UNIVERSITY OF TROMSØ UIT

FACULTY OF HEALTH SCIENCES
DEPARTMENT OF COMMUNITY MEDICINE

Human exposure to perfluorinated compounds

Concentrations, dietary impact and molecular signatures

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A dissertation for the degree of Philosophiae Doctor

May 2010

ISM skriftserie nr. 108

ISM skriftserie
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ISBN 13: 978 - 82 - 90263 - 22 - 0
2010

Human exposure to perfluorinated compounds

Concentrations, dietary impact and molecular signatures

By

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May 2010

"The truth is out there..."

-X-files-

Preface

Being a PhD student is very similar to go on a trip in a roller coaster. It goes up and it goes down, and it goes up and down again, and up again and down again and it sometimes feels that it will continues forever. You almost reach the sky, but then you are suddenly at ground level again, very quickly. I was lucky to get a ticket for a trip and somehow I managed to hang on for four years (even though I had my periods with indisposition). On my way I have had many fantastic (and some not that fantastic) experiences that I am very grateful for taking part of. Many people have contributed with their knowledge, experience, listening abilities and encouraging skills and of course with their friendship.

For sure, this trip had never taken place if it wasn't for Torkjel Sandanger, my main supervisor. I am extremely grateful to you for always believing in me, encouraging me and not always listening to me when my mood and inspiration have failed. You are a fantastic supervisor, always having time for questions and inspirational talks and always being able to guide me, even though you had now idea what I was doing. I have felt very privileged for working together with you for several years. I owe you also many thanks for giving me the opportunity to work in Vietnam and South Africa even though this was way out of my original tasks. I am also very grateful to Eiliv Lund, my co-supervisor. Thank you for your guidance and for sharing all your knowledge. I have really appreciated your ability to always see "the big picture" and for always being open minded.

The chemistry team on NILU is incredible! Sandra Huber and Morten Moe have spent lots of time to get my samples running through the LC-MS instrument. Mikael Harju has contributed with valuable knowledge in multivariate data analysis. Eldbjørg Heimstad and Dorte Herzke are always extremely supportive and willing to share their knowledge. Silje Winnem has helped me out with picture editing, travel arrangements and lots of other administrative work. Nicholas Warner has been my living English dictionary the last years. Helena Falk made all my hours in the lab much more structured and, most important, much more fun. Linda Hanssen, has shared all my ups and down during the last years. I was very lucky to get you as my fellow PhD student at NILU. To all the others at NILU Tromsø; I am very grateful to you for creating such an incredibly good working environment.

The microarray article would never have been finished if it wasn't for Vanessa Dumeaux. Thank you for introducing me to R, guiding me through the mysteries of gene expressions and always being willing to answer questions very quickly.

Magritt Brustad was one of the first persons I met when I arrived in Tromsø soon five years ago and she welcomed me to the "contaminant-food-health" group with opened arms. During all this years she has also been incredibly supportive.

I owe a lot to my fellow PhD students at the university and at the Polar Environmental Centre. Especially thanks to Marit Waaseth, who is always willing to answer questions and discuss epidemiology assignments, microarray problems or just general PhD issues. Your phone will now become silent! Thank you Heli, for all nice evenings in your cabin discussing R, ski trips and other aspects of life over a cup of hot chocolate. To all my other colleagues: thank you for always being willing to support a young woman in need.

I'd like to express my gratitude to the staff at the Nha Trang Pasteur Institute in Vietnam and the National Institute of Occupational Health in Johannesburg, South Africa for giving me a wonderful time when I visited you. Working together with you have inspired me a lot and motivated me for the endless days in front of the computer!

My parents and sisters are always standing by my side and I am very grateful for that. A special thanks to my mother who gave me the curiosity for knowledge and taught me never to accept a "truth". Thanks to Dad, who has given me the independency and stubbornness to finish this thesis.

I am very lucky to have many good friends, always willing to go skiing, canoeing, dog mushing, eating sushi, knitting or just having a very nice talk. I really appreciate you all! A special thanks to Marit and Meri for accommodating me during these years. Also, thanks to Johan, for proof reading my thesis in the very last minute.

Finally I like to thank Aslak for always helping me see things from another perspective, but most important, for cheering up my days!

This thesis would never had been written if it wasn't for all the study participants who donated both their time and blood to future research. The medical and administrative staff have made an enormous job with data collection. Thank you all! This work was supported by the University of Tromsø, the Norwegian Institute for Air Research (NILU), the Norwegian Institute for Nutrition and Seafood Research (NIFES) and the Norwegian Research Council.

Charlotta Rylander

Tromsø, March 2010

Summary

Perfluorinated compounds (PFCs) are man-made chemicals extensively used in industry and in consumer products due to their unique property of repelling both water and fat. As a consequence, many PFCs are frequently detected in the environment and in human blood samples. In general, perfluorooctane sulfonate (PFOS) is the dominating compound in human blood followed by perfluorooctanoate (PFOA), perfluorohexane sulfonate (PFHxS) and perfluorononanoate (PFNA). Health effects of PFCs have been assessed among occupationally exposed individuals, however, few have investigated the effects of background concentrations in the general population. Gene expression profiles of human blood may have large implications in epidemiology as early biomarkers of disease or through investigations of the etiology of diseases. In the context of environmental pollutants, gene signatures have the potential of elucidating the effects of contaminants on human health.

The main objectives of this doctoral thesis were to assess concentrations, dietary impact and molecular signatures of PFCs in blood samples from the general population in Norway. In order to in depth explore the effect of lactation, residency and age, PFC levels in delivering women from Vietnam were assessed. These women had considerably different group characteristics and living conditions than those of the Norwegians.

The current thesis is based on samples from three different study groups: The Norwegian women and Cancer study (NOWAC, women only, n=326, mean age 56 years), the Andenes study (men and women, n=56, mean age 44 years) and the Vietnamese study group (women only, n=91, mean age 26 years).

Concentrations of the most common PFCs were 3-7 times higher in the Norwegian samples than in the Vietnamese, indicating a more extensive exposure to PFCs in Norway. The linear PFOS isomer constituted 69-70% of total PFOS in the Norwegian samples, which was considerably lower than in the Vietnamese blood samples (83%), indicating differences in exposure between the two countries.

In the Norwegian study groups, "fish eaters" (people who consumed fish and seafood) were identified as having higher concentration of a number of PFCs. The identification of dietary predictors was highly dependent on the dietary habits in the study group due to the ubiquitous presence of PFCs and especially PFOS in many foodstuffs. To exemplify, the Norwegians were in general high consumers of marine food and consequently fish and seafood became the significant dietary predictor for several PFCs in those study groups.

The magnitude of human exposure to PFCs has varied considerably during the last 50 years, due to changing production quantities. As a consequence, people that were born before the substantial increase in PFC production around 1970 have experienced the same life time exposure. Nevertheless, in the Norwegian study groups, older women had higher concentrations of PFOS, PFHxS, PFNA and/or perfluoroheptane sulfonate (PFHpS). There was no age trend among men. The observed increase in PFC concentrations with age among women was explained by age dependent differences in diet and/or time since last childbearing. There are strong evidences for lactation reducing a women's body burden of PFCs. Nevertheless, no effect of parity (strongly correlated to lifetime lactation) was observed in the younger low contaminated Vietnamese study group. Low blood concentrations of PFCs may result in low partitioning of the compounds into the breast milk and, thus, low "body loss". The lack of association could also be a result of an increasing exposure due to the current production of PFCs in China.

Blood gene profiles from the general population indicated that the glucose metabolism was affected by PFOS exposure. Several diseases e.g. Diabetes II and Alzheimer's' disease have been associated with metabolic disorders, emphasizing the need for a more thorough understanding of the effects of PFOS on human health. No genes or gene sets were differentially expressed in relation to PFOA or PFHxS concentration.

Sammendrag

Perfluorerte forbindelser (PFCer) er en stor gruppe menneskeskapte forbindelser som brukes som overflateaktive stoffer og er både fett- og vannavstøtende. På grunn av de unike egenskapene har disse forbindelsene blitt hyppig brukt både i industrien og i mange forbrukerprodukter. Dessverre finner man disse igjen i miljøet og i blodet hos mennesker. Tidligere forskning tyder på at matinntak er en viktig eksponeringsvei til PFCer for mennesker. Generelt dominerer perfluoroktansulfonat (PFOS) i humant blod, men også perflouoroaktanoat (PFOA), perfluoroheksansulfonat (PFHxS) og perfluorononanoat (PFNA) er hyppig forekommende. Studier på eventuelle helseeffekter av PFCer er blitt gjort blant yrkeseksponerte mennesker. Derimot har det vært lite fokus på effekten av bakgrunnseksponering blant den generelle befolkningen. Genekspresjonsanalyse av humant blod har stor potensial som et verktøy for å finne tidlige markører for sykdom eller til å undersøke årsaken til sykdom, og potensialet er stort for å undersøke effekten av miljøgifter på mennesker.

Det overordnede formålet med avhandlingen var å undersøke nivåer av perfluorerte forbindelser i blodet hos den generelle norske befolkningen og finne kostholdets betydning for disse nivåer. Hensikten var videre å undersøke effekten av disse miljøgifter på genuttrykket i blodet hos norske kvinner. For at få dypere innsikt i sammenhengen mellom PFC konsentrasjonen og alder, amming og boplass, ble en studie av PFC nivåer blant gravide kvinner i Vietnam gjennomført. Disse kvinnene hadde helt annen alderssammensetning og levevilkår enn de norske.

Avhandlingen baserer seg på blodprøver fra tre forskjellige studier: Kvinner og kreft (bare kvinner, n=315, gjennomsnittsalder=56 år), Andenes-studiet (menn og kvinner, n=56, gjennomsnittsalder=44 år) og Vietnamstudiet (bare kvinner, n=91, gjennomsnittsalder=26 år)

Konsentrasjonene av de vanligste PFCer var 3-7 ganger høyere i de norske prøvene enn i de vietnamesiske. En større andel av forbindelsene var også påvist i de norske prøvene, noe som tyder på at den norske befolkningen har vært mer eksponert for PFCer enn

befolkningen i Vietnam. Den lineære PFOS isomeren utgjorde 69-70% av total PFOS i de norske prøvene. Dette var betydelig lavere enn i de Vietnamesiske blodprøvene (83%), hvilket tyder på forskjellig type eksponering i landene.

I de norske studiene var samlebetegnelsen "fiskespisere" assosiert med høyere konsentrasjoner av flere PFCer. Man finner likevel PFCer i mange type mat og populasjonens kostholdsvaner vil være avgjørende for hvilke matartikler som blir identifisert som viktige PFC kilder. Den norske befolkningen spiser generelt mye fisk sammenlignet med i andre land, og derfor ble "fiskespisere" assosiert med høyere konsentrasjoner. I en populasjon som spiser mye kjøtt, blir kjøtt identifisert som den viktigste kilden til PFCer.

Den humane eksponeringen til PFCer har variert betydelig de siste 50 årene grunnet variasjoner i produksjonsmengder. Dette innebærer at mennesker som ble født før 1970, hvor produksjonsmengden økte betraktelig, har blitt noenlunde likt eksponert. Likevel fant man i de norske populasjonsgruppene at eldre kvinner hadde høyere nivåer enn yngre kvinner av PFOS, PFHxS. PFNA og/eller perfluoroheptansulfonat (PFHpS). Blant menn var det ikke noen alderssammenheng. Økningen med alder blant kvinner kunne forklares av aldersavhengige forskjeller i kosthold, samt av tiden passert siden siste barnefødsel. Det er sterke indikasjoner at amming medfører en reduksjon av kvinners kroppsbelastning av PFCer, som for andre organiske miljøgifter. Likevel hadde antall barn, i den yngre Vietnamesiske studiegruppen, ingen effekt. Disse damene hadde imidlertid lave nivåer av PFCer og dette kan innebære lav utskilling av disse forbindelsene i brystmelken og således liten reduksjon av kroppsnivåene. Det kan også tyde på at eksponeringen er økende i Vietnam.

Resultatene fra genekspresjonsanalysene viste effekter på sitronsyresyklusen i de som var høy eksponert sammenlignet med lav eksponert, hvilket tyder på at glukose metabolismen påvirkes av PFOS eksponering. Det er flere sykdommer (for eksempel diabetes 2 og alzheimer's sykdom) som henger sammen med metabolske forstyrrelser og dette taler for at effekten av lav-dose eksponering til PFOS bør undersøkes grundigere i

lignende studier. I dette studiet var det ikke noen gener eller gensett som ble påvirket av PFOA eller PFHxS eksponering.

List of papers

This thesis is based on four papers, referred to in the text by their roman numerals.

I. **Dietary predictors and plasma concentrations of perfluorinated compounds in a coastal population from northern Norway.** Rylander, C., Brustad, M., Falk, H., Sandanger, T.M. Journal of Environmental and Public Health, 2009, doi: 10.1155/2009/268219.

II. **Dietary patterns and plasma concentrations of perfluorinated compounds in 315 Norwegian women: the NOWAC postgenome study.** Rylander, C., Sandanger, T.M., Frøyland, L., Lund, E. *Submitted.*

III. **Perfluorinated compounds in delivering women from south central Vietnam.** Rylander, C., Phi, D.T., Odland, J.Ø., Sandanger, T.M. Journal of Environmental Monitoring 2009; 11: 2002-8.

IV. **Perfluorinated compounds and blood gene signatures in postmenopausal women: the NOWAC postgenome study.** Rylander, C., Dumeaux, V., Standahl Olsen, K., Waaseth, M., Sandanger, T.M., Lund, E. *Submitted.*

Abbreviations

ANOVA	analysis of variance
APFO	ammonium perfluorooctanoate
BMI	body mass index
DNA	Deoxyribonucleic acid
DNBC	Danish national birth cohort
ECF	electrochemical fluorination
FFQ	food frequency questionnaire
HDL	high-density lipoprotein
HPLC-QTOF-MS	high performance liquid chromatography-quadrupole time of flight- mass spectrometer
LOD	limit of detection
LOQ	limit of quantification
mRNA	messenger RNA
NHANES	National health and nutrition examination survey
NILU	Norwegian institute for air research
NOWAC	Norwegian women and cancer study
PCR	polymerase chain reaction
PFASs	perfluoroalkyl sulfonates
PFCs	perfluorinated compounds
PFCAs	perfluoroalkyl carboxylates
PFHpA	perfluoroheptanoate
PFHpS	perfluoroheptane sulfonate
PFHxS	perfluorohexane sulfonate
PFNA	perfluorononanoate
PFOA	perfluorooctanoate
PFOS	perfluorooctane sulfonate
PFOSA	perfluorooctanesulfonamide
PLS	partial least square regression
POPs	persistent organic pollutants

POSF	perfluorooctane sulfonyl fluoride
RNA	ribonucleic acid
rRNA	ribosomal RNA
SMR	standard mortality ratio
tRNA	translation RNA

1. Introduction

1.1 Production and use of perfluorinated compounds

Perfluorinated compounds (PFCs) are a large group of man-made chemicals that have been produced for more than 50 years [1]. Two of the main sub-groups among the PFCs are the perfluoroalkyl sulfonates (PFAS) and the perfluoroalkyl carboxylates (PFCAs). All PFCs consist of a non-polar, fully fluorinated carbon back-bone and a polar functional group (a sulfonate group for the PFASs and a carboxylate group for the PFCAs). Perfluorooctane sulfonate (PFOS, Figure 1) is the most common PFC in nature and in human blood samples. Perfluorooctanoate (PFOA), perfluorohexane sulfonate (PFHxS) and perfluorononanoate (PFNA) are also frequently detected in various concentrations.

Figure 1. The chemical structure of perfluorooctane sulfonate (PFOS)

PFCs have been extensively used in industrial applications and as constituents of consumer products due to their unique property of repelling both water and fat [2]. The main building block for many fluorochemicals is perfluorooctane sulfonyl fluoride (POSF) which can degrade to PFOS during hydrolysis [3]. 3M, the major producer of POSF-related chemicals started the production in 1949, although, production volumes before 1970 were estimated to be low [4]. From 1975 to 1990 the production of POSF-chemicals multiplied several times before staying relatively constant until year 2000, when 3M voluntarily phased out PFOS and related chemicals from production [4] (Figure 2). The production of deliberated PFOS has been low and only estimated to 470 tons [4]. Thus the widespread occurrence of PFOS in nature is mainly a result of PFOS being an

impurity in POSF based chemicals and/or the degradation of POSF and other precursor PFCs.

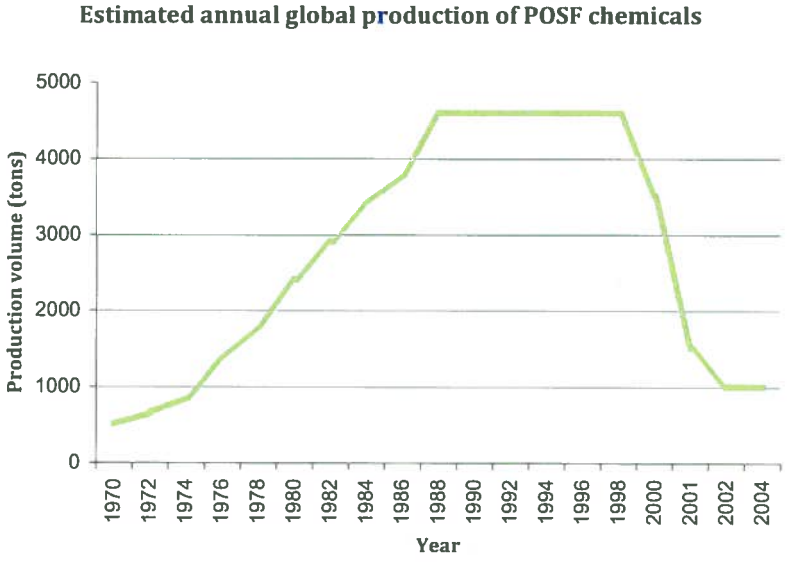

Figure 2. Estimated annual global production of POSF chemicals, adapted from Paul et al, 2009.

The total global production of POSF products from the start of production until 2002 has been estimated to 96 000 tons in addition to 26 500 tons of POSF-containing waste [4]. Besides from 3Ms production plants in the U.S and Belgium, production facilities for fluoropolymers have been based in the U.K, Switzerland, Italy, Japan, India, the Russian Federation, China and Brazil [5]. Currently, China is considered the world's leading producer of POSF/PFOS, however, production volumes are not known [6].

One of the major production processes of PFCs has been electrochemical fluorination (ECF). During that process both straight chain POSF (approximately 70%), linear homologs (4-9 carbons atoms) and branched POSF derived impurities (30 %) were produced, thus, the commercialized POSF products were not pure chemicals [4]. POSF/PFOS products have been important components of surface treatment products for water and grease repellants for textiles, furniture, paper, leather etc [3]. In addition, PFOS

has been a constituent in aqueous fire fighting foam and has been used in hydraulic fluids and in the photographic and electronic industry [2]. In Norway, PFOS has mainly been a component in fire fighting foam on oil rigs [7]. The estimated historic release of PFOS and related compounds in Norway were 58 tons and remaining quantities were approximately 22 tons [8].

POSF can react with methyl or ethyl amine and form N-methyl or N-ethyl-perfluorooctanesulfonamide (FOSA) that is further converted to PFCAs e.g. PFOA (Figure 3). FOSA can subsequently be combined with ethylene carbonate and form N-methyl/N-ethyl perfluorooctanesulfonamideethanol (FOSE) which is the precursor to fluortelemer alchohols (FTOHs). In turn, FTOHs may undergo atmospheric or biological degradation to PFCA [9]. FTOHs are also produced by telomerization which only yields straight chain isomers. Perfluorooctanesulfoneamide (PFOSA) is another chemical intermediate, produced from POSF and used in surface treatment products and in fire fighting foam [5]. PFOSA can further degrade to PFOS in the environment.

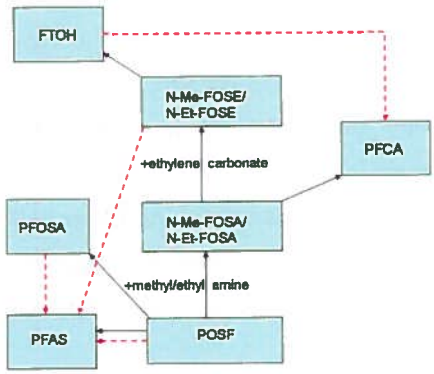

Figure 3. Industrial synthesis of PFAS, PFCA, PFOSA and FTOH by ECF (black, solid lines) and possible degradation routes (red dashed lines).

PFOA and PFNA has been extensively used as a processing aids in the production of fluoropolymers (Teflon for example) [1]. The global historical emissions (1951-2004) of PFCAs from direct or indirect sources have been estimated to 3200-7300 tons [1]. There are currently, no known sources of PFOA in Norway although, it has been estimated that 1.3 kg of PFOA enter Norway each year as a result of importation of paper products with PFOA containing coating [10]. Du Pont, a major producer of PFOA in the U.S has committed to phase out PFOA and PFOA precursors before 2015 [11].

In May 2009, PFOS and POSF were listed as Persistent Organic Pollutants (POPs) under the Stockholm Convention on POPs [12].

