Time Trends and the Influence of Age and Gender on Serum Concentrations of Perfluorinated Compounds in Archived Human Samples

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Fifty-seven pooled archived human serum samples were analyzed to assess the time trends as well as influence of age and gender on selected perfluorinated compounds (PFCs) in Norwegian residents. The study comprised determinations of 19 PFCs in serum samples pooled according to year of collection from 28 years in the period 1976 to 2007. A 9-fold increase in the serum concentrations of perfluorooctyl sulfonate, perfluorooctanoic acid, and perfluoroheptyl sulfonate was measured for men (40−50 years) from 1977 to the mid 1990s where the concentrations reached a plateau before starting to decrease around year 2000. A similar trend was also seen for perfluorohexyl sulfonate, perfluorononanoic acid, perfluorodecanoic acid, and perfluoroundecanoic acid, but no clear decline was observed for these PFCs in the recent years. No statistically significant difference was observed between the PFC levels in the male and female serum pools, though the statistical power is low due to few data points. For most PFCs, the concentrations in the human serum samples were found to increase with age in the pools from 2007, while the results for 1976, 1987, and 1998 were more varying. Several PFCs were significantly intercorrelated.

Introduction

Polyfluorinated compounds comprise a diverse class of anthropogenic compounds consisting of an alkyl chain which is partially or fully fluorinated (perfluorinated), with different functional groups. Their physicochemical characteristics such as chemical and thermal stability, surface tension-lowering properties, and the ability to create stable foams have led to widespread use of polyfluorinated compounds in a multitude of industrial and consumer products for more than 50 years (*1*). Concerns about the persistence and bioaccumulative properties of polyfluorinated compounds were raised when the widely used surfactant perfluorooctylsulfonate (PFOS) was found to be ubiquitously distributed in wildlife and human populations worldwide (*2*−*5*). In 2000, 3M announced the phase-out of production of "perfluorooctanyl" compounds (*6*). Subsequently, the US EPA requested eight manufacturers to voluntarily eliminate perfluorooctanoic acid (PFOA), its precursors, and related chemicals (*7*). Recently, several other perfluorinated compounds (PFCs) have been shown to be persistent, to biomagnify, and to be transported to remote areas (*8*).

Several PFCs have been detected in human blood from populations in North and South America, Asia, Australia, and Europe (*9*). Usually PFOS, PFOA, and perfluorohexyl sulfonate (PFHxS) are the PFCs found most frequently and in the highest amount (low ng/mL level for PFOA and PFHxS and somewhat higher for PFOS), although several other PFCs have also been reported (*9*). In recent time, more has become known about exposure in the general population (*10*−*14*). PFCs have been shown to bind to blood proteins and accumulate in the liver of exposed mammalian organisms (*2, 15*). These properties favor the use of blood concentrations as a measure of internal dose, and PFOS concentrations in blood have been suggested as an integrated measure of exposure from various sources (*16*). The toxicity of PFCs have been studied in laboratory animals showing hepatotoxicity, developmental toxicity, and immunotoxicity as well as hormonal effects (*9*). Several studies have been conducted on occupationally exposed workers, but no consistent associations between PFC levels and adverse health effects have been found (*9*). So far few studies of impacts on human health of general populations have been conducted, but comparison of benchmark dose−response and biomonitoring data have generally indicated large margins of exposure (*17*).

Retrospective time trend studies are a valuable tool for judging the development of an environmental pollution situation. So far, only a few studies have explored the historical trends of PFCs in human samples, whereof some have observed clear trends (*5, 18*−*23*) and others have not (*24, 25*). For several so-called legacy persistent organic pollutants, e.g., polychlorinated biphenyls (PCBs), the concentrations in human serum increase with age (*26*−*28*) and the concentrations are higher in males than females (*26*). But for the PFCs, the results seem to be more varying. This difference may be explained by the fact that PCBs are lipophilic and accumulate with time in the lipid stores of the body, while the PFCs are protein-bound and not stored in lipids. Thus, a strong association between PFCs and age is not expected. Data from the National Health and Nutrition Examination Survey (NHANES, United States) of 1999−2000 showed higher concentrations of PFOA, PFHxS, and PFOS in males than in females (*29*), which is in accordance with an Australian study (*28*). In the same Australian study an increase in the PFOS concentrations in human serum with increasing age for both genders was observed (*28*). In contrast, Kannan et al. in general found no significant difference in PFC concentrations between the sexes and no association with age (*4*).

The objective of this study was to investigate the time trends and the role of age and sex for the concentrations of 19 different PFCs in pooled serum samples from the general Norwegian population.

Experimental Section

Chemicals. Perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), PFOA, perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA), perfluorotridecanoic acid (PFTrDA), perfluorotetradecanoic acid (PFTeDA), perfluorobutane sulfonic acid (PFBS), PFHxS,

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TABLE 1. Concentrations of PFCs (ng/mL) in Pooled Serum Samples from Men, Age 40−50 Years, in the Period 1977 to 2006[a]

	PFPeA	PFHpA	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTrDA	PFBS	PFHxS	PFHpS	PFOS	PFOSA
1977	0.25	<0.050	0.58	<0.050	<0.050	<0.050	<0.050	0.067	0.074	0.10	<0.050	3.8	<0.050
1980	0.47	0.11	1.3	0.077	<0.050	0.069	0.056	<0.050	<0.050	0.29	<0.050	6.1	0.06
1981	0.22	<0.050	1.4	0.12	<0.050	0.097	<0.050	0.073	0.18	0.49	<0.050	9.4	0.14
1982	0.63	<0.050	1.4	0.12	0.074	0.13	0.055	0.088	0.074	0.56	<0.050	11	0.095
1983	0.20	<0.050	1.5	0.21	0.065	0.28	<0.050	0.090	<0.050	0.52	0.19	10	0.34
1985	<0.050	<0.050	2.2	0.36	0.10	0.27	<0.050	0.13	0.082	0.80	0.19	16	0.68
1986	<0.050	0.064	2.6	1.7	0.12	0.61	0.062	0.18	0.071	0.80	0.22	15	0.37
1988	<0.050	0.11	2.7	0.67	0.13	0.19	0.051	0.085	0.065	0.82	0.22	18	0.60
1989	0.10	0.089	3.1	0.79	0.22	0.31	<0.050	0.088	0.082	1.3	0.37	22	0.69
1990	<0.050	<0.050	3.3	0.55	0.19	0.29	<0.050	0.11	0.096	1.1	0.34	20	0.37
1991	<0.050	0.11	3.4	0.72	0.22	0.17	0.054	0.072	0.11	1.3	0.30	23	0.59
1993	<0.050	0.11	5.2	0.71	0.25	0.27	0.051	0.11	0.087	1.7	0.47	33	0.57
1994	<0.050	<0.050	4.1	0.66	0.20	0.22	<0.050	0.092	0.082	1.9	0.42	24	0.32
1995	<0.050	0.084	4.4	0.93	0.26	0.21	<0.050	0.13	0.069	1.4	0.38	31	0.31
1996	<0.050	<0.050	4.0	0.58	0.25	0.20	<0.050	0.10	0.083	1.4	0.35	25	0.28
1997	<0.050	0.088	4.2	0.70	0.26	0.21	<0.050	0.15	0.086	1.8	0.47	31	0.35
1999	0.052	<0.050	4.0	0.71	0.28	0.17	0.070	0.13	0.096	1.6	0.37	29	0.32
2000	<0.050	0.14	4.5	0.67	0.26	0.24	0.084	0.12	0.12	3.4	0.43	30	0.56
2001	0.17	0.083	4.9	1.2	0.25	0.24	0.054	0.16	<0.050	1.6	0.10	27	0.15
2002	0.28	0.061	3.9	0.75	0.30	0.21	0.053	0.11	<0.050	2.2	0.19	27	0.081
2003	0.29	0.092	3.8	0.72	0.23	0.23	<0.050	0.13	<0.050	1.7	0.11	19	<0.050
2004	0.18	0.14	3.4	0.78	0.31	0.18	0.063	0.11	<0.050	1.4	0.12	18	<0.050
2005	0.30	0.10	3.5	0.85	0.39	0.25	0.065	0.20	<0.050	1.6	0.11	21	<0.050
2006	0.11	0.078	2.7	0.55	0.22	0.14	<0.050	0.071	<0.050	1.4	0.055	12	<0.050

[a] <0.050: concentration below the limit of quantification.

perfluoroheptane sulfonic acid (PFHpS), PFOS, perfluorodecane sulfonic acid (PFDS), perfluorooctane sulfonamide (PFOSA), *N*-methylperfluorooctane sulfonamide (MeFOSA), *N*-ethylperfluorooctane sulfonamide (EtFOSA), perfluoro-n-[1,2,3,4-$^{13}C_4$]butanoic acid (MPFBA), perfluoro-n-[1,2-$^{13}C_2$]hexanoic acid (MPFHxA), perfluoro-n-[1,2,3,4-$^{13}C_4$]octanoic acid (MPFOA), perfluoro-n-[1,2,3,4,5-$^{13}C_5$]nonanoic acid (MPFNA), perfluoro-n-[1,2-$^{13}C_2$]decanoic acid (MPFDA), perfluoro-n-[1,2-$^{13}C_2$]dodecanoic acid (MPFDoDA), sodium perfluoro-1-hexane[$^{18}O_2$]sulfonate (MPFHxS), sodium perfluoro-1-[1,2,3,4-$^{13}C_4$]octanesulfonate (MPFOS), *N*-methyl-d_3-perfluoro-1-octanesulfonamide (*d*-N-MeFOSA), and *N*-ethyl-d_5-perfluoro-1-octanesulfonamide (*d*-N-EtFOSA) were purchased from Wellington Laboratories (Guelph, Ontario, Canada). The other chemicals used are described elsewhere (*30*).

Serum Samples. The study was performed on 57 pooled serum samples from a biobank at the Norwegian Institute of Public Health. The serum had been sampled from patients at different county hospitals in Norway regardless of the disease and the reason for hospitalization, during the period 1976 to 2007, and stored in polyethylene containers at −20 °C. Five hundred microliters of serum from each individual ($n \geq 20$) was pooled and stored in containers made of polyethylene at −20 °C. The pooling of the samples was carried out at two different time points. The pools representing the years 1976 to 2002 have been prepared previously and used for other investigations (*26*), while the remaining pools (2003−2007) were prepared in an identical manner in spring 2008.

The first series comprised 24 pools (one pool per year) from 24 different years between 1977 and 2006 (see Table 1). These samples were restricted to serum from men of age 40−50 years, to limit variation of body burden with gender and age. For the 1997 pool, serum was sampled from 14 individuals only because of limited sample volumes available from this year.

A second sample series was prepared for studying PFC concentrations in groups with different age and gender in a total of 33 pooled samples from 1976, 1987, 1998, and 2007. In the serum bank, the samples had been divided into eight age groups. One single pool was prepared for each year/gender/age group combination (e.g., females, age 25−59 from 1998), and serum from 20 individuals or more was included in each pool (see Table 2). For the age group 0−4 years, only 250 μL was taken from each individual sample, as sample volumes were limited. For the age group 0−4 years in 1998, no serum samples were available.

Determination of PFCs. Calibration solutions were prepared in serum from newborn calves, which has proven to be an acceptable surrogate matrix for human serum in a thorough method validation (*30*). The samples and standards were prepared and analyzed according to a previously described method (*30*). In brief, 150 μL of either serum from newborn calves or human serum was transferred to a centrifugation tube, and internal standards, native PFCs (only calibration solutions), and methanol were added to make up a total volume of 150 μL of methanol for precipitation of proteins and then mixed using a whirl mixer. The samples were then centrifuged, and the supernatant was transferred to a glass autosampler vial, and 0.1 M formic acid was added and mixed on a whirl mixer. The extracts were analyzed by injection of 400 μL on a column switching liquid chromatography system coupled to a triple quadrupole mass spectrometer as described in detail elsewhere (*30*). The LOQ was limited by the lowest calibration point and set to 0.050 ng/mL for all PFCs except PFBA which was 0.10 ng/mL. In the statistical analyses the concentration of all PFCs found to be below LOQ was set to LOQ divided by the square root of two (*29*). For quantification of PFOS, the total area of the linear and branched isomers was integrated.

The method for determination of PFCs in serum has recently been validated and found satisfactory (*30*). The high accuracy of the method was assured by use of ^{13}C-labeled internal standards and matrix-matched calibration solutions. To ensure that the sample containers as well as the other laboratory equipment used did not contain any traces of PFCs, method blanks were prepared ($n = 3$). For preparation of method blanks, purified water was added to sample containers and further prepared identical to that of the pooled serum samples. The method blanks did not contain any of the PFCs above LOQ. The quality of the determinations in the present study was also controlled by analyzing quality control samples simultaneously to the samples. These

TABLE 2. Concentrations of PFCs (ng/mL) in Pooled Serum Samples from Different Age Groups and Genders (*m* = male, *f* = female)[a]

		PFPeA	PFHpA	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTrDA	PFBS	PFHxS	PFHpS	PFOS	PFOSA
1976	0−4	<0.050	<0.050	0.68	<0.050	<0.050	0.089	<0.050	<0.050	<0.050	0.053	<0.050	1.8	<0.050
	5−14	<0.050	<0.050	0.64	<0.050	<0.050	0.098	<0.050	<0.050	0.053	0.14	<0.050	2.6	<0.050
	15−24 m	0.088	<0.050	0.51	<0.050	<0.050	0.078	<0.050	<0.050	0.072	0.098	<0.050	2.7	<0.050
	15−24 f	0.11	<0.050	0.49	<0.050	<0.050	0.31	<0.050	<0.050	0.071	0.096	<0.050	2.5	0.065
	25−59 m	0.15	<0.050	0.65	<0.050	<0.050	0.13	<0.050	<0.050	0.073	0.30	0.12	4.3	0.052
	25−59 f	0.12	<0.050	0.46	<0.050	<0.050	0.067	<0.050	0.051	0.067	0.069	<0.050	2.3	0.061
	60+ m	0.22	<0.050	0.31	<0.050	<0.050	0.054	<0.050	<0.050	<0.050	0.061	<0.050	2.9	0.070
	60+ f	0.13	<0.050	0.36	<0.050	<0.050	0.081	<0.050	0.055	<0.050	0.053	<0.050	3.0	0.067
1987	0−4	<0.050	0.31	5.7	0.35	0.097	0.14	<0.050	0.062	<0.050	2.7	0.67	28	0.36
	5−14	0.10	0.15	3.0	0.63	0.11	0.18	<0.050	0.066	0.062	0.74	0.28	19	0.44
	15−24 m	0.11	0.13	2.3	0.44	0.11	0.19	<0.050	0.068	0.062	0.53	0.15	13	0.27
	15−24 f	0.075	<0.050	1.9	0.35	0.083	0.17	<0.050	0.082	0.078	0.39	0.15	13	0.23
	25−59 m	0.075	<0.050	2.8	0.83	0.19	0.46	<0.050	0.11	0.069	1.6	0.29	23	0.50
	25−59 f	0.11	0.11	2.2	0.69	0.16	0.64	<0.050	0.11	0.072	0.89	0.20	15	0.29
	60+ m	0.098	0.098	1.5	0.54	0.12	0.37	<0.050	0.075	0.073	0.81	0.24	16	0.27
	60+ f	0.17	0.10	2.4	0.85	0.19	0.48	<0.050	0.074	0.083	1.2	0.28	21	0.54
1998	5−14	<0.050	0.19	5.4	0.50	0.16	0.14	<0.050	0.060	0.068	3.1	0.40	24	0.76
	15−24 m	<0.050	0.059	4.2	0.42	0.16	0.18	<0.050	0.054	0.071	3.1	0.27	21	0.30
	15−24 f	<0.050	0.14	4.1	0.47	0.15	0.093	<0.050	0.054	0.091	4.7	0.34	25	0.47
	25−59 m	<0.050	<0.050	4.3	0.67	0.24	0.22	<0.050	0.081	<0.050	1.9	0.43	31	0.42
	25−59 f	<0.050	0.085	3.1	0.46	0.17	0.19	<0.050	0.070	<0.050	1.2	0.21	15	0.38
	60+ m	<0.050	0.064	3.8	0.78	0.25	0.32	0.067	0.12	0.084	3.0	0.46	28	0.37
	60+ f	<0.050	0.063	3.5	0.55	0.20	0.18	<0.050	0.070	0.072	1.1	0.36	24	0.33
2007	0−1	<0.050	0.069	1.6	0.55	0.075	0.072	<0.050	<0.050	0.065	0.31	0.058	4.0	<0.050
	1−4	<0.050	0.24	2.6	0.70	0.17	0.090	<0.050	0.060	<0.050	0.73	0.11	6.4	<0.050
	5−14 m	<0.050	0.10	2.3	0.67	0.18	0.15	0.060	0.054	<0.050	2.0	0.12	7.3	<0.050
	5−14 f	<0.050	0.11	2.1	0.83	0.17	0.10	<0.050	0.054	0.062	2.1	0.091	6.5	<0.050
	15−24 m	<0.050	0.070	2.3	0.63	0.17	0.11	<0.050	0.058	<0.050	0.84	0.18	11	<0.050
	15−24 f	<0.050	0.13	2.1	0.91	0.23	0.13	<0.050	0.082	<0.050	0.65	0.12	7.7	<0.050
	25−59 m	<0.050	0.052	2.1	0.62	0.19	0.14	<0.050	0.077	<0.050	0.75	0.18	10	<0.050
	25−59 f	<0.050	<0.050	1.9	0.94	0.23	0.19	<0.050	0.11	<0.050	0.94	0.17	10	<0.050
	60+ m	<0.050	<0.050	2.8	1.1	0.37	0.30	0.064	0.10	0.055	1.4	0.39	22	0.11
	60+ f	<0.050	0.069	2.3	0.74	0.23	0.15	<0.050	0.11	<0.050	1.0	0.29	15	0.084

[a] <0.050: concentration below the limit of quantification.

samples were prepared in a manner the same as that used for the pooled serum samples. As reference samples, three pooled human serum samples from an interlaboratory comparison study organized by Institute national de santé publique du Québec (Canada) for the Arctic Monitoring and Assessment Programme (AMAP) (*31*) were used. In addition, one internal quality control sample of serum was analyzed in duplicate. The results of the reference samples were within −5.7 to + 6.2% of the assigned value.

Results and Discussion

Two series of pooled serum samples were analyzed to examine whether there had been a change in the concentrations of the 19 PFCs in the period 1976 to 2007, and to clarify the role of age and gender in the human serum levels. The concentrations of PFCs in the different serum pools are given in Tables 1 and 2. PFOS was found in highest concentrations in all samples, followed by PFOA and PFHxS. In most samples, PFNA, PFDA, PFUnDA, PFTrDA, PFHpS, and PFOSA were detected, while PFPeA, PFHpA, PFDoDA, and PFBS were found less frequently. PFBA, PFHxA, PFTeDA, PFDS, MeFOSA, and EtFOSA were not observed above the limit of quantification (LOQ) in any of the samples. The concentration ranges of PFCs in serum pools found in the present study for the period 2000−2006 are in agreement with results reported worldwide for samples collected in this time period (*9*). The current concentrations (men, age 40−50, in 2006) of the most prominent PFCs, PFOS, PFOA, and PFHxS, were found to be 12, 2.7, and 1.4 ng PFC/mL serum, respectively.

Different time trends were observed for concentrations of the various PFCs in the 24 serum samples from men of age 40−50 years pooled by year of collection over a period of almost 30 years (Table 1 and Figure 1). An approximately 9-fold increase in the serum concentrations of PFOA, PFHpS, and PFOS was seen from 1976 to the mid 1990s where the concentrations reached a plateau before they started to decrease around year 2000. The PFOS and PFOA concentrations observed in serum around year 2000 were approximately two times higher than what was found in 2006 while the corresponding decrease for PFHpS was about eight times. A similar trend was seen for PFOSA, although the highest concentrations were reached between 1985 and 1993 for this compound. A clear initial increase in the PFC concentrations was also observed for PFHxS, PFNA, PFDA, and PFUnDA from 1976 to the early 1990s where they have stabilized. The concentration of PFNA in the 1986 pool was considerabely higher than expected from the results of preceding and following years. It was not was possible to reanalyze this pool. However, close inspection of the chromatograms obtained for PFNA and comparison of the PFNA results with the concentrations found for the other PFCs in the pool revealed no obvious analytical errors. This high concentration observed might be due to one or more individuals in this particular pool having an extraordinary concentration of PFNA in their serum. Nevertheless, the remaining PFNA data fit well to the curve. For PFPeA, PFHpA, PFDoDA, PFTrDA, and PFBS, the concentrations in the serum pools showed no obvious tendencies for change over time; however, the concentrations of these PFCs were close to the LOQ. The observed increase in PFOS and PFOA serum concentrations until the mid 1990s are in accordance with the increasing use of products containing these PFCs, while the decreasing

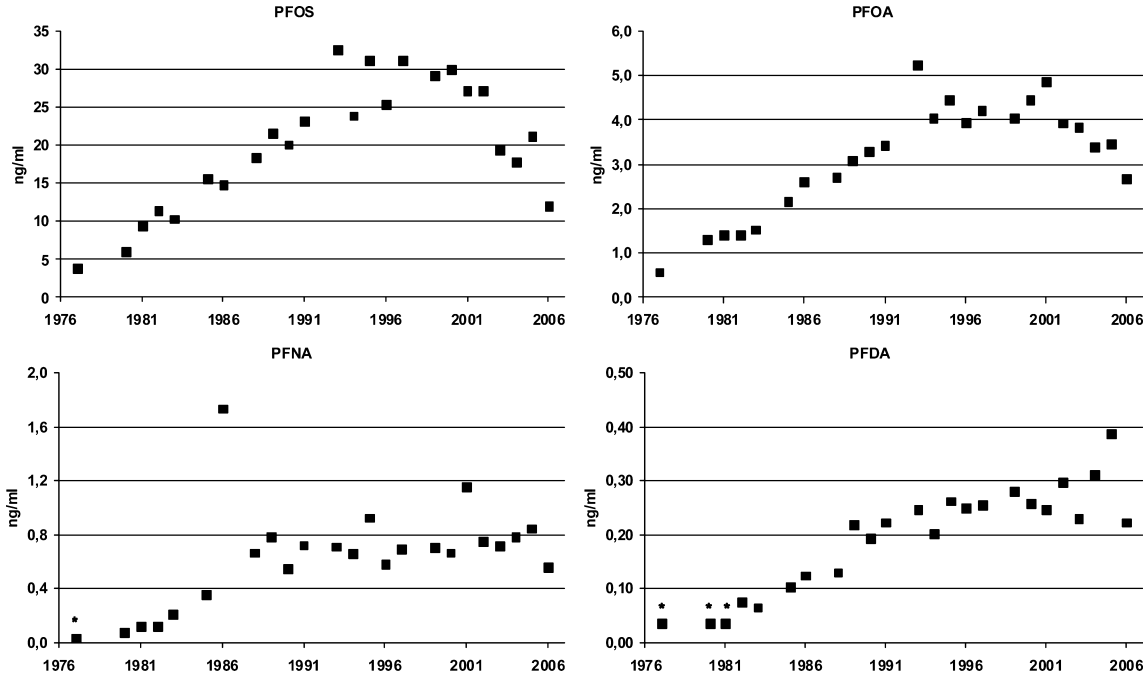

FIGURE 1. Concentrations in ng/mL of PFOS, PFOA, PFNA, and PFDA in pooled serum samples from men, age 40−50 years, in the period 1977 to 2006. Data points marked with an asterisk were below the LOQ (0.050 ng/mL serum) and are set to LOQ divided by the square root of two.

concentrations observed the past few years are consistent with the phase-out of these compounds.

As this study was perfomed as a cross-sectional study, it was not possible to examine changes of PFC concentrations in the individuals at different points of time. Nevertheless, the data were examined by looking at possible 'cohort' effects, assuming that for instance the age group 0−4 years in 1976 was 5−14 years in 1987, 15−24 years in 1998, and 25−59 years in 2007. This examination confirmed the time trends described above except that a decrease in the concentrations of PFHxS was found between 1998 and 2007, and the decrease of PFHpS between 1998 and 2007 was approximately two times compared to the eight times reduction found in the time trends. This may indicate that the reductions of the concentrations of PFHxS and PFHpS in this time period are in the same range as for PFOA and PFOS which is expected as the half-lives of PFHxS, PFHpS, PFOS, and PFOA are in a similar range (*32*).

This is the first time temporal trends of PFCs in human populations are studied in almost annual intervals over a time period of nearly 30 years. Several other studies have reported similar time trends in serum PFC levels, however, covering shorter time periods or with larger intervals between sampling points. PFOS concentration in human serum from a study conducted in Japan tended to increase until the late 1980s when a plateau was reached, while PFOA was observed to increase steadily throughout the whole study period from 1983 to 2004 (*20*). In one study from the city of Shenyang, China, an increase in the concentration of both PFOS and PFOA from 1987 to 2002 has been observed (*21*). A decline in PFOS and PFOA concentrations from 1985 to 2004 has been reported from a study of students from Germany (*22*). In the United States one study of serum samples of adults from Maryland have reported an increase in both PFOA and PFOS concentrations between 1974 and 1989 (*5*). In addition, a study of serum and plasma samples from six American Red Cross blood donor centers as well as a study of serum samples from the NHANES survey has shown a decrease in the concentrations from 2000 to 2006 (*23, 29*). This is in close agreement with what was found in the present study. It must be emphasized that only the study based on serum samples from the NHANES survey (*29*) is nationally representative.

TABLE 3. Spearman's Rank Correlation Coefficients between Average Age and PFC Concentrations in Human Serum Pools from Four Decades

	1976	1987	1998	2007
PFPeA	0.90[b]	0.60		
PFHpA		−0.70	−0.80	−0.90[b]
PFOA	−0.90[b]	−0.90[b]	−1.00[a]	0.30
PFNA		0.80	0.80	1.00[a]
PFDA		0.80	0.80	1.00[a]
PFUnDA	−0.30	0.90[b]	0.80	0.90[b]
PFDoDA			0.78	0.45
PFTrDA	0.89[b]	0.70	0.80	1.00[b]
PFBS	0.10	1.00[a]	0.00	−0.67
PFHxS	0.30	−0.20	−0.60	0.40
PFHpS	0.35	−0.60	0.40	1.00[a]
PFOS	0.90[b]	−0.50	0.20	1.00[a]
PFOSA	0.98[a]	0.20	−0.80	0.71

[a] Correlation was significant at the 0.01 level (two-tailed).
[b] Correlation was significant at the 0.05 level (two-tailed).

The serum levels of PFCs were also investigated with respect to age and gender using 33 pooled samples (second sample series) according to Table 2. The PFC concentrations found for the group 'men 25−59 years' from these four years (1976, 1987, 1998, and 2007) fit well in the time trends described above (Table 1). To examine possible differences in PFC concentrations related to gender, two data sets were established for each PFC, one set for males and one set for females. Each data set comprised PFC concentrations for the age groups 15−24, 25−59, and 60+ from the four different years 1976, 1987, 1998, and 2007, and in addition the age group 5−14 for year 2007 (two times 13 data points in total per PFC). To neutralize the possible effect of age and year of collection, corresponding pools (female vs male) were compaired pairwise using a paired-sample *t* test. No statistically significant difference in PFC serum concentrations for men and women was found, although the statistically power

TABLE 4. Spearman's Rank Correlation Coefficients between Concentrations of Different PFCs in Pooled Serum Samples (men, 40−50 years) from 1977 to 2006

	PFPeA	PFHpA	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTrDA	PFBS	PFHxS	PFHpS	PFOS
PFHpA	−0.052											
PFOA	−0.46[b]	0.31										
PFNA	−0.21	0.45[b]	0.59[a]									
PFDA	−0.14	0.38	0.77[a]	0.68[a]								
PFUnDA	−0.40	0.040	0.31	0.46[b]	0.15							
PFDoDA	0.19	0.46[b]	0.14	0.34	0.36	−0.088						
PFTrDA	−0.20	0.081	0.58[a]	0.64[a]	0.59[a]	0.57[a]	0.28					
PFBS	−0.56[a]	−0.17	0.19	−0.23	−0.099	−0.005	−0.083	−0.072				
PFHxS	−0.27	0.29	0.89[a]	0.50[b]	0.81[a]	0.25	0.18	0.54[a]	0.087			
PFHpS	−0.81[a]	0.18	0.70[a]	0.35	0.42[b]	0.48[b]	−0.078	0.33	0.52[b]	0.60[a]		
PFOS	−0.53[a]	0.27	0.95[a]	0.57[a]	0.75[a]	0.32	0.12	0.53[a]	0.31	0.85[a]	0.81[a]	
PFOSA	−0.74[a]	0.064	0.18	0.11	−0.15	0.53[a]	−0.085	0.058	0.56[a]	0.028	0.68[a]	0.35

[a] Correlation was significant at the 0.01 level (two-tailed). [b] Correlation was significant at the 0.05 level (two-tailed).

is low because of few data points. Some publications in the literature have reported statistically significant differences in PFC levels between sexes (*19, 23, 29, 33*−*35*), while others have not (*4, 36, 37*). This inconsistency might be due to differences in the design of the studies, such as size, selection of the populations, etc.

To investigate the relationship between age and PFCs serum levels, an average age had to be assigned to each pool. For the serum pools from 1976, 1987, and 1998, information on each individual donors' age was not available; thus, the median of the age interval was used, e.g., the age group 5−14 years was assigned an age of 9.5. For the 2007 samples, the average age of the pools were calculated from each individual's age. The relationship between this assigned age and concentrations of PFCs was examined. The mean of both genders for each age group was calculated as no significant difference in PFC concentrations was found between men and women.

In Table 3 the Spearman's rank correlation coefficients are shown. In general, statistically significant positive associations were observed for most PFCs in 2007, while the results from 1976, 1987, and 1998 were more varying. For PFNA, PFDA, and PFUnDA as well as for PFTrDA, the concentration of PFCs in serum increased with age for all three years where these PFCs were detected, though the correlation coefficients varied. The strongest correlations were observed for the PFCs with the longest carbon chain lengths which might be explained by an increasing half-life with increasing chain length. However, one should keep in mind the limitations in these calculations when using assigned age of the serum donors and pooled samples. Similar to the inconsistency in the results of published studies investigating the differences in PFC concentrations between genders, the role of age for the PFC body burden is also not yet clarified. One study found an indication of correlations between age and PFC concentrations (*28*), while others have not (*4, 23, 29, 35, 38, 39*).

The relationships between the concentrations of different PFCs were examined by using Spearman's rank correlation (Table 4). PFBS, PFOSA, PFUnDA, and PFDoDA were not correlated to any other PFCs with a few exceptions, while PFOS and PFOA were significantly correlated to each other as well as to PFHxS, PFHpS, PFNA, PFDA, and PFTrDA. As reported by others, the correlations between the perfluorinated alkyl carboxylic acids, e.g. PFOA, and the perfluorinated alkyl sulfonates, e.g. PFOS, are interesting as they cannot convert directly into each other (*4, 38*). This points to common sources for human exposure of these two PFC classes, such as food, dust, and air.

In the second samples series a chromatographic separation between the linear PFOS isomer and what was assumed to be a mixture of branched PFOS isomers was obtained. Unfortunately, it was not possible to confirm the identity of the peaks, as no single isomer standards were available; however, it has been demonstrated that the branched isomers elute from the reversed-phase HPLC column prior to the dominant linear isomer. The relative abundance (area) of the linear PFOS (L-PFOS) varied between 53 and 78% of the total PFOS area. This is in close agreement with a Swedish study reporting 58−70% of the L-PFOS (*40*). The mean relative abundance of L-PFOS for the pools from 1976, 1987, 1998, and 2007 was found to be 68, 64, 60, and 57%, respectively. Whether this continuous decline in the proportion of L-PFOS is due to changes in production methods of the PFCs or due to different half-lives of linear vs branched isomers remains to be determined.

In conclusion, for eight PFCs, including PFOS and PFOA, increasing concentrations were observed in serum samples from the general population of Norway between 1977 to the mid 1990s. Further, a clear decline in the PFOA, PFHpS, and PFOS concentrations from around year 2000 confirms findings reported by Olsen (*23*). For the serum samples from year 2007, the PFC concentrations were increasing significantly with age for most PFCs except the ones with shortest chain length. Our study demonstrates the importance of establishing human biomonitoring specimen banks for identifying emerging environmental pollutants which may pose potential threats to human health and for monitoring the efficacy of measures to reduce the environmental pollution.

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