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Fluorinated compounds, including a novel oxidizable fraction in blood of Norwegian women and their predictors – the Norwegian Women and Cancer Study

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Sammendrag

Per- og polyfluorinerte forbindelser (PFAS) er persistente organiske miljøgifter (POP's) og det finnes mer enn 4000 av disse forbindelser. De blir brukt i blant annet fettsikkert papir, i brannskum og har flekkavissende egenskaper på grunn av sine hydrofobe og lipofobe egenskaper. PFOS, en perfluoralkyl sulfonat syre (PFSA) og PFOA en perfluoroalkyl karboksyl syre (PFCA) er de to forbindelsene som er mest kjent og identifisert i miljøet. Kostholdet er den mest dominerende miljøeksponeringen og man finner PFAS i ulike matvaregrupper som fisk, kjøtt, egg, frukt og grønnsaker. Denne studien ble utført for å undersøke konsentrasjonene av konvensjonelle fluorinerte forbindelser (PFASs) og deres prediktorer i plasma fra 316 middel aldrende kvinner som deltok i den norske Kvinner og Kreft studien og i en subgruppe konsentrasjonene av nye oksiderbare forbindelser med hjelp av den totale oksiderbare prekursor assay (TOPA). Fødselsår var den viktigste prediktor og eldre kvinner hadde høyere verdier av de fleste fluorforbindelser. PFOS (median 19,9 ng/mL), PFOA (2,9 ng/mL), PFPeA (0,9 ng/mL), PFUnDA (0,4 ng/mL) and PFHxS (1 ng/mL) ble funnet i mer enn 99 % av prøvene. PLS regresjons plots viste at de fleste PFASs i denne studien var assosiert med fiskeinntak og økte verdier av PFOA, PFOS, PFHxS, PFNA, PFDA og PFDoDA ble funnet i kvinner som spiste mest fisk sammenlignet med de som spiste ingen eller lite fisk. PFPeA var ikke assosiert med fiskeinntak, men med bakevarer. TOPA metoden i subgruppen indikerte i gjennomsnitt dobbelt så høye verdier av den totale PFAAs summen etter oksidasjon og indikerer at vi er eksponert av ukjente oksiderbare forløpere. Denne fraksjonen var dårlig korrelert med de konvensjonelle fluorforbindelsene og indikerer at kildene er annerledes.

Abstract

Per and polyfluoroalkyl substances (PFASs) are persistent organic pollutants (POPs) and more than 4000 different PFASs can be found today and are used in several industrial applications (greaseproof paper, firefighting foam, stain repellents) because of their hydrophobic and lipophobic characteristics. PFOS (a perfluoroalkyl sulfonic acid (PFSA)) and PFOA (a perfluoroalkyl carboxylic acid (PFCA)) are the two compounds most detected in the environment. The diet is the most dominant environmental exposure route and PFASs are found in various food products, like fish, meat, eggs, and vegetables.

This study was conducted to assess the concentrations of conventional PFASs compounds and their predictors in plasma of 316 middle-aged women of the Norwegian women and Cancer Study (NOWAC) and in a subgroup the concentrations of novel oxidizable compounds with help of the total oxidizable precursor assay (TOPA).

Birthyear was found to be the strongest predictor, where older women had elevated levels of most of the investigated PFASs.

PFOS (median 19.9 ng/mL), PFOA (2.9 ng/mL), PFPeA (0,9 ng/mL), PFUnDA (0,4 ng/mL) and PFHxS (1 ng/mL) were detected in more than 99 % of the plasma samples. The PLS regression plots indicated that a cluster of marine foods were associated with almost all the investigated PFASs. High consumers of total marine foods were found to have significant elevated levels of PFOA, PFOS, PFHxS, PFNA, PFDA and PFDoDA. PFPeA was not associated with marine foods, but with pastries.

The TOPA method on the subgroup indicated on average a twice as high sum of PFCAs after oxidation and indicates that we are exposed to unknown oxidizable precursor compounds. The fraction was poorly correlated to the traditional PFASs and indicates that the sources are different.

Abbreviations

4:2 FTS – 4: 2 Fluorotelomer sulfonic acid

6:2 FTS – 6:2 Fluorotelomer sulfonic acid

8:2 FTS – 8: 2 Fluorotelomer sulfonic acid

AFFF - aqueous firefighting foam

ANCOVA – Analysis of covariance

EFSA – European Food Safety Authority

FFQ – Food Frequency questionnaire

FOSA – perfluoroocatane sulfonamide

FTOHs - fluorotelomer alcohols

HDL-C - high density lipoprotein cholesterol

ISTD - internal standard

LC/MS-MS – Liquid Chromatography/Mass Spectrometry

LDL-C - low-density lipoprotein cholesterol

LOD – limit of detection

MeOH – methanol

MTBE – methyl tert-butyl ether

NaOH – Sodium hydroxide

NILU – Norsk Institutt for luftforskning/ Norwegian Institute for Air Research

NOWAC – Norwegian Women and Cancer Study

OECD - Organisation for Economic Co-operation and Development

PFAA - Perfluoroalkyl acids

PFASs – Per and polyfluoroalkyl substances

PFBA – Perfluorobutanoic acid

PFBS – Perfluorobutane sulfonic acid

PFCA - Perfluoroalkyl carboxylic acid

PFDA- Perfluorodecanoic acid

PFDoDA – Perfluorododecanoic acid

PFDS – Perfluorodecanoic sulfonic acid

PFESA – Per or poly fluoralkyl ether sulfonic acid

PFHpS – Perfluoroheptane sulfonic acid

PFHpA – Perfluoroheptanoic acid

PFHxA – Perfluorohexanoic acid

PFHxS – Perfluorohexane sulfonic acid

PFNA – Perfluorononanoic acid

PFNS – Perfluorononane sulfonic acid

PFOA - perfluorooctanoic acid

PFOS - perfluorooctane sulfonic acid

PFPeA – Perfluoropentanoic acid

PFPS – Perfluoropentane sulfonic acid

PFSA – Perfluoroalkyl sulfonic acids

PFTeDA – Perfluorotetradecanoic acid

PFTrDA – Perfluorotridecanoic acid

PFUnDA – Perfluoroundecanoic acid

PLS – Partial least square

POP – persistent organic pollutant

POSF – perfluorooctanesulfonyl fluoride

RSTD – recovery standard

TOPA - total oxidizable precursor assay

TWI – tolerable weekly intake

VIP – variable important to projection

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1 Introduction

1.1 Background

For many decades we have been exposed to all sorts of pollutants through our diet, inhalation of particles, and absorption through our skin(1). Many toxicological studies have been conducted on these pollutants and what the consequences are for both humans and animals(1). Many of the pollutants we encounter daily can bioaccumulate, are persistent in the environment, and are toxic for both animals and humans. They also have the potential for long range transport(2) These are called persistent organic pollutants (POPs) (1). One group of POPs are per-and polyfluoroalkyl substances (PFASs) and potentially other fluorinated compounds which is the topic of this thesis.

1.2 What are PFASs?

PFASs consists of perfluoroalkyl substances where all the H atoms attached to C atoms have been replaced by F atoms, except the ones in the functional groups, and polyfluoroalkyl substances for which all H atoms attached to at least one (but not all) C atoms have been replaced by F atoms as shown in Figure 1.

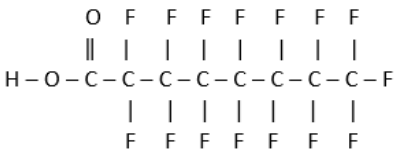


Figure 1: Systematic model of a PFAS.

Polyfluoroalkyl substances can be transformed into perfluoroalkyl substances (3). PFASs are used to define a broad class of fluorinated substances in the environment and more than 4000 different PFASs can be found today. Because of their strong and stable C-F bond and both hydrophobic and lipophobic nature, they are therefore used for several industrial applications, like textile and stain repellents, grease proof papers, firefighting foam (AFFF's) and other additional applications like in the metal plating industry (3).

There has been a lot of focus on perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) because of its early presence in wildlife and numerous samples of human blood purchased from biological supply companies (3). The production of PFOS and PFOA started in the 1950s and used in several applications. The presence of PFOA was already confirmed in human blood in the late 1960s in individuals that were not occupationally exposed (3). Between 2000 and 2002, 3M, one of the largest producers of PFASs, voluntarily phased out its production of PFOS and PFOA after international concerns of the effects of PFAS exposures. Shortly after, PFOS was added to the Stockholm Convention's list of globally restricted Persistent Organic Pollutants (POPs)(2) and restricted the use of PFOS in applications where there is no alternative compound.

Two classes of PFASs are the perfluoroalkyl sulfonic acids (PFSA's) like PFOS and perfluoroalkyl carboxylic acids (PFCA's) like PFOA(4). PFOS is usually found in the highest concentrations, while PFOA is the most frequently detected.

Still more research is needed on other PFASs, especially the total consequences and health outcomes when different PFAS interact with each other and accumulate.

1.3 Exposure pathways

For environmental exposures routes, the diet has been found to be the dominant route of exposure for many PFASs(5). PFASs are found in various food products like vegetables, dairy products, beverages, meat products, eggs shellfish, and fish. (6, 7). Elevated serum concentrations of PFASs were found in several coastal populations where seafood is regularly found on the menu (2, 8, 9). Other studies found elevated concentrations of PFASs in other food groups like meat, eggs and vegetables(10).

Other exposure pathways are through intake of contaminated drinking water, inhalation of indoor air and contact with media that have been contaminated (2).

Exposure to PFASs does not only occur through direct pathways as mentioned above, but also through indirect exposure where PFASs can biotransform into other PFASs like PFOS and PFOA(5). This biotransformation could happen in the environment, wildlife, and humans.

A study from Nilsson et.al indicates that fluorotelomer alcohols (FTOHs), made by telomerization which is a manufacturing process for perfluoroalkyl substances, can bio transform to perfluoroalkyl carboxylic acids (PFCA) in humans(11). PFCAs are highly persistent, bioaccumulative, and have been detected in biotic and abiotic environments, food, and humans and therefore it is important to find its sources and reduce exposure (3, 12). A known and often studied PFCA is PFOA(3).

1.4 Health effects of PFAS exposure

Most of the epidemiological studies that were conducted on the effects of PFASs in humans found associations between elevated serum PFASs and elevated total cholesterol and low-density lipoprotein cholesterol (LDL-C) or reduced high density lipoprotein cholesterol (HDL-C) (2). Other studies that examined the effects of PFASs on hypertension and vascular diseases were inconsistent(2). A nested case-control study within a population-based US prospective cohort found a significant increased risk of renal cell carcinoma and PFOA exposure, also after adjusting for other PFASs(13).

Rappazzo et al. found positive associations between PFASs exposure and several health effects like dyslipidemia, renal function, and age at menarch in articles based on children(14). Another study on children from the Faroe Islands showed that PFASs exposure could lead to decreases in tetanus antibody concentrations at age 5(15).

1.5 Temporal trends

The production of PFASs started in the 1950s and had its peak in the 1980s-1990s. Between 2000 and 2002, 3M Company, the largest producer of POSF (perfluorooctanesulfonyl) based materials; raw material for PFOS, PFOA and related compounds, phased out the production of these chemicals (16). In 2009, PFOS was added to the persistent organic pollutant list of the Stockholm convention, and these compounds were then only allowed for specific and

acceptable use. This restriction lead to a reduced production of these compounds(16). Several studies in several countries showed that the PFAS concentrations in people increased from the 1970s to the 1990s, where a decrease of PFOS and PFOA was found during 2001-2008. Despite the downward trend of PFOS and PFOA, an upward trend of longer-chained PFAS (PFNA, PFDeA) were found in other studies(16, 17). This indicates an ongoing exposure of these long-chained PFASs.

After the restriction of PFOS, alternative PFASs were produced and its detection in the environment is increasing (18). For example, GenX (HFPO-DA) has been used since 2010 as a replacement for PFOA and was detected for the first time in surface water in 2015(18).

1.6 Precursors of novel fluorinated compounds

The phase out of PFOS and PFOA has led to a substantial increase in production and use of alternatives to especially these two compounds(18) and much less is known about novel compounds. Only recently there has been an interest in researching these alternative PFASs because of its detection in wildlife, sediments, water, sewage, and in humans (18).

As mentioned earlier are the most studied PFASs the perfluoroalkyl acids (PFAA) that consists of the perfluorocarboxylic acids (PFCA's) and perfluoroalkyl sulfonates (PFSA's)(4).

Of special interest are the PFAA precursors that can be potentially transformed abiotically or biotically into PFCA or PFSA end products (4). They could be the original alternative PFAS products coming from a manufacturing plant or it could be intermediates that were formed during other transformation processes. The biotransformation abilities of PFAA precursors can influence the fate and transport of PFCA's or PFSA's in the environment (4).

According to the Organisation for Economic Co-operation and Development (OECD) the definition of a precursor is "a substance that has been recognized as having the potential to degrade to perfluorocarboxylic acids (PFCA's) with a carbon chain length of 8 and higher (including PFOA) or perfluoroalkyl sulfonates with a carbon length of 6 and higher (including

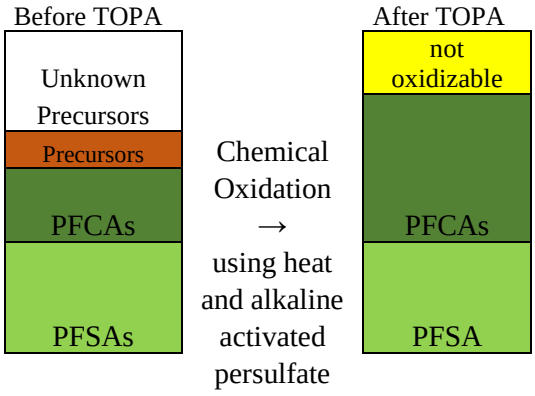
PFHxS and PFOS)(19). The presence of precursor PFAS and the potential to biotransform into PFCA's or PFSA should therefore be further investigated to get a better understanding.

1.6.1 Total oxidizable precursors assay

The total oxidizable precursor assay (TOPA) is a quantification method that can analyse PFAA precursors that are challenging to analyse as individual precursors, that are known measurable precursors and unknown precursors(20). Because of lack of knowledge on the compound identity and/or missing authentic and mas-labelled standards, only few of the PFAA precursors can be analysed with existing methods(4). Precursors are aggressively oxidized to PFCAs and PFSAs by using hydroxyl radicals created by the thermolysis of persulfate ($S_2O_8^{2-}$) under basic pH conditions (20).

To measure the presence of total oxidizable precursors we measure the PFAAs after oxidation minus the PFAAs before oxidation(20). TOPA is not a method mimicking the metabolic process in a human body but rather a method to identify the presence and amount of unknown PFASs humans are exposed to. The TOPA method is a step towards identifying the total PFAS load in humans. Figure 2 gives an overview on compounds before and after TOPA after analysis on the LC/MS-MS. In this thesis, the oxidizable fraction concerns only compounds after oxidation.

Figure 2: Overview of the TOPA method



Adapted from Dora Chiang (21)

1.7 Aims and Objectives

The aim is to assess concentrations of conventional PFASs compounds and their predictors in plasma of participants of the Norwegian women and Cancer Study (NOWAC).

Furthermore, in a subgroup the concentrations of novel oxidizable fluorinated compounds will be assessed and compared to the conventional PFASs compounds. This will include some preliminary considerations of sources of exposure to these compounds.

2 Material and methods

2.1 Study population

Data and blood samples from the Norwegian Women and Cancer Study (NOWAC) were used in this master thesis. Data collection of this Norwegian cohort study started in 1991 and has information about smoking, physical activity, anthropometry, reproductive history, screening for breast cancer and other relevant background information of around 172.000 Norwegian women between 30 and 70 years old. This study is one of the few studies that are representative for the Norwegian female population of their age group. All the participants were randomly selected from the Norwegian Central Person Register. Detailed Food Frequency Questionnaire (FFQ's) and blood samples were sent in between 1998 and 2006 (n=50.000) (22). The NOWAC study has been found to be nationally representative for Norwegian women in this age group and the questionnaire has been validated several times (22-24).

2.1.1 Food Frequency Questionnaire

Participants of the NOWAC study received a 4- 8-page long FFQ depending on which serial number the participants belonged to. Questions about drinking habits (water, juice, coffee/tea, and milk), bread and cereals, fruit and vegetables, rice, spaghetti, porridge and soup, meat intake and other food habits like intake of desserts, chocolate and salty snacks were asked. Specific questions about fish intake, like fatty and lean fish, fish products, preparation way of fish and what kind of fat they had along their fish meals were also in the questionnaire. Shellfish and fish liver and roe intake was also a part of the questionnaire. Other questions were about alcohol consumption and what food habits they had in their childhood.

2.1.2 Study sample

The study sample consisted of 316 participants of the NOWAC study that had previously been selected as age matched controls in two nested lung and breast cancer studies. Blood samples of these participants were donated between 2003 and 2006 and shipped overnight to the Institute of Community Medicine, University of Tromsø, prepared, and stored at – 70

degrees Celsius. The participants also received a detailed FFQ of 8 pages. There is no reason to expect any bias in terms of diet and contaminants.

2.2 Blood analysis

Plasma samples were analysed at the Norwegian Institute for Air Research (NILU) in Tromsø, Norway by Liquid Chromatography/Mass Spectrometry (LC/MS).

2.2.1 Analysis of conventional PFAS

For the conventional PFASs analysis, the following method was used:

First the plasma samples were vortexed one by one before 250 µl of thawed plasma of each sample was transferred in an Eppendorf tube. 20 µl of internal standard PFAS mix (ISTD) and 0.5 ml methanol was added to the same Eppendorf tubes. For every batch (n =19) one blank was added. The blanks received 250 µl of methanol instead of plasma but otherwise the same procedure as with the plasma samples. The Eppendorf tubes were vortexed for a couple of seconds. After the vortex, it went through a 3 times 10 min ultrasonic bath and between every cycle vortexed for a couple of seconds. After the third ultrasonic homogenization the samples were centrifuged at 10.000 rpm for 10 minutes until sedimentation of the solution.

The next step was the transfer of all the extract without the sediment to 2 ml vials and vortexed for a couple of seconds. An aliquot of 50 µl of the extract was put in separate vials with screw caps and 20 µl of RSTD (recovery PFAS standard solution) was added. The rest of the extract was used for the total oxidizable precursor assay (TOPA).

For the conventional PFAS analysis, the vials with the extract and RSTD were vortexed and 25 µl was transferred into a glass vial with insert. Prior to the analysis in the LC-MS (MS) system, 25 µl of MeOH buffer was added to the vials with insert and vortexed before going into the LC/MS-MS system.

As a reference, 10 samples with known PFAS concentrations and one blank were prepared in the same way as the samples from the NOWAC study and analysed on the LC/MS-MS system.

2.2.2 Total oxidizable precursor assay

Total oxidizable precursor assay or TOPA is a way to quantify the measurable PFSA and PFCA concentrations after an oxidation process that transforms PFAA precursors abiotically into PFCA's and PFSA's(4). Due to methodological challenges only 49 samples were oxidized.

The following procedure was used:

The subsample that was earlier kept apart from the PFAS analysis was transferred into 2 ml vials where the MeOH was evaporated by using the MiVac (1 hour 30 minutes, 20 mbar, preheating at 40 ℃). This was to prevent the MeOH to act as a radical scavenger during the oxidation process.

500 µl of $Na_2S_2O_8$ 0.8 M and 80 µl of NaOH 10N solution was added to the vials, capped with tape and vortexed.

For the oxidation process to start, the vials were placed into an oven at 85℃ for 24 hrs.

After 24 hrs, the vials were cooled down and 50 µl of concentrated HCl was added and vortexed. The pH was checked to reach a pH 1-2. 20 µl of the ISTD mix (internal standard) was added and 0,5 ml of MTBE, vortexed and put in an ultrasonic bath for 10 minutes.

This resulted in 2 separate layers in the vial where 100 µl of the MTBE extract (the top layer) was taken out and put in a vial with insert together with 20 µl 0.1 ng/µl RSTD (recovery standard) and vortexed. 30 µl 2% NH_3 in MeOH was added. The next step was the evaporation of the MTBE where the caps were taken off and waited for around two hours for the MTBE to evaporate. Prior to the analysis in the LC/MS-MS system 50 µL of 2 mM NH4OAc 100% MeOH buffer (buffer B) was added.

The focus in this thesis is the difference between concentrations of sumPFCA's after oxidation and concentrations of sumPFCA's before oxidation and is defined here as $TOPA_{diff}$.

The ratio $TOPA_{diff}$/PFCAs is the sum of $TOPA_{diff}$ divided with the total sum of PFCAs from the 44 samples.

2.2.3 Quality control analysis conventional PFASs

For every 19 samples, one blank and one reference sample were prepared and analysed on the LC/MS/MS system in batches with the study samples. Reference samples are used as a measure for quality control of the method. The reference samples that were used in the analysis contain PFASs in known concentrations. To check whether the method is working appropriately, the known concentrations are compared with the calculated concentrations after sample processing and quantification. These should be between 80 and 120 % compared to the known PFASs concentrations with a margin of 20 %. Blanks were used to see whether there was contamination happening during the analysis.

The limit of detection (LOD) was calculated by quantification software at NILU. For most of the compounds LODs are calculated from the blanks. PFPeA, PFHxA, PFHpA, PFOA, PFNA were detected in a few blank samples. LOD is the average concentration in the blanks plus three times the standard deviation of the blanks. The compounds that do not see a signal in the blanks, the concentration was calculated as three times the signal to noise ratio given by the quantification software.

2.3 Statistical analysis

Statistical analyses were performed using SPSS statistic software version 26 (IBM SPSS Inc.) with the partial least square extension module (Python extension module). PFASs with detection frequencies of 30% over LOD were examined in the statistical analyses where the values below LOD were replaced with LOD/$\sqrt{2}$.

PLS regressions were used as an exploratory method for data reduction and to identify relevant predictors of interest. All collected demographic and dietary variables were centered and included as continuous independent variables, whereas PFASs were centered and included as dependent variables. Variables with variable importance to projection (VIP) values of > 0,6 were included in the final models to enhance the predictability of the models.

The demographic and dietary predictors that were identified from the PLS regressions were further investigated by linear regression and analysis of covariance (ANCOVA). Assumptions for regression analyses were checked by residual plots and test of homoscedasticity. A significance level of 0,05 were used.

3 Results

3.1 Demographic descriptives

The 316 Norwegian women from the study population were born between 1943 and 1957. The mean age was 55 years. From the 288 women that gave birth to one or more children, the mean breastfeeding was 14 months. The participants were enrolled in the NOWAC study between 20th Nov 1998 and 12th Dec 2005. Blood drawing dates were between 27th May 2003 and 13th Nov 2006. The mean years since last birth at the blood drawing date was 26,5 years for the 288 women that gave birth. 90 women smoked and 220 were non-smokers at the time of the study. More details about the demographic characteristics can be found in Table 1.

Table 1: Demographic descriptives of the study group (n=316)

Variables	Mean	SD	Median	Percentiles (5, 95)
Birthyear	1949	4.3	1948	1943, 1957
Age	54.5	4.3	55	47, 61
Parity (children)	2.2	1.1	2	0, 4
Breastfeeding (in months)	N= 287 13.9	12.1	11	0, 38.60
Years since last birth at blood draw	(n=288) 26.5	7.2	27	14.5, 37.6
BMI (kg/m$^{2)}$	(n=307) 25.1	3.6	24.8	19.6, 32.0
Smoking (yes/no)	N=310 (90/220)	N/A	N/A	N/A

3.2 Dietary intake

From the 115 dietary variables that were included in the dataset, 30 main groups were included in the statistical analyses and are listed in Table 2. This table represents the median food intake of the study sample. Two (0,6%) of the 316 women reported no fish intake and four women (1,3%) were vegetarian. The highest calorie intake was 3789 kcal.
The median intake calories per day was 1684 kcal for this study group.

Table 2: Dietary intake of the study group (n=316)

Foodstuff	N%	Dietary intake g/day Median	Percentiles (5, 95)
Alcohol	N/A	N/A	N/A
Beer	N=316	0	0, 72
Wine	N=316	10	0, 94
Spirits	N=316	0	0, 6
Bread and cereals	N=316	132.6	45, 240
Butter and margarine	N=316	12.5	0, 40
Cheese	N=316	22.5	4, 88
Chocolate	N=316	3.1	0, 33
Coffee	N=316	533.8	0, 1470
Eggs	N=316	16.8	0, 32
Fish oil	N=316	0	0, 11
Fish	N=316	85.8	26, 194
Fat fish[a]	N=316	12.9	0, 48
Fish liver	N=316	0	0, 1
Fish spread[b]	N=316	8.7	0, 34
Lean fish[c]	N=316	22	0, 82
Processed fish[d]	N=316	24.9	0, 75
Roe	N=316	0,6	0, 3
Shellfish	N=316	3,5	0,9
Fruits	N=316	204.5	25, 502
Jam	N=316	5,7	0, 50
Meat	N=316	108.6	32, 207
Reindeer meat	N=313	0	0, 5.00
Milk products (milk and yoghurt)	N=316	128.7	0, 618
Pasta	N=316	12.1	0, 52
Pastry[e]	N=316	31.9	0, 83
Potato	N=316	63	19 ,189
Rice	N=316	10.1	0, 42.9
Salty snacks	N=316	3	0, 16.5
Soft drinks	N=316	0	0, 525
Vegetables	N=316	151	43, 371
Calories per dag (kcal/day)	N=316	1683.5	1004, 2517

[a] Include wolffish, salmon, mackerel, and herring.
[b] Include mackerel in tomato sauce, caviar, smoked salmon, herring, and other fish spread.
[c] Include boiled and fried cod.
[d] Include fish cakes, fish au gratin and deep-fried fish.
[e] Include cakes, pancakes, waffles, sweet pastries.

3.3 Conventional PFAS concentrations in the study population

A total of 23 PFASs; 11 perfluorocarboxylates (PFCAs, C_5-C_{14}): PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA, 8 perfluorosulfonates (PFSA's, C_4-C_{10})): PFBS, PFPS, PFHxS, PFHpS, PFOS linear and branched, PFNS, PFDS and 4 sulfonate telomers (or PFAA precursors) 4:2 FTS, 6:2 FTS, 8:2 FTS, FOSA were analyzed on the LC/MS-MS system.

Fourteen PFASs analytes had a detection rate of more than 30% and were taken further for statistical analyses. The concentrations and detection rates are presented in Table 3. Total PFOS (linear and branched) was the most dominant compound, followed by PFOA, PFHxS, PFPeA and PFUnDA as shown in Table 3. Of the sulfonate telomers 8:2 FTS was detected in 23 samples. Both 6:2 FTS and FOSA were detected in one sample and 4:2 FTS not detected and therefore all excluded in the statistical analyses.

Table 3: PFAS concentrations (ng/mL) in the study group with detection rate of > 30 %

PFAS concentration (ng/mL)	Median	n= 316 Range	LOD	%>LOD
PFPeA	0.86	<LOD-5.85	0.21	99.7
PFHxA	0.40	<LOD-1.08	0.10	94.9
PFHpA	0.06	<LOD-0.80	0.06	52.2
PFOA	2.89	0.49-9.08	0.13	100
PFNA	0.63	<LOD-1.95	0.24	98.4
PFDA	0.22	<LOD-1.08	0.02	54.1
PFUnDA	0.36	<LOD-1.50	0.02	99.7
PFDoDA	0.04	<LOD-0.38	0.02	80.4
PFTrDA	0.05	<LOD-0.34	0.07	36.1
PFBS	0.03	<LOD-0.16	0.04	33.2
PFHxS	1.02	<LOD-10.8	0.11	99.7
PFHpS	0.26	<LOD-0.88	0.03	97.8
PFOS lin	11.5	3.28-41.8	0.02	100
PFOS br	8.05	1.54-23.6	0.26	100
∑PFOS	19.9	5.26-64.0	0.02	
∑PFAS	26.7	8.95-75.5		

LOD, limit of detection; %>LOD % of samples with concentrations above LOD; PFPeA, Perfluoropentanoic acid; PFHxA,Perfluorohexanoic acid; PFHpA, perfluoroheptanoic acid; PFOA, perfluoroctannoic acid; PFNA, perfluorononanoic acid; PFDA, perfluorodecanoic acid; PFUndA, perfluoroundecanoic acid; PFDoDA,perfluorododecanoic acid; PFTrDA, perfluorotridecanoic acid; PFBS, perfluorobutane sulfonic acid; PFHxS*, perfluorohexane sulfonic acid; PFHpS, perfluoroheptane sulfonic acids; PFOS br, perfluorooctane sulfonic acids branched; PFOS lin, perfluorooctane sulfonic acids linear, ∑PFAS, total sum of the PFASs with a detection rate of 30 % and more.

Internal correlations between the analysed PFASs are presented in Table 4 and indicated that total PFOS and PFHpS concentrations were strongly correlated (r = 0.81), and medium strong correlated with PFHxS (r= 0.69) and PFOA (r= 0.68) with PFOS. Long chained PFASs like PFUnDA were medium strong correlated with other long chained PFASs like PFNA (r=0.64). PFPeA was not correlated with any of the other short chained PFAS.

Table 4: Correlation coefficients (Spearmans rho) for all analysed PFASs

	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTrDA	PFBS	PFHxS	PFHpS	Total PFOS
PFPeA	1												
PFHxA	0.03	1											
PFHpA	-0.01	0.11	1										
PFOA	-0.05	**0.25****	**0.41****	1									
PFNA	0.03	0.08	**0.32****	**0.56****	1								
PFDA	0.02	0.03	**0.25****	**0.24****	**0.58****	1							
PFUnDA	-0.01	0.02	**0.17****	**0.24****	**0.64****	**0.56****	1						
PFDoDA	0.09	-0.01	**0.24****	**0.20****	**0.39****	**0.35****	**0.55****	1					
PFTrDA	0,02	0.07	**0.23****	**0.18****	**0.41****	**0.33****	**0.52****	**0.49****	1				
PFBS	**-0.11***	**0.15****	**0.13***	**0.14***	-0.06	-0.17	-0.05	0.05	-0.05	1			
PFHxS	-0.01	**0.12***	**0.19****	**0.65****	**0.56****	**0.25****	**0.39****	**0.21****	**0.23****	0.06	1		
PFHpS	0.07	0.08	**0.24****	**0.69****	**0.57****	0.28	**0.33****	**0.17****	**0.13***	0.02	**0.68****	1	
Total PFOS	0.11	**0.17****	**0.19****	**0.68****	**0.67****	**0.41****	**0.52****	**0.26****	**0.25****	0.03	**0.69****	**0.81****	1

* $p < 0.05$
** $p < 0.01$
PFPeA, Perfluoropentanoic acid; PFHxA,Perfluorohexanoic acid; PFHpA, perfluoroheptanoic acid; PFOA, perfluoroctannoic acid; PFNA, perfluorononanoic acid; PFDA, perfluorodecanoic acid; PFUndA, perfluoroundecanoic acid; PFDoDA,perfluorododecanoic acid; PFTrDA, perfluorotridecanoic acid; PFBS, perfluorobutane sulfonic acid; PFHxS*, perfluorohexane sulfonic acid; PFHpS, perfluoroheptane sulfonic acids; Total PFOS, sum linear and branched perfluorooctane sulfonic acids

3.4 Predictors of PFASs

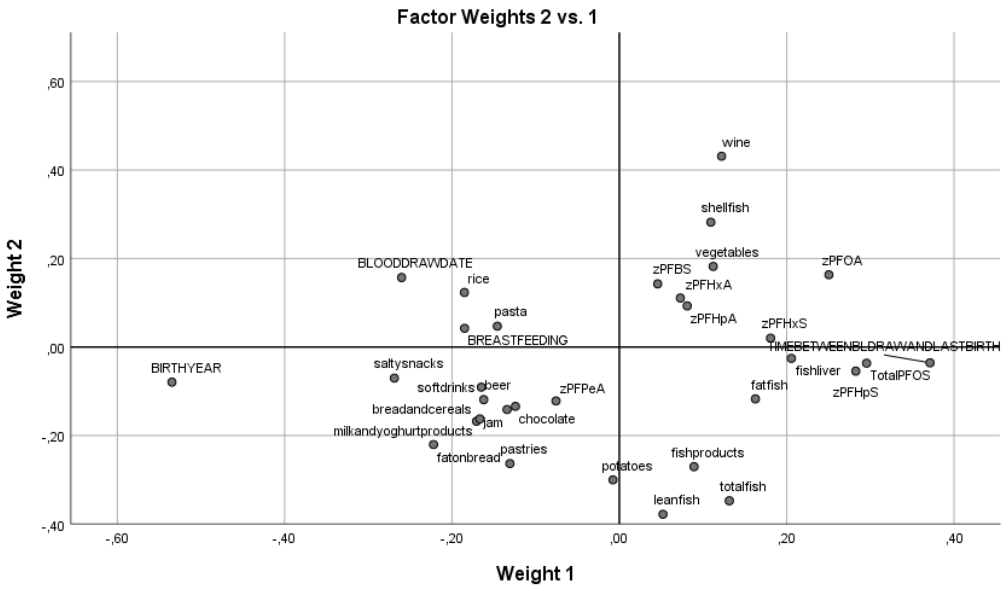
In the PLS regression analyses, PFASs covaried according to chain length. Predictors explored for short (C_4-C_8) and long chained (C_9-C_{13}) PFASs are therefore presented in two different PLS plots, Figure 3, and Figure 4, respectively.

Short chained PFASs (PFPeA, PFHxA,PFHpA,PFOA, PFBS, PFHxS, PFHpS and total PFOS), showed that PFBS, PFHxA, PFHpA were negatively associated with birth year, blood draw date and total months of breastfeeding. PFHpS, total PFOS, PFHxS also covaried with each other and were also negatively associated with birth year, blood draw date and total months of breastfeeding (Figure 3). Years since blood draw date and last birth was positively associated for all PFASs in this plot. A cluster of marine foods (total fish, lean fish, fish products, fatty fish, shellfish, and fish liver), vegetables, and wine were all positively

associated, while rice, pasta, bread and cereals, fat on bread, jam, milk and yoghurt products, salty snacks, chocolate, pastries, beer, and soft drinks were all negatively associated (Figure 3).

PFPeA was negatively associated with birth year, breastfeeding and blood draw date in the same PLS regression. Years since blood draw date and last birth was positively associated. The PLS regression plot showed that PFOA and PFPeA did not covary with the other short chained PFAS in the PLS regression plot (Figure 3).

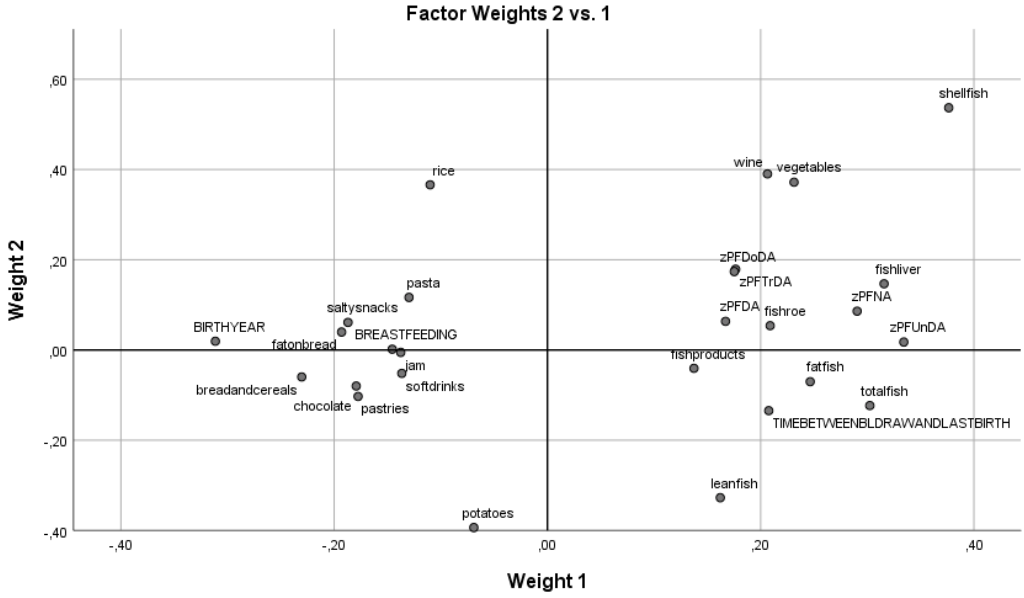
Figure 3: Loading plot for shortchained PFASs (PFPeA, PFHxA,PFHpA,PFOA,, PFBS, PFHxS, PFHpS and total PFOS



zPFOA = PFOA, perfluoroctannoic acid; zPFBS = PFBS, perfluorobutane sulfonic acid; zPFHxA = PFHxA, Perfluorohexanoic acid; zPFHxS = PFHxS, perfluorohexane sulfonic acid; Total PFOS, sum branched and linear PFOS perfluorooctane sulfonic acids; zPFHpS = PFHpS, perfluoroheptane sulfonic acids; zPFPeA = PFPeA, Perfluoropentanoic acid; zPFHpA = PFHpA, perfluoroheptanoic acid.

The PLS regression plot for the long chained PFCAs (PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA) in Figure 4 showed a positive association with these PFASs and years between blood draw and last birth, while breastfeeding and birthyear were negatively associated. A cluster of marine foods were positive associated, together with wine and vegetables. Rice, pasta, potatoes salty snacks, chocolate, fat on bread, jam and bread and cereals were all negatively associated.

Figure 4: Loading plot for long chained PFASs (PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA)



zPFNA = PFNA, perfluorononanoic acid; zPFDA= PFDA, perfluorodecanoic acid; zPFUnDA = PFUndA, perfluoroundecanoic acid; zPFDoDA = PFDoDA,perfluorododecanoic acid; zPFTrDA = PFTrDA, perfluorotridecanoic acid;

Overall, the two PLS regression plots showed that birthyear was the strongest demographic predictor and a cluster of marine foods the dietary predictor that was most associated with most of the investigated compounds.

3.4.1 Demographic predictors

Demographic variables like breastfeeding, parity, birthyear, years between blood draw date and last birth, blood draw date, smoking status and BMI were evaluated as possible predictors.

3.4.1.1 Birthyear

The PLS regressions indicated that birth year was the strongest demographic predictor for most of the PFASs. Linear regressions indicated significant decreasing concentrations with increasing birthyear and predicted for every increasing birthyear a mean reduction of 0,09 ng/ml for PFOA and for total PFOS (linear and branched) a mean reduction of 0,80 ng/ml as listed in Table 6. The ANCOVA models demonstrated decreasing concentrations for the different birthyear groups; where the highest birthyear group (born in 1953 or later) had a significant 41% lower mean PFOA concentration compared to women born in 1945 and earlier. This was 84% for PFHxS, 81 % for PFHpS, 65% for total PFOS, 55% for PFUnDA and 32% for PFNA. The explained variance was 21 % for total PFOS, 17% for PFHpS, 13% for PFOA, 9 % for PFHxS, 8 % PFUnDA and 8 % for PFNA when only adjusted for significant covariates. Table 5 shows the effect of birthyear on selected PFASs concentrations.

Table 5: The effect of significant predictor birth year on PFASs concentrations across birthyear intervals adjusted for covariates[a], group differences, 95 % confidence intervals (CI) and p values.

	PFOA[b]					**Total PFOS[c]**				
Predictor	N	mean conc	diff	95 % CI	p	N	mean conc	diff	95 % CI	p
Birthyear										
<1945	83	**3.57**	.	.	.	83	**25.7**	.	.	.
1946-1948	78	3.29	0.28	(-0.255, 0.760)	1	78	23.6	2.08	(-1.554, 5.722)	0.78
1949- 1952	77	2.92	0.65	(0.161, 1.179)	**0.003**	77	19.6	6.06	(2.418, 9.718)	**0.001**
1953+	78	2.54	1.03	(0.551, 1.566)	**0.0001**	78	15.6	10.1	(6.446, 13.721)	**0.001**
R^2 = 13 %						**R^2= 21 %**				

	PFUnDA[d]					PFNA[e]				
Predictor	N	mean conc	diff	95 % CI	p	N	mean conc	diff	95 % CI	p
Birthyear										
<1945	83	**0.51**	.	.	.	83	0.78	.	.	.
1946-1948	78	0.42	0.09	(-0.004, 1.750)	0.073	78	0.70	0.08	(-0.020, 0.189)	0.20
1949- 1952	77	0.38	0.13	(0.035, 0.215)	**0.002**	77	0.63	0.15	(0.049, 0.260)	**0.001**
1953+	78	0.33	0.18	(0.081, 0.261)	**0.0001**	78	0.59	0.19	(0.085, 0.295)	**0.001**
R^2 = 8 %						**R^2 = 8 %**				

	PFHxS[f]					PFHpS[g]				
Predictor	N	mean conc	diff	95 % CI	p	N	mean conc	diff	95 % CI	p
Birthyear[b]										
<1945	78	**1.51**	.	.	.	83	**0.38**	.	.	.
1946-1948	74	1.31	0.20	(-0.247, 0.579)	1	78	0.33	0.05	(-0.013, 0.125)	0.185
1949- 1952	70	0.99	0.52	(0.040, 0.885)	**0.023**	77	0.26	0.12	(0.046, 0.184)	**0.0001**
1953+	65	0.82	0.69	(0.195, 1.059)	**0.001**	78	0.21	0.17	(0.090, 0.228)	**0.0001**
	R^2= 9 %					**R^2 = 17 %**				

a

b Parity is included as a significant covariate. Birthyear as fixed factor.

c Blood draw date is included as significant covariate. Birthyear as fixed factor

d No significant covariates included. Birthyear as fixed factor

e No significant covariates included. Birthyear as fixed factor

f Breastfeeding is included as significant covariate. Birthyear as fixed factor. N = 287

g Blood draw date is included as significant covariate. Birthyear as fixed factor

Birthyear was found to be a significant covariate for PFHpA, but no significant differences when divided in birthyear groups and no other significant covariates were in the model. Birth year did not seem to be an important significant demographic predictor for PFPeA, PFDoDA PFDA, PFBS and PFHxA. This was also visible in the linear regression models listed in Table 6.

Table 6: Linear regression models for the investigated fluorinated compounds and demographic predictors[d]

	Total PFOS	**PFOA**	**PFHxS**	**PFUnDA**	**PFNA**	**PFHpS**	**PFHxA**	**PFDA**	**PFHpA**	**PFDoDA**	**PFBS**	**PFTrDA**	**PFPeA**
Constant	945,95	8,07	4,02	1,059	1,468	16,565	0,632		0,157		-1,21	0,176	
Predictors[a]	Regression coefficients (β-values)												
Birthyear	-0.804	-0.092	-0.055	-0.013	-0.016	-0.013	-0.005		-0.002			-0.001	
Breastfeeding (months)			-0.011										
BMI at blood draw												-0.002	
Smoking status											0.004		
Years between blood draw													
Blood draw date	0					0					0		
Parity		-0.222											
R^{2c}	19	12	8	6	7	15	1.5		1		3	3.5	

[a] Predictors in this model are continuous variables.

[b] β values describe the change in PFASs concentrations (ng/mL) per unit increase (year, kg/m^2, months, child)

[c] R^2 express the proportion of the variance of the PFASs concentrations explained by the demographic predictors.

[d] Not adjusted for food variables.

3.4.1.2 Parity and breastfeeding

Parity was not an important significant predictor for the investigated PFASs. Only PFOA mean concentration was significantly different according to linear regressions, demonstrating decreasing concentrations (0,22 ng/ml) with increasing parity after adjusting for birthyear as shown in Table 6. Also, in ANCOVA models was parity significant different when divided in four parity groups where nulliparous women had a higher mean concentration of PFOA compared to the group that were multiparous as shown in Table 7.

Table 7: The effect of parity on the concentrations of PFOA in ANCOVA models

	PFOA				
Predictor	N	Mean conc	Diff	95 % CI	p
Number of children (parity)[a]					
0	28	**3.46**	.	.	.
1	27	3.24	0.22	(-0.405, 1.352)	0.921
2	145	3.18	0.28	(-0.235, 1.105)	0.516
3+	116	2.85	0.61	(0.113, 1.483)	**0.013**

[a] adjusted for birthyear

Also, years between blood draw date and last birth was not a significant predictor. Breastfeeding was only a significant predictor for PFHxS in the linear regression models (See Table 6), and unadjusted in the ANCOVA model. Different breastfeeding groups (duration in months) where the longest breastfeeding group (22 months and longer) had a 57 % reduction of mean concentration of PFHxS compared to the group with the shortest duration of breastfeeding in months. This was however unadjusted for other covariates or food groups.

3.4.1.3 Smoking status and BMI

Smoking status was only a significant predictor for PFBS and was adjusted for significant covariate blood draw date in linear regression model. Smokers versus non-smokers had significant higher mean concentration of PFBS, also after adjusting for significant covariate blood draw date. Smoking status was not an important predictor for the other investigated PFASs.

Mean concentration of PFTrDA and body mass index (BMI) were significant different where the highest BMI group had the lowest PFTrDA mean concentration compared to the low BMI group. This was unadjusted for other covariates or food variables and was not significant when divided in BMI groups.

3.4.1.4 Blood draw date

For the majority of the investigated PFASs, concentrations decreased with time during the blood draw period. However, this trend was not significant for most of the PFASs, except PFHxA, PFBS, and PFOS (linear and branched) after adjusting for birthyear (PFHpS, PFOS), smoking status (PFBS) and breastfeeding and birthyear (PFHxS). Median concentrations for the investigated compounds in the period 2003-2006 can be found in Table 8.

Table 8: Median PFASs concentrations per sampling year 2003-2006

PFAS concentration (ng/mL)	2003 (n= 59) Median	2004 (n= 66) Median	2005 (n= 108) Median	2006 (n= 83) Median
PFPeA	1.03	0.77	0.87	0.86
PFHxA	0.43	0.33	0.43	0.40
PFHpA	0.04	0.06	0.04	0.06
PFOA	3.22	2.86	2.97	2.75
PFNA	0.61	0.62	0.62	0.65
PFDA	0.30	0.26	0.13	0.20
PFUndA	0.34	0.38	0.37	0.34
PFDoDA	0.04	0.05	0.04	0.05
PFTrDA	0.05	0.05	0.05	0.05
PFBS	0.03	0.03	0.03	0.03
PFHxS	1.15	1.07	0.98	0.97
PFHpS	0.36	0.30	0.24	0.22
PFOS lin	15.2	11.7	10.5	11.2
PFOS br	9.93	7.71	7.69	7.28
∑PFOS	26.2	19.6	18.7	18.1
∑PFAS	33.6	26.9	26.3	24.6

PFPeA, Perfluoropentanoic acid; PFHxA,Perfluorohexanoic acid; PFHpA, perfluoroheptanoic acid; PFOA, perfluoroctannoic acid; PFNA, perfluorononanoic acid; PFDA, perfluorodecanoic acid; PFUndA, perfluoroundecanoic acid; PFDoDA,perfluorododecanoic acid; PFTrDA, perfluorotridecanoic acid; PFBS, perfluorobutane sulfonic acid; PFHxS*, perfluorohexane sulfonic acid; PFHpS, perfluoroheptane sulfonic acids; PFOS br, perfluorooctane sulfonic acids branched; PFOS lin, perfluorooctane sulfonic acids linear, ∑PFAS, total sum of the PFASs with a detection rate of 30 % and more.

3.4.2 Dietary predictors

PLS regression plots (Figure 3 and Figure 4) showed that several PFASs were positively associated with a cluster of marine foods (fatty fish, lean fish, other kinds of fish, processed fish products, fish spread, fish liver and shellfish). PFNA, PFHxS, PFOS and PFOA were detected in more than 98% samples as shown in Table 3 and selected in Table 9.

After adjusting for significant covariates (birthyear and blood draw date) mean concentrations of PFNA were significant different between low and high total marine food intake in ANCOVA models as presented in Table 9. The ANCOVA models for different marine foods intake indicated that the concentrations of PFNA, PFHxS, PFOS and PFOA were higher in the high intake groups compared to the lower intake groups. Only for PFNA was this difference significant. However, the differences in concentrations were small for all compounds, except for PFOS (5,6 ng/mL).

More details about confidence intervals and p-values for the different intake groups can be found in Appendix I.

Table 9: PFASs concentrations of selected PFASs in four different intake groups of total marine foods according to ANCOVA models[a]

	0-60 g/day (n=79)	61-86 g/day (n=79)	87-122 g/day (n=79)	>123 g/day (n=79)			
	Mean	Mean	Mean	Mean	Diff[e]	95 % CI[b]	P[b]
PFNA (ng/mL)	0.60	0.66	0.69	0.75	0.15	(-0.238, -0.027)	**0.006**[c]
PFHxS (ng/mL)	1.16	1.06	1.27	1.20	0.04	(-0.372, 0.479)	1[d]
PFOS (ng/mL)	18.9	21.1	20.2	24.5	5.60	(-7.101, 0.292)	0.09[c]
PFOA	3.08	3.02	3.07	3.18	0.10	(-0.499, 0.539)	1[c]
Birthyear	1949	1949	1949	1948			**0.128**[f]

a Predicted mean concentration of selected PFASs with intake of total marine foods which include fatty fish, lean fish, other kinds of fish, processed fish products, fish spread, fish liver and shellfish, fatty fish and lean fish.

b 95 % confidence intervals and p values are between the lowest and highest intake groups of total marine food intake

c Birthyear and blood draw date included as significant covariates. Intake of total marine foods included as fixed factor

d Birthyear and breastfeeding included as significant covariates. Intake of total marine foods included as fixed factor

e Difference mean concentrations between lowest and highest intake groups of total marine foods.

f No significant covariates included. Total fish intake included as fixed factor. Birthyear overall significant p<0,03, but not significant when divided in 4 intake groups.

Total marine foods were also found significant different between low and high intake for PFUnDA (35% difference between the highest and lowest intake groups) and PFDA (41%) but in much lower concentrations than shown in Table 10.

High fatty fish consumers had significant elevated concentrations, after adjusting for significant covariates, of PFUnDA (23% difference between the highest and the lowest intake groups) and PFNA (16%), and for lean fish, PFUnDA (29%)

Table 10: Parameter estimates, group differences, 95 % confidence intervals and p values for PFASs[a] and total intake of marine foods, fatty fish, and lean fish.

	PFUnDA[b]					PFNA[c]					PFDA[d]				
Predictor	N	mean conc	Diff[e]	95 % CI	p	N	mean conc	Diff	95 % CI	p	N	mean conc	Diff	95 % CI	p
Intake of marine foods[a]															
0-60 g/day	79	**0.33**	.	.	.	79	**0.60**	.	.	.	79	0.17	.	.	.
61-86 g/day	79	0.38	0.05	(-0.140, 0.036)	0.719	79	0.66	0.06	(-0.172, 0.038)	0.541	79	0.23	**0.06**	(-0.162, 0.046)	0.841
87-122 g/day	79	0.42	0.09	(-0.184, -0.008)	**0.025**	79	0.69	0.09	(-0.198, 0.011)	0.111	79	0.26	0.09	(-0.186, 0.021)	0.213
>123 g/day	79	0.51	0.18	(-0.255, -0.077)	**0.0001**	79	0.75	0.15	(-0.238, -0.027)	**0.006**	79	0.29	0.12	(-0.222, -0.014)	**0.016**
Intake of fatty fish[a]															
<8 g/day	106	**0.36**	.	.	.	106	**0.62**	.	.	.					
9-19 g/day	104	0.41	0.05	(-0.119, 0.022)	0.302	104	0.66	0.04	(-0.115, 0.050)	1					
20+ g/day	106	0.47	0.11	(-0.169, -0.028)	**0.003**	106	0.74	0.12	(-0.190, -0.026)	**0.005**					
Intake of lean fish[a]															
<16 g/day	103	**0.35**	.	.											
17-32 g/day	92	0.40	0.05	(-0.122, 0.022)	0.294										
33+ g/day	93	0.49	0.14	(-0.194, -0.048)	**0.001**										

a Predicted PFASs concentrations per selected food variables divided in intake groups: Total marine foods: fatty fish, lean fish, other kinds of fish, processed fish products, fish spread, fish liver and shellfish.

b Birthyear included as significant covariate. Intake of total marine foods, fatty fish and lean fish included as fixed factor.

c Birthyear and blood draw date included as covariate, Intake of total marine foods and fatty fish included as fixed factor.

d No significant covariates included in the model.

PFUndA, perfluoroundecanoic acid;PFNA, perfluorononanoic acid; PFDA, perfluorodecanoic acid;

Looking into other individual fish intake variables indicated that high consumers of fish liver had 17 % higher PFOS concentrations compared to low consumers, 20 % higher for PFHxS and 41 % higher for PFDA concentrations as shown in Table 11.

Table 11: PFASs mean concentrations in two different intake groups of fish liver[a]

	<0 g/day (n=214)[a]	1+ g/day (n=102)[a]			
	Mean conc.	Mean conc.	Diff.	95% CI	p
Total PFOS (ng/mL)	**19.9**	24	4.1	(-4.938, -0.887)	**0.005[b]**
PFHxS (ng/mL)	**1.09**	1.37	0.28	(-0.461, -0.014)	**0.037[c]**
PFDA (ng/mL)	**0.20**	0.32	0.12	(-0.182, -0.068)	**0.001[d]**

a Predicted PFASs concentrations in low and high intake of fish liver.
b Birthyear included as significant covariate. Fish liver included as fixed factor.
c Birthyear included as significant covariate. Fish liver include as fixed factor.
d No significant covariate included. Fish liver included as fixed factor.
PFDA, perfluorodecanoic acid; PFHxS, perfluorohexane sulfonic acid; PFOS perfluorooctane sulfonic acids

Dividing shellfish consumers into two groups indicated significant higher mean concentrations PFOA, PFNA, PFDA, PFUnDA, PFDoDA and PFTrDA in the highest intake group as shown in Table 12.

Table 12: PFASs mean concentrations in two different intake groups of shellfish[a]

	>4 g/day (n=120)[a]	5+ g/day (n=196)[a]			
	Mean conc.	Mean conc.	Diff [n].	95 % CI	p
PFOA (ng/mL)	**2.83**	3.25	0.42	(-0.706, -0.160)	**0.002[b]**
PFNA (ng/mL)	**0.61**	0.71	0.10	(-0.167, -0.055)	**0.001[d]**
PFDA (ng/mL)	**0.18**	0.27	0.09	(-0.152, - 0.041)	**0.001[e]**
PFUnDA (ng/mL)	**0.34**	0.45	0.11	(-0.163, -0.067)	**0.001[c]**
PFDoDA (ng/mL)	**0.04**	0.06	0.02	(-0.028, -0.008)	**0.001[f]**
PFTrDA (ng/mL)	**0.06 (n=117)**	0.08 (n=195)	0.02	(-0.032, -0.012)	**0.0001[g]**

a Predicted PFASs concentrations in low and high intake of shellfish
b Birthyear and parity included as significant covariates. Intake of shellfish included as fixed factor
c Birthyear included as significant covariate. Intake of shellfish included as fixed factor
d Birthyear included as significant covariate. Intake of shellfish included as fixed factor
e No significant covariates included. Intake of shellfish included as fixed factor
f No significant covariates included. Intake of shellfish included as fixed factor
g Birthyear and BMI included as significant covariates. Intake of shellfish included as fixed factor
h Diff is the difference between high and low intake groups.

PFOA, perfluoroctannoic acid; PFNA, perfluorononanoic acid; PFDA, perfluorodecanoic acid; PFUndA, perfluoroundecanoic acid; PFDoDA,perfluorododecanoic acid;

Other findings were significant elevated concentrations between low and high intake of wine for PFOA (16% difference between the highest and lowest intake groups), and PFTrDA (28%) as shown in the Appendix I, Table II.

PLS regressions plot in Figure 3, indicated that PFPeA behaved differently than the other compounds and ANCOVA models indicated a significant difference between low and high intake (30 % difference) of pastries as shown in Table 13.

Table 13: Parameter estimates, group differences, 95 % confidence intervals and p values for PFPeA and intake of pastries unadjusted

		PFPeA[b]			
Predictor	N	mean conc	Diff	95 % CI	p
Intake of pastries[a]					
<16 g/day	79	**0.76**	.	.	.
17-32 g/day	79	0.95	0.19	(-0.391, 0.023)	0.113
33-45 g/day	79	1.00	0.24	(-0.439, -0.023)	**0.02**
46+ g/day	79	1.09	0.33	(-0.532, -0.118	**0.001**

a Predicted PFASs concentrations per intake groups of pastries (includes cakes, pancakes, waffles and sweet pastries)

b No significant covariates included. Intake of pastries included as fixed factor

PFPeA, Perfluoropentanoic acid

Table 14 shows linear regression models for the investigated PFASs with demographic and dietary predictors.

Table 14: Linear regression models with demographic and dietary predictors for the investigated PFASs

	Models Total PFOS	PFOA	PFHxS	PFUnDA	PFNA	PFHpS	PFHxA	PFDA	PFHpA	PFDoDA	PFBS	PFTrDA	PFPeA
Constant	770.3	7,75		0,854	1,432	16,277			0,064	0,088	-1,371	0,162	0,848
Predictors[a]	Regression coefficients (β-values)[b]												
Birthyear	-0.852	-0.094		-0.011	-0.017	-0.013				-0.001		-0.001	
Breastfeeding (months)	0												
BMI at blood draw												-0.001	
Smoking status													
Blood draw date											0		
Years between blood draw													
Parity		-0.2											
R^{2} [c]	19	12	8	6	7	15	1.5				8	3.5	
Coffee	0.002										0		
Eggs													
Fatty fish				-0.004		0.001							
Fish liver	3.7			0.118	0.104								
Fish oil													
Fish products	0.052												
Fish roe													
Fish spread				-0.004									
Shellfish		0.075			0.018					0,003		0.003	
Lean fish				-0.004									
Total fish				0.005									
Pastries													0.003
Potatoes	-0.021												
Reindeer					0.008								
Rice													
Spirits													
Vegetables										0			
Wine		0.004							0			0	
R^{2}[d]	24	20		20	20	17	1,5		3	8	3	12	3

[a] Predictors in this model are continuous variables.

[b] β values describe the change in PFASs concentrations (ng/mL) per unit increase (year, kg/m^2, months, child).

[c] R^2 expresses the proportion of the variance of the PFASs concentrations explained by the demographic predictors.

[d] R^2 expresses the proportion of the variance of the PFASs concentrations explained by demographic and dietary predictors.

3.5 Oxidizable compounds in the study population

Only 49 random samples from the 316 samples were further oxidized with the TOPA method as described in the methods. Five samples were lost during the oxidation method and had to be excluded, resulting in n=44 of TOPA results. Long chained PFCAs like PFTrDA and PFTeDA were present only in low concentrations, hence the low n of detected cases. None of the perfluorosulfonates (PFSAs) increased after oxidation and were left out for further analysis. The short chained PFCAs (PFBA- PFHpA) were not fulfilling the QA/QC requirements and are not reported here. The PFCA and $TOPA_{diff}$ concentrations in the subgroup are presented in Table 15, describing PFCA concentrations determined after the TOPA (PFCAs in Table 15) and the concentration change ($TOPA_{diff}$ in Table 15), see method chapter for calculation of $TOPA_{diff.}$

Table 15: PFCAs and $TOPA_{diff}$ concentrations

(ng/ml)	PFCAs Median	TOPA diff Median	PFCAs Range min-max	$TOPA_{diff}$ Range min-max	Median Ratio $TOPA_{diff}$/PFCAs	Mean Ratio $TOPA_{diff}$/PFCAs	Min-Max range ratio $TOPA_{diff}$/PFCAs
PFOA (n=39)	2.45	1.49	0.64-4.69	0.25- 8.07	0.61	0.70	0.39-1.72
PFDA	0.24	1.59	0.02-0.81	0.41- 6.34	2.05	9.10	20.5 - 7.8
PFNA	0.59	1.53	0.24-1.75	0.24- 6.24	6.63	2.47	1.00- 3.56
PFUnDA	0.32	0.82	0.08-0.86	0.16-3.83	1.81	2.31	2.00- 4.45
PFDoDA	0.03	0.88	0.02-0.18	0.18-6.04	29.3	23.9	9.00- 33.6
PFTrDA (n=33)	0.07	0.09	0.07-0.34	0.01-0.47	1.29	1.29	0.14- 1.38
PFTeDA (n=9)	0.09	0.10	0.07-0.86	0.01-0.47	1.19	1.19	0.14- 3.61
Total PFCA	3.56	5.22	0.83-7.06	1.54-30.9	1.35	1.98	1.86- 4.37

LOD % limit of detection, PFOA, perfluorooctanaoate, PFNA, perfluorononanoate, PFDA, perfluorodecanoate, PFUnDA, perfluoroundecanoate, PFDoDA, perfluorododecanoate, PFTrDA, perfluorotridecanoate, PFTeDA, perfluorotetradecanoate, TOPA, total oxidizable precursors assay, PFAAs, perfluoroalkyl acids

[a] PFAAs concentrations before oxidation

[b] $TOPA_{diff\ =}$ after TOPA – before TOPA

Correlation coefficients in Table 16 between PFCAs and $TOPA_{diff}$ indicated that PFTeDA concentrations was highly correlated with PFNA, PFDA and PFUnDA concentrations, while $TOPA_{diff}$ PFTeDA was only correlated with PFTeDA.

Table 16: Correlation coefficients (Spearmans rho) between PFCAs[a] and $TOPA_{diff}$ [b] (n=44)

	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTrDA	PFTeDA	**TOPA diff PFOA**	**TOPA diff PFNA**	**TOPA diff PFDA**	**TOPA diff PFUnDA**	**TOPA diff PFDoDA**	**TOPA diff PFTrDA**	**TOPA diff PFTeDA**
PFOA (n=39)	1													
PFNA	**0.52****	1												
PFDA	0.20	**0.64****	1											
PFUnDA	0.02	**0.64****	**0.49****	1										
PFDoDA	-0.06	-0.04	-0.07	0.23	1									
PFTrDA(n=33)	-0.02	0.33	0.42*	**0.52****	0.34	1								
PFTeDA (n=9)	0.39	**0.94****	**0.85****	**0.77***	0.13	0.67	1							
TOPA PFOA (n=39)	-0.14	0.02	-0.07	0.08	-0.05	-0.31	-0.31	1						
TOPA diff PFNA	0.08	0.13	-0.15	0.10	0.17	-0.08	-0.09	**0.88****	1					
TOPA diff PFDA	0.08	0.09	-0.23	0.04	0.18	-0.14	-0.12	**0.87****	**0.97****	1				
TOPA diff PFUnDA	0.04	0.06	-0.15	0.01	0.07	-0.18	-0.09	**0.86****	**0.91****	**0.92****	1			
TOPA diff PFDoDA	0.17	0.16	-0.09	0.14	-0.02	-0.16	-0.14	**0.87****	**0.93****	**0.92****	**0.91****	1		
TOPA diff PFTrDA (n=33)	0.01	0.29	0.13	**0.41***	0.16	0.17	0.70	**0.47****	**0.51****	**0.53****	**0.50****	**0.49****	1	
TOPA diff PFTeDA (n=9)	-0.64	-0.65	-0.34	-0.52	-0.13	-0.11	**-0.71***	0.21	0.46	0.35	0.46	0.44	-0.11	1

**p < 0,01

* p < 0,05

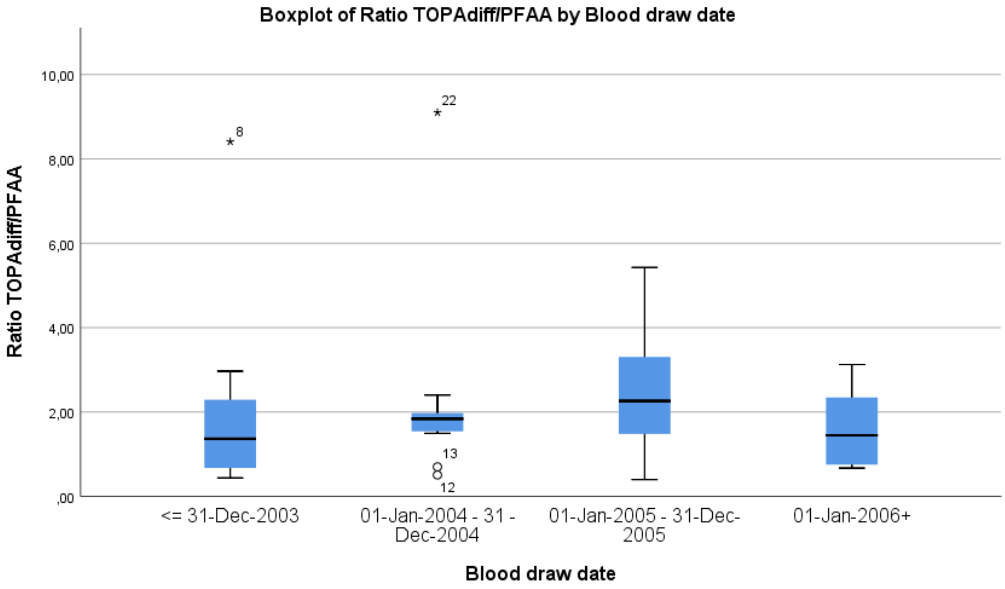
[a] Conventional PFAAs (before oxidation)

[b] $TOPA_{diff}$: after TOPA – before TOPA

PFOA, perfluorooctanaoate, PFNA, perfluorononanoate, PFDA, perfluorodecanoate, PFUnDA, perfluoroundecanoate, PFDoDA, perfluorododecanoate, PFTrDA, perfluorotridecanoate, PFTeDA, perfluorotetradecanoate, TOPA, Total oxidizable precursor assay, PFCAs, perfluorocarboxylic acids

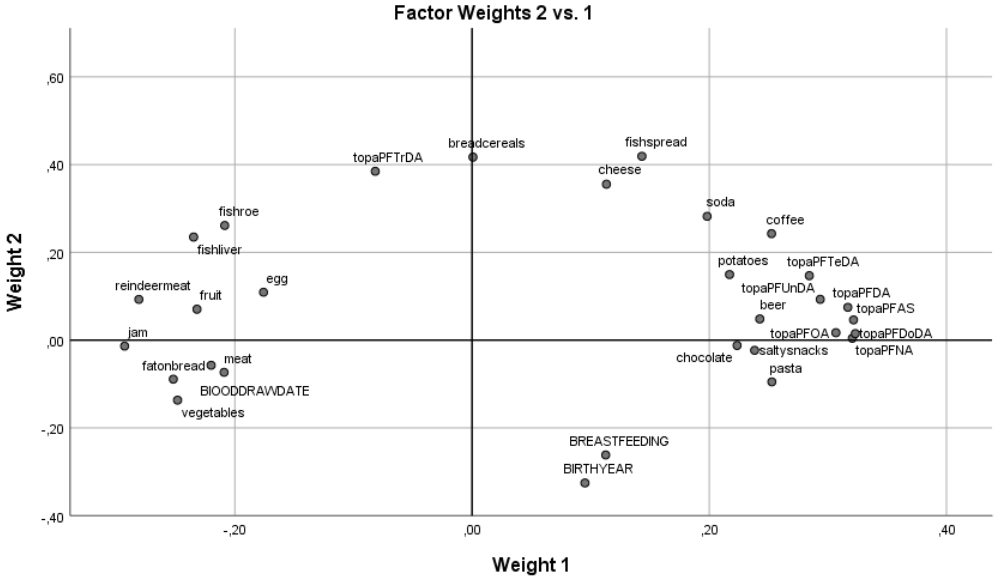
Figure 6 shows the distribution of the ratio sum $TOPA_{diff}$ and PFAA between 2003 og 2006 after the TOPA method for the 44 samples that were included in the analysis.

Figure 6: Boxplot with ratio sum $TOPA_{diff}$/PFAA by Blood draw date 2003 (n=8), 2004 (n=11), 2005 n=17) and 2006 (n=8)



PLS regression plots in Figure 7 and Figure 8, indicate different demographic and dietary predictors between $TOPA_{diff}$ and PFCAs before the TOPA method for the 44 samples. Birthyear and breastfeeding were positively associated with $TOPA_{diff}$, while they were negatively associated in the plot with PFCAs.

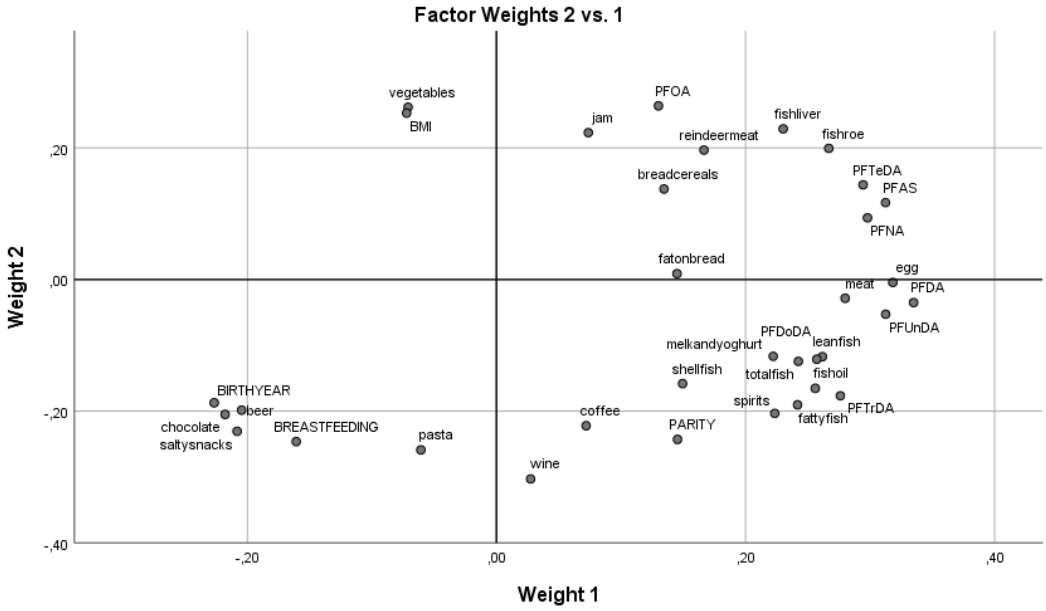
Figure 7: Partial least square loading plot for $TOPA_{diff}$ compounds (n=44)



topaPFOA, perfluorooctanaoate, topaPFNA, perfluorononanoate, topaPFDA, perfluorodecanoate, topaPFUnDA, perfluoroundecanoate, topaPFDoDA, perfluorododecanoate, topaPFTrDA, perfluorotridecanoate, topaPFTeDA, perfluorotetradecanoate, $TOPA_{diff}$ = after TOPA – before TOPA, PFCAs, perfluorocarboxyl acids
All TOPA compounds in the plot are $TOPA_{diff}$ (concentrations difference)

PLS plot in Figure 7 indicated that a cluster of marine foods were positively associated with PFCAs for the 44 samples while $TOPA_{diff}$ compounds were mostly associated with salty snacks, chocolate, and coffee as shown in Figure 6.

Figure 8: Partial least square loading plot for PFCAs (n=44) (PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA and PFTeDA)



PFOA, perfluorooctanaoate, PFNA, perfluorononanoate, PFDA, perfluorodecanoate, PFUnDA, perfluoroundecanoate, PFDoDA, perfluorododecanoate, PFTrDA, perfluorotridecanoate, PFTeDA, perfluorotetradecanoate, $TOPA_{diff}$ = before TOPA – after TOPA, PFCAs, perfluorocarboxyl acids

4 Discussion

4.1 Main findings

For the first time, we report data on precursor PFAAs in blood after oxidizing these precursors to perfluoroalkyl carboxylates (PFCAs) using the total oxidizable precursor assay (TOPA). Comparing the concentrations of PFCA's of 44 samples before and after oxidation indicates that the concentration is on average doubled after oxidation. This is a clear indication that humans are exposed to considerable amounts of fluorinated precursor compounds. The fraction was poorly correlated to the traditional PFCA's which indicates that the sources of exposure are different, and the PLS plots indicated that these compounds clustered differently.

Birthyear was found to be the strongest demographic predictor for most of the conventional PFASs where older individuals had higher concentrations. A cluster of marine foods was indicated as the main dietary predictor in the study population for PFOS, PFNA, PFHxS, PFUnDA, PFTrDA, PFDoDA, and PFDA.

4.2 PFAS and demographic predictors

PFOA and PFOS were the dominating fluorinated compounds and detected in all samples. These were followed by PFPeA, PFUnDA, and PFHxS and detected in 99,7 % of the samples.

This study used samples from four different sampling years and since PFASs concentrations are decreasing with years this could push the average lower for the compounds that are decreasing in 2005 and 2006. We therefore compared the median PFASs concentrations from samples taken in 2004 (n=66) with a study with women in the same cohort with all samples from 2004 (n=315) as shown in Table 8. The concentrations of the conventional PFASs in the current study were comparable to what was reported earlier in the same cohort for the same blood draw year (2004)(9); PFOS; 19,6 ng/mL (2004) compared to 20 ng/mL (2004), PFHxS; 1,1 ng/mL compared to 1 ng/mL. PFOA and PFNA were lower in the current study (PFOA; 2,9 ng/mL compared to 4,4 ng/mL, PFNA; 0,62 ng/mL (2004) compared to 0,81 ng/mL)(9). Total median concentrations of PFASs in the current study for 2004 were also

comparable with the same cohort (26,9 ng/mL compared to 26,7 ng/mL, while they were higher for the period 2003-2006 (27,6 ng/mL).

Another study reported similar or higher concentrations than the current study in 41 women from a coastal area in Northern Norway with a median age of 44 years and blood draw date in September 2005 (PFOS; 18.7 ng/mL in the current study (2005) compared to 24 ng/mL, PFOA; 3 ng/mL in the current study (2005) compared to 3.4 ng/mL)(8).

PFOS concentrations in Swedish women aged 45-50+ years with blood draw year in 2006 were much less than in the current study (PFOS; 18,1 ng/mL in the current study (2006) compared to 10,4 ng/mL and PFOA; 2,8 ng/mL in the current study compared to 2,5 ng/mL) (25). PFOS concentrations were slightly lower in another Norwegian study (median 10,6 ng/mL 2007) but included female participants that were younger (30-year-olds) than in the current study, while PFOA had similar concentrations (2,37 ng/mL 2007)(26).

This shows how sensitive these comparisons are for blood draw year when concentrations are changing in the environment.

For most of the investigated PFASs, mean concentrations decreased with time during the blood draw period 2003-2006 (Table 8), however, this trend was only significant for PFOS (sum linear and branched), PFHxA and PFBS.

Birthyear was used as a demographic predictor and not age, because it is the birthyear that matters for the concentrations of PFASs and other contaminants in blood. However, since the blood draw year varied with three years, age at the blood draw date would have given a somewhat more precise estimate although this would not change the overall findings.

The participating women were born between 1943 and 1957 which was the same period when the production of PFASs, like PFOA and PFOS started. Large- scale production of these PFAS did not start until the 1980’s-1990s and was phased out between 2000 and 2002. Most of these women gave birth in the 1970s and 1980s and a long period has passed since giving birth and breastfeeding. This could be a reason why there was no observed effect for

breastfeeding and parity. Rylander et.al did not find an effect for breastfeeding and parity either for the same study population in the same blood draw period (2004)(9).

Birthyear explained more of the variance in the data compared to total marine food intake. As seen in PFOS concentrations that differed 10 ng/mL between younger and older women as shown in Table 5, while low and high intake of total marine foods differed 5 ng/mL for PFOS (although not significant different) as shown in Table 9.

4.3 Dietary predictors of PFAS

PLS regression plots as shown in Figure 7 and 8 indicated that fish variables were mostly associated with most of the PFASs except for PFPeA. Most of the fish variables were grouped with each other in the PLS plots and were hard to disentangle. Women with a high fish intake eat more of most types of fish and makes it difficult to distinguish whether there are specific fish variables or the combination that cause the effects. Because of covariance, the focus here is on total marine food intake, where elevated concentrations of PFHxS (low intake 1,16 ng/mL compared to high intake 1,20 ng/mL) PFOA (3,1 ng/mL vs 3,2 ng/mL), PFOS (18,9 ng/mL vs 24,5 ng/mL), PFNA, PFDA and PFUnDA were found, although only significant different for PFNA, PFDA and PFUnDA as shown in Table 9 and 10.

There were minor differences in age for the high consumers of fish compared to women that ate less fish (Table 9). The PLS plots shows that there are other single dietary items that are positively or negatively associated with the investigated PFASs, but further exploration of their effect on the actual concentrations of the different PFASs shows that they play a minor role. Wine intake was found significant different between the lowest and highest intake groups for PFOA (low intake 2,8 ng/mL compared to high intake 3,4 ng/mL), PFDoDA (0,04 ng/mL compared to 0,06 ng/mL) and PFTrDA (0,07 ng/mL compared to 0,09 ng/mL) PFASs are found in most foods and different dietary patterns will therefore give different main predictors(27).

Several studies reported that shellfish and fish had the largest concentrations of PFOA and PFOS in other countries like the Netherlands, Spain, Germany, Sweden (28). These studies investigated concentrations in different food items.

Even though total marine foods intake was seen as the most important dietary predictor in this study group and the highest concentrations of the investigated PFASs have been reported in marine foods (Table 9 and 10), different dietary items will dominate in populations with other dietary preferences as shown in a Danish birth cohort. This cohort with 1076 pregnant Danish women reported that red meat, animal fat and snacks intake were associated with elevated levels of PFOS and PFOA(29). Median fish intake for these women was 21 grams per day versus 86 grams per day in the current study. Other contributors of PFASs are egg and egg products and vegetables(10) but these were not associated with elevated concentrations of PFASs in the current study.

The shorter chain PFPeA was detected in 99,7 % of the samples with a median concentration of 0,9 ng/mL. Considering that this compound was not detected in the study from Rylander et al. from 2010(9) indicates that the sensitivity of the analytical methodology has improved.

Even though PFPeA was detected in almost all the samples and the lack of an association with the other investigated PFASs, and fish variables (as shown in Figure 2), shows that sources are different. This would be as expected since short chain PFASs are considered not bioaccumulative (30-32) PFPeA is found as a dominating compound in wastewater in wastewater treatment plants (33, 34) and is a microbial degradation product from 6:2 FTH(35).

4.4 PFCA precursors assessed through the TOPA assay

The differences in concentration after and before oxidation show that there are elevated concentrations of PFCA precursors. Even though the analytical methods used are still uncertain, this does indicate that we are exposed to substantial amounts of these precursors. Due to methodological challenges, we obtained data from the TOPA assay for 44 samples only. As these PFCA precursors were highly correlated, it is impossible to distinguish them

after oxidation and thus they are treated as one fraction- the sum of the individual compounds. The ratio $TOPA_{diff}$/PFCA shows that high amounts are found in some individuals. However, by using a ratio, the same amount of oxidizable precursors could end up having different ratios because of different concentrations before the oxidation.

$TOPA_{diff}$ was not correlated to the PFCAs in the subsample which indicates different exposure sources. However, based on the low subsample (n=44) and the uncertainties in the TOPA analysis, it is challenging to elucidate the sources of exposure properly.

The perfluorosulfonates (PFSAs) did not increase after oxidation and were left out for further analysis. This was also confirmed in a study from Casson et.al that the oxidation process only converts PFAA precursors into PFCAs only(4). Whether TOPA would result into more branched and linear PFOS is unknown and therefore were dietary and demographic predictors for linear and branched PFOS not further investigated in the conventional PFASs analysis, but only the sum of these together. The TOPA method does not reproduce the biotransformation processes that actually occur in the human body but oxidation is done under aggressive non metabolic conditions and we therefore can see the TOPA method as a “worst-case” scenario(4).

4.5 Strengths of the current study

The food frequency questionnaire that was used in the NOWAC study consisted of detailed dietary data of more than 100 food products and had an emphasis on fish consumption. The FFQ has been thoroughly validated and retested(22-24) and therefore seen as a strength. The participants of the NOWAC study represent middle-aged women from whole Norway which is a great advantage(22).

Even though this novel modified TOPA method for human plasma has some analytical limitations and uncertainties, it is a useful tool to assess the total PFAS burden for humans better(36). The preliminary results of the subgroup are useful and looking at this one fraction gives a very strong indication that we are exposed to other fluorinated compounds not been described before.

The preliminary results of the subgroup in the current study were quite promising and more research and adjusting of the analysis could lead to an even better understanding and quantification of PFAA precursors.

4.6 Limitations of the current study

There are some limitations of the TOPA method used for this master thesis. First, there are no standardized TOPA methods. The method that was used in the current study is based on the research of Houtz (20) and alterations had to be made to use the TOPA method on plasma to receive satisfying results. There were some issues with the loss of the internal standard for the five missing samples.

The TOPA results do not identify or quantify the structures from individual PFAA precursors. It can be used as an estimation of the significance of PFAA precursors in the plasma where normal analytical methods do not detect these compounds. The TOPA method cannot either detect the intermediates that might have been formed(4).

4.7 Public health considerations

High fish consumption has several positive health effects like reduced risk of cardiovascular diseases, strokes, and a stable vitamin D status. It is also a good source of vitamin B12, iodine, and selenium(37). The Norwegian Directorate of Health recommends a weekly intake of 300-450 grams of fish per week of which 200 gram fatty fish (37). The actual differences in the investigated compounds for total marine foods and the fact that these are small differences are unlikely to have a detectable effect. However, the European Food Safety Authority (EFSA) has set a new tolerable weekly intake (TWI) of 4,4 ng per kg bodyweight per week which is lower than the TWI from 2018(13 ng/kg body weight per week for PFOS and 6 ng/kg body weight per week for PFOA(38)) and combines four PFASs (PFOA, PFOS, PFNA and PFHxS)(39). For most of the guidelines like TWI's, an increase in knowledge leads to a further reduction in intake values. The more we understand, the more we realise that compounds cause an effect at lower concentrations than previously believed.

5 Conclusion

Results from the total oxidizable precursor assay in a subgroup indicated that we are exposed to more fluorinated compounds than the conventional PFASs and that demographic and dietary sources are different than for the conventional PFASs. However, due to the low subsample we were not able to fully explore predictors in this thesis.

For the traditional PFASs, marine foods were mostly associated with elevated concentrations of several PFASs for the study group, where birthyear was the strongest predictor.

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Appendix I

Table I: Parameter estimates, group differences, 95 % confidence intervals and p values for selected PFASs and total marine food intake

Predictor		PFNA[b]					PFHxS[c]			
Intake of total marine foods[a]	N	Mean conc	Diff	95 % CI	p	N	Mean conc	Diff	95 % CI	p
0-60 g/day	79	**0,60**	.	.	.	79	**1,16**		.	.
61-86 g/day	79	0,66	0,06	(-0,172, 0,038)	0,541	79	1,06	-0,10	(-0,324, 0,534)	1
87-122 g/day	79	0,69	0,09	(-0,198, 0,011)	0,111	79	1,27	0,11	(-0,538, 0,304)	1
>123 g/day	79	0,75	0,15	(-0,238, -0,027)	**0,006**	79	1,20	0,04	(-0,372, 0,479)	1
		Total PFOS[d]					PFOA[e]			
Predictor	N	mean conc	Diff	95 % CI	p	N	mean conc	Diff	95 % CI	p
Intake of total marine foods[a]										
0-60 g/day	79	**18,9**				79	**3,08**	.	.	.
61-86 g/day	79	21,1	2,2	(-5,386, 1,828)	1	79	3,02	-0,06	(-0,460, 0,569)	1
87-122 g/day	79	20,2	1,3	(-4,533, 2,717)	1	79	3,07	0,01	(-0,538, 0,490)	1
>123 g/day	79	24,5	5,6	(-7,101, 0,292)	0,09	79	3,18	0,10	(-0,499, 0,539)	1

a Predicted PFASs concentrations per selected food variables divided in intake groups: Total marine foods: fatty fish, lean fish, other kinds of fish, processed fish products, fish spread, fish liver and shellfish.

b Birthyear and blood draw date included as covariate, Intake of total marine foods included as fixed factor.

c Birthyear and breastfeeding included as covariate. Intake of total marine foods included as fixed factor.

d Birthyear and blood draw date included as covariate. Intake of total marine foods included as fixed factor.

e Birthyear and blood draw date included as covariate. Intake of total marine foods included as fixed factor.

Table II: Parameter estimates, group differences, 95 % confidence intervals and p values for PFOA, PFDoDa and PFTrDA and wine intake

		PFOA[b]					PFTrDA[c]					PFDoDA[d]			
Predictor	N	mean conc	Diff	95 % CI	p	N	mean conc	Diff	95 % CI	p	N	mean conc	Diff	95 % CI	p
Intake of wine[a]															
<4 g/day	117	**2,81**	.	.	.	115	**0,066**	.	.	.	117	**0,041**	.		
5-17 g/day	103	3,15	0,34	(-0.719,0.064)	0,14	102	0,074	0,008	(-0,022, 0,007)	0,60	103	0,053	0,012	(-0,027, 0,002)	0,122
18+ g/day	96	3,36	0,55	(-0.898, -0.098)	**0,009**	95	0,092	0,026	(-0,039, -0,010)	**0,001**	96	0,061	0,020	(-0,035, -0,005)	**0,004**

a Predicted PFASs concentrations per wine intake variable divided in intake groups.

b Birthyear and parity included as significant covariates, Intake of wine included as fixed factor.

c Birthyear and BMI included as significant covariates, Intake of wine included as fixed factor.

d No significant covariates included. Intake of wine included as fixed factor.