1.2 PFCs in the environment

PFOS, PFOA and PFHxS have been shown to bioaccumulate and biomagnify in Arctic marine food webs [13, 14]. Several PFASs and PFCAs have also been detected in human blood samples from remote regions [15], indicating a global transport of these compounds to isolated areas. As they are non-volatile chemicals with high water solubility, the global distillation principle that describes atmospheric transport of legacy POPs is not applicable for PFASs and PFCAs. Direct and indirect transport pathways for the PFCs have therefore been identified. The direct pathways include discharges from fluoropolymer plants, leakage from landfills and sewage treatment plants into the water body, followed by riverine and oceanic transport [6] (Figure 4). Indirect transport result from emissions of volatile precursors, e.g. fluorotelomer alcohols (FTOH) that are transported long-distances with atmospheric currents, followed by atmospheric biological degradation to PFCAs [6, 9].

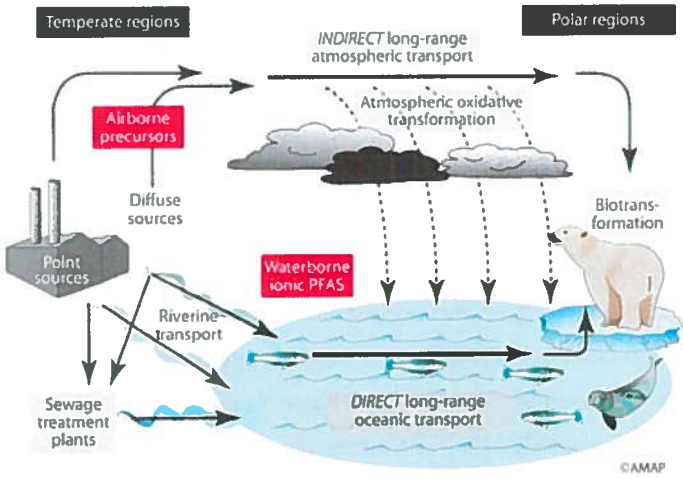

Figure 4. Direct and indirect transport pathways for PFCs. Copyright AMAP 2009

1.3 Human exposure to PFCs

From the mid 1990's PFCs has been monitored in blood samples from workers in fluoropolymer industries. Over the last 15 years, numerous publications about human PFC levels have confirmed that PFCs are human contaminants. A main difference from the legacy POPs is that PFCs are mainly distributed in blood and are bound to serum albumin [16, 17]. Concentrations found in human blood samples are therefore several times higher on volume basis than concentrations of the fat-soluble legacy POPs.

Large geographical variations in human blood concentrations of PFCs have been reported with higher concentrations in some of the industrialized countries than in parts of the developing world [18, 19]. The current exposure situation may, however, change with time as POSF/PFOS are still in production in China. Recently, a decline in human PFOS and PFOA concentrations was reported among U.S. citizens after the 3M phase out in 2000 [20]. A similar trend has been observed in pooled samples from Norway [21].

PFCs have been detected in various kinds of food [22-24] (meat, fish, diary products, potatoes etc.) as well as in drinking water [25], household dust [26] and consumer

products [27] indicating a large number of potential exposure routes to humans. The diet has been considered the major exposure pathway [28]. Several studies have identified fish and/or marine food as major contributors to increased blood levels of some PFCs [25, 29, 30]. However, Halldorsson et al. [31] identified red meat, snacks and animal fat as dietary predictors for PFOS and PFOA.

Male sex has been associated with increased plasma concentrations of PFCs [32, 33], although some contradictory studies suggest that gender has no effect [19]. The impact of age is so far conflicting. Some studies have reported increasing levels of PFC with age [25] (some for women only), whereas others found no effect of age [32, 34]or decreasing concentrations for some PFCs [33] with increasing age. Toms et al. [35] suggested recently that the concentrations of several PFCs are relatively constant from the age of 10, except for PFOS that increase with age.

The effect of parity has only been evaluated by a limited number of studies with contradictory findings [18, 36]. Nevertheless, several PFCs have been detected in breast milk [37, 38], suggesting that lactation reduce a woman's body burden of PFCs. The effect of BMI on blood concentrations of PFCs is so far not fully characterized.

1.4 Toxicological effects of PFCs

PFOS and PFOA have been characterized as potent peroxisome proliferators in rodents and chickens [39]. Gene expression analysis has shown that genes related to a large number of biological pathways, including the fatty acid metabolism [40, 41], cell communication [42], apoptosis [43] and hormone regulation [40, 41] were affected following PFOS and /or PFOA exposure among test animals. Increased liver weight and reduced serum cholesterol levels [44], changes in cell membrane fluidity [45], indicators of oxidative stress [46] and neurotoxic effects [47] have also been observed in *in vivo* studies following PFC exposure. Additionally, increased mortality among rat pups was reported when pregnant dams were exposed to PFOS [44].

While the effects of PFOS and PFOA have been extensively investigated, less focus have been on the other PFCs. Sprague Dawley rats showed reductions in serum cholesterol levels, decreased triglycerides and increased levels of albumin following exposure to PFHxS [48]. It has to be emphasized that these test animals were exposed to several times higher concentrations of the selected PFCs than concentrations found in the general population. Also the half-life of the different compounds varies between species and is much shorter in rats than in humans [44].

1.5 Human health effects of PFCs

A number of health effects among workers in fluoropolymer industries have been investigated in relation to place of work as an exposure marker. Alexander et al. [49] investigated the mortality in a cohort of 2083 workers that had worked at the fluoropolymer facility for at least one year. The overall mortality for the total cohort was lower than what was expected in the general population, however, there was an increased risk of dying from bladder cancer in the total cohort and all cases (n=3) occurred in the high exposure group. Whether there was a causal relationship between exposure to fluoropolymers and bladder cancer, or if the cases were attributable to other factors, was not elucidated. A later study investigated the association between bladder cancer and PFOS exposure in 1895 fluoropolymer workers and found no association [50]. The relationships between PFOS exposure and cancer of breast, prostate, colon and melanoma, non cancer- conditions such as liver diseases and birth weight have also been assessed among fluoropolymer workers (1137 males and 263 females) [51]. No association between any of these self-reported medical conditions and exposure was found. Lundin et al. [52] investigated recently the relationship between exposure to a precursor substance (ammonium perfluorooctanoate, APFO) to PFOA and mortality in 3993 occupationally exposed workers (divided into "non exposed", "probably exposed" and "definite exposed"). They found no association between liver, pancreatic and testicular cancer, liver cirrhosis and exposure to APFO. Risk of death from diabetes mellitus was elevated in the "probably" exposure group (standard mortality ratio, SMR 2.0 (1.2-3.2) compared to a standard population (no deaths from diabetes mellitus had

occurred in the "definite" exposure group so SRM was impossible to calculate for that group). Exposure to APFO was also associated with prostate cancer and cerebrovascular diseases when comparing within the study group, but not when comparing with an external reference population.

Less focus has been on the potential health effects of background exposure to PFCs among the general population. In 2009, Lin et al. [53] reported that serum PFCs were associated with glucose homeostasis and indicators of metabolic syndrome. More specifically, serum samples from 474 adolescents and 969 adults participating the National Health and Nutrition and Examination Survey (NHANES) in the U.S were analyzed for PFOS, PFHxS, PFOA and PFNA and assessed in relation to a number of indicators of metabolic syndrome. PFOS levels in adults were positively associated with increased blood insulin levels, homeostasis model of insulin resistance and β-cell function and increased risk of HDL cholesterol levels above the definition for metabolic syndrome. Increased PFNA levels were associated with lower risk of metabolic syndrome in adolescents and increased risk of elevated glucose levels and decreased HDL cholesterol. The results were somehow inconsistent, but the authors suggested that PFCs interfere with the glucose metabolism in the general population.

The relationship between PFOS and PFOA levels and pregnancy outcome and especially birth weight has been evaluated by several studies with contradictory results. Only one study found a significant association between increased PFOA levels and decreased birth weight [18, 54, 55].

1.5 Genes, proteins and gene expression analysis

Proteins are major components of the human body as they serve as building material for muscles and as enzymes to catalyze chemical reactions. They are further important for cell signaling and the immune system. Production of proteins occurs in the cell nucleus and in the cytoplasm with the help of the DNA. A sequence of the DNA is called a gene and each gene encode for a specific protein. During protein synthesis, the DNA strand is

transcribed into messenger RNA molecules (mRNA) that in turn are transported to the cytoplasm and translated into proteins in the ribosome with the help of tRNA and rRNA.

During blood withdrawal for gene expression analysis a special blood collection tube containing a buffer that preserves the total RNA in the blood is used. Total RNA is afterwards extracted and amplified before going through reverse transcription into complimentary DNA (cDNA) [56]. cDNA is further labeled with a fluorescent dye (Figure 5) and hybridized to a microarray platform where each spot displays gene specific probes [56]. Signal intensities are later monitored using a chemilumniscence detector. The stronger the intensity, the more expressed the gene. Thus, gene signatures in human blood represent the genes that were activated or deactivated at the time for blood withdrawal, i.e. what proteins were produced or not produced at that time.

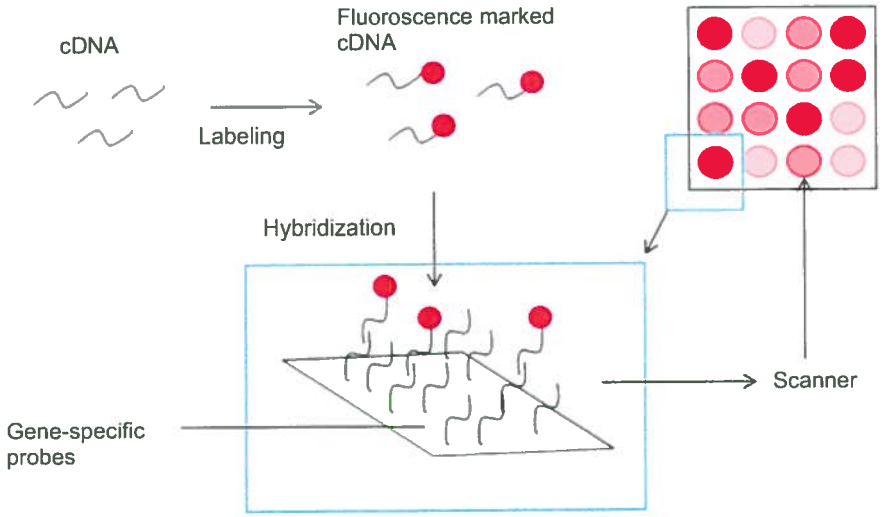

Figure 5. Labeling and hybridization of cDNA to a microarray platform. Copyright Wikimedia Common.

A number of parameters may alter the blood gene expression, including current health status, diet, age and medication use [57]. Technical variables such as batch number, amplification date and storage time can also influence gene signatures [58]. These variables have therefore to be taken into account and corrected for in the subsequent

statistical analysis. Dumeaux et al. [57] reported recently that smoking, medication use, hormone therapy use and body mass index were reflected in the blood gene expression among participants in the Norwegian Women and Cancer Study (NOWAC) although technical noise was present in the analyses. Gene signatures have large potential for evaluating the effects of environmental exposures on the general population and also for detecting early biomarkers of disease [59].Gene signatures have been used for evaluating the effects on human health of a number of environmental exposures, e.g., ionizing radiation [60], dioxin [61] and benzene exposure [62], smoking [63] and metal fumes [64].

Until now, no one has evaluated the effects of organic pollutants on blood gene signatures from the general population.

2. Objectives

The main objectives of this doctoral thesis were to assess concentrations, dietary impact and molecular signatures of PFCs in the general population in Norway. In order to in depth explore the effect of lactation, residency and age, PFC levels in delivering women from Vietnam were assessed. These women had considerably different group characteristics than those of the Norwegians.

Specific aims:

- Provide information about human plasma concentrations of the most common PFCs in Norway (I and II)
- Investigate the dietary impact on plasma concentrations of PFCs (I and II)
- Assess the impact of age, gender, parity, BMI and place of residence on plasma concentrations of PFCs (I, II and III)
- Evaluate plasma concentrations of selected PFCs in delivering women from Vietnam. (III)
- Investigate the PFOS isomer distribution in human blood samples in order to elucidate sources of exposure (I, II and III).
- Investigate blood gene expressions as a potential tool for assessing the effects of PFOS, PFOA and PFHxS exposure on the general population (IV)

3. Material and methods

3.1 Study populations

This thesis was based on samples from three different study populations.

The Andenes study (Paper I)

The Andenes study group consisted of 60 people (16 men and 44 women, aged 26 to 60) that were residents of the island Andøya in Northern Norway (69° north). This study was initiated in 2004 with the main objective to study UV-light and vitamin D status in a northern community. The participants were recruited by an advertisement in the local newspaper and inclusion criteria were age between 20 and 60 years and being a permanent resident on the island Andøya. Blood samples for Vitamin D analysis were collect each month during a year. The samples used for PFC analysis were drawn in August and September 2005.

The Norwegian women and cancer study (NOWAC) (Paper II &IV)

The Norwegian Women and Cancer study is a population based prospective study that was initiated in 1991 with the aim of investigating the etiology of cancer among Norwegian women [65]. NOWAC consists currently of more than 170 000 women (30-70 years) that have answered a detailed questionnaire regarding current health status, medication use and dietary intake. The participating women were randomly chosen from the central person registry in Norway and invited to participate in the survey through an invitational letter sent to their home address. Each 4-6 years the women are followed up through repetitive questionnaires. NOWAC receives regularly updates from the Norwegian Cancer Registry, making it possible to assess lifestyle-associated factors in relation to several cancer types among the participants. The external validity of the NOWAC study has been thoroughly validated [66].

The NOWAC postgenome cohort [67] consists of more than 50 000 participants (born between 1943 and 1957) from the original NOWAC study that in addition to answering a questionnaire, also have delivered a blood sample. Paper II and IV are based on randomly chosen blood samples from the NOWAC postgenome study (Figure 6).

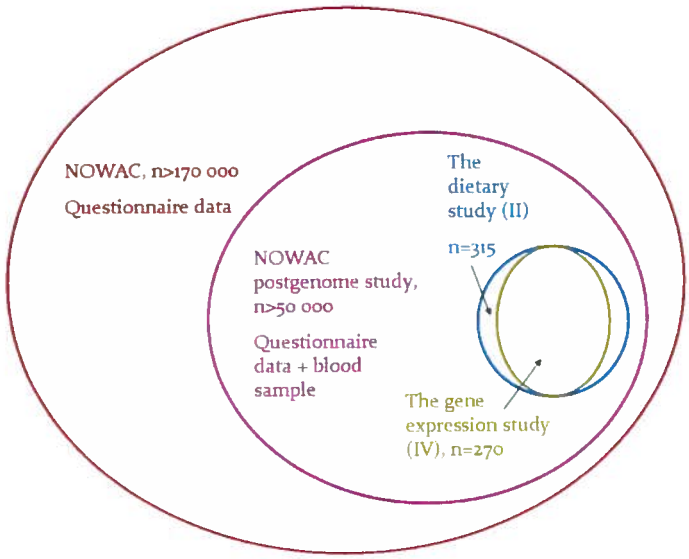

Figure 6. A description of the NOWAC cohort and the samples used in paper II and IV.

The Vietnamese study group (Paper III)

The Vietnamese study group consisted of 189 delivering women from the Khanh Hoa province in south central Vietnam. All participating women were pregnant and recruited from their local hospital at the time for delivery. Criteria for being included in the study were being a resident for at least five years in the coastal city Nha Trang or in the inland district Dien Khanh. A standard form, registering the women's name, weight, height and data regarding the pregnancy and delivery, was filled out by the medical personnel at the hospital. Additionally, all women answered a standard questionnaire regarding health status, diet and living conditions. The information from those questionnaires was, however, incomplete and only data on parity was used in paper III. Of the 91 women (18-40 years of age), randomly chosen for PFC analysis as budget limitations did not allow

for more samples, 37 were residents in Nha Trang, 36 were from Dien Khanh and 18 had an unknown home address.

3.2 Food frequency questionnaire (FFQ)

Paper I and II focus on the dietary impact on plasma levels of selected PFCs. All participants answered the NOWAC FFQ (Appendix I) where they were asked to record how often they consumed more than 90 different foodstuffs during the preceding year. The FFQ has special emphasis on fish consumption. Questions about portion size were also included and by using the Norwegian table for household measures and weights for foods [68] the amount consumed of each foodstuff per day was calculated. The NOWAC FFQ has been validated thoroughly by 24h recalls [69], a test-retest study [70] and against serum phospholipids levels as biomarkers for fish consumption [71].

3.3 Analysis of perfluorinated compounds

Plasma concentrations of PFCs were analyzed using a modified method by Powley et al. [72]. The preparation methods and instrumental settings used are described in detail in paper I and III. In short, sonication facilitated liquid-liquid extraction using methanol as a solvent was used for extraction of PFCs from the matrix. The extracts were cleaned-up using activated charcoal and the samples were analyzed on HPLC-QTOF-MS. Labelled internal standards were used during the analysis and reference samples were analyzed continuously to assure accurate analyses.

3.4 Analysis of fatty acids

Analysis of fatty acids was performed at the National Institute of Nutrition and Seafood Research (NIFES) in Bergen using a method described in detail elsewhere [73].

3.5 Gene expression analysis

Microarray analysis was performed using the Applied Biosystems expression array system. All methods for RNA extraction, data capturing and preprocessing of data is described in Paper IV and by Dumeaux et al. [57].

3.6 Statistical analyses

Statistical analyses were performed using the freely available software R version 2.8.1 (http://www.cran.r-project.org) in paper I-IV. Additionally, the multivariate data analysis software "the Unscrambler 7.0" (CAMO, Oslo, Norway) was used in paper II. Details regarding the statistical analyses are described in the respective papers.

4. Results- summary of papers

Paper I

Dietary predictors and plasma concentrations of perfluorinated compounds in a coastal population from northern Norway

The objectives of this study were to assess PFC concentrations in a small Norwegian coastal population (44 women and 16 men, 26-60 years) and evaluate the dietary impact on PFC levels. People from this area of Norway are known for having a high intake of marine food.

PFOS (median 29 ng/ml), PFOA (3.9 ng/ml), PFHxS (1.1 ng/ml), PFNA (0.81ng/ml) and PFHpS (0.46 ng/ml) were detected in more than 95% of all samples. Age, male sex, low intake of fruit and vegetables and a high intake of fatty fish were associated with increased concentrations of PFOS and PFHpS. The significant result for fatty fish intake was based on one person only. Although that person was not identified as an outlier, the results should be interpreted with care. Age and gender predicted the concentration of PFHxS while only gender influenced the concentration of PFOA. In the total study group, linear PFOS contributed with 69% of the total PFOS concentration. Men had significantly lower percentage of linear PFOS than women (67% vs. 69%).

Paper II

Dietary patterns and plasma concentrations of perfluorinated compounds in 315 Norwegian women: the NOWAC postgenome study

Paper II investigated the impact of a large number of dietary- and lifestyle-related variables on plasma concentrations of selected PFC in a large, representative group of 315 Norwegian middle-aged women. PFOS (median 20 ng/ml), PFOA (4.4 ng/ml), PFHxS (1.0 ng/ml) and PFNA (0.81 ng/ml) were detected in more than 90% of the samples. "Fish eaters" (women who consumed fish and shellfish) were identified as having the highest concentrations of PFOS, PFHxS and PFNA. Younger women with a

large household and with a "western" diet consisting of rice, pasta, water, white and red meat, pastries and chocolate had lower concentrations of the same compounds. The magnitude of the difference in PFOS concentrations between high and low consumers of marine food was however, marginal (21g/ml vs 19 ng/ml). No specific food cluster was associated with increased PFOA concentrations, although a large proportion of the variations in PFOS, PFOA, PFHxS and PFNA concentrations were explained by the dietary- and lifestyle-associated variables. This study confirmed that the diet is a major exposure route to PFCs for humans. It also emphasized that the identification of dietary predictors is highly dependent on the dietary habits within the population studied due to the ubiquitous presence of PFCs in all kinds of food. Linear PFOS constituted 70% of sum PFOS concentration in the current study group.

Paper III

Perfluorinated compounds in delivering women from south central Vietnam

This study was undertaken to provide more information about human exposure to PFCs in south east Asia and to explore the relationship between age, parity, BMI, place of residence and plasma concentrations of selected PFCs in a group of 91 delivering women (18-40 years).

PFOS, PFOA and PFHxS were detected in 98-100% of all samples. PFOS (median 3.2 ng/ml) was the most common compound followed by PFOA (1.6 ng/ml), PFHxS and PFNA (both 0.7ng/ml). There was a strong correlation between PFOS and PFHxS (r=0.94). Women residing in the coastal city Nha Trang had significantly higher concentrations of all investigated compounds than those of women from the inland district Dien Khanh. Even though the two study locations are situated only 10 km apart, the dietary habits were slightly different. The diet in Nha Trang is based on marine food whereas people in Dien Khanh consume more land-locked fish and produces more of their own food. Marine fish is also frequently consumed in Dien Khanh. Another potential difference between the two areas is the higher family income in Nha Trang (not confirmed) that may affect the quality of housing and the use of consumer products. Age,

parity and BMI had no impact on the concentrations of PFCs. The current study group had a median linear PFOS percentage of 83% related to sum PFOS.

Paper IV

Perfluorinated compounds and blood gene signatures in postmenopausal women: the NOWAC postgenome study

The impact of PFOS, PFOA and PFHxS on the blood gene expressions was assessed in a representative group of 270 healthy, postmenopausal Norwegian women (48-60 years). Forty-eight gene sets, curated from the literature, the Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) and previously linked to PFC exposure were explored in relation to the selected PFCs. Two gene sets, both related to the citric acid cycle were differentially expressed between the PFOS high (> 30 ng/ml, n=42) and the PFOS low group (<30 ng/ml, n=228). Eight of the nine core genes (genes that were most expressed) encoded central enzymes in the citric acid cycle. All except for one core gene were down-regulated following PFOS exposure. Our results suggest that PFOS interfere with the glucose metabolism in the general population, which is consistent with other studies. None of the investigated gene sets were significantly enriched in the PFOA or PFHxS groups. The results also advocate that blood gene signatures is a promising tool for investigating the impact of pollutants on human health.

5. Discussion

Over the last decade there has been a rapid development in analytical performance of PFC analyses. The limit of detection has decreased dramatically as a result of better instrumentation and improved sample preparation techniques. The availability of mass labeled and native standards has also increased during the last years, resulting in improved accuracy. Despite that, inter-laboratory calibration studies still indicate that the uncertainties of analyses both between and within a laboratory often vary +/- 20 % , with larger uncertainties among the less common PFCs [74, 75]. One major problem is that there are few laboratories that participate in these programs and the standard of comparison is therefore weak. It is, however, evident that the accuracy and reproducibility are improving, but there are likely large variations in analytical performance between the earliest and the latest publications. Additionally, a number of factors, including study group characteristics, choice of sample matrix and year of sampling influence the analytical results and complicate comparisons between studies.

5.1 Limitations and challenges of PFC analyses

5.1.1 Number of participants and statistical power

During the last 10 years there have been an increasing number of publications concerning human blood concentrations of PFCs. The majority of them focus on PFOS and PFOA and mainly come from industrialized countries. Geographical differences in human PFC concentrations has been observed, although the number of participants in the different studies is highly variable, e.g. Denmark n=1399 [18] and Belgium n=4 [19]. Thus, observed differences between countries could be a result of random variation rather than a real distinction. As only population based studies (DNBC, NOWAC, NHANES) are nationally representative, results from smaller studies, with a non-representative study group should be interpreted carefully. Large in-country differences have also been reported from China [76], Japan [77], Sri Lanka [78] and Germany [34, 79], indicating that the study group recruitment is essential for achieving reliable results for the PFC load within a country.

Often ignored is the statistical power of a test, which is the probability of rejecting a false null hypothesis [80]. For example, let us assume that the null hypothesis is "There is no difference in PFOS concentration between women from Norway and Sweden". If the statistical power is 80%, there is a 20% risk of concluding that there is *no* difference in PFOS concentration between women from Norway and Sweden when there *is* a difference. Power calculations could be used for calculating necessary sample size for detecting a difference of a certain size, when standard deviation and significance level is known. A power of 80% is acceptable with the significance level of 0.05. One of the main results in the Vietnamese study (III) was that there was a significant difference in PFC concentration between the two study locations. With that sample size (n=37 and 36) and the significance level of 0.05, the statistical power for detecting the observed differences in PFOS concentration was 60%. It has to be emphasized that a study group of 73 persons is larger than many other studies that has been published the last 10 years.

Investigations of environmental pollutants in human blood samples are often limited by costs, logistics (in some countries) and the availability of samples. The Vietnamese study was not only restricted by the budget but also by plasma volumes. Thus, it is not always possible to perform a study with large enough samples size to achieve 80% power. Yet, the statistical power of a study should be kept in mind when comparing differences in contaminant concentration between groups/studies.

5.1.2 Sample matrix

Currently, most studies analyze PFCs in serum or plasma. Choice of sample matrix, i.e. plasma, serum or full blood, may affect the results as the conversion factor between plasma/serum and full blood is not fully characterized. Ehresman et al. [81] found a median conversion factor from full blood to plasma/serum of approximately 2 for PFOS, PFOA and PFHxS, however, among the 18 individuals tested, the ratio varied between 1.7 and 3.7. Kärrman et al. [82] reported a conversion factor of 1.2 for PFOS based on five individuals, indicating that some PFCs are present in the red blood cells or that there are large individual variations in hematocrit. The advantages of analyzing PFCs in

plasma/serum are the reduced risk of matrix effects. However, when sample preparation techniques are improving, the risk of matrix effects becomes less important and full blood could therefore be the choice of preference. Some of the earliest investigations used full blood as sample matrix and comparisons between these studies and studies with plasma/serum as matrix may therefore be misleading as the conversion factor may vary between individuals. Considering this uncertainty and the lack of knowledge regarding the mechanisms for distribution, more effort should be spent on elucidating that relationship.

5.1.3 Year of sampling

The year of sampling is crucial as Haug et al. [21] reported a clear decreasing time trend in pooled Norwegian blood samples starting in year 2000, the same year as PFOS was phased out of production by 3M. Comparing samples collected before 2000 with samples collected after 2000 may therefore lead to biased conclusions if the year of sampling is not considered. All samples in the current thesis were collected in 2005.

5.1.4 Isomer specification

Many PFCs consists of branched and linear isomers. For PFOS specifically, the branched isomers constitute 20-50% of total PFOS concentrations. It is evident that the earliest studies reported the sum of the linear and the most common branched PFOS isomers. However, as a result of improved equipment and sample preparation techniques, it is now possible to separate the linear and the branched isomers of PFOS. Despite that, very few studies state whether they report the linear PFOS concentrations or the sum of all isomers. This may result in erroneous conclusions regarding the world-wide exposure to PFOS. There is also limited knowledge about how the isomer distribution changes with age, time and gender. Our results indicate a gender difference but the mechanisms behind such a difference are not understood.

5.2 Predictors of PFCs

5.2.1 Gender, parity and BMI

The effect of gender and parity was evaluated in Paper I, II and III. Only the Andenes study (I) consisted of both male and female participants. Males had higher concentrations of PFOS, PFHxS and PFHpS, whereas there was no difference in PFOA concentrations between genders. Some studies have reported gender-related differences for PFOS, PFOA and PFHxS [25, 32, 79] whereas others have not [19]. PFOS and PFOA have been detected in cord blood [83] and several PFCs have also been measured in human milk [37], thereby indicating a transfer of PFCs to the child. Kärrman et al. [37] suggested that infants are exposed to 200 ng of PFC per day through lactation. Nine month of nursing, will thus reduce a women's body burden of PFC with more than 50% assuming that a women had a total PFC concentration of 20 ng/ml. Tao et al. [38] detected 9 different PFCs in breast milk from the U.S with PFOS as the most common compounds followed by PFOA. The authors calculated an average daily intake of PFC of 23.5 ng/kg bw for infants. Thus, childbearing and lactation could explain the different body burdens of PFCs between men and women. Another gender specific aspect to consider is that a woman loose approximately 400 ml of blood each year through her menstrual cycle. This will result in an exchange of the body's total blood volume in 12 years and a possible dilution effect of PFCs. Harada et al. [84] suggested that the elimination rate of PFCs in females with active menstrual cycle is two times higher than in males. On the other hand, being a blood donor in Norway implies the donation of 1 L of blood each year which result in an exchange of total blood volume every 5 years. Blood donation could therefore confound the analysis even though it is not likely that the gender related effect is due to blood donation. Unfortunately, no information about blood donor practices among the study participants was available.

The NOWAC participants (II & IV) were born between 1943 and 1957 with a mean age of 56 years (born in 1949). Most of them had their children and nursed in the 1970's or in the beginning of 1980's, thus, before or during the start up phase of the extensive production of PFCs (Figure 2). No effect or parity or breastfeeding was observed in this

study group, nor was it expected as most of these women nursed before they were exposed. The younger Vietnamese study group (mean age 27 in 2005) was therefore more suitable for investigating the effect of lactation on PFC levels as they were potentially exposed to PFCs before childbearing. There was a very strong correlation (r=0.94) between parity and total months of nursing among the Vietnamese women [85], indicating that parity reflected total lactation. Due to sometimes insufficient lactation data, the effect of parity was evaluated. Despite that the Vietnamese women breastfeed their children for a considerably time period [85], no significant effect of parity on PFC blood levels was observed (Figure 7).

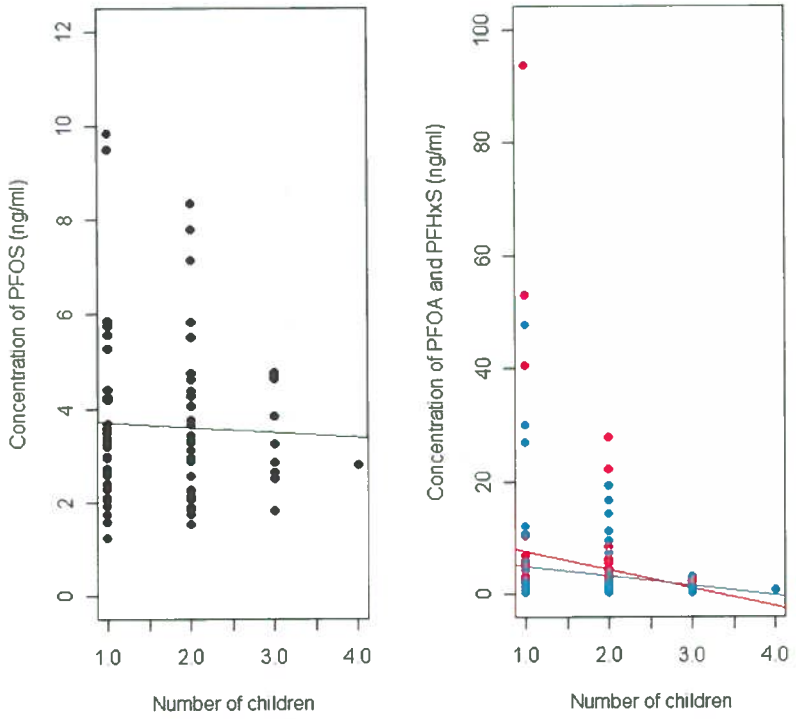

Figure 7. Concentration of PFOA (black), PFOA (red) and PFHxS (blue) versus parity in the Vietnamese study group.

Kärrman et al. [37] reported a strong correlation between the concentration of PFCs in matched serum and human milk samples and suggested that the concentration in milk was 1% of the woman's serum level. The blood concentrations of PFCs in the Vietnamese study group were 3-7 times lower than in those of the Norwegians. Low plasma concentrations of PFCs may result in low partitioning of these compounds into the breast milk and thus, low "body loss". The current production of PFOS in China could imply increased PFC exposure in south east Asia. Effects of lactation will then not be detected. Finally, only few women had more than two children. Small study groups reduce the chances of detecting an effect of lactation. Only a limited number of studies that have investigated the association between parity and blood PFC levels and the results are contradictory. Fei et al. [18] reported decreasing levels of PFOS and PFOA with increasing parity among 1400 Danish mothers [18], whereas Hanssen et al. [36] found no effect of parity in a group of delivering women from South Africa (n=71). However, the breastfeeding traditions are highly variably between countries, and parity does not always correlate to total time of nursing. To be able to address the effect of breastfeeding on PFC levels, good data on lifetime lactation is required as well as a large study group with many children. Time past since last childbearing is also crucial to consider.

BMI had no effect on PFC levels in any of the studies included in this thesis. This result on top of the limited number of studies investigating BMI makes it hard to conclude what possible effect BMI is likely to have. Fei et al. [18] observed higher concentrations of PFOS and PFOA in overweight and obese women than in those of normal or underweight women. BMI should therefore always be included as a covariate in the initial statistical analyses to avoid the risk of residual confounding.

5.2.2 Age

The magnitude of human PFC exposure has varied during the last 50 years as a result of changing production quantities. This aspect needs to be considered when evaluating the relationship between age and blood concentrations of PFCs. Before 1970, the production of PFCs was low. It increased rapidly after 1975 with the peak period during the 1990's,

followed by a sudden decrease in year 2000 [4] (Figure 2). As a consequence, people being born before 1970 have experienced the same lifetime exposure. The higher concentrations of PFOS, PFNA and PFHxS in older NOWAC women (II) were therefore explained by age dependent differences in dietary intake. Older women (>56 years, mean age) consumed more marine food than younger women (96g per day vs. 82 g each day, respectively) and "fish eaters" were identified as having higher concentrations of several PFCs in that same study group (II). Higher intake of fish among older women was also the case for the whole NOWAC cohort [86]. Thus, the observed age-related effect was confounded by dietary differences.

Within the Andenes study group (I), higher concentrations of PFOS, PFHxS and PFHpS was observed among females only (26-60 years, data not shown in I). This finding is supported by Fromme et al. [79] and Harada et al. [84] who also reported an increase in PFC levels with age among women only. Considering that there are strong evidences for lactation influencing a women's body burden of PFCs, time past since last childbearing is crucial to consider when evaluating the relationship between age and human PFC concentrations. Older women gave birth and nursed before the massive increase in PFC production started, whereas younger women were exposed to PFCs during their childhood and adolescence and reduced their body burden of PFCs through lactation. The observed age-related effects among females (I) were thus explained by time since last childbearing and/or age dependent differences in dietary habits.

Among the younger Vietnamese women an age related effect was expected as a result of time past since last childbearing. Nevertheless, there was no relationship between age and any of the investigated PFC, which could indicate increasing PFC exposure due to the current production and use in south east Asia

5.2.3 Geographical differences between and within countries

Geographical differences in human PFC concentrations have been observed worldwide. As discussed in Chapter 5.1, there are many factors that may affect the PFC results and

comparisons between studies should therefore be made with caution. The initial emphasis is therefore put on studies that can be considered as nationally representative. Nevertheless, due to the lack of such investigations, comparisons between countries had also to include smaller studies.

The NOWAC women (II) had comparable concentrations of PFOS (20 ng/ml vs 18.4 ng/ml), PFOA (4.4 ng/ml vs 3.5 ng/ml) and PFNA (0.81 ng/ml vs 0.9 ng/ml) to the female participants in the U.S NHANES study (n=1041, collected in 2003-2004) [32] but lower PFOS concentrations than in delivering women from Denmark (35 ng PFOS/ml and 5.6 ng PFOA/ml, n=1399, collected in 1996-2002) [18]. PFOA concentrations were more uniform between the three studies (5.6 ng/ml, DNBC). The female participants in the NHANES study were above 12 years old but there was no information about mean age, whereas the Danish women were considerable younger than the NOWAC participants (mean age 56 years).

The Vietnamese women (mean age 27 years) were also younger than the NOWAC participants and they had distinctly lower concentrations of PFOS (3.2 ng/ml) and PFOA (1.6 ng/ml), whereas the body burden of PFHxS and PFNA were more similar. There was a higher percentage of detection of the seven investigated PFCs in the Norwegian study group, indicating a more extensive exposure to PFCs in Norway than in Vietnam. Nevertheless, a large variation in PFOA (0.55-94 ng/ml) and PFHxS (0.19-69 ng/ml) concentration was observed in the Vietnamese study group, suggesting large in-country differences and/or exposure to point sources. For the NOWAC women, the concentrations were more uniform (PFOA <LOD-21 ng/ml and PFHxS <LOD-13 ng/ml). This could be a result of the homogeneity of the Norwegian study group.

Figure 8 provide the human blood concentrations of PFOS, PFOA, PFHxS and PFNA among women from different countries, including the three study groups from the current thesis. With few exceptions, lower concentrations of PFCs are reported from less developed countries (Figure 8). It has to be emphasized that there is a lack of data from the southern hemisphere with results from South Africa [36], Peru [87], Brazil [19] and

Australia [35] only. For better understanding of the human exposure to PFCs, this knowledge gap needs to be filled, especially in relation to the current production of PFOS in China.

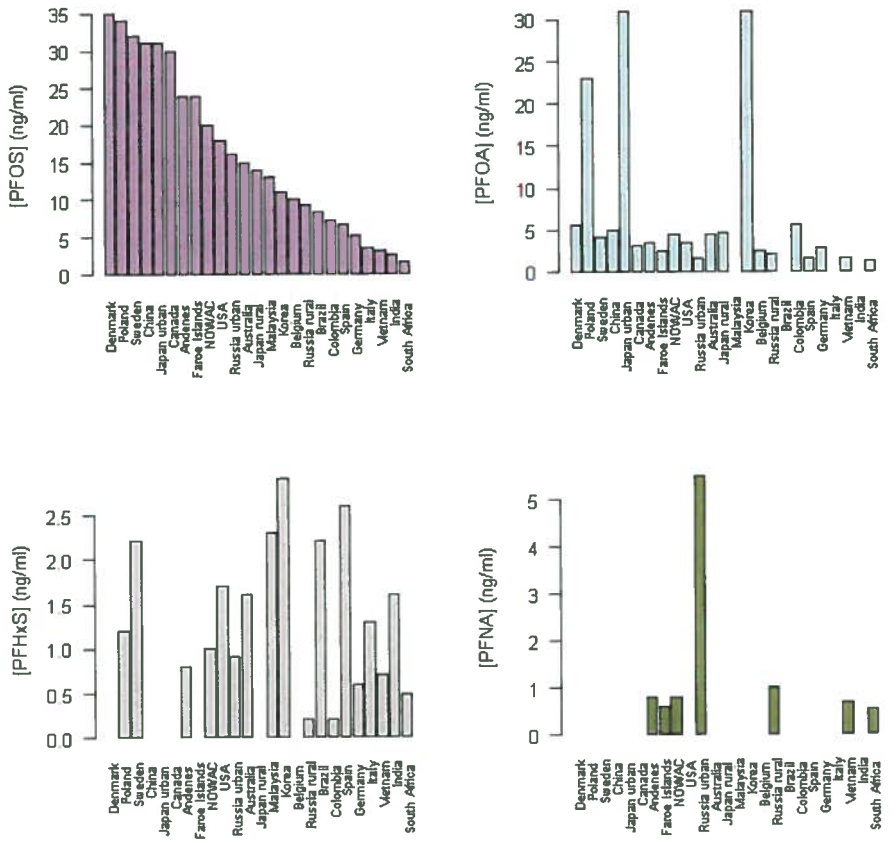

Figure 8. Plasma, serum or full blood levels of PFOS, PFOA, PFHxS and PFNA in women from different countries. Denmark (n=1400) [18], Poland (n=15) [19], Sweden (n=26) [82], Japan urban (n=20) [88], China (n=83) [89], Canada (n=21) [90], Andenes (n=44) (Paper II), NOWAC (n=326, paper III), Faroe Islands (n=12) [30], USA (n=1072) [32], Russia urban (n=12) [15], Australia (pooled samples) [35], Japan rural (n=20) [88], Malaysia (n=7) [19], Korea (n=25) [19], Belgium (n=4) [19], Russia rural (n=12) [15] Brazil (n=17) [19], Columbia (n=25) [19], Spain (n=24) [33], Germany (n=153) [25], Italy (n=8) [19], Vietnam (n=91, Paper I), India (n=11) [19], South Africa (n=71) [36]

It is important to note that the number of participants is highly variable (Figure 8). Due to possible inter-laboratory differences, it should be emphasized that the Norwegian (I and II) and Vietnamese samples (III) were prepared in the same laboratory and with the same methods as samples from South Africa and Russia (urban and rural, Figure 5). The Vietnamese and South African samples had considerable lower concentrations of all four investigated PFCs. The Russian samples are not further discussed as there were only 12 samples from each location and the chance of biased conclusions is therefore large.

Significant differences in PFC concentration between geographical regions were observed within Vietnam (III) and Norway (II). Women from northern Norway had significantly higher concentrations of PFOS than women from southern Norway ($p<0.05$, data not shown in II). There were also clear geographical variations in dietary habits within that same study group (Figure 9). Women from northern Norway were characterized by a high intake of fish and fish products, fish liver, rice-porridge (traditional Saturday dinner), potatoes, reindeer meat and bread, i.e. traditional Norwegian food. There were indications that the two study sites in Vietnam had slightly different dietary habits as well. In public health research, place of residence often reflects dietary habits and distinct geographical differences in PFC concentrations could reflect different dietary pattern.

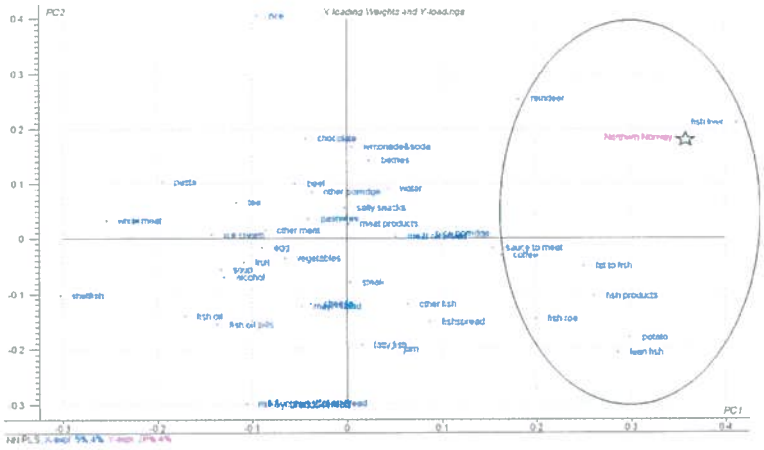

Figure 9. Geographical differences in dietary habits. Coming from Northern Norway is marked with a star.

The current three studies, in addition to previous investigations, indicate that the magnitude of PFC exposure varies between and within countries. It is likely that "high-consumer populations" historically has been more exposed to PFCs as these chemicals have been used in many consumer products, such as packaging material, carpets, other furniture and cooking equipment etc. Food originating from industrialized countries could also contain higher PFC loads.

5.2.4 Diet

Paper I and II investigated the dietary impact on plasma concentrations of selected PFCs. Two different statistical approaches were used. In both studies intake of fish and marine food was significantly associated with increased concentrations of PFOS (I &II), PFHpS (I), PFHxS (II) and PFNA (II), although the result in paper I was somehow inconsistent. There was no significant association between plasma levels of omega 3 fatty acids (as a biomarker for fatty fish intake) and PFC concentrations in paper II, suggesting that total marine food, not only fatty fish intake were important for the body burden of the selected PFCs. Younger women with a "western" diet consisting of rice and pasta, red and white

meat and desserts had lower concentrations of PFOS, PFHxS and PFNA (II). Paper II focused on food clusters, not single foodstuffs.

Within the Danish National Birth Cohort (DNBC) red meat, animal fat and snacks were identified as dietary predictors of PFOS and PFOA in a large group of pregnant women (N=1076) [31]. Considering the size and representativeness of that study group it has to be considered as the most comprehensive dietary investigation in relation to PFC levels so far. The Danish women were younger and consumed more meat (69g red meat/day, not further defined, vs. 42g of beef and processed meat products/day in NOWAC) than the NOWAC women. Additionally, only 19% of the Danish women consumed >30g marine food/day whereas 33% of the NOWAC women ingested >100g/day. The Norwegian women could therefore be characterized as high consumers of marine food compared to the Danish. These facts suggest that the identification of dietary predictors is highly dependent on the dietary habits due to the ubiquitous presence of PFC in food and especially in animal products. Consequently, fish will be identified as a dietary source of PFCs in a fish eating population whereas meat will become significant in a group of high-consumers of meat. This idea is supported by Ostertag et al. [91] who investigated the dietary impact of PFC among Canadian Inuit, based on food sample analyses and interviews. The authors found that reindeer meat and arctic char were important sources of PFCs for Inuit. Nevertheless, the reindeer meat had comparable concentrations of PFCs to store-bought meat. Also cookies and processed cheese were identified as possible sources, indicating that intake amounts affect the identification of dietary predictors.

Fish/seafood has previously been identified as an important source of PFOS and/or other PFCs in some other study groups [25, 29, 30], although none of them took the total diet into account. Hølzer et al. [25] focused on intake of fish (yes/no), whereas Weihe et al. [30] investigated the relationship between intake of pilot whale dinners and plasma concentrations of selected PFCs. Falandyz et al. [29] compared PFC levels in a small group of self-declared high consumers of fish from the Baltic Sea to a reference group with unknown dietary habits. These studies may to a large extent be subjected to

confounding as they have not controlled for other co-varying dietary factors. Furthermore, there are many advantages in investigating dietary impact on PFC concentrations in a population based study like NOWAC, as the results are nationally representative.

A number of studies have also investigated the concentrations of PFCs in different food stuffs [22-24]. The results show no clear trend although animal products seem to contain higher levels of PFOS than vegetarian products. Mainly PFOS was detected in the different food items. Analysis of food samples and subsequent calculations of daily intake of PFCs indicate the magnitude of exposure. Nevertheless it is important to compare the actual blood concentrations of PFCs in high and low consumers of that specific foodstuff in order to evaluate the effect on a person's body burden. To exemplify, when comparing the actual plasma levels of PFOS in high and low consumers of marine food among the NOWAC women, the differences were marginal (21 ng/ml PFOS in high consumers vs. 19 ng/ml in low consumers).

No specific food groups or food clusters were identified as being highly correlated to PFOA concentrations (I & II). Despite that, a large proportion of variation (21%) in PFOA concentration was explained by the investigated variables (II), indicating that these dietary and lifestyle factors were important for PFOA levels. In line with these findings, Vestergren et al. [92] suggested that the diet is the major human exposure route for PFOA. However, PFNA is often more common than PFOA in environmental samples as a result of different bioaccumulation properties [14]. The opposite trend is observed in human blood, indicating that other sources than the diet may be of great importance for human PFOA levels. In addition, no age-related effect was observed for PFOA (I & II), illustrating that the age-dependent dietary differences did not affect PFOA concentrations as was the case for PFOS, PFHxS and PFNA (II). With few exceptions, human PFOA concentrations are uniform between countries (Figure 8), which also has been indicated by others [92]. Vestergren et al. suggested that humans have been exposed to an unknown historical source of PFOA. This hypothesis could explain the narrow concentration range of human PFOA levels worldwide.

As the diet and especially marine food has been identified as a major source of some PFCs for Norwegians, the question remains if this is something to worry about. There are many well-documented health benefits from a fish-rich diet e.g. stable vitamin D status [93] and reduced risk of cardiovascular diseases and incident heart failure [94, 95]. Considering that cardiovascular diseases are a common cause of death in Norway, the general population should be more concerned about a "no fish diet" rather than the marginal increased PFC concentrations a fish-rich diet could imply, especially since the levels of many PFCs are currently decreasing in nature.

5.3 PFOS isomer distribution in human plasma

Technical mixtures of POSF contain 67% to 79% of the linear isomers [96]. In the current project, isomers specification was only performed for PFOS, where the linear isomer clearly dominated. The branched PFOS isomers were not structurally elucidated, but identified as a peak of co-eluted compounds with shorter retention time than the linear isomer (Figure 10). However, Kärrman et al. [97] have suggested that the mono-methylated PFOS isomers are most common in human blood samples. In the following discussion, "% linear PFOS" refers to the proportion linear PFOS in relation to the sum of the linear and branched isomers.

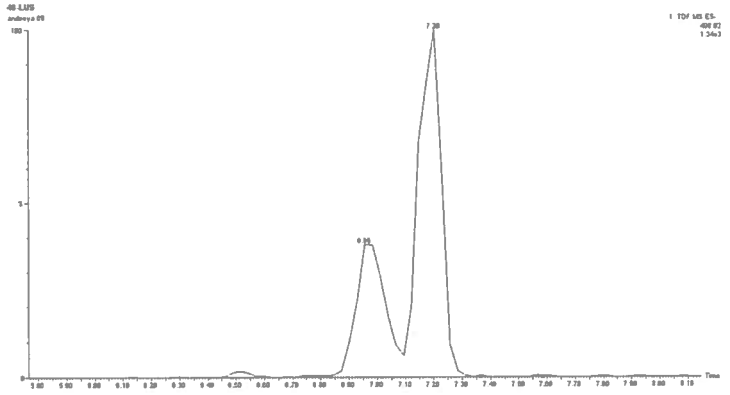

Figure 10. Chromatogram of branched (RT = 6.96) and linear PFOS (RT = 7.20) in a human plasma sample.

Linear PFOS constituted 83% of total PFOS concentration in the Vietnamese study group (III), whereas the median percentage was 69% for the total Andenes study group (I). There was a significant but small difference between genders, with men having 67% linear PFOS and women 69%. PFOS isomer distribution in breast milk has not been evaluated but selective excretion of the different isomers could result in different isomer distribution between genders. The NOWAC women (II) had a median percentage of 70% linear PFOS, thus, the Norwegian samples were comparable but they were distinctly different from the Vietnamese samples. In addition to different living standards and dietary habits, the Norwegian women were older than the Vietnamese. There was no significant association between age and percentage linear PFOS within each of the study groups.

Kärrman et al. [97] reported 68% linear PFOS in Swedish blood samples, thus comparable to the results in paper I and II. Samples from the UK and Australia both contained 59% linear PFOS [97] whereas 58% linear PFOS was reported in blood samples from delivering women in South Africa [36]. Haug et al. [21] observed a

decreasing trend of linear PFOS over time in Norwegian samples. In 1976, 68% was found whereas 64% was observed in 1987, 60% in 1998 and 57% in samples from 2007. The authors suggested that differences could be a result of various half-lifes of the branched and linear isomers, i.e. that the branched isomers are more persistent than the linear. Nevertheless, this hypothesis does not explain why the isomer composition differs between countries. In addition, it is not consistent with the findings of Benskin et al. who reported that all mono-methylated branched PFOS isomers, except for 1-perfluoromethyl-PFOS, were less persistent than linear PFOS in rats [98].

Powley et al. [14] suggested recently that the bioaccumulation potentials or elimination rates of PFOS isomers are species-specific and that the percentage linear PFOS does not reflect the concentration of these isomers in the lower trophic level. Differences in bioaccumulation potential/elimination rates could result in different isomers composition of food that, in turn, would affect the exposure to humans. If the branched PFOS isomers are more persistent in animal products than in vegetables, various dietary habits could explain the observed geographical differences. Swedish and Norwegian people have similar dietary habits and similar percentage of linear PFOS. As Scandinavians in general have similar living conditions, differences in PFOS isomer patterns could also reflect other exposure routes, such as use of packaging material containing precursor substances. The current EU-project PERFOOD which aims to investigate PFCs in different kinds of food will hopefully fill several knowledge gaps regarding PFOS isomer distribution in foodstuffs.

To conclude, it is evident that the linear percentage of PFOS varies worldwide. Whether this is a result of dietary differences, time past since exposure or different exposure routes are yet to be explained. With decreasing global production of PFOS, isomers patterns could be useful for identifying possible point sources. Before that is possible it is necessary with more background data for the population in general. Isomer patterns of PFOA and PFNA in human blood samples could be useful for identifying sources of human exposure as these compounds both have been produced by ECF and act as

degradation endpoints of FTOH that are produced through telomerization. The latter process yields only straight chain isomers.

5.4 Molecular effects of PFCs and implications for public health

Potential health effects of PFCs have been thoroughly evaluated using *in vitro* experiments or animals model studies. Several epidemiological investigations on health effects among highly exposed workers in fluoropolymer industries have also been performed [49-52], but only a limited number of studies have looked into the potential health effects of PFCs among the general population. The effect of low-dose long term exposure to pollutants is often hard to investigate as the mechanism of action in humans is not characterized. The time between exposure and outcome are often long and the potential outcomes may have large normal variability (e.g. birth weight) or being a complex diseases (e.g. cancer), making the causal relationship hard to evaluate. Thus, there is a need for sensitive methods to investigate the effects of background concentrations of pollutants on human health. Peripheral blood gene signatures have large potential for detecting early effects of contaminants and for elucidating which biological pathways that are being affected by pollutants. By using gene expression analyses it is possible to detect a unique set of genes, differentially expressed by the specific pollutant or mixtures of contaminants. Before that is possible, large amounts of data of gene signatures among healthy individuals is needed in order to explore normal variability. In the new research project "Transcriptomics In Cancer Epidemiology " (TICE), several thousands of blood gene expression profiles from healthy NOWAC women are being produced. Blood samples from the same individuals are available for further studies, opening up new research avenues for studying the effects of pollutants and other environmental exposures.

In paper IV, blood gene signatures were used for evaluating the effect of background concentrations of PFCs by comparing gene profiles between participants with "high" and "low" plasma concentrations of PFOS, PFOA and PFHxS respectively. The study group was identical to that of paper II (Figure6), however, 45 women were excluded from the

original group of 315 due to insufficient RNA quality, purity or integrity, signal to noise values, use of diabetes medicine or menopausal status. Therefore, the final study group consisted of 270 healthy, postmenopausal Norwegian women (48 to 62 years of age). The results from paper IV advocate for gene expression analyses having large potential for elucidating which biological pathways that are being affected by environmental pollutants. Technical noise was present and corrected for in that study as described in detail by Dumeaux et al. [57]. It is evident that the microarray platforms currently go through rapid development. Future platforms will be less sensitive to technical variables which in turn will ascertain improved gene expression results and wider applications for gene signatures.

Despite that technical variables influenced the gene expression result (IV), genes encoding for enzymes within the citric acid cycle were identified as being differentially expressed between women with "high" PFOS concentrations (>30 ng/ml) and women with "low" PFOS concentrations (<30 ng/ml). The result indicates that the glucose metabolism is affected by background concentrations of PFOS among the general population. Many other factors, for example the diet, affect the glucose metabolism within the human body. The "high" PFOS group was significantly older and had lower ratio of n6/n3 fatty acids than the "low" group. The lower n6/n3 fatty acid ratio indicates dietary differences and a possible higher consumption of fatty fish in the "high" PFOS group, which is in line with the findings in paper II. There was no difference in smoking or fasting status or BMI between the "high and "low" PFOS groups. When adjusting the analyses for age and fatty acid ratio, the citric acid cycle remained differentially expressed between the groups. Eight of the nine most expressed genes within the citric acid cycle gene set were down-regulated in the "high" group, whereas one gene was up-regulated. The effect of the direction of the expressed genes has to be elucidated in future research.

Lin et al. [53] reported recently that PFOS and PFNA were associated with indicators of metabolic syndrome in a group of adults from the general population, which support our finding. A number of diseases, e.g. diabetes II and Alzheimer's disease [99] have been

linked to citric acid cycle disorders, emphasizing the need for better understanding of the mechanism of action for PFOS. Additionally, increased mortality of diabetes was observed among workers "probably" exposed to APFO (precursor to PFOA) although that study was not consistent as there was no death of diabetes in the "definite" exposure group [52]. Four women were excluded from the analysis in IV as they were using diabetes II medicine. The median PFOS concentration among those women was 24 ng/ml (mean 20 ng/ml) whereas the corresponding median concentration was 19 ng/ml (mean 21 ng/ml) for the total study group (n=270). Although the diabetes group was too small for statistical purposes, the potential difference in PFOS concentration between diabetes patients and healthy individuals should be further assessed. This could easily be performed using a case control study design. Important to keep in mind is that a case control study only estimates the strength of association between exposure and outcome and does not tell anything about the causal relationship between exposure and disease. The mechanism of action for PFOS on the citric acid cycle should therefore be thoroughly investigated. Results from paper IV should be confirmed by polymerase chain reaction (PCR) and also repeated with improved microarray techniques.

The current study (IV) is the first investigating the effects of PFCs on blood gene signatures in the general population. Although PFCs were present in background concentrations representing Norwegian postmenopausal women, effects indicated by previous research were mirrored in the blood of these healthy participants. Currently, it is too early to conclude what consequences changes in enzyme levels within the citric acid cycle will have on public health. None of the epidemiological studies on occupationally exposed workers has concluded with any severe health effects of PFCs. Nevertheless, there has been an enormous increase in diabetes II incidence during the same periods as high PFOS exposure has been present. The current result indicates a possible link between PFOS exposure and metabolic syndrome/diabetes II which should be further investigated.

6. Conclusions

The blood concentrations of PFCs from the general Norwegian population are comparable to those of other industrialized countries. As the human exposure to PFCs has varied considerably over the last 50 years, birth cohort effects are observed that easily could be interpreted incorrectly as a continuous increase in PFC concentrations with age.

The overall diet is a major contributor to human PFC levels. Yet, dietary predictors vary between populations as a result of different dietary habits and the fact that PFCs are present in many kinds of food.

Gene signatures of peripheral blood cells are applicable in the search for biological pathways affected by environmental contaminants and for detecting early effects of pollutants on human health. Gene expression analysis indicated that PFOS interfere with the glucose metabolism in the general population. Thus, future research on the possible link between PFOS exposure and metabolic disorders is encouraged.

7. Future perspectives

- Differences between plasma, serum and full blood levels of PFCs should be further investigated to assess the distribution of PFCs in blood.

- The isomer distribution of PFCs in different environmental matrices (food, blood, breast milk etc) seems promising for gaining insight into human exposure routes, potential sources and isomer specific bioaccumulation/elimination of these compounds.

- There is a lack of knowledge regarding human PFC levels in the southern hemisphere and in developing countries.

- Non-dietary exposure routes for PFCs should be investigated, especially for PFOA.

- In order to assess the dietary importance for human PFC concentrations, focus on dietary patterns and intake amounts are necessary rather than single food items.

- A study on the effect of PFCs on blood gene signatures should be repeated as the microarray techniques have improved during the last years.

- The potential relationship between PFOS and metabolic disorders should be further investigated.

- Future monitoring of fluorinated compounds in human blood should also include "newer" compounds such as the polyfluoroalkyl phosphate surfactants (PAPS).

8. References

1. Prevedouros K, Cousins IT, Buck RC, Korzeniowski SH. Sources, fate and transport of perfluorocarboxylates. Environ Sci Techn 2006; 40: 32-44.

2. OECD. Results of survey on production and use of PFOS, PFAS and PFOA related substances and products/mixtures containing these substances. Report no ENV/JM/MONO(2005)1 2005.

3. 3M. The Science of organic fluorochemistry. 3M Company. 1999. Ducument no.AR226-0547.

4. Paul AG, Jones KC, Sweetman AJ. A First Global Production, Emission, And Environmental Inventory For Perfluorooctane Sulfonate. Environ. Sci. Techn 2009; 43: 386-92.

5. OECD. Cooperation on existing chamicals. Hazard Assesment of Perfluorooctane sulfonate (PFOS) and its salts. 2002. Report no ENV/JM/RD(2002)17/FINAL

6. AMAP. Arctic Pollution 2009. Oslo: Arctic Monitoring and Assessment Programme; 2009.

7. Herzke D, Schlabach M, Mariussen E, Uggerud H, Heimstad E. A literature survey on selected chemical compounds. 2007. Report no TA-2238/2007. Available from: http://www.klif.no/publikasjoner/2238/ta2238.pdf Accessed 18 March 2010

8. Climate and Pollution Agency. Kartlegging av PFOS i brannskum, [Mapping of PFOS in fire fighting foam]. 2005.Report no TA-2139/2005 Available from: http://www.klif.no/publikasjoner/kjemikalier/2139/ta2139.pdf Accessed 18 March 2010

9. Ellis DA, Martin JW, De Silva AO, Mabury SA, Hurley MD, Sulbaek Andersen MP, et al. Degradation of Fluorotelomer Alcohols: A Likely Atmospheric Source of Perfluorinated Carboxylic Acids. Environ Sci Techn 2004; 38: 3316-21.

10. Climate and Pollution Agency. PFOA in Norway. 2007. Report no. TA-2354/2007. Available from: http://www.klif.no/publikasjoner/2354/ta2354.pdf Accessed 18 March 2010

11. DuPont. DuPont Progress Report on PFOA Phase Out. 2008. Available from: http://www2.dupont.com/PFOA2/en_US/assets/downloads/pdf/PFOAProgressReport20081212.pdf Accessed 18 March 2010

12. Stockholm Convention on POPs. The 9 new POPs under the Stockholm Convention. 2009. Available from: http://chm.pops.int/Programmes/NewPOPs/The9newPOPs/tabid/672/language/en-US/Default.aspx Accessed 18 March 2010.

13. Haukas M, Berger U, Hop H, Gulliksen B, Gabrielsen GW. Bioaccumulation of per- and polyfluorinated alkyl substances (PFAS) in selected species from the Barents Sea food web. Environ Pollut 2007; 148: 360-71.

14. Powley CR, George SW, Russell MH, Hoke RA, Buck RC. Polyfluorinated chemicals in a spatially and temporally integrated food web in the Western Arctic. Chemosphere. 2008; 70: 664-72.

15. Climate and Pollution Agency. Kartlegging av "nye" miljøgifter i humane blodprøver fra Nord-Norge, Nord-Vest Russland og Sibir, [Mapping of "new" environmental pollutants in human blood samples from northern Norway, north west Russia and Siberia.]. 2006. Report no. TA-2184/2006. Available from: http://www.klif.no/publikasjoner/overvaking/2184/ta2184.pdf Accessed 18 March 2010

16. Wu LL, Gao HW, Gao NY, Chen FF, Chen L. Interaction of perfluorooctanoic acid with human serum albumin. BMC Struct Biol 2009; 9: 31. doi: 10.1186/1472-6807-9-31.

17. Zhang X, Chen L, Fei XC, Ma YS, Gao HW. Binding of PFOS to serum albumin and DNA: insight into the molecular toxicity of perfluorochemicals. BMC Mol Biol 2009;10: 16. doi: 10.1186/1471-2199-10-16

18. Fei CY, McLaughlin JK, Tarone RE, Olsen J. Perfluorinated chemicals and fetal growth: A study within the Danish National Birth Cohort. Environ Health Perspect 2007; 115: 1677-82.

19. Kannan K, Corsolini S, Falandysz J, Fillmann G, Kumar KS, Loganathan BG, et al. Perfluorooctanesulfonate and related fluorochemicals in human blood from several countries. Environ Sci Techn 2004; 38: 4489-95.

20. Olsen GW, Mair DC, Church TR, Ellefson ME, Reagen WK, Boyd TM, et al. Decline in perfluorooctanesulfonate and other polyfluoroalkyl chemicals in American Red Cross adult blood donors, 2000-2006. Environ Sci Techn 2008; 42: 4989-95.

21. Haug LS, Thomsen C, Becher G. Time Trends and the influences of age and gender on Serum Concentrations of Perfluorinated compounds in archieved human samples. Environ Sci Technol 2009; 43: 2131-36

22. Tittlemier SA, Pepper K, Seymour C, Moisey J, Bronson R, Cao XL, et al. Dietary exposure of Canadians to perfluorinated carboxylates and perfluorooctane sulfonate via consumption of meat, fish, fast foods, and food items prepared in their packaging. J Agric Food Chem 2007; 55: 3203-10.

23. Ericson I, Marti-Cid R, Nadal M, Van Bavel B, Lindstrom G, Domingo JL. Human exposure to perfluorinated chemicals through the diet: Intake of perfluorinated compounds in foods from the Catalan (Spain) Market. J Agric Food Chem 2008; 56: 1787-94.

24. U.K. Food Standards Agency. Fluorinated Chemicals: UK dietary intakes. Food Survey Information Sheet 11/06. 2006. Available from: http://www.food.gov.uk/multimedia/pdfs/fsis1106.pdf Accessed 18 March 2010

25. Hölzer J, Midasch O, Rauchfuss K, Kraft M, Reupert R, Angerer J., et al. Biomonitoring of perfluorinated compounds in children and adults exposed to perfluorooctanoate-contaminated drinking water. Environ Health Perspect 2008;116: 651-57.

26. Kato K, Calafat AM, Needham LL. Polyfluoroalkyl chemicals in house dust. Environ. Research 2009; 109: 518-23.

27. Kannan K Perfluorinated compounds: From frying pans to polar bears. Reprod Toxicol 2009; 27: 413-413.

28. Fromme H, Tittlemier SA, Völkel W, Wilhelm M, Twardella D. Perfluorinated compounds - Exposure assessment for the general population in western countries. Int J Hyg Environ Health 2009; 212: 239-70.

29. Falandysz J, Taniyasu S, Gulkowska A, Yamashita N, Schulte-Oehlmann U. Is fish a major source of fluorinated surfactants and repellents in humans living on the Baltic coast? Environ Sci Techn 2006; 40: 748-51.

30. Weihe P, Kato K, Calafat AM, Nielsen F, Wanigatunga AA, Needham LL, et al. Serum concentrations of polyfluoroalkyl compounds in Faroese whale meat consumers. Environ Sci Techn 2008; 42: 6291-95.

31. Halldorsson TI, Fei CY, Olsen J, Lipworth L, McLaughlin JK, Olsen SF. Dietary Predictors of Perfluorinated Chemicals: A Study from the Danish National Birth Cohort. Environ Sci Techn 2008; 42: 8971-77.

32. Calafat AM, Wong LY, Kuklenyik Z, Reidy JA, Needham LL. Polyfluoroalkyl chemicals in the US population: Data from the National Health and Nutrition Examination Survey (NHANES) 2003-2004 and comparisons with NHANES 1999-2000. Environ Health Perspect 2007; 115: 1596-1602.

33. Ericson I, Gomez M, Nadal M, van Bavel B, Lindstrom G, Domingo JL. Perfluorinated chemicals in blood of residents in Catalonia (Spain) in relation to age and gender: A pilot study. Environ Int 2007; 33: 616-23.

34. Midasch O, Schettgen T, Angerer J. Pilot study on the perfluorooctanesulfonate and perfluorooctanoate exposure of the German general population. Int J Hyg Environ Health 2006; 209: 489-96.

35. Toms LML, Calafat AM, Kato K, Thompson J, Harden F, Hobson P, et al. Polyfluoroalkyl Chemicals in Pooled Blood Serum from Infants, Children, and Adults in Australia. Environ Sci Techn 2009; 43: 4194-99.

36. Hanssen L, Röllin H, Odland JO, Moe MK, Sandanger TM. Perfluorinated compounds in maternal serum and cord blood from selected areas of South Africa: results of a pilot study. *In Press*. J Environ Monit 2010.

37. Kärrman A, Ericson I, van Bavel B, Darnerud PO, Aune M, Glynn A. et al. Exposure of perfluorinated chemicals through lactation: Levels of matched human milk and serum and a temporal trend, 1996-2004, in Sweden. Environ Health Perspect 2007; 115: 226-30.

38. Tao L, Kannan K, Wong CM, Arcaro KF, Butenhoff JL. Perfluorinated compounds in human milk from Massachusetts, USA. Environ Sci Techn 2008; 42: 3096-3101.

39. Wolf CJ, Takacs ML, Schmid JE, Lau C, Abbott BD. Activation of mouse and human peroxisome proliferator-activated receptor alpha by perfluoroalkyl acids of different functional groups and chain lengths. Toxicol Sci 2008; 106: 162-71.

40. Guruge KS, Yeung LWY, Yamanaka N, Miyazaki S, Lam PKS, Giesy JP, et al. Gene expression profiles in rat liver treated with perfluorooctanoic acid (PFOA). Toxicol Sci 2006; 89: 93-107.

41. Hu W, Jones PD, Celius T, Giesy JP. Identification of genes responsive to PFOS using gene expression profiling. Environ Toxicol Pharmacol 2005;19: 57-70.

42. Yeung LWY, Guruge KS, Yamanaka N, Miyazaki S, Lam PKS. Differential expression of chicken hepatic genes responsive to PFOA and PFOS. Toxicology 2007; 237: 111-25.

43. Shi X, Du Y, Lam PKS, Wu RSS, Zhou B. Developmental toxicity and alteration of gene expression in zebrafish embryos exposed to PFOS. Toxicol App Pharmacol 2008; 230: 23-32.

44. Lau C, Butenhoff JL, Rogers JM. The developmental toxicity of perfluoroalkyl acids and their derivatives. Toxicol App Pharmacol 2004; 198: 231-41.

45. Hu WY, Jones PD, DeCoen W, King L, Fraker P, Newsted J, et al. Alterations in cell membrane properties caused by perfluorinated compounds. Comp Biochem Physiol C Toxicol Pharmacol 2003; 135: 77-88.

46. Liu C, Yu K, Shi X, Wang J, Lam PKS, Wu RSS, et al. Induction of oxidative stress and apoptosis by PFOS and PFOA in primary cultured hepatocytes of freshwater tilapia (Oreochromis niloticus). Aquat Toxicol 2007; 82: 135-43.

47. Sato I, Kawamoto K, Nishikawa Y, Tsuda S, Yoshida M, Yaegashi K, et al. Neurotoxicity of perfluorooctane sulfonate (PFOS) in rats and mice after single oral exposure. J. Toxicol Sci 2009; 34: 569-74.

48. Butenhoff JL, Chang S-C, Ehresman DJ, York RG. Evaluation of potential reproductive and developmental toxicity of potassium perfluorohexanesulfonate in Sprague Dawley rats. Reprod Toxicol 2009; 27: 331-47

49. Alexander BH, Olsen GW, Burris JM, Mandel JH, Mandel JS. Mortality of employees of a perfluorooctanesulphonyl fluoride manufacturing facility. Occup. Environ Med 2003; 60: 722-29.

50. Alexander BH, Olsen GW. Bladder Cancer in Perfluorooctanesulfonyl Fluoride Manufacturing Workers. Ann Epidemiol 2007; 17: 471-78.

51. Grice MM, Alexander BH, Hoffbeck R, Kampa DM. Self-reported medical conditions in perfluorooctanesulfonyl fluoride manufacturing workers. J Occup Environ Med 2007; 49: 722-29.

52. Lundin JI, Alexander BH, Olsen GW, Church TR. Ammonium Perfluorooctanoate Production and Occupational Mortality. Epidemiology 2009; 20: 921-28.

53. Lin CY, Chen PC, Lin YC, Lin LY. Association Among Serum Perfluoroalkyl Chemicals, Glucose Homeostasis, and Metabolic Syndrome in Adolescents and Adults. Diabetes Care 2009; 32: 702-7.

54. Inoue K, Okada F, Ito R, Kato S, Sasaki S, Nakajima S, et al. Perfluorooctane sulfonate (PFOS) and related perfluorinated compounds in human maternal and cord blood samples: Assessment of PFOS exposure in a susceptible population during pregnancy. Environ Health Perspect 2004;112: 1204-7.

55. Apelberg BJ, Witter FR, Herbstman JB, Calafat AM, Halden RU, Needham LL, et al. Cord serum concentrations of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in relation to weight and size at birth. Environ Health Perspect 2007; 115: 1670-76.

56. Applied Biosystems. Applied Biosystems 1700 Chemiluminescent Microarray Analyzer: Chemistry Guide. 2004.

57. Dumeaux V, Olsen, KS, Nuel G, Paulssen RH, Børresen-Dale A-L, Lund E. Deciphering normal blood gene expression - The NOWAC postgenome study. PLoS Genet 2009; 6: e1000873. doi:10.1371/journal.pgen.1000873

58. Tanner MA, Berk LS, Felten DL, Blidy AD, Bit SL, Ruff DW. Substantial changes in gene expression level due to the storage temperature and storage duration of human whole blood. Clin. Lab. Haematol 2002; 24: 337-41.

59. Lund E, Dumeaux V. Systems Epidemiology in Cancer. Cancer Epidemiol. Biomarkers Prev 2008; 17: 2954-57.

60. Amundson SA, Do KT, Shahab S, Bittner M, Meltzer P, Trent J, et al. Identification of potential mRNA biomarkers in peripheral blood lymphocytes for human exposure to ionizing radiation. Radiat Res 2000;154: 342-46.

61. McHale CM, Zhang L, Hubbard AE, Zhao X, Baccarelli A, Pesatori AC, et al. Microarray analysis of gene expression in peripheral blood mononuclear cells from dioxin-exposed human subjects. Toxicology 2007; 229: 101-13.

62. McHale CM, Zhang LP, Lan Q, Li GL, Hubbard AE, Forrest MS, et al. Changes in the peripheral blood transcriptome associated with occupational benzene exposure identified by cross-comparison on two microarray platforms. Genomics 2009; 93: 343-49.

63. Lampe JW, Stepaniants SB, Mao M, Radich JP, Dai HY, Linsley PS, et al. Signatures of environmental exposures using peripheral leukocyte gene expression: Tobacco smoke. Cancer Epidemiol Biomarkers Prev 2004; 13: 445-53.

64. Wang ZX, Neuburg D, Li C, Su L, Kim JY, Chen JC, et al. Global gene expression profiling in whole-blood samples from individuals exposed to metal fumes. Environ Health Perspect 2005; 113: 233-41.

65. Lund E, Dumeaux V, Braaten T, Hjartaker A, Engeset D, Skeie G, et al. Cohort profile: The Norwegian women and cancer study - NOWAC - Kvinner og kreft. Int. J. Epidemiol 2008; 37: 36-41.

66. Lund E, Kumle M, Braaten T, Hjartåker A, Bakken K, Eggen E, et al. External validity in a population-based national prospective study - the Norwegian Women and Cancer Study (NOWAC). Cancer Causes Control 2003; 14: 1001-8.

67. Dumeaux V, Borresen-Dale AL, Frantzen JO, Kumle M, Kristensen VN, Lund E. Gene expression analyses in breast cancer epidemiology: the Norwegian Women and Cancer postgenome cohort study. Breast Cancer Res 2008;10: 9. doi: 10.1186/bcr1859

68. Blaker B, Aarsland M. Mål og vekt for matvarer [Measures and weights for foods], National Association for Nutrition and Health, Oslo, Norway, 1995.

69. Hjartåker A, Andersen LF, Lund E. Comparison of diet measures from a food-frequency questionnaire with measures from repeated 24-hour dietary recalls. The Norwegian Women and Cancer Study. Public Health Nutr 2007; 10: 1094-1103.

70. Parr C, Veierød M, Laake P, Lund E, Hjartåker A. Test-retest reproducibility of a food frequency questionnaire (FFQ) and estimated effects on disease risk in the Norwegian Women and Cancer Study (NOWAC). Nutr J 2006; 5: 4. doi: 10.1186/1475-2891-5-4.

71. Hjartåker A, Lund E, Bjerve K. Serum phospholipid fatty acid composition and habitual intake of marine foods registered by a semi-quantitative food frequency questionnaire. Eur J Clin Nutr 1997; 51: 736-42.

72. Powley CR, George SW, Ryan TW, Buck RC. Matrix effect-free analytical methods for determination of perfluorinated carboxylic acids in environmental matrixes. Anal Chem 2005; 77: 6353-58.

73. Araujo P, Nguyen T-T, Frøyland L, Wang J, Kang JX. Evaluation of a rapid method for the quantitative analysis of fatty acids in various matrices. J Chromatogr A 2008; 1212: 106-13.

74. AMAP. AMAP Ringtest for Persistent Organic Pollutants in human Serum 2009-3. Avalable from: http://www.inspq.qc.ca/ctq/page/amap/rapports/AMAP-2009-3.pdf Accessed 18 March 2010

75. Lindström G, Kärrman A,van Bavel B. Accuracy and precision in the determination of perfluorinated chemicals in human blood verified by interlaboratory comparisons. J Chromatogr A 2009; 1216: 394-400.

76. Yeung LWY, So MK, Jiang G, Taniyasu S, Yamashita N, Song M, et al. Perfluorooctanesulfonate and Related Fluorochemicals in Human Blood Samples from China. Environ Sci Techn 2006; 40: 715-20.

77. Harada K, Koizumi A, Saito N, Inoue K, Yoshinaga T, Date C, et al. Historical and geographical aspects of the increasing perfluorooctanoate and perfluorooctane sulfonate contamination in human serum in Japan. Chemosphere 2007; 66: 293-301.

78. Guruge KS, Taniyasu S, Yamashita N, Wijeratna S, Mohotti KM, Seneviratne HR, et al. Perfluorinated organic compounds in human blood serum and seminal plasma: a study of urban and rural tea worker populations in Sri Lanka. J. Environ Monit 2005; 7: 371-77.

79. Fromme H, Midasch O, Twardella D, Angerer J, Boehmer S, Liebl B. Occurrence of perfluorinated substances in an adult German population in southern Bavaria. Int Arch Occup Environ Health 2007; 80: 313-19.

80. Crawley M. The R book. London: John Wiley & Sons; 2007.

81. Ehresman DJ, Froehlich JW, Olsen GW, Chang S-C, Butenhoff JL. Comparison of human whole blood, plasma, and serum matrices for the determination of perfluorooctanesulfonate (PFOS), perfluorooctanoate (PFOA), and other fluorochemicals. Environ Research 2007; 103: 176-84.

82. Kärrman A, van Bavel B, Järnberg U, Hardell L, Lindström G. Perfluorinated chemicals in relation to other persistent organic pollutants in human blood. Chemosphere 2006; 64: 1582-91.

83. Midasch O, Drexler H, Hart N, Beckmann MW, Angerer J. Transplacental exposure of neonates to perfluorooctanesulfonate and perfluorooctanoate: a pilot study. Int Arch Occup Environ Health 2007; 80: 643-48.

84. Harada K, Inoue K, Morikawa A, Yoshinaga T, Saito N, Koizumi A. Renal clearance of perfluorooctane sulfonate and perfluorooctanoate in humans and their species-specific excretion. Environ Research 2005; 99: 253-61.

85. Hansen S, Odland JO, Phi DT, Nieboer E, Sandanger TM. Maternal levels of organochlorines in two communities in southern Vietnam. Sci Total Environ 2009; 408: 225-32.

86. Engeset D, Alsaker E, Ciampi A, Lund E. Dietary patterns and lifestyle factors in the Norwegian EPIC cohort: The Norwegian Women and Cancer (NOWAC) study. Eur J Clin Nutr 2005; 59: 675-84.

87. Calafat AM, Needham LL, Kuklenyik Z, Reidy JA, Tully JS, Aguilar-Villalobos M, et al. Perfluorinated chemicals in selected residents of the American continent. Chemosphere 2006; 63: 490-96.

88. Kärrman A, Harada, KH, Inoue K, Takasuga T, Ohi E, Koizumi A. Relationship between dietary exposure and serum perfluorochemical (PFC) levels--A case study. Environ Int 2009; 35: 712-17.

89. Jin YH, Saito N, Harada KH, Inoue K, Koizumi A. Historical trends in human serum levels of perfluorooctanoate and perfluorooctane sulfonate in Shenyang, China. Tohoku J Experiml Med 2007; 212: 63-70.

90. Kubwabo C, Vais N, Benoit FM. A pilot study on the determination of perfluorooctanesulfonate and other perfluorinated compounds in blood of Canadians. J Environ Monit 2004; 6: 540-45.

91. Ostertag SK, Tague BA, Humphries MM, Tittlemier SA, Chan HM. Estimated dietary exposure to fluorinated compounds from traditional foods among Inuit in Nunavut, Canada. Chemosphere 2009; 75: 1165-72.

92. Vestergren R, Cousins IT.. Tracking the Pathways of Human Exposure to Perfluorocarboxylates. Environ Sci Techn 2009; 43: 5565-75.

93. Brustad M, Edvardsen K, Wilsgaard T, Engelsen O, Aksnes L, Lund E. Seasonality of UV-radiation and vitamin D status at 69 degrees north. Photochem Photobiol Sci 2007; 6: 903-8.

94. Mozaffarian D. Fish and n-3 fatty acids for the prevention of fatal coronary heart disease and sudden cardiac death. Am J Clin Nutr 2008; 87:1991S-6S.

95. Mozaffarian D, Bryson CL, Lemaitre RN, Burke GL, Siscovick DS. Fish Intake and Risk of Incident Heart Failure. J Am Coll Cardiol. 2005; 45: 2015-21.

96. Arsenault G, Chittim B, McAlees A, McCrindle R, Riddell N, Yeo B. Some issues relating to the use of perfluorooctanesulfonate (PFOS) samples as reference standards. Chemosphere 2008; 70: 616-25.

97. Kärrman A, Langlois I, van Bavel B, Lindström G, Oehme M. Identification and pattern of perfluorooctane sulfonate (PFOS) isomers in human serum and plasma. Environ Int 2007; 33: 782-88.

98. Benskin JP, De Silva AO, Martin LJ, Arsenault G, McCrindle R, Riddell N, et al. Disposition of perfluorinated acid isomers in Sprague-dawley rats; part 1: single dose. Environ Toxicol Chem 2009; 28: 542-54.

99. Brooks WM, Lynch PJ, Ingle CC, Hatton A, Emson PC, Faull RLM, et al. Gene expression profiles of metabolic enzyme transcripts in Alzheimer's disease. Brain Research 2007; 1127: 127-35.

Errata

Paper III

First page, second column, line 4.

The sentence

"Also, two of the most common contaminants in nature and in humans; perfluorooctane sulfonate (PFOS) and perfluoroctanoate (PFOA) are potent peroxisome proliferators in rodents and in human hepatocytes"

should be changed to

"Also, two of the most common contaminants in nature and in humans; perfluorooctane sulfonate (PFOS) and perfluoroctanoate (PFOA) are potent peroxisome proliferators in rodents and in human cell models".

Paper I

Hindawi Publishing Corporation
Journal of Environmental and Public Health
Volume 2009, Article ID 268219, 10 pages
doi:10.1155/2009/268219

Research Article

Dietary Predictors and Plasma Concentrations of Perfluorinated Compounds in a Coastal Population from Northern Norway

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Received 15 May 2009; Revised 2 September 2009; Accepted 5 October 2009

Recommended by Pam R. Factor-Litvak

Dietary intake, age, gender, and body mass index were investigated as possible predictors of perfluorinated compounds in a study population from northern Norway (44 women and 16 men). In addition to donating a blood sample, the participants answered a detailed questionnaire about diet and lifestyle. Perfluorooctane sulfonate (PFOS) (29 ng/mL), perfluorooctanoate (PFOA) (3.9 ng/mL), perfluorohexane sulfonate (PFHxS) (0.5 ng/mL), perfluorononanoate (PFNA) (0.8 ng/mL), and perfluoroheptane sulfonate (PFHpS) (1.1 ng/mL) were detected in more than 95% of all samples. Of the dietary items investigated, fruit and vegetables significantly reduced the concentrations of PFOS and PFHpS, whereas fatty fish to a smaller extent significantly increased the levels of the same compounds. Men had significantly higher concentrations of PFOS, PFOA, PFHxS, and PFHpS than women. There were significant differences in PFOS isomer pattern between genders, with women having the largest proportion of linear PFOS. PFOS, PFHxS, and PFHpS concentrations also increased with age.

1. Introduction

As a result of many years of production and use in industry and consumer products, perfluorinated compounds (PFCs) are frequently found in the environment as well as in human blood world wide [1–4]. Due to their unique properties to repel both water and oil, PFCs have been important components of surface protectants for different materials and in fire-fighting foams and chemicals [5]. The predominant PFC in human samples is perfluorooctane sulfonate (PFOS), although other frequently detected compounds are perfluorooctanoate (PFOA), perfluorohexane sulfonate (PFHxS), and perfluorononanoate (PFNA) [1, 3]. In Norway, PFOS has mainly been used as a component in fire-fighting foams on oil rigs, although there is no information available about the use of PFHxS or PFOA [6]. Perfluorocarboxylates (PFCAs), for example, PFOA, and perfluoroalkyl sulfonates (PFASs), for example, PFOS, exist as branched and linear isomers. The linear isomer of PFOS is most common in technical mixtures and also in human samples [7, 8]. Differences in proportion of linear PFOS levels between inhabitants of three different countries have been reported and the authors concluded that this indicated different exposure sources [7]. However, there is limited information on isomer distribution in humans world wide.

PFOS and PFHxS have been reported to bioaccumulate and magnify in the food chain [9, 10], even though PFCs behave differently from the legacy persistent organic pollutants (POPs) in the environment. A number of toxic effects, including alterations in fatty acid metabolism, enlargement of the liver, reduced birth weight, altered growth and development, and increased mortality in newborns, have been demonstrated in PFOS- and PFOA-exposed rodents [11–14]. Epidemiological studies on humans and health effects of PFCs are so far limited. Occupationally exposed workers in PFC industries have been studied in relation to morbidity, bladder cancer, and self-reported medical conditions [15–17]. No association between exposure and outcome was found. Several PFCs are found in human cord blood, indicating that these compounds easily cross the placental barrier. Results from human studies investigating the association between birth weight and PFC concentration

Table 1: Study group characteristics.

	Total $n = 56$[a] (100%)	Women $n = 41$ (73%)	Men $n = 15$ (27%)
	Median (range)	Median (range)	Median (range)
Age (years)	44 (26–60)	44 (26–60)	44 (32–57)
BMI (kg/m^2)	25 (18–43)	24 (18–43)	25 (21–33)
Intake of meat (kg/year)[b]	46 (5.2–119)	42 (5.2–119)	63 (12–83)
Intake of dairy products (kg/year)	65 (40–299)	54 (4.0–299)	138 (8.9–235)
Intake of bread and cereals (kg/year)	48 (14–114)	46 (14–108)	78 (21–114)
Intake of vegetables and fruit (kg/year)[c]	151 (51–363)	154 (52–363)	136 (51–305)
Intake of fatty fish (kg/year)[d]	3.2 (0–39)	3.1 (0–18)	3.4 (0–39)
Intake of lean fish (kg/year)[e]	15 (0–63)	14 (0–63)	18 (5.1–38)
Intake of other kinds of fish (kg/year)[f]	2.1 (0–19)	2.1 (0–19)	2.1 (0–19)
Intake of fish products (kg/year)[g]	15 (1.7–53)	15 (1.7–41)	20 (4.5–53)
Intake of shellfish (kg/year)	0 (0–3.2)	0 (0–3.2)	0 (0–3.2)

[a]Four people were excluded due to poor chromatography and the total number of study participants was reduced to 56.
[b]Include meat and meat products.
[c]Include potatoes, vegetables, juice, jam, and fruit.
[d]Include intake of salmon, mackerel, wolfish, and herring.
[e]Include intake of boiled and fried cod.
[f]Include intake of other kinds of fish not included in above two categories.
[g]Include intake of fish cakes, fish au gratin, deep-fried fish, and fish spread.

are so far conflicting [18, 19]. In 2001, 3 M, one of the major producers of PFOS, voluntarily started to withdraw PFOS from production due to its persistent and toxic properties in the environment [20]. By 2015 or earlier, DuPont, which is a large producer of PFOA, is committed to totally phasing out PFOA and PFOA production precursors [21].

Even though PFCs have recently received a great deal of attention, routes of human exposure to PFCs are still unclear. Different pathways have been considered, such as diet, contaminated drinking water, household dust, and outdoor and indoor air [22]. Some studies have found diet to be the major pathway, whereas others suggest that diet is part of a more complex exposure scenario [22–25]. Five different studies have investigated PFC concentrations in food samples from Germany, Canada, the UK, Spain, and the Netherlands, and the results deviate [23–27]. None of the studies detected PFCs in all samples analyzed and several of them investigated only selected foodstuffs, not the whole diet. The UK food survey found the highest concentrations of PFOS in potatoes and potato products, such as french fries, hash browns, and potato salads [26]. The Spanish study [25] reported the highest concentrations of PFOS in fish and dairy products whereas the Canadian study [23] found the highest concentration of PFOS in beefsteak and salt water fish. De Voogt et al. reported the highest PFOS concentrations in beef, cod, and milk [27]. A study on seafood from Chinese fish markets observed detectable but low concentrations of PFOS (0.33–13.9 ng/g) in all species investigated, with the highest level in mantis shrimp [28]. Recently, the National Institute of Nutrition and Seafood Research in Norway reported low levels of PFCs in capelin (2–3.5 ng/g) and shrimp (<1–10 ng/g) and levels below the limit of detection (LOD) (<3 ng/g) in filets of farmed salmon [29, 30]. Only a few previous studies have investigated the relationship between self-reported dietary intake and plasma concentrations of PFCs [31–33]. Two of the studies [31, 33] concluded that locally caught fish significantly increased the body burden of PFOS. The third study [32] observed a positive association between PFOS and PFOA and consumption of red meat, snacks, and animal fat, and a negative relationship to intake of fruit, vegetables, and poultry.

The aim of this study was to determine the background concentrations of PFCs in a Norwegian coastal population in relation to age, gender, body mass index (BMI), and dietary habits, with special emphasis on fish consumption. This study also adds more information about the PFOS isomer pattern in human blood samples.

2. Materials and Methods

2.1. Study Participants. Study participants were recruited by an advertisement in the local newspaper through the survey "UV-light in Northern Norway and D-vitamin production in skin", a research project on Andøya Island, described elsewhere [34]. The people on Andøya are known to have a high consumption of various kinds of seafood. Criteria for being included in the study were age between 20 and 60 years and living in the municipality of Andenes at 69°N. The participants were 44 women and 16 men of various ages (26–60 years) (Table 1). Concentrations of POPs in the same study group have been reported elsewhere [35].

2.2. Food Frequency Questionnaire. The participants answered the Norwegian Women and Cancer Study's (NOWAC) food frequency questionnaire. Study participants were asked to record how often they consumed 86 different foodstuffs including alcohol. Questions about portion size were also answered. The questionnaire differentiates between different kinds of fish and meat consumed. These variables were

later grouped together (Table 1). The questionnaire has been validated and described in detail elsewhere [36, 37].

2.3. Blood Samples. Blood samples were collected in August to September 2005. Blood was drawn in BD Vacutainer blood collection tubes (BD, NJ, USA) containing EDTA buffer (10.8 mg) and mailed overnight to the University of Tromsø. The samples were centrifuged at 3000 rev./min for 15 min and the plasma was collected. Plasma samples were stored at −80°C until analysis.

2.4. Chemical Analysis. Samples were extracted and cleaned up using a modified method from Powley et al. [38]. In short, 0.2 g plasma was weighed into a 50 mL polypropylene centrifuge tube (Nalgene, Rochester, NY, USA). Twenty-five microliters of $^{13}C_4$-labeled PFOS and $^{13}C_4$-labeled PFOA (0.1 ng/μL) (Wellington laboratories, Guelph, ON, Canada) and 4 g methanol (Merck, Darmstadt, Germany) were added to the test tube as internal standards and extraction solvents. Samples were extracted for 3 × 10 minutes in an ultrasonic bath (Branson Ultrasonics BV, Soest, the Netherlands). In between each extraction, samples were mixed thoroughly with a vortex mixer (VWR, Wester Chester, PN, USA). Samples were then centrifuged at 2000 rev./min for 5 min using a Jouan A14 centrifuge (Thermo Scientific, Waltham, MA, USA). The supernatant was collected before volume reduction to 1 g on a Rapid Vap evaporation unit (Labconco, Kansas City, MO, USA). The extract was transferred to a 1.5 mL microcentrifuge tube (Brand GMBH, Wertheim, Germany) containing 25 mg ENVI-Carb 120/400 (Supelco, PN, USA) and 50 μL glacial acetic acid (KEBOLab, Kalbakken, Norway). The solution was mixed thoroughly and then centrifuged for 10 min at 10 000 rev./min. The supernatant was weighed and transferred to a 1.8 mL glass tube, and 20 μL 3,5-bis(trifluoromethyl)phenyl acetic acid (BTPA) (0.1 ng/μL) (Wellington Laboratories, Guelph, ON, Canada) were added as recovery standard. Before analysis, 100 μL extract was mixed with 100 μL water containing 2 mmol/L NH_4OAc (BDH Laboratory Supplies, Leicestershire, England).

PFCs were analyzed using a quadrupole time-of-flight-mass spectrometer, Q-TOF micro, equipped with a 2777 autosampler and a binary HPLC pump (1525) from Waters (Milford, MA, USA). The method used is slightly modified from that of Berger and Haukås [39]. Mobile phases consisted of (a) 2 mmol/L NH_4OAc in water and (b) 2 mmol/L NH_4OAc in methanol (Merck, Darmstadt, Germany). Before analysis, mobile phases were degassed using an ultrasonic bath (Branson Ultrasonics BV, Soest, the Netherlands). A 50 μL sample was injected into an ACE 3 C18 reversed-phase column, with particle size 3 μm and length 150 mm (ACT, Aberdeen, Scotland). The flow rate of the mobile phase was 0.2 mL/min. The following gradient settings were applied for elution of the target analytes from the column: 0 min to 50% B; 0–5 min linear gradient to 85% B; 5–10 min to 85% B; 10-11 min linear gradient to 99% B; 11–20 min to 99% B; 20-21 min linear gradient to 50% B; 21–28 min to 50% B. The QTOF-MS was operated by the Mass Lynx 4.1 software in negative electrospray ionization mode (ESI) in the m/z range 100–725. Settings were optimized before analysis as follows: capillary voltage, −3 kV; sample cone voltage, 50 V (0.5–10.3 min), 35 V (0.5–20.0 min), and 20 V (10.3–20.0 min); desolvation and source temperature, 350 and 120°C, respectively; nitrogen was used as cone gas at a flow of 20 l/min, as nebulizer gas maximum flow, and as desolvation gas at 600 l/min. Target analytes and analytical standards, their abbreviation, quantification masses and cone voltages are listed in supplementary Table S1 in Supplementary Material available online at doi:10.1155/2009/268219.

The quantification was conducted by the QuanLynx software, version 3.5 (Waters, Milford, MA, USA).

The linear PFOS isomer was chromatographically separated from the branched isomers and quantified, both separated and as the sum of all isomers. The coelution of branched isomers (one peak) was not structurally elucidated but rather identified as eluting earlier than the linear PFOS, as indicated in supplementary Figure S1. Isomer specification was not performed for the other PFCs, where the linear isomer clearly dominated. Data presented as "PFOS" consist of the sum of the linear and the coeluted peak of branched isomers. Similar response factors have been reported for branched and linear isomers of PFOS [40], so the mass labeled "internal standard for linear PFOS" was used for quantification of the branched isomer as well.

2.5. Quality Control of Chemical Analysis. The quality of the analysis was assured through repetitive analysis of blank samples and reference samples obtained from previous international comparison programs. For each batch of 30 samples, one reference material and two blank samples were prepared. Three times a year, the present laboratory also participates in the AMAP Ringtest for Persistent Organic Pollutants in Human Serum, an international comparison program, organized by Institut National de Santé Publique du Québec, Canada. Results from interlaboratory comparisons indicate that the uncertainties of our analysis are well within ±30% of the assigned values. Four samples were excluded from all analysis due to poor chromatography. Therefore, the total number of study participants was reduced to 56. Recovery rates varied between 60% and 120%.

The LOD was automatically calculated by the quantification software from the signal-to-noise level in each sample. The individual LODs were comparable for all samples and an average is reported for each analyte in Table 2. PFOA was detected in a few blank samples. If the concentration of PFOA in these samples was larger than the software-determined LOD for that batch of samples, LOD was determined from the concentration of PFOA in the blanks. All samples were well within the linear range of the instrument.

2.6. Statistical Analysis. Statistical analysis was performed using the freely available software R, version 2.8.1 (http://www.cran.r-project.org/). Statistics for PFOSA, which had more than 20% of the observations below LOD, were performed with the NADA package for R. Summary statistics for PFOSA were calculated using the maximum likelihood estimation (MLE) according to Helsel [41]. PFHpA had

Table 2: PFC concentrations (ng/mL) in the study group.

Concentration (ng/mL plasma)	Total $n = 56$					Men $n = 15$			Women $n = 41$		
	Median	AM	Range	LOD	% >LOD	Median	AM	Range	Median	AM	Range
PFOSA	0.08	0.11	<LOD–0.7	0.03	79	0.11	0.11	<LOD–0.35	0.08	0.10	<LOD–0.71
PFHxS	1.1	1.8	0.40–13	0.07	100	1.8	3.5	0.95–13	0.8	1.2	0.40–3.8
PFHpS	0.46	0.57	<LOD–1.9	0.13	96	0.70	0.89	0.31–1.9	0.35	0.45	<LOD–1.3
PFOS branched	9.4	10	<LOD–31	0.22	96	12	15	<LOD–31	7.1	8.3	<LOD–26
PFOS linear	20	23	4.7–69	0.40	100	24	33	14–69	17	19	4.7–47
PFOS	29	33	6.9–99			43	48	28–99	24	27	6.9–67
PFHpA	NA	NA	NA	0.26	0	NA	NA	NA	NA	NA	NA
PFOA	3.9	4.4	1.4–9.6	0.30	100	5.1	5.4	3.0–8.8	3.4	4.0	1.4–9.6
PFNA	0.81	0.95	<LOD–2.9	0.26	98	0.94	1.1	0.40–2.9	0.77	0.88	<LOD–2.4
% linear PFOS	69	69	49–100			67	67	49–100	69	70	56–100

AM, arithmetic mean; LOD, method detection limit; % >LOD, percentage of samples in which the analyte was detected; NA, not available; PFOSA, perfluorooctane sulfonic acid; PFHxS, perfluorohexane sulfonate; PFHpS, perfluoroheptane sulfonate; PFOS, sum of branched and linear isomers of PFOS (perfluorooctane sulfonate); PFHpA, perfluoroheptanoate; PFOA, perfluorooctanoate; PFNA, perfluorononanoate; % linear PFOS, percentage linear PFOS related to PFOS.

more than 95% of the observations below LOD and were not evaluated statistically.

Possible predictors of PFC concentrations investigated were age, gender, BMI, and nine different categories of foodstuff (see Table 1). The impact of these predictors on PFOS, PFOA, PFHxS, PFHpS, PFNA, and percentage linear PFOS was investigated using linear models on log-transformed variables or Wilcoxon's rank sum test. For the censored data (PFOSA), the nonparametric Peto-Prentice test, as well as the nonparametric Akritas-Thiel-Sen slope in the NADA package for R, was used. The censored methods are described by Helsel [41]. Model assumptions for the linear models were evaluated using diagnostic plots of the residuals. Parameter estimates (β) with 95% confidence interval and the levels of significance (P values) for the final regression models are reported in Table 3. The parameter estimates (β) are back-transformed logresults and should be interpreted as the number of times that the response variable increased/decreased by one unit in explanatory variable. Wilcoxon's test estimator (W) and the corresponding P values are reported in the text. The P values <.05 were considered to be significant.

3. Results

Median, arithmetic mean, range, LOD, and percentage of samples with values > LOD of the eight monitored PFCs are provided in Table 2. PFOS, PFOA, PFHxS, PFHpS, and PFNA were detected in more than 95% of all samples.

PFOS and PFHpS concentrations were strongly correlated ($r = 0.93$) (Figure 1), as well as PFOS and PFNA ($r = 0.70$) and PFOSA and PFNA ($r = 0.72$) (Figure 1). The remaining PFCs were medium strong or weakly correlated (supplementary Table S2). There were four significant predictors for PFOS concentration in this study group: gender, age, intake of fruit and vegetables, and intake of fatty fish (Table 3). Men had 75% higher concentrations of PFOS than those of women and the PFOS concentration increased by 2% per year of age (for the whole group) (Table 3). An additional serving (150 g) of fruit and vegetables per day during the last year decreased the concentrations of PFOS by 16%, whereas an extra meal (150 g) of fatty fish per week during the last year resulted in an increase in PFOS concentrations (22%) (Table 3). Age, gender, consumption of fatty fish, and consumption of fruit and vegetables explained 57% of the variation in PFOS concentration (Table 3). Fatty fish alone accounted for 4%, fruit and vegetables explained 16%, and age and gender 37%. There was no correlation between intake of fatty fish and intake of fruit and vegetables ($r = -0.00014$).

Male sex was clearly associated with increased plasma concentrations of PFOA (44%), PFHxS (172%), and PFHpS (107%) (Table 3). There was a positive relationship between age and PFHxS and PFHpS, both indicating a 3% increase in concentrations per year of age (Table 3). An additional intake of 150 g (one serving) of fatty fish per week, over the last year, resulted in increased concentrations of PFHpS (32%), whereas an additional serving (150 g) of fruit and vegetables each day, over the last year, decreased the concentration of PFHpS by 17% (Table 3). None of the investigated variables influenced the concentration of PFNA or PFOSA significantly. Two people were excluded from the PFOS and PFHpS models because they were strongly influential. The results were the same, before and after exclusion, but the refined models explained more of the variation in the dataset. Intake frequencies of fatty fish and fruit/vegetables, and the corresponding concentrations of PFOS and PFHpS, are reported in Table S3. The person who consumed two to three servings of fatty fish per week (67 ng/mL PFOS, supplementary Table S3) was not indicated as an outlier by the diagnostic plots of the residuals, although, on removal of that person from the dataset, fatty fish were no longer a significant predictor ($P > .05$) for

Table 3: Back-transformed parameter estimates, 95% confidence interval (CI) and P values for the final regression models of selected PFCs and significant predictors.

	PFOS		PFOA		PFHxS		PFHpS	
	β (95% CI)	P	β (95% CI)	P	β (95% CI)	P	β (95% CI)	P
Male sex	1.75 (1.43, 2.14)	<.001	1.44 (1.12, 1.85)	.009	2.72 (1.86, 3.92)	<.001	2.07 (1.57, 2.74)	<.001
Age (years)	1.02 (1.01, 1.03)	<.001	1.01 (0.998, 1.02)	.096	1.03 (1.01, 1.03)	<.001	1.03 (1.01, 1.04)	<.001
Consumption of fatty fish (150 g per week during a year)	1.22 (1.02, 1.45)	.029					1.32 (1.03, 1.68)	.027
Consumption of fruit and vegetables (150 g per day during a year)	−1.16 (−1.08, −1.25)	<.001					−1.17 (−1.06, −1.29)	.002
R^2 (%)	57		17		41		50	

β, parameter estimates (back-transformed logresults); R^2, coefficient of determination, that is, the proportion of variability in the dataset that is explained by the model; PFOS, perfluorooctane sulfonate; PFOA, perfluorooctanoate; PFHxS, perfluorohexane sulfonate; PFHpS, perfluoroheptane sulfonate.

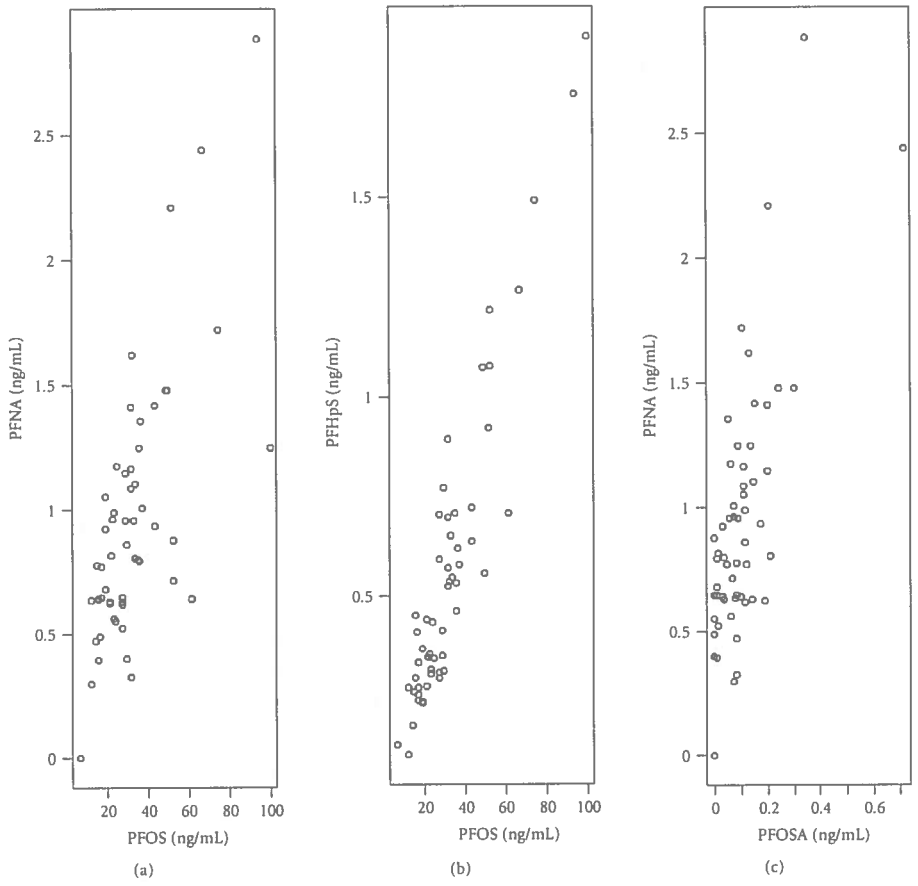

Figure 1: Correlation between perfluorooctane sulfonate (PFOS) and perfluorononanoate (PFNA) (r = 0.70) and PFOS and perfluoroheptane sulfonate (PFHpS) (r = 0.93), as well as perfluorooctane sulfonic acid (PFOSA) and PFNA (r = 0.72).

PFOS or PFHpS concentrations. The result for fruit and vegetables, age, and gender remained the same, before and after exclusion. In the final model (Table 3), the person was, however, included because of the indication of not being an outlier.

The study group had an average proportion of linear PFOS of 69% (range 49%–100%) (Table 2). Women had a significantly larger percentage of linear PFOS than that of men (70% versus 67%, $W = 415.5$, $P = .047$). The proportion of linear PFOS varied between 49% and 100% for men and between 56% and 100% for women (Table 2).

4. Discussion

We have shown that PFOS and PFHpS concentrations in the current study decreased with intake of fruit and vegetables and increased with intake of fatty fish. An additional serving of fruit and vegetables (150 g) per day, over the last year, gave an estimated decreased body burden of PFOS and PFHpS of 16% (95% confidence interval (CI): 8%–25%) and 17% ((CI): 6%–29%), respectively. On the other hand, an additional meal of fatty fish per week (150 g), over the last year, resulted in 22% (CI: 2%–45%) and 32% (CI: 3%–68%) higher concentrations of PFOS and PFHpS, respectively. Intake of fatty fish and intake of fruit and vegetables were not correlated and the observed effect cannot be explained by co-variation. The maximum intake of fruit and vegetables was 6.6 servings per day and 2.3 servings of fatty fish per week (supplementary Table S3). No conclusions outside these intake ranges can be made from this dataset.

Surprisingly, consumption of fruit and vegetables decreased PFOS concentrations in the current study. Supporting and contradictory findings have been reported by others. Halldorsson et al. [32] found that intake of fruit and vegetables decreased PFOS and PFOA concentrations in the Danish Birth cohort ($n = 1076$), but the effect could be partly explained by lower intake of red meat, animal fats, and snacks (positively associated to PFOS) among the high consumers of fruit and vegetables. Emmet et al. [42] reported a positive association between locally grown vegetables and PFOA concentration, whereas Holzer et al. [31] observed no effect from intake of locally grown fruit and vegetables on PFOA, PFOS, or PFHxS concentrations in a group of 521 participants. The different findings may reflect different study designs and/or presence of confounding factors. In the Norwegian Women and Cancer Study (NOWAC), dietary patterns and lifestyle factors were investigated in 35 554 Norwegian women [43]. "Healthy eaters" had the highest intake of fruit, vegetables, skimmed milk, juice, instant coffee, crisp bread, rice, chicken, and cod liver oil, which is frequently used as a food supplement in Norway. They were also characterized by high education, high income, few current smok, and high activity. They were more likely to use dietary supplements than the other women and most of them lived in the southern or eastern part of Norway. This study indicates that health awareness is characterized not only by diet but also by many lifestyle factors. Consequently, our findings of decreasing PFOS and PFHpS concentrations with increasing intake of fruit and vegetables may be explained by a large number of confounding variables that characterize the lifestyle of "healthy eaters", rather than a high fruit and vegetable intake. It is nevertheless an important finding that needs to be investigated further.

In the current study, 20% of the observed variation in PFOS concentration were explained by intake of fruit and vegetables (16%) and intake of fatty fish (4%). These findings support the previous hypothesis that fish intake contributes to increased body burdens of PFOS [31, 33]. However, only one person (67 ng/mL, supplementary Table S3) was responsible for the significant association of fatty fish ($P < .05$), and interpretation of this result should therefore be made with care. In addition, of the participants' total intake of seafood, only 9% consisted of fatty fish. The remaining 91% were intake of lean fish, fish products, and other kinds of fish that had no impact on PFC concentrations. Thus, the main source of seafood did not contribute to increased body burdens of PFOS or PFHpS. The health effects of seafood consumption have been frequently debated. Benefits from a fish-rich diet, for example, lower risk of cardiovascular diseases, has been weighed up against the possible drawbacks of increased body burden of environmental pollutants [44]. A substantial increase of one meal of fatty fish per week over a year resulted in only a 22% increase in PFOS concentration, which corresponds to 6 ng/mL (from 29 ng/mL (median) to 35 ng/mL). Furthermore, no associations between a number of outcomes and occupational exposure to PFCs have been found in a study group exposed to higher concentrations than the general population [15–17]. It is therefore unlikely to expect adverse effects from intake of fatty fish. The protective effects of seafood on several health outcomes are clear and well documented [45] and the benefits of a fish-rich diet should by far outweigh the concerns. PFCs are, in addition, detected in all kinds of food [23, 25, 26, 28] and also in household dust [46], drinking water [42], and consumer products [47], indicating that the exposure is complex and could not be explained just by single foodstuffs. This was also recently pointed out by Halldorsson et al. [32] that suggested that the overall diet is likely to be more important for PFC exposure than single foodstuffs and results from individual food groups should therefore be interpreted with care. Kärrman et al. [48] concluded also that the importance of dietary intake of PFCs may differ between regions.

Fatty fish and fruit/vegetables were also significant predictors for PFHpS concentrations in this study group. This finding is most probably an artifact, explained by the strong correlation between PFOS and PFHpS ($r = 0.93$). However, there was a lack of association between the investigated dietary predictors and plasma concentrations of PFOA, PFHxS, PFNA, and PFOSA in this study group. This may point to the diet being a less important pathway for these compounds due to low concentrations present in food. The UK food survey detected PFOS in some of their composite food samples, whereas the concentrations of PFOA were below LOD (<0.5–10 ng/g) in the same samples [26]. Ericson et al. [25] and Tittlemier et al. [23] detected PFOS in nearly all of their composite food samples, although only a few had detectable concentrations of PFOA and/or PFHpA and PFNA. PFOSA was not found in any of these

food surveys. Another explanation for the lack of association between the dietary variables and PFOA, PFHxS, PFNA, and PFOSA could be the fact that human blood concentrations of these compounds are lower and more uniform. Also, the current study had fewer participants, that is, lower chance of detecting weak effects.

All the investigated PFCs (except for PFOSA versus PFOA and PFHxS) were medium strong or highly correlated (supplementary Table S2), with the strongest correlation being between PFOS and PFHpS ($r = 0.93$). The correlation between PFCs seems to vary between countries/studies. Olsen et al. [49] reported the strongest correlation between PFOS and PFOA ($r = 0.63$) in American Red Cross blood donors. Similar results were provided by Haug et al. [50] that reported PFOS and PFOA being highly correlated ($r = 0.95$) in pooled Norwegian samples. Ericson et al. [51] observed the strongest correlation between PFOS and PFHxS ($r = 0.23$) in a study group from Spain while Rylander et al. [52] reported the strongest correlation between PFOS and PFHxS ($r = 0.94$) in delivering women from Vietnam. Differences may be attributable to a number of factors, including different sample sizes, age and gender compositions of the study groups, different exposure sources, or analytical challenges.

Men had significantly higher concentrations of PFOS, PFOA, PFHxS, and PFHpS than those of women in this study group. Several other studies have reported gender-related differences for the same compounds, with men having higher concentrations than those of women [31, 51, 53–55]. PFOS and PFOA have been found to cross the placental barrier and have been detected in human breast milk, in addition to PFHxS, PFOSA, and PFNA [18, 19, 56–60]. Differences between genders may therefore be partly explained by transplacental transfer and excretion through breastfeeding, which seem likely in this rather young study group. The differences attributable to childbearing are expected to decrease over time due to continuous exposure to contaminants. Another possible explanation for the gender-related differences could be a higher dietary intake among men. Menopause status, use of contraceptives, and blood donor practices among the study participants could also affect the results through the possible blood loss, but this was not taken into account in the current study. These variables have, however, not been identified as confounding factors in other studies.

Despite the fact that men had higher concentrations of PFOS than those of women in the current study, female blood contained a significantly larger proportion of linear PFOS compared with male blood (70% versus 67%). The major production process of PFOS, electrochemical fluorination (ECF), produced 70%–80% linear PFOS [8]. Our samples contained on average 69% linear PFOS (range 49%–100%). This is comparable to results from Sweden (68%, range 50%–70%) [7] and a previous study in Norway (50%–78%) [50], but higher than in samples from Australia (59%) [7] and the UK (60%) [7] and considerably lower than in samples from Vietnam (83%) [52]. Powley et al. [38] showed recently that the proportion of branched PFOS differed between Arctic cod and ringed seal (which feed on Arctic cod), indicating differences in elimination rates between species. Thus, the dietary contributions of PFOS isomers may vary between countries/continents due to different dietary habits. Also, the importance of different exposure routes, for example, diet, dust, and consumer products, could vary between countries. In the current study, the branched isomers of PFOS were not chromatographically separated but quantified as the sum of a mixture of branched isomers. Kärrman et al. [7] as well as Benskin et al. [61] identified several of the branched isomers of PFOS in human blood, and both studies indicate that the monoperfluoromethyl-substituted isomers are most abundant in human blood samples. However, in order to make correct comparisons of PFOS distribution world wide, it is important to specify whether linear PFOS or the sum of all PFOS isomers is reported, especially as the branched isomers may contribute as much as 30% to the total.

PFOS concentrations in this study group were comparable to samples from Denmark, Sweden, Australia, the USA, Canada, Poland, Korea, and Belgium, although samples from Spain, Germany, Colombia, Brazil, Italy, India, Sri Lanka, Vietnam, and Malaysia were on average up to 20 times less contaminated [3, 31, 51–53, 62, 63]. Poland, Korea, and Japan have reported high PFOA concentrations, whereas studies from China and Spain reported low levels [2, 51, 54]. Our samples were in the midrange. There were smaller differences in PFHxS concentrations between countries and only a few studies reported the concentrations of the other PFCs. Vietnamese women, for example, had considerable lower concentrations of PFHpS but comparable levels of PFNA to the current study group [52]. Considerable regional differences in PFC concentrations within countries [48, 54] have also been observed, indicating that a large population-based study is needed to achieve the full picture of the exposure pattern within a country.

We observed a positive relationship between age and PFOS, PFHxS, and PFHpS concentrations. Previous studies show contradictory results regarding age-related differences in PFC concentrations [2, 31, 53], which point to no clear age trend for the PFCs.

5. Conclusions

Intake of fruit/vegetables and fatty fish affected the concentration of PFOS and PFHpS in this study group. The reason for the decreased PFOS and PFHpS concentrations with increased intake of fruit and vegetables has yet to be explained. A larger population based study is suggested to evaluate the effect of fatty fish consumption on PFOS/PFHpS concentrations thoroughly since uncertainties were present in the current study (only one person responsible for the significant effect). Dietary predictors should, however, be interpreted with care since dietary habits also reflect differences in lifestyle (not adjusted for in the current analysis) which may affect PFC concentrations. Future research should focus on dietary patterns instead of single food groups and lifestyle factors should also be taken into account. Men had significantly higher concentrations of PFOS, PFOA, PFHxS, and PFHpS than those of women, and PFOS, PFHxS, and

PFHpS increased with age. Women had a larger proportion of linear PFOS than those of men (70% versus 67%). However, the overall average of linear PFOS in this study group was comparably to previous studies from Sweden and Norway but considerable higher than in samples from Vietnam. In future studies the PFOS isomers should be reported separately in order to help identify differences between populations and different sources of exposure.

Acknowledgments

The authors gratefully acknowledge all participants in the study "UV-light in Northern Norway and D-vitamin production in skin" and Tonje Braaten for statistical support.

References

[1] A. M. Calafat, L. L. Needham, Z. Kuklenyik, et al., "Perfluorinated chemicals in selected residents of the American continent," *Chemosphere*, vol. 63, no. 3, pp. 490–496, 2006.

[2] K. Kannan, S. Corsolini, J. Falandysz, et al., "Perfluorooctanesulfonate and related fluorochemicals in human blood from several countries," *Environmental Science & Technology*, vol. 38, no. 17, pp. 4489–4495, 2004.

[3] A. Kärrman, B. van Bavel, U. Järnberg, L. Hardell, and G. Lindström, "Perfluorinated chemicals in relation to other persistent organic pollutants in human blood," *Chemosphere*, vol. 64, no. 9, pp. 1582–1591, 2006.

[4] O. Midasch, T. Schettgen, and J. Angerer, "Pilot study on the perfluorooctanesulfonate and perfluorooctanoate exposure of the German general population," *International Journal of Hygiene and Environmental Health*, vol. 209, no. 6, pp. 489–496, 2006.

[5] E. Kissa, *Fluorinated Surfactants and Repellants*, vol. 97, Marcel Dekker, New York, NY, USA, 2nd edition, 2001.

[6] D. Herzke, M. Schlabach, E. Mariussen, H. Uggerud, and E. Heimstad, "A literature survey on selected chemical compounds," Tech. Rep. TA-2238/2007, 2007, http://www.sft.no/publikasjoner/2238/ta2238.pdf.

[7] A. Kärrman, I. Langlois, B. v. Bavel, G. Lindström, and M. Oehme, "Identification and pattern of perfluorooctane sulfonate (PFOS) isomers in human serum and plasma," *Environment International*, vol. 33, no. 6, pp. 782–788, 2007.

[8] I. Langlois and M. Oehme, "Structural identification of isomers present in technical perfluorooctane sulfonate by tandem mass spectrometry," *Rapid Communications in Mass Spectrometry*, vol. 20, no. 5, pp. 844–850, 2006.

[9] R. Bossi, F. F. Riget, R. Dietz, et al., "Preliminary screening of perfluorooctane sulfonate (PFOS) and other fluorochemicals in fish, birds and marine mammals from Greenland and the Faroe Islands," *Environmental Pollution*, vol. 136, no. 2, pp. 323–329, 2005.

[10] M. Haukås, U. Berger, H. Hop, B. Gulliksen, and G. W. Gabrielsen, "Bioaccumulation of per- and polyfluorinated alkyl substances (PFAS) in selected species from the Barents Sea food web," *Environmental Pollution*, vol. 148, no. 1, pp. 360–371, 2007.

[11] C. Lau, J. R. Thibodeaux, R. G. Hanson, et al., "Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse. II: postnatal evaluation," *Toxicological Sciences*, vol. 74, no. 2, pp. 382–392, 2003.

[12] C. Lau, J. L. Butenhoff, and J. M. Rogers, "The developmental toxicity of perfluoroalkyl acids and their derivatives," *Toxicology and Applied Pharmacology*, vol. 198, no. 2, pp. 231–241, 2004.

[13] C. Lau, J. R. Thibodeaux, R. G. Hanson, et al., "Effects of perfluorooctanoic acid exposure during pregnancy in the mouse," *Toxicological Sciences*, vol. 90, no. 2, pp. 510–518, 2006.

[14] M. B. Rosen, J. R. Thibodeaux, C. R. Wood, R. D. Zehr, J. E. Schmid, and C. Lau, "Gene expression profiling in the lung and liver of PFOA-exposed mouse fetuses," *Toxicology*, vol. 239, no. 1-2, pp. 15–33, 2007.

[15] B. H. Alexander, G. W. Olsen, J. M. Burris, J. H. Mandel, and J. S. Mandel, "Mortality of employees of a perfluorooctanesulphonyl fluoride manufacturing facility," *Occupational and Environmental Medicine*, vol. 60, no. 10, pp. 722–729, 2003.

[16] B. H. Alexander and G. W. Olsen, "Bladder cancer in perfluorooctanesulfonyl fluoride manufacturing workers," *Annals of Epidemiology*, vol. 17, no. 6, pp. 471–478, 2007.

[17] M. M. Grice, B. H. Alexander, R. Hoffbeck, and D. M. Kampa, "Self-reported medical conditions in perfluorooctanesulfonyl fluoride manufacturing workers," *Journal of Occupational and Environmental Medicine*, vol. 49, no. 7, pp. 722–729, 2007.

[18] R. Monroy, K. Morrison, K. Teo, et al., "Serum levels of perfluoroalkyl compounds in human maternal and umbilical cord blood samples," *Environmental Research*, vol. 108, no. 1, pp. 56–62, 2008.

[19] C. Fei, J. K. McLaughlin, R. E. Tarone, and J. Olsen, "Perfluorinated chemicals and fetal growth: a study within the Danish National Birth Cohort," *Environmental Health Perspectives*, vol. 115, no. 11, pp. 1677–1682, 2007.

[20] OECD, "Hazard assesment of perfluorooctane sulfonate (PFOS) and its salts," Tech. Rep. ENV/JM/RD(2002)17/FINAL, 2002, http://www.oecd.org/dataoecd/23/18/2382880.pdf.

[21] DuPont, "DuPont progress report on PFOA phase out," December 2008, http://www2.dupont.com/PFOA2/en_US/assets/downloads/pdf/PFOAProgressReport20081212.pdf.

[22] H. Fromme, S. A. Tittlemier, W. Völkel, M. Wilhelm, and D. Twardella, "Perfluorinated compounds—exposure assessment for the general population in western countries," *International Journal of Hygiene and Environmental Health*, vol. 212, no. 3, pp. 239–270, 2009.

[23] S. A. Tittlemier, K. Pepper, C. Seymour, et al., "Dietary exposure of Canadians to perfluorinated carboxylates and perfluorooctane sulfonate via consumption of meat, fish, fast foods, and food items prepared in their packaging," *Journal of Agricultural and Food Chemistry*, vol. 55, no. 8, pp. 3203–3210, 2007.

[24] H. Fromme, M. Schlummer, A. Möller, et al., "Exposure of an adult population to perfluorinated substances using duplicate diet portions and biomonitoring data," *Environmental Science & Technology*, vol. 41, no. 22, pp. 7928–7933, 2007.

[25] I. Ericson, R. Martí-Cid, M. Nadal, B. van Bavel, G. Lindström, and J. L. Domingo, "Human exposure to perfluorinated chemicals through the diet: Intake of perfluorinated compounds in foods from the Catalan (Spain) market," *Journal of Agricultural and Food Chemistry*, vol. 56, no. 5, pp. 1787–1794, 2008.

[26] UK Food Standards Agency, "Fluorinated chemicals: UK dietary intakes," *Food Survey Information Sheet*, June 2006, http://www.food.gov.uk/multimedia/pdfs/fsis1106.pdf.

[27] P. de Voogt, F. W. M. van der Wielen, J. Westerveld, W. D'Hollander, and L. Bervoets, "Determination of perfluorinated organic compounds in food and dust," in *Proceedings of the 28th International Symposium on Halogenated Persistent Organic Pollutants (Dioxin '08)*, vol. 70 of *Organohalogen Compounds*, p. 714, Birmingham, UK, August 2008.

[28] A. Gulkowska, Q. Jiang, M. K. So, S. Taniyasu, P. K. S. Lam, and N. Yamashita, "Persistent perfluorinated acids in seafood collected from two cities of China," *Environmental Science & Technology*, vol. 40, no. 12, pp. 3736–3741, 2006.

[29] National Institute of Nutrition and Seafood Research, "Low levels of perfluorinated compounds in seafood," August 2009, http://www.nifes.no/index.php?page_id=126&article_id=2750&lang_id=2.

[30] National Institute of Nutrition and Seafood Research, "Seafood data," August 2009, http://www.nifes.no/index.php?page_id=137&lang_id=2.

[31] J. Hölzer, O. Midasch, K. Rauchfuss, et al., "Biomonitoring of perfluorinated compounds in children and adults exposed to perfluorooctanoate-contaminated drinking water," *Environmental Health Perspectives*, vol. 116, no. 5, pp. 651–657, 2008.

[32] T. I. Halldorsson, C. Fei, J. Olsen, L. Lipworth, J. K. Mclaughlin, and S. F. Olsen, "Dietary predictors of perfluorinated chemicals: a study from the Danish National Birth Cohort," *Environmental Science & Technology*, vol. 42, no. 23, pp. 8971–8977, 2008.

[33] J. Falandysz, S. Taniyasu, A. Gulkowska, N. Yamashita, and U. Schulte-Oehlmann, "Is fish a major source of fluorinated surfactants and repellents in humans living on the Baltic coast?" *Environmental Science & Technology*, vol. 40, no. 3, pp. 748–751, 2006.

[34] M. Brustad, K. Edvardsen, T. Wilsgaard, O. Engelsen, L. Aksnes, and E. Lund, "Seasonality of UV-radiation and vitamin D status at 69 degrees north," *Photochemical & Photobiological Sciences*, vol. 6, no. 8, pp. 903–908, 2007.

[35] C. Rylander, T. M. Sandanger, and M. Brustad, "Associations between marine food consumption and plasma concentrations of POPs in a Norwegian coastal population," *Journal of Environmental Monitoring*, vol. 11, no. 2, pp. 370–376, 2009.

[36] A. Hjartåker, L. F. Andersen, and E. Lund, "Comparison of diet measures from a food-frequency questionnaire with measures from repeated 24-hour dietary recalls. The Norwegian Women and Cancer Study," *Public Health Nutrition*, vol. 10, no. 10, pp. 1094–1103, 2007.

[37] A. Hjartåker, E. Lund, and K. S. Bjerve, "Serum phospholipid fatty acid composition and habitual intake of marine foods registered by a semi-quantitative food frequency questionnaire," *European Journal of Clinical Nutrition*, vol. 51, no. 11, pp. 736–742, 1997.

[38] C. R. Powley, S. W. George, M. H. Russell, R. A. Hoke, and R. C. Buck, "Polyfluorinated chemicals in a spatially and temporally integrated food web in the Western Arctic," *Chemosphere*, vol. 70, no. 4, pp. 664–672, 2008.

[39] U. Berger and M. Haukås, "Validation of a screening method based on liquid chromatography coupled to high-resolution mass spectrometry for analysis of perfluoroalkylated substances in biota," *Journal of Chromatography A*, vol. 1081, no. 2, pp. 210–217, 2005.

[40] G. Arsenault, B. Chittim, A. McAlees, R. McCrindle, N. Riddell, and B. Yeo, "Some issues relating to the use of perfluorooctanesulfonate (PFOS) samples as reference standards," *Chemosphere*, vol. 70, no. 4, pp. 616–625, 2008.

[41] R. D. Helsel, *Nondetects and Data Analysis: Statistics for Censored Environmental Data*, John Wiley & Sons, Hoboken, NJ, USA, 2005.

[42] E. A. Emmett, F. S. Shofer, H. Zhang, D. Freeman, C. Desai, and L. M. Shaw, "Community exposure to perfluorooctanoate: relationships between serum concentrations and exposure sources," *Journal of Occupational and Environmental Medicine*, vol. 48, no. 8, pp. 759–770, 2006.

[43] D. Engeset, E. Alsaker, A. Ciampi, and E. Lund, "Dietary patterns and lifestyle factors in the Norwegian EPIC cohort: the Norwegian Women and Cancer (NOWAC) study," *European Journal of Clinical Nutrition*, vol. 59, no. 5, pp. 675–684, 2005.

[44] D. Mozaffarian, "Fish intake, contaminants, and human health: evaluating the risks and the benefits part 2- health risks and optimal intakes," *CardiologyRounds*, vol. 10, no. 9, 2006.

[45] B. M. Yashodhara, S. Umakanth, J. M. Pappachan, S. K. Bhat, R. Kamath, and B. H. Choo, "Omega-3 fatty acids: a comprehensive review of their role in health and disease," *Postgraduate Medical Journal*, vol. 85, no. 1000, pp. 84–90, 2009.

[46] K. Kato, A. M. Calafat, and L. L. Needham, "Polyfluoroalkyl chemicals in house dust," *Environmental Research*, vol. 109, no. 5, pp. 518–523, 2009.

[47] K. Kannan, "Perfluorinated compounds: from frying pans to polar bears," *Reproductive Toxicology*, vol. 27, no. 3-4, p. 413, 2009.

[48] A. Kärrman, K. H. Harada, K. Inoue, T. Takasuga, E. Ohi, and A. Koizumi, "Relationship between dietary exposure and serum perfluorochemical (PFC) levels—a case study," *Environment International*, vol. 35, no. 4, pp. 712–717, 2009.

[49] G. W. Olsen, T. R. Church, J. P. Miller, et al., "Perfluorooctanesulfonate and other fluorochemicals in the serum of American Red Cross adult blood donors," *Environmental Health Perspectives*, vol. 111, no. 16, pp. 1892–1901, 2003.

[50] L. S. Haug, C. Thomsen, and G. Becher, "Time trends and the influence of age and gender on serum concentrations of perfluorinated compounds in archived human samples," *Environmental Science & Technology*, vol. 43, no. 6, pp. 2131–2136, 2009.

[51] I. Ericson, M. Gómez, M. Nadal, B. van Bavel, G. Lindström, and J. L. Domingo, "Perfluorinated chemicals in blood of residents in Catalonia (Spain) in relation to age and gender: a pilot study," *Environment International*, vol. 33, no. 5, pp. 616–623, 2007.

[52] C. Rylander, P. Duong Trong, J. O. Odland, and T. M. Sandanger, "Perfluorinated compounds in delivering women from south central Vietnam," *Journal of Environmental Monitoring*, vol. 11, pp. 2002–2008, 2009.

[53] A. M. Calafat, L. Y. Wong, Z. Kuklenyik, J. A. Reidy, and L. L. Needham, "Polyfluoroalkyl chemicals in the U.S. population: data from the National Health and Nutrition Examination Survey (NHANES) 2003-2004 and comparisons with NHANES 1999-2000," *Environmental Health Perspectives*, vol. 115, no. 11, pp. 1596–1602, 2007.

[54] L. W. Y. Yeung, M. K. So, G. Jiang, et al., "Perfluorooctanesulfonate and related fluorochemicals in human blood samples from China," *Environmental Science & Technology*, vol. 40, no. 3, pp. 715–720, 2006.

[55] A. Kärrman, J. F. Mueller, B. van Bavel, F. Harden, L.-M. L. Toms, and G. Lindström, "Levels of 12 perfluorinated chemicals in pooled Australian serum, collected 2002-2003, in relation to age, gender, and region," *Environmental Science & Technology*, vol. 40, no. 12, pp. 3742–3748, 2006.

[56] L. Tao, K. Kannan, C. M. Wong, K. F. Arcaro, and J. L. Butenhoff, "Perfluorinated compounds in human milk from Massachusetts, U.S.A.," *Environmental Science & Technology*, vol. 42, no. 8, pp. 3096–3101, 2008.

[57] W. Völkel, O. Genzel-Boroviczény, H. Demmelmair, et al., "Perfluorooctane sulphonate (PFOS) and perfluorooctanoic acid (PFOA) in human breast milk: results of a pilot study," *International Journal of Hygiene and Environmental Health*, vol. 211, no. 3-4, pp. 440–446, 2008.

[58] A. Kärrman, I. Ericson, B. van Bavel, et al., "Exposure of perfluorinated chemicals through lactation: levels of matched human milk and serum and a temporal trend, 1996–2004, in Sweden," *Environmental Health Perspectives*, vol. 115, no. 2, pp. 226–230, 2007.

[59] O. Midasch, H. Drexler, N. Hart, M. W. Beckmann, and J. Angerer, "Transplacental exposure of neonates to perfluorooctanesulfonate and perfluorooctanoate: a pilot study," *International Archives of Occupational and Environmental Health*, vol. 80, no. 7, pp. 643–648, 2007.

[60] B. J. Apelberg, F. R. Witter, J. B. Herbstman, et al., "Cord serum concentrations of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in relation to weight and size at birth," *Environmental Health Perspectives*, vol. 115, no. 11, pp. 1670–1676, 2007.

[61] J. P. Benskin, M. Bataineh, and J. W. Martin, "Simultaneous characterization of perfluoroalkyl carboxylate, sulfonate, and sulfonamide isomers by liquid chromatography-tandem mass spectrometry," *Analytical Chemistry*, vol. 79, no. 17, pp. 6455–6464, 2007.

[62] K. S. Guruge, S. Taniyasu, N. Yamashita, et al., "Perfluorinated organic compounds in human blood serum and seminal plasma: a study of urban and rural tea worker populations in Sri Lanka," *Journal of Environmental Monitoring*, vol. 7, no. 4, pp. 371–377, 2005.

[63] C. Kubwabo, N. Vais, and F. M. Benoit, "A pilot study on the determination of perfluorooctanesulfonate and other perfluorinated compounds in blood of Canadians," *Journal of Environmental Monitoring*, vol. 6, no. 6, pp. 540–545, 2004.

Supplemental material for the article

Dietary predictors and plasma concentrations of perfluorinated compounds in a coastal population from northern Norway

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Table S1. Target analytes and native analytical standards, their abbreviation, quantification masses and cone voltages.

Compound	Abbreviation	Quantification mass (*m/z*) (cone voltage (V))
3,5-Bis(trifluoromethyl)phenyl acetic acid[a]	BTPA	227.03 (35)
Mass labeled perfluorooctanoate[b]	[$^{13}C_4$]PFOA	372.98 (35)
Mass labeled perfluorooctane sulfonate[b]	[$^{13}C_4$]PFOS	502.93 (50)
Perfluorooctane sulfonic acid[c]	PFOSA	497.95 (35)
Perfluorohexane sulfonate[c]	PFHxS	398.94 (50)
Perfluoroheptane sulfonate[c]	PFHpS	449.12 (50)
Perfluorooctane sulfonate branched isomers	PFOS branched	498.93 (50)
Perfluorooctane sulfonate linear isomer[c]	PFOS linear	498.93 (50)
Perfluoroheptanoate[c]	PFHpA	318.98 (35)
Perfluorooctanoate[c]	PFOA	368.98 (35)
Perfluorononanoate[c]	PFNA	418.97 (35)

[a]Used as recovery standard.
[b]Used as internal standard.
[c]Used as native analytical standard.

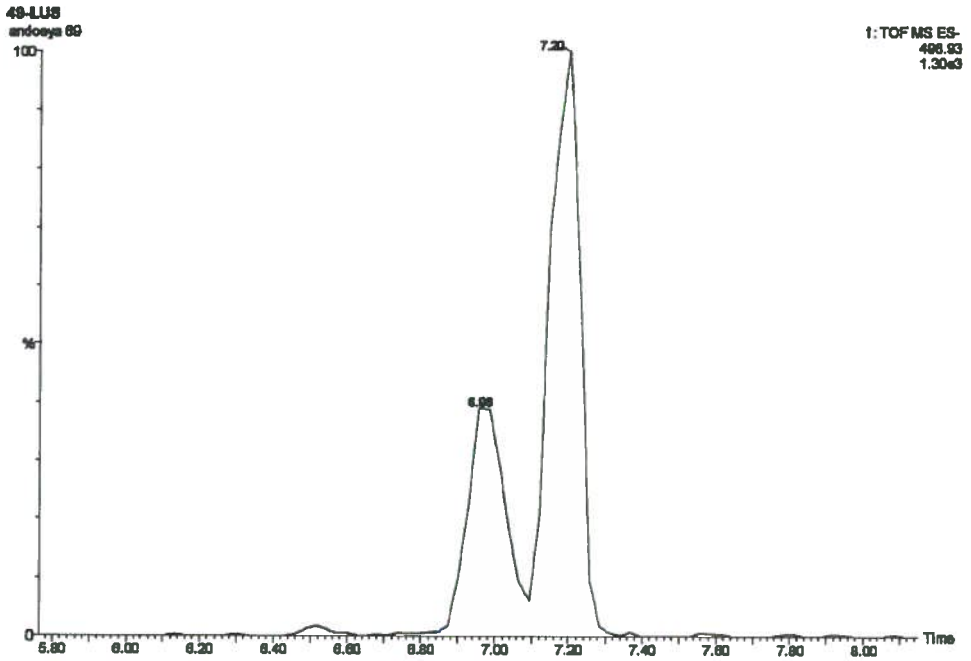

Figure S1. Chromatogram of branched (RT = 6.96) and linear PFOS (RT = 7.20) in a human plasma sample.

Table S2. Correlation coefficients for the investigated PFCs.

	PFOS	PFOA	PFHxS	PFHpS	PFNA	PFOSA
PFOS	-	0.56***	0.59***	0.93***	0.70***	0.45***
PFOA	0.56***	-	0.53***	0.56***	0.37**	0.18
PFHxS	0.59***	0.53***	-	0.56***	0.46***	0.23
PFHpS	0.93***	0.56***	0.56***	-	0.65***	0.41**
PFNA	0.70***	0.37**	0.46***	0.65***	-	0.72***
PFOSA	0.45***	0.18	0.23	0.41**	0.72***	-

***p <0.001.
**p <0.01.
*p <0.05.
PFOS, perfluorooctane sulfonate; PFOA, perfluorooctanoate; PFHxS, perfluorohexane sulfonate;PFHpS perfluoroheptane sulfonate; PFNA perfluorononanoate; PFOSA, perfluorooctane sulfonic acid.

Table S3. Intake frequencies of fatty fish and fruit and vegetables and corresponding median PFOS and PFHpS concentration (ng/ml) in the study group.

Servings (150 g) of fatty fish /week	Intake frequency*	Median PFOS (ng/ml) (range)	Median PFHpS (ng/ml) (range)
0	15	28 (12–52)	0.52 (0.10–1.2)
<1	33[a]	29 (6.9–99)	0.44 (0.12–1.9)
1–2	6	25 (16–33)	0.41(0.27–0.89)
2–3	1	67	1.3
3–4	0	–	–
4–5	0	–	–
5–6	1[a]	93	1.8
Servings (150 g) of fruit and vegetables/day	**Intake frequency***	**Median PFOS (ng/ml) (range)**	**Median PFHpS (ng/ml) (range)**
0	0	–	–
<1	3	37 (37–99)	0.62 (0.58–1.9)
1–2	11	24 (14–52)	0.43 (0.17–1.2)
2–3	21[a]	29 (6.9–93)	0.52 (0.12–1.8)
3–4	13	30 (16–52)	0.45 (0.25–0.92)
4–5	4	28 (12–31)	0.47 (0.10–0.89)
5–6	2	34 (19–50)	0.39 (0.23–0.50)
6–7	2	14 (12–15)	0.26 (0.26–0.27)

*Two outliers were excluded from the statistical analysis. [a] indicates which groups the outliers belonged to.

Paper II

Dietary patterns and plasma concentrations of perfluorinated compounds in 315 Norwegian women: the NOWAC postgenome study

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Brief

The relationship between plasma concentrations of perfluorinated compounds and self-reported dietary patterns was assessed in a representative group of middle-aged women.

Abstract

This study was undertaken to assess the impact of self-reported dietary habits and lifestyle on the plasma concentration of selected perfluorinated compounds (PFCs) in a representative group of 315 middle-aged Norwegian women (48-62 years of age). Perfluorooctane sulphonate (PFOS; median: 20 ng/ml), perfluorooctanoate (PFOA; 4.4 ng/ml), perfluorohexane sulphonate (PFHxS; 1.0 ng/ml) and perfluorononanoate (PFNA; 0.81ng/ml) were detected in more than 90% of the samples. By using multivariate data analysis, women who ate fish or "fish eaters" (high consumers of fish and shellfish) were identified as having increased plasma concentrations of PFOS, PFNA and PFHxS. Younger women with a larger household and a "western" diet consisting of rice, pasta, water, white and red meat, chocolate, ice cream, cheese and pastries had lower concentrations of the same compounds. No specific food cluster was associated with increased PFOA concentrations, indicating that the dietary impact on PFOA concentrations was different from that of the other investigated PFCs. This study confirms that the total diet is a major contributor to human body burdens of selected PFCs, but the identification of dietary predictors is highly dependent on the dietary habits within the population studied due to the ubiquitous presence of PFCs in many kinds of food.

Introduction

In 1999, Olsen et al. reported high serum levels of perfluorooctane sulphonate (PFOS) from workers in fluoropolymer industries [1]. Previous investigations had detected organofluorine in human blood samples already in the 1960's, but no compound specific analyses were made until the 1990's. Over the last decade, numerous articles have been published on perfluorinated compounds (PFCs) in human blood, providing evidence that PFCs are widespread pollutants. PFOS is usually the major PFC in human blood samples but perfluorooctanoate (PFOA), perfluorohexane sulphonate (PFHxS) and perfluorononanoate (PFNA) are also frequently detected. PFCs have unique chemical properties (repelling both water and fat) and have therefore been widely used as surfactants and surface treatment products in industry and in consumer products. In Norway, PFOS has mainly been used in fire fighting foam on oil rigs although there is limited information available about the use of the other PFCs [2]. Concerns were raised since several PFCs were found to bioaccumulate and magnify in the food chain [3]. Toxicological findings showed that PFOS and PFOA act as peroxisome preliferators and interfere with fatty acid metabolism in rodents [4]. Yet, no association was found for place of work (as an exposure marker), bladder cancer, mortality and self-reported health in workers from fluorochemical industries [5-7]. However, PFOA exposure was associated with increased mortality of prostate cancer, cerebrovascular disease and diabetes among occupationally exposed workers, although the results were inconclusive [8]. Some of the PFCs have also been associated with indicators of metabolic syndrome in a general population [9]. After more than 40 years of production, 3M, the major producer of PFOS voluntarily withdrew it from production in year 2000 [10]. Other producers followed, and the phasing out of PFOA and PFOA precursors has begun [11].

Higher concentrations of PFCs have been reported from some industrial countries than from parts of the developing world, indicating that PFCs are lifestyle related pollutants [12]. The exposure scenario for humans are, however, complex since PFCs have been detected in many kinds of food [13], house hold dust [14], a number of consumer products [15] and drinking water [16]. The diet has recently been considered the major pathway [17] and animal products seem to contribute to a larger extend than vegetarian foodstuffs [18] [13]. There are only a limited number of studies that investigate the relationship between self-reported dietary intake and plasma concentrations of PFCs, and the findings are contradictory [19] [20]. Intake of fish has been identified as a possible source [20], but also red meat, snacks and animal fat were associated with increased plasma concentrations of some PFCs [19]. So far, focus has mainly been on specific foodstuffs, while dietary patterns (food clusters) have not been evaluated. This study was undertaken to assess the impact of a large number of self-reported dietary and lifestyle related variables on plasma concentrations of PFCs using food clusters. Plasma concentrations of marine-derived omega-3 fatty acids were also assessed as a biomarker for fatty fish intake.

Materials and Methods

Study population and collection of blood samples

The women taking part in the current study were all participants of the Norwegian Women And Cancer Study (NOWAC), which consists of 172 471 Norwegian women aged 30-70 years [21]. All participants answered a detailed questionnaire about diet and lifestyle and approximately 50 000 also donated a blood sample. The latter constitute the NOWAC postgenome cohort, and detailed information about the study group and the blood collection procedures have been reported elsewhere [22]. At the time of blood sampling, the participants filled out a two-page questionnaire regarding current health status. From the 50 000 women who delivered a blood sample, a random sample of 500 women (born between 1943-1957, blood drawn in 2005) was used for analysis of fatty acids, gene expressions, sex hormones and environmental contaminants. In total, 326 samples fulfilled the inclusion criteria and were analyzed for PFCs (mean age 56 years). Study group characteristics and the self-reported dietary intake are reported in Table S1 and S2 in the Supporting Information. Eleven women had missing values on the omega-3 fatty acid concentration, so the total number included in the statistical analysis was 315.

Analysis of PFCs

Perfluorinated compounds were determined in the plasma samples using sonication-facilitated liquid-liquid extraction, activated charcoal clean-up and analysis on high performance liquid chromatography (HPLC)-QTOF-MS (Waters, Milford, MA, USA). Mass labeled internal standards were used in the analysis. The method is described in detail by Rylander et al. [20].

Analysis of fatty acids

The fatty acid compositions of total lipids in plasma were analyzed by a modified gas chromatography method described in detail by Araujo et al. [23].

Quality control of chemical analysis

The quality of the PFC analysis was assured through repetitive analysis of blank samples and reference samples from previous rounds of international comparison programs. For each batch of 30 samples, one reference material and two blank samples were prepared. Three times a year, our laboratory participates in the AMAP Ringtest for Persistent Organic Pollutants in Human Serum, an international comparison program, organized by Institut National de Santé Publique du Québec, Canada. Results from interlaboratory comparisons indicate that the uncertainties of our analysis are well within +/- 30% of the assigned values. Recovery rates of the internal standard varied between 60% and 150% due to matrix induced ion suppression of the recovery standard signal.

The limit of detection (LOD) was automatically calculated by the quantification software. PFOS, PFOA and PFHxS were detected in a few blank samples. If the concentration of these compounds in the blank samples were larger than the software determined LOD for that batch of samples, LOD would be determined from three times the concentration of the analytes in the blanks.

The linear PFOS isomer was chromatographically separated from the branched isomers and quantified, both separately and as the sum of all isomers. The co-elution of branched isomers (one peak) was not structurally elucidated but rather identified as eluting earlier than the linear PFOS. This has been described in detail elsewhere [20]. Isomer specification was not performed for the other PFCs, where the linear isomer clearly dominated. Data presented as "PFOS" consist of the sum of the linear and the co-eluted peak of branched isomers. Similar response factors have been reported for branched and linear isomers of PFOS [24], so the mass labeled "internal standard for linear PFOS" was used for quantification of the branched isomer as well.

The quality of the fatty acid analysis method has been comprehensively evaluated through a factorial design involving all critical steps and by the use of certified reference materials [23].

Statistical analysis

Statistical analysis were performed using the freely available software R version 2.8.1 (http://www.cran.r-project.org) with the NADA package for R and the multivariate data analysis software "the Unscrambler", version 7.5 (Camo Software Inc., NJ, USA). Censored statistics (Kaplan-Meier models) were used for finding central tendency (Table 1) for analytes that were detected in less than 90% of the samples (PFOSA and PFHpS). Fifty different food stuffs and lