factor and age as a random term. Selected graphical displays of age-period-cohort effects in Fig. 4 present longitudinal and cross-sectional organizations of PFOA, PFUnDA and PFOS concentrations (those for PFHpA, PFNA, PFDA and PFHxS are presented in Supplemental Material, Fig. S4). The change in concentrations of PFOS from 2001 to 2007 was not significantly different across age quartiles (Kruskal-Wallis rank sum test, $p>0.05$).

Fig. 4: APC plots: Longitudinal (age and period effects are confounded) and cross-sectional (age and birth year effects are confounded) organization of PFOS and PFOA concentrations (medians for each quartile in each sampling year, in ng/ml wet weight). The asterisk indicates significant birth cohort differences (Kruskal-Wallis rank sum test, $p<0.05$).



4. Discussion

4.1. Time trends in PFAS concentrations

The observed longitudinal trends of PFASs in a population experiencing background exposure during a thirty year period covering the years of most intense production of PFOS-related compounds provide new insight. Overall, time trends for PFOA, PFOS and FOSA (Fig. 1) are in harmony with the global production history of long-chain PFASs (Paul et al., 2009; Prevedouros et al., 2006). The relatively rapid response in serum concentrations of PFOA and PFOS observed in this and other studies following reduced production during 2000-2002 is somewhat remarkable considering their long human half-lives (Olsen et al., 2007) and the absence of concurrent and distinct decreases in PFOA and PFOS concentrations in wild-life studies (Butt et al., 2010; Holmström et al., 2010). Although a levelling off or slight decrease in PFOS concentrations was observed in wild-life in Northern Norway during this period (Ahrens et al., 2011; Verreault et al., 2007), the observed declines in human sera in this study cannot be explained by concurrent decreases in PFAS exposures through human food-chains. Further, the stable ratio and correlation between PFOS and PFOA during 1979-2007 suggest that their exposure pathways have changed little or done so concomitantly during this period. One hypothesis, which has been proposed to explain the concurrent decrease in serum concentrations of PFOA and PFOS in cross-sectional studies in other countries, states that consumer products made a significant contribution to the total exposure (direct or through degradation of precursors) to these compounds prior to year 2000 (D'eon and Mabury, 2011; Jackson and Mabury, 2012; Olsen et al., 2008; Vestergren and Cousins, 2009). As their production ceased during 2000-2002 human serum concentrations of PFOA and PFOS might be expected to converge as diet-linked environmental pathways would become increasingly important in a post ban situation (Vestergren and Cousins, 2009).

Indications of an earlier peak in PFOA concentrations relative to those of PFOS in some individuals (Fig. 2 and S2) likely reflect the somewhat longer human elimination half-life of PFOS compared to PFOA (4.6 and 3.4 years, respectively, estimated median in fluorochemical production workers by Olsen et al. (2007)). Concentrations of PFHxS did not display a significant decrease from 2001 to 2007 (Fig. 1) despite that production of this compound was phased out at the same time as PFOS (Kannan et al., 2004). The diverging time trends of PFHxS and PFOS could be due to the longer elimination half-life of PFHxS (7.1 years; Olsen et al., 2007) relative to PFOS and a relatively higher exposure of PFHxS through the food-chain exposure pathway suggested throughout the study period compared to PFOS. Concentrations of FOSA were quite stable from 1986 to 2001 and decreased to 2007. This compound has been shown to be a precursor of PFOS (Xu et al., 2004) as well as a metabolite of other precursor compounds (Benskin et al., 2007). The time trend of FOSA as a precursor compound is as such interesting, and the decrease from 2001 to 2007 could contribute to the observed decline in PFOS concentrations. Furthermore, the FOSA decline could also reflect the decline of other precursor compounds, which is in line with such trends reported for two German cities (Yeung et al., 2013a,b).

PFNA, PFDA and PFUnDA concentrations increased from 1979 to 2007 (not significantly so for every sampling year) which could be due to their continued production after 2001 (Armitage et al., 2009) along with longer elimination half-lives and bioaccumulation ability compared to shorter-chain PFCAs (Conder et al., 2008; Zhang et al., 2013). As opposed to for PFOA and PFOS, time trends of these compounds in humans are more in accordance with those observed in wild-life biomonitoring (Ahrens et al., 2011; Butt et al., 2010; Holmström et al., 2010; Verreault et al., 2007). Inter-correlations between PFNA, PFDA and PFUnDA as well as their strengthened

correlations to PFOS over the same period suggest a gradual co-exposure through environmental background concentrations rather than consumer products (Vestergren and Cousins, 2009). Although human exposure pathways of C>8 PFCAs are not well understood, recent dietary intake studies (e.g. Vestergren et al., 2013) and biomonitoring studies (e.g. Brantsæter et al., 2013) demonstrate that human concentrations of these compounds are currently linked to the diet. The increasing time trends signify the concern towards human body burdens of longer-chain PFCAs.

Cross-sectional time trend studies involve testing different subjects at each sampling point, while our longitudinal study follow the same aging individuals. Comparing our PFAS time trends to those in pooled sera from Norwegian men (aged 40-50 at the time of each collection) during 1977-2006 (Haug et al., 2009) revealed interesting similarities and differences. The concentrations and temporal changes of PFOA observed were comparable between the studies and demonstrate a uniform exposure to PFOA during these years (Supplemental Material, Fig. S5). Furthermore, concentrations of PFOS in 1979 were similar to those in Haug et al., but the subsequent incline to later years was steeper in our study and the decline from 2001 was less pronounced (-23% during 2001-2007 in this study, and -55% during 2001-2006 in Haug et al. (2009)). PFNA, PFDA and PFUnDA increased more during the study period in men from Northern Norway compared to those in Haug et al. (Supplemental Material, Fig. S5). The observed differences in time trends could partly be explained by the enhanced and prolonged exposure to these compounds in the Northern Norwegian men, possibly related to their expected higher fish consumption (Alexander et al., 2006; Johansson and Solvoll, 1999; Nøst et al., 2013). However, the different study designs and resulting age group differences (intraindividual versus interindividual age differences in longitudinal and cross-sectional studies, respectively), could also contribute to the discrepancies. It may also be noted that environmental concentrations of

PFOS in northern latitudes are expected to respond more slowly to changes in their production and use due to the slow transport of PFOS with ocean currents (Armitage et al., 2009).

The decline in PFOS from 2001 to 2007 was also less pronounced in the Northern Norwegian men when compared to cross-sectional studies in Germany and U.S.A. although time trends for PFOA, PFNA and PFHxS in 2001 and 2007 were comparable (Supplemental Material, Fig. S5) (Kato et al., 2011; Olsen et al., 2012; Schröter-Kermani et al., 2012). Further, concentrations were generally higher for PFOS, slightly lower for PFOA, and comparable for PFNA and PFHxS in the Northern Norwegian men. Again this likely reflects the difference in study designs and characteristics of the exposure experienced by the different populations.

4.2. Changing correlations between subsequent measurements

Correlations between subsequent measurements varied across the sampling period but became stronger in 2001-2007 relative to the earlier years. Notably, correlations for PFUnDA became stronger throughout. The varying correlations could reflect changing intensities and pathways of human PFAS exposures during 1979-2007. It is likely that in the earliest years, human exposure pathways were various and intensities increased (Paul et al., 2009; Prevedouros et al., 2006). In accordance with this, the widest concentration ranges of PFOA and PFOS were observed in the years of highest concentrations (1994 and 2001; Fig. 2 and S2), and likely reflect large individual variation in exposures when intensities peaked. After 2001, exposure intensities decreased and contribution of the human food chain likely increased. Indeed, diet is suspected to be the major current exposure route of PFASs for humans (Egeghy and Lorber, 2011; Fromme et al., 2009; Haug et al., 2011; Lorber and Egeghy, 2011; Rylander et al., 2009; Vestergren and Cousins, 2009).

4.3.Time trends in relative compositions

Relative production and use of the different PFASs, environmental persistence, human half-lives, precursor chemistry and exposure pathways must all be kept in mind when considering human compositional PFAS patterns over time. PFOS was most abundant in all years, but PFOA and PFHxS also contributed substantially to the PFAS burdens. The relative contribution to the sum of PFASs by PFOA and PFOS were stable during the study (Fig. 3), whereas those for PFNA, PFDA and PFUnDA increased. Higher bioaccumulation potentials have been proposed for longer chain PFCAs (Conder et al., 2008), and could explain the prolonged exposure to these compounds from the environment. The PFOA/PFNA ratio decreased from 1979 to 2007, and the relative decrease in PFOA could reflect the declining production and use of PFOA and the increasing influence of food-chain related exposure as PFNA>PFOA in wildlife (Vestergren and Cousins, 2009; Butt et al., 2010). This is also likely valid for the similar trends in PFOA/PFDA and PFOA/PFUnDA ratios. The relative concentration of FOSA decreased during the study period, but the proportions reported are underestimated in this study as 80-90% of FOSA is associated with the cell fraction discarded from plasma/serum (Kärrman et al., 2006; Hanssen et al., 2013).

No consistent trend was observed among subjects for the relative percentages of linear and branched isomers of PFOS over time (Fig. S3). However, it should be noted that there were individual variations in the percentage of branched PFOS. Enriched profiles of branched PFOS (>30% branched) has been suggested as a biomarker of exposure to PFOS precursors (Martin et al., 2010). In line with Martin et al. (2010), the relative constant contribution from branched PFOS of around 30% indicate direct exposure to PFOS, rather than exposure to PFOS precursors for the studied population. Differences in the quantification procedure (Berger et al., 2011;

Riddell et al., 2009) and individual differences in PFOS isomer profiles should therefore be further investigated to clarify the relative importance of PFOS precursors for human exposure. Qualitative confirmation of presence of PFBA and PFPeA in three random samples (from different years) indicates that these compounds should be investigated in future monitoring of PFASs. Fluorotelomer sulphonic acids (FTSAs; 4:2, 6:2 and 8:2), perfluorobutane sulphonic acid (PFBS), perfluorodecane sulphonic acid (PFDcS) or C12-14 and C16 PFCAs were not detected in any sampling year and indicate that the past and recent exposure to these compounds has been low or their elimination rate high relative to the exposure.

4.4. Age-period-cohort effects for PFOA, PFUnDA and PFOS

Calendar year of sampling was the strongest predictor of PFAS concentrations and the calendar year trends reveal that human concentrations reflect overall historic trends in production and use of PFASs (see Section 4.1). Although time trends differed between PFASs, the influence of calendar year was evident for most compounds.

Including age or birth cohort predictors in addition to sampling year in mixed models for PFOA and PFOS did not improve model fits and indicates that these variables were of less importance compared to sampling year. The persons in the current study were all born before the onset of large-scale PFAS production and the exposure (duration and intensity) to all persons was expected to be similar at the times of sampling. Indeed, concentrations were not significantly different between age/birth cohort quartiles other than for PFOA in 1994, with the youngest quartiles having the highest concentrations compared to the older quartiles. Furthermore, when the results were organised cross-sectionally, age-associations were variable between years and indicate that these associations must be understood in relation to historic production and use (Fig.

4 and S4). Variable associations of PFASs to age between sampling years were also reported in the pooled, cross-sectional data from Norway (Haug et al., 2009). Compared to PFOA and PFOS, the mixed model fit improved for PFUnDA when including birth year quartiles as fixed factors and age as a random effect. This suggests that experienced exposure or elimination rates could differ between birth year quartiles.

Associations between POP concentrations and age in a population are not only affected by historic production and use relative to the sampling time, age structure of the study population, and compound persistence (Quinn and Wania, 2012; Ritter et al., 2009), but also by exposure pathways, and age-dependent PFAS intake rates related to e.g. dietary habits (Haug et al., 2010) relative to elimination rates. In post-ban exposure scenarios, the so-called legacy POPs have often been reported to increase with age in cross-sectional studies. This association likely reflects birth-cohort differences in duration and intensity of exposure to these compounds (Nøst et al., 2013; Quinn and Wania, 2012; Ritter et al., 2009). However, reports of correlations of PFASs to age are not consistent in cross-sectional studies (Calafat et al., 2007a, 2007b; Harada et al., 2007; Haug et al., 2009; Kato et al., 2011; Olsen et al., 2008; Yeung et al., 2006), and may be due to similar exposures for all age groups/birth cohorts due to recent or ongoing production and use. Age-differentiated intake rates (e.g. Tittlemier et al., 2007) or toxicokinetic properties could influence individual trends of PFASs over time, although differences in internal kinetics appear not to be strong in the general population (Harada et al., 2005). Furthermore, the present results suggest that the coarse features of temporal trends relate to changes in production and use. It is anticipated that diet-linked environmental exposures and time passed since peak production will render associations with age more pronounced in post-ban years due to age-dependent total PFAS intakes.

4.5. Study limitations and future perspectives

The longitudinal serum data for PFASs in the present study allowed an improved understanding for how human concentrations of these compounds have changed in relation to production and use patterns. Further studies of longitudinal evaluation of additional precursor compounds such as listed by Martin et al. (2010), Calafat et al. (2007a), and Yeung et al. (2013a,b), and isomer-specific analyses could have offered additional knowledge of the relative importance of PFOS precursors in PFAS time trends and pathway tracking. However, the analytical methodology employed in this study did not allow for that.

FOSA concentrations were presented as a sum of isomers due to high analytical variation between the branched and linear peaks in parallel sample injections. It should be mentioned that SRM results indicated that concentrations of PFHpA, PFDA and PFUnDA were underestimated (see Section 2.3.1.). Thus the reported concentrations of these analytes might constitute a low estimate although the time trends would not be affected by this. The limitations regarding statistical approaches to assess APC effects in POP concentrations in the current study group are described in Nøst et al. (2013).

5. Conclusion

This study describes past and current exposure to PFASs in the same men in a coastal population experiencing background exposure. The nearly 30-year time trends of PFAS concentrations in the repeated measurements from men in Northern Norway suggested unique time trends for the different PFASs quantified. We have demonstrated that human concentrations of PFASs have followed overall trends in production and use although compound differences in global transport mechanisms, bioaccumulation potentials and a varying extent of consumer exposures influence the observed trends. PFOA and PFOS concentrations decreased after 2001, as opposed to the increasing trends of PFNA, PFDA and PFUnDA throughout the study period.

The assessments of age-period-birth cohort effects demonstrated that calendar time was the dominating influence on PFAS concentrations, and associations to age/birth cohorts were variable between sampling years and not significant.

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APPENDIX

Table A.1: Concentrations (ng/ml) of PFASs[a] analysed in repeated serum samples from men in Northern Norway.

	1979 N = 53			1986 N = 52			1994 N = 48			2001 N = 49			2007 N = 52			LOD[c]
Compound	Median (Range)	AM	% > LOD[b]	Median (Range)	AM	% > LOD[b]	Median (Range)	AM	% > LOD[b]	Median (Range)	AM	% > LOD[b]	Median (Range)	AM	% > LOD[b]	
PFHxA	- (<LOD-0.1)	0.1	32	- (<LOD-0.2)	0.1	42	- (<LOD-0.2)	0.1	27	- (<LOD-0.1)	0.1	25	- (<LOD-0.2)	0.1	35	0.05
PFHpA	0 (<LOD-0.1)	0	60	0 (<LOD-0.1)	0	75	0.1 (<LOD-0.7)	0.1	95	0.1 (<LOD-0.7)	0.1	77	0.1 (<LOD-0.2)	0.1	87	0.03
PFOA	0.9 (0.4-2.5)	1	100	2.5 (1.4-4.5)	2.5	100	3.9 (2-9.6)	4.6	100	4.2 (1.8-9.9)	4.4	100	3.1 (1.3-6.8)	3.2	100	0.13
PFNA	0.1 (<LOD-0.2)	0.1	81	0.5 (0.2-1.3)	0.6	100	0.8 (0.2-1.6)	0.8	100	1.1 (0.5-1.9)	1.2	100	1.5 (0.7-3.4)	1.6	100	0.05
PFDA	0.1 (<LOD-0.2)	0.1	91	0.3 (0.1-0.7)	0.3	100	0.5 (0.1-1.1)	0.5	100	0.7 (0.2-1.7)	0.7	100	0.8 (0.2-1.8)	0.8	100	0.03
PFUnDA	0.1 (<LOD-0.7)	0.1	87	0.8 (0.3-3)	0.9	100	0.8 (0.1-2.4)	0.9	100	1.1 (0.4-4.5)	1.3	100	1.3 (0.1-3.9)	1.4	100	0.04
PFHxS	0.2 (0.1-3)	0.3	100	0.7 (0.4-5.3)	1	100	1.5 (0.3-4.8)	1.5	100	2 (0.6-12)	2.6	100	1.9 (0.6-12)	2.3	100	0.03
PFHpS	0.2 (<LOD-1.9)	0.3	92	0.5 (<LOD-1.9)	0.5	96	0.6 (0.2-2)	0.7	100	0.8 (<LOD-1.7)	0.8	98	0.7 (0.2-1.5)	0.7	100	0.10
Branched PFOS	2.7 (1.1-20)	3	100	7.2 (3.2-27)	7.8	100	11 (2.8-21)	11	100	12 (<LOD-25)	13	98	8.7 (3.1-18)	9.4	100	0.08
Linear PFOS	5.7 (2.4-18)	6.3	100	16 (7.2-43)	17	100	25 (9.2-52)	25	100	30 (12-70)	33	100	23 (6.5-47)	24	100	0.07
PFOS	8.6 (3.7-38)	9.3	100	23 (10-61)	25	100	37 (13-73)	37	100	43 (20-90)	46	100	33 (11-65)	33	100	0.15
FOSA	0.3 (0-1.1)	0.4	100	0.9 (0.3-4.2)	1.2	100	1 (0.2-6.8)	1.2	100	1 (0.1-13)	1.4	100	0.2 (0-1.3)	0.3	100	0.02
% linear PFOS	69 (48-79)	69		69 (56-77)	69		68 (57-79)	68		71 (60-82)	71		72 (56-80)	71		

[a]For compound abbreviations, see Table S1. Censored summary statistics are presented for compounds with detection frequencies less than 80%: PFHxA and PFHpA. Results are not presented for compounds detected in <20% of samples in a subset of 20 samples: PFBA, PFPeA, PFDoDA, PFTrDA, PFTeDA, PFHxDA, PFBS, PFDcS, and FTSAs (4:2, 6:2 and 8:2).

[b]% > LOD = Percentage of samples in which analyte was detected.

[c]LOD = Limit of detection (mean concentrations in blanks) in ng/ml.

SUPPLEMENTAL MATERIAL

Repeated measurements of per- and polyfluoroalkyl substances (PFASs) from 1979 to 2007 in males from Northern Norway: Assessing time trends, compound correlations and relations to age/birth cohort

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Contents:

748 **Table S1:** Abbreviations of chemical names for the PFASs analysed

Abbreviation	Compound
4:2 FTSA	4:2 fluorotelomer sulphonic acid
6:2 FTSA	6:2 fluorotelomer sulphonic acid
8:2 FTSA	8:2 fluorotelomer sulphonic acid
PFBA	Perfluorobutanoic acid
PFPeA	Perfluoropentanoic acid
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
PFHxDA	Perfluorohexadecanoic acid
PFBS	Perfluorobutane sulphonic acid
PFHxS	Perfluorohexane sulphonic acid
PFHpS	Perfluoroheptane sulphonic acid
PFOS	Perfluorooctane sulphonic acid
PFDcS	Perfluorodecane sulphonic acid
FOSA	Perfluorooctane sulfonamide

749

750

751 **Table S2:** Internal standard mixture

Labeled compound	Concentration
13C4 PFBA	0.1 ng/µl
13C5 PFPeA	0.1 ng/µl
13C5 PFHxA	0.1 ng/µl
13C4 PFHpA	0.1 ng/µl
13C4 PFOA	0.1 ng/µl
13C5 PFNA	0.1 ng/µl
13C6 PFDA	0.1 ng/µl
13C7 PFUnDA	0.1 ng/µl
13C2 PFDoA	0.1 ng/µl
13C3 PFHxS	0.0946 ng/µl
13C4 PFOS	0.0956 ng/µl
13C8 FOSA	0.1 ng/µl

752

Fig. S1: Examples of chromatograms displaying branched and linear isomers of PFOS (A) and FOSA (B) in serum samples. The branched isomers were identified as eluting earlier than the linear isomers.

A) B)

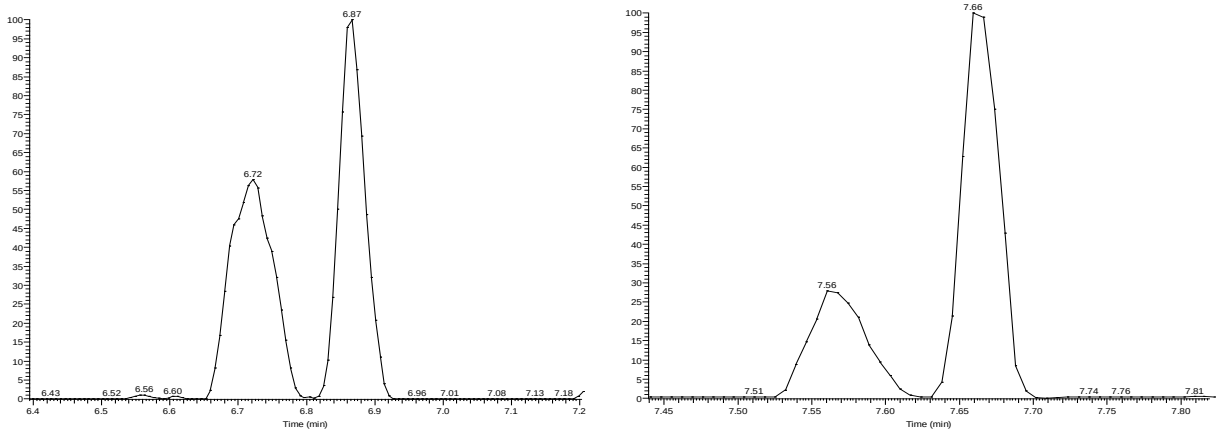

Fig. S2: Individual trend lines for PFOA concentrations in repeated measurements in 1979, 1986, 1994, 2001 and 2007 in serum samples of men from Northern Norway. Results are separated into birth year quartiles.

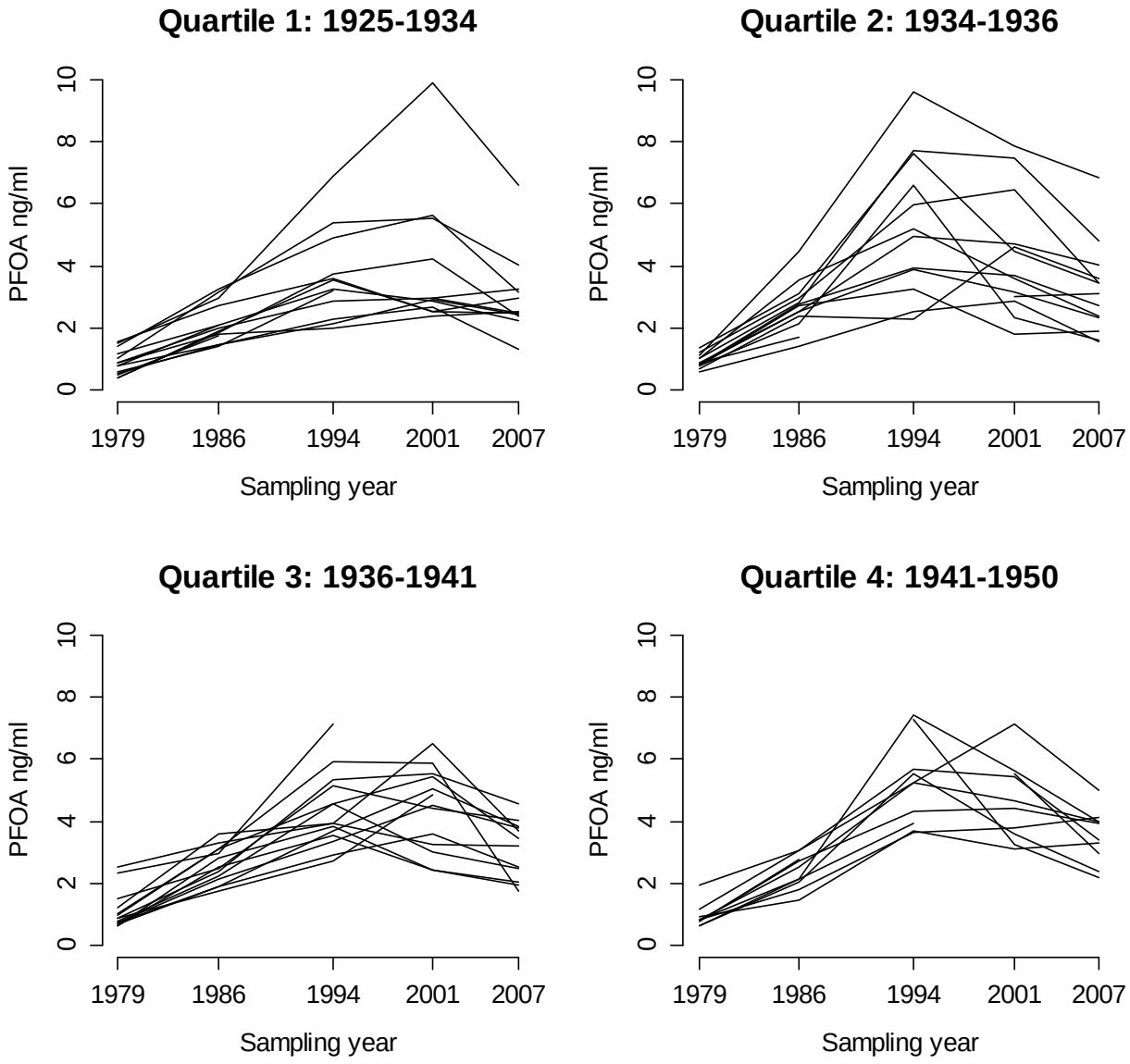

Fig. S3: Individual trend lines for percent of the linear PFOS isomer of summed PFOS concentrations in repeated measurements in 1979, 1986, 1994, 2001 and 2007 in serum samples of men from Northern Norway. Results are separated into birth year quartiles.

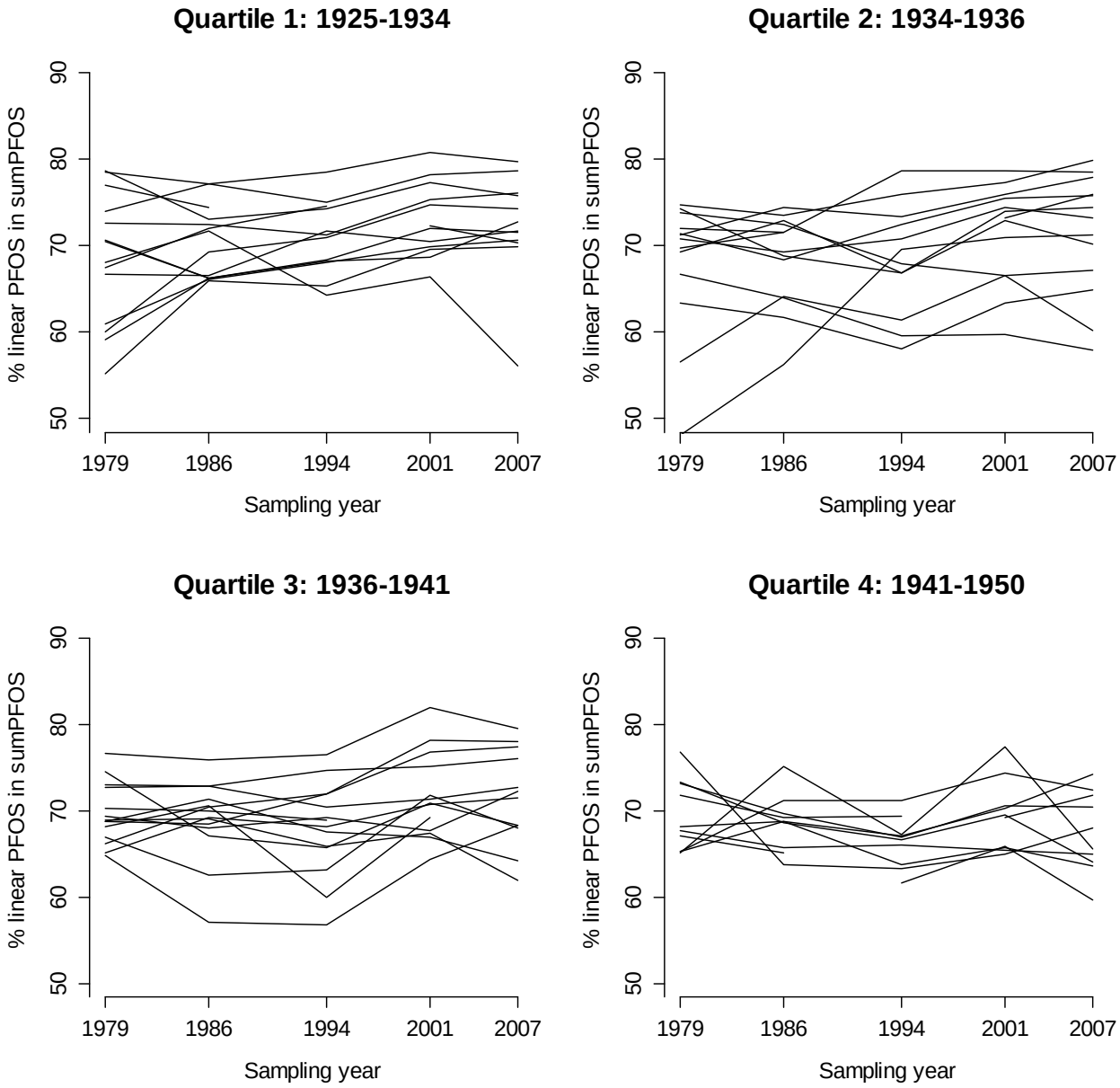

Table S3: Correlation coefficients (Spearman`s ρ) for associations between PFASs presented separately for the sampling years. Numbers in bold are significant at the level $p<0.05$.

1979	PFHpA	PFHxS	PFHpS	PFOA	PFNA	PFDA	PFUnDA	PFOS
PFHpA								
PFHxS	0.12							
PFHpS	0.12	0.23						
PFOA	0.18	**0.29**	0.04					
PFNA	0.12	**0.29**	-0.11	**0.34**				
PFDA	0.15	-0.13	-0.16	-0.17	0.19			
PFUnDA	-0.13	0.06	-0.09	-0.05	**0.32**	**0.38**		
PFOS	0.15	**0.73**	0.09	**0.41**	**0.61**	0.12	**0.27**	
FOSA	0.09	0.13	-0.14	0.17	**0.3**	0.24	0.22	**0.3**

1986	PFHpA	PFHxS	PFHpS	PFOA	PFNA	PFDA	PFUnDA	PFOS
PFHpA								
PFHxS	0.04							
PFHpS	0.03	**0.3**						
PFOA	0.15	**0.31**	**0.31**					
PFNA	-0.15	**0.52**	0.18	0.18				
PFDA	-0.02	**0.31**	0.16	-0.03	**0.57**			
PFUnDA	-0.05	**0.31**	-0.06	-0.12	**0.59**	**0.62**		
PFOS	-0.13	**0.72**	**0.31**	**0.32**	**0.7**	**0.39**	**0.54**	
FOSA	-0.03	0.2	0.16	0.26	**0.35**	0.12	0.25	**0.47**

1994	PFHpA	PFHxS	PFHpS	PFOA	PFNA	PFDA	PFUnDA	PFOS
PFHpA								
PFHxS	0.01							
PFHpS	0.06	**0.32**						
PFOA	**0.41**	0.22	0.24					
PFNA	0.09	**0.65**	0.22	**0.38**				
PFDA	0.06	**0.57**	0.09	0.23	**0.8**			
PFUnDA	-0.08	**0.55**	0.06	-0.07	**0.65**	**0.74**		
PFOS	-0.12	**0.68**	**0.36**	**0.31**	**0.63**	**0.61**	**0.64**	
FOSA	0.25	**0.31**	0.25	**0.5**	**0.54**	**0.48**	**0.4**	**0.55**

2001	PFHpA	PFHxS	PFHpS	PFOA	PFNA	PFDA	PFUnDA	PFOS
PFHpA								
PFHxS	0.08							
PFHpS	0.07	**0.64**						
PFOA	**0.49**	**0.48**	**0.47**					
PFNA	0.18	**0.55**	**0.48**	**0.44**				
PFDA	0.21	**0.29**	0.25	0.25	**0.71**			
PFUnDA	0.12	0.25	0.13	0.03	**0.54**	**0.84**		
PFOS	0.1	**0.66**	**0.62**	**0.32**	**0.67**	**0.62**	**0.62**	
FOSA	0.24	0.08	-0.04	0.09	**0.3**	0.26	**0.37**	**0.29**

2007	PFHpA	PFHxS	PFHpS	PFOA	PFNA	PFDA	PFUnDA	PFOS
PFHpA								
PFHxS	-0.02							
PFHpS	-0.07	**0.53**						
PFOA	0.13	**0.43**	**0.55**					
PFNA	-0.18	**0.41**	**0.39**	**0.44**				
PFDA	-0.23	**0.36**	**0.28**	0.25	**0.74**			
PFUnDA	-0.22	0.26	0.06	-0.04	**0.57**	**0.89**		
PFOS	-0.23	**0.61**	**0.5**	**0.35**	**0.68**	**0.77**	**0.72**	
FOSA	0.05	0.18	0.21	0.02	**0.31**	0.12	0.16	0.18

Table S4: Mixed effect model estimates[a] (coefficients and 95% CI) of PFOA concentrations (ng/ml) in men from Northern Norway from 1979 to 2007 with age, calendar period and birth cohort as predictors.

Predictors and coefficients	Model 1: Period only (fixed effects)		Model 2: Period and age (fixed effects)		Model 3: Period and age (fixed effects) plus birth cohort (random effects)[b]		Model 4: Period and birth cohort (fixed effects)		Model 5: Period and birth cohort (fixed effects) plus age (random effect)[b]	
Period[c]										
1979	Ref	-	Ref	-	Ref	-	Ref	-	Ref	-
1986	1.5	(1,2.1)	1.4	(0.9,2.1)	1.7	(1.3,2.3)	1.3	(0.7,2.1)	1.3	(0.8,1.8)
1994	3.4	(2.4,4.7)	3.2	(2,4.8)	4.5	(3.4,5.9)	3.0	(1.9,4.6)	2.6	(1.7,3.8)
2001	3.1	(2.1,4.3)	2.9	(1.7,4.7)	4.5	(3.4,5.9)	2.8	(1.7,4.3)	2.6	(1.9,3.6)
2007	2.1	(1.4,3)	2.0	(1,3.4)	3.3	(2.3,4.7)	1.8	(1.1,3)	1.8	(1.1,2.6)
Age[d]										
29-47	-		Ref	-	Ref	-	-		-	
47-57	-		0.0	(-0.2,0.3)	-0.1	(-0.2,0)	-		-	
57-66	-		0.1	(-0.2,0.5)	-0.2	(-0.3,-0.2)	-		-	
66-82	-		0.0	(-0.3,0.5)	-0.3	(-0.4,-0.1)	-		-	
Birth cohort[e]										
1925-1934	-		-		-		Ref	-	Ref	-
1934-1936	-		-		-		0.2	(-0.2,0.7)	0.2	(-0.1,0.7)
1936-1941	-		-		-		0.1	(-0.2,0.7)	0.2	(-0.1,0.7)
1941-1950	-		-		-		0.1	(-0.2,0.7)	0.2	(-0.1,0.6)
AIC[f]	132		149		152		143		145	

[a]Coefficients are back-transformed from log-estimates of fixed effect variables. All models included a subject-specific random term and a random slope for sampling year. Age and birth cohort variables were divided into quartiles.

[b]Variables were added to models as random terms to allow for random variation in individuals.

[c]Coefficients express change for PFOA concentrations in ng/ml across sampling years with 1979 as reference.

[d]Coefficients express change in PFOA concentrations in ng/ml across age quartiles with the youngest (29-47) as reference.

[e]Coefficients express change in PFOA concentrations in ng/ml across birth cohort quartiles with the oldest (1925-1934) as reference.

[f]Akaike's information criterion. Lower numbers indicate better model fit when comparing models.

Table S5: Mixed effect model estimates[a] (coefficients and 95% CI) of PFUnDA concentrations (ng/ml) in men from Northern Norway from 1979 to 2007 with age, calendar period and birth cohort as predictors.

Predictors and coefficients	Model 1: Period only (fixed effects)		Model 2: Period and age (fixed effects)		Model 3: Period and age (fixed effects) plus birth cohort (random effects)[b]		Model 4: Period and birth cohort (fixed effects)		Model 5: Period and birth cohort (fixed effects) plus age (random effect)[b]	
Period										
1979	Ref	-	Ref	-	Ref	-	Ref	-	Ref	-
1986	0.74	(0.5,1.2)	0.68	(0.4,1.1)	0.65	(0.4,1)	0.89	(0.5,1.6)	0.94	(0.5,1.7)
1994	0.73	(0.4,1.2)	0.60	(0.3,1.1)	0.63	(0.4,1.1)	0.88	(0.5,1.6)	0.85	(0.5,1.5)
2001	1.04	(0.6,1.6)	0.80	(0.4,1.5)	0.82	(0.5,1.4)	1.26	(0.7,2.3)	1.33	(0.7,2.4)
2007	1.05	(0.6,1.7)	0.78	(0.4,1.6)	0.91	(0.5,1.7)	1.27	(0.6,2.4)	1.31	(0.7,2.4)
Age										
29-47	-		Ref	-	Ref	-	-		-	
47-57	-		0.01	(0,0.1)	0.00	(0,0)	-		-	
57-66	-		0.02	(0,0.1)	0.02	(0,0.1)	-		-	
66-82	-		0.03	(0,0.1)	0.00	(0,0.1)	-		-	
Birth cohort										
1925-1934	-		-		-		Ref	-	Ref	-
1934-1936	-		-		-		-0.02	(-0.1,0.1)	-0.02	(-0.1,0.1)
1936-1941	-		-		-		-0.02	(-0.1,0.1)	-0.01	(-0.1,0.1)
1941-1950	-		-		-		-0.03	(-0.1,0)	-0.04	(-0.1,0)
AIC	364		375		393		370		352	

[a]Coefficients are back-transformed from log-estimates of fixed effect variables. All models included a subject-specific random term and a random slope for sampling year. Age and birth cohort variables were divided into quartiles.

[b]Variables were added to models as random terms to allow for random variation in individuals.

[c]Coefficients express change for PFUnDA concentrations in ng/ml across sampling years with 1979 as reference.

[d]Coefficients express change in PFUnDA concentrations in ng/ml across age quartiles with the youngest (29-47) as reference.

[e]Coefficients express change in PFUnDA concentrations in ng/ml across birth cohort quartiles with the oldest (1925-1934) as reference.

[f]Akaike's information criterion. Lower numbers indicate better model fit when comparing models.

Table S6: Mixed effect model estimates[a] (coefficients and 95% CI) of PFOS concentrations (ng/ml) in men from Northern Norway from 1979 to 2007 with age, calendar period and birth cohort as predictors.

Predictors and coefficients	Model 1: Period only (fixed effects)		Model 2: Period and age (fixed effects)		Model 3: Period and age (fixed effects) plus birth cohort (random effects)[b]		Model 4: Period and birth cohort (fixed effects)		Model 5: Period and birth cohort (fixed effects) plus age (random effect)[b]	
Period										
1979	Ref	-	Ref	-	Ref	-	Ref	-	Ref	-
1986	14.9	(10.2,20.8)	14.4	(9.3,21.1)	15.4	(10.8,21.1)	14.8	(8.2,24)	15.2	(8.4,24.8)
1994	25.3	(17.5,35.4)	23.5	(14.7,35.6)	25.1	(16.8,36.2)	25.1	(14.6,40.3)	25.9	(15.6,40.6)
2001	33.3	(23.7,45.7)	29.9	(18.3,46.4)	32.0	(20.8,47.5)	33.0	(20.1,51.8)	32.8	(20.1,51.1)
2007	22.2	(14.9,31.8)	18.9	(9.8,32.6)	20.6	(11.4,34)	22.0	(12.3,36.4)	22.1	(12.4,36.4)
Age										
29-47	-		Ref	-	Ref	-	-		-	
47-57	-		0.2	(-1.8,2.9)	0.1	(-1.7,2.4)	-		-	
57-66	-		0.7	(-1.9,4.5)	0.5	(-1.9,3.7)	-		-	
66-82	-		1.2	(-2.1,6)	0.8	(-2.2,5.1)	-		-	
Birth cohort										
1925-1934	-		-		-		Ref	-	Ref	-
1934-1936	-		-		-		0.4	(-3.5,7.6)	0.1	(-3.8,7.3)
1936-1941	-		-		-		0.4	(-3.5,7.4)	0.3	(-3.6,7.4)
1941-1950	-		-		-		-0.6	(-4.2,6)	-0.9	(-4.3,5.2)
AIC	116		133		150		128		154	

[a]Coefficients are back-transformed from log-estimates of fixed effect variables. All models included a subject-specific random term and a random slope for sampling year. Age and birth cohort variables were divided into quartiles.
[b]Variables were added to models as random terms to allow for random variation in individuals.
[c]Coefficients express change for PFOS concentrations in ng/ml across sampling years with 1979 as reference.
[d]Coefficients express change in PFOS concentrations in ng/ml across age quartiles with the youngest (29-47) as reference.
[e]Coefficients express change in PFOS concentrations in ng/ml across birth cohort quartiles with the oldest (1925-1934) as reference.
[f]Akaike's information criterion. Lower numbers indicate better model fit when comparing models.

Fig. S4: APC plots: Longitudinal and cross-sectional organization of concentrations of PFHpA, PFNA, PFDA and PFHxS (medians for each quartile in each sampling year in ng/ml wet weight). Further details are provided in the figure legend of Fig. 4 and Section 4.4. of the article.

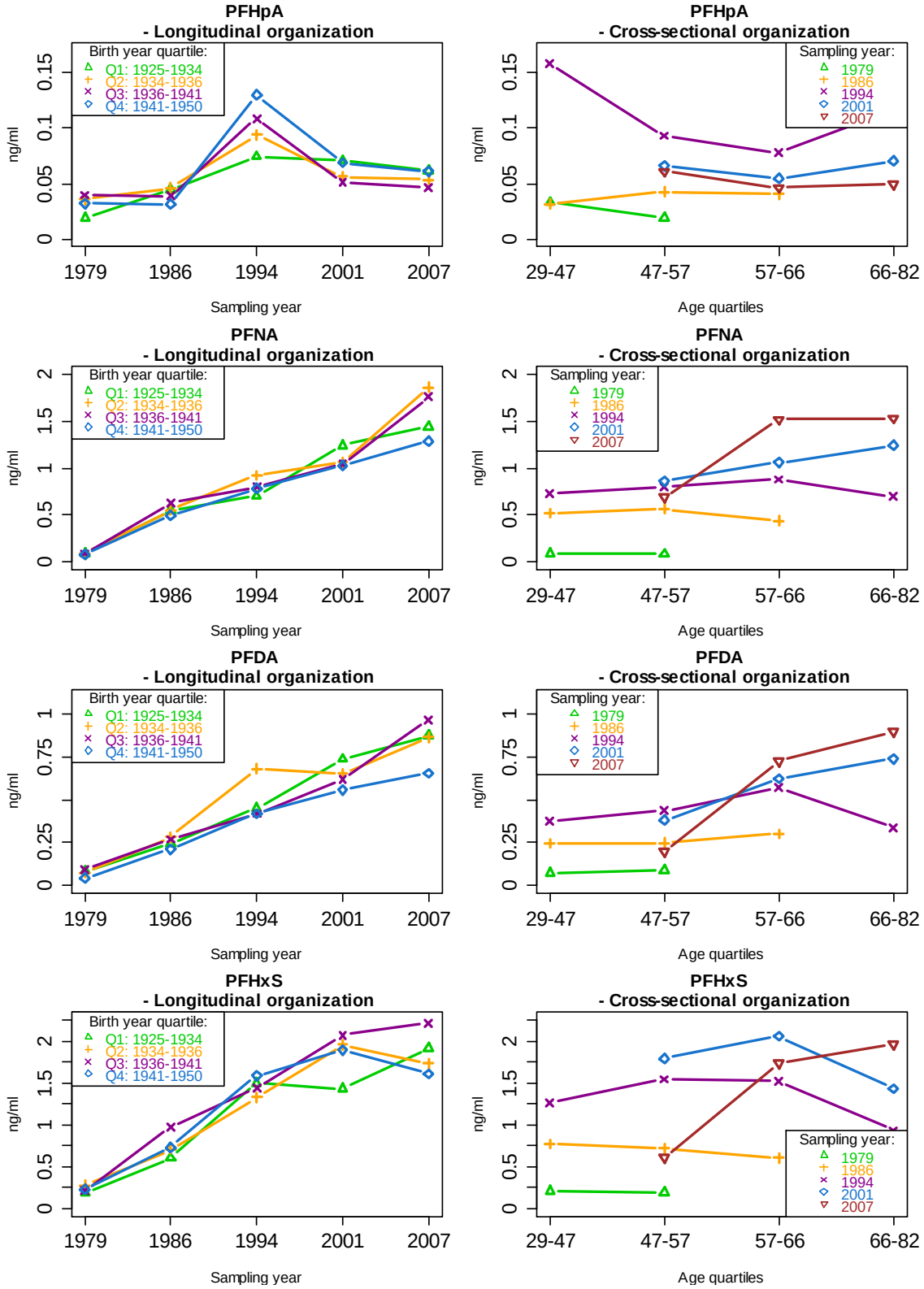

Fig. S5: Graphical display of concentrations between 1975 and 2010 from this study (●) and other studies: Haug et al., 2007 (■); Kato et al., 2011 (▲); Olsen et al., 2012 (●); Schröter-Kermani et al., 2012 (◆). See Section 4.1. of the article for discussion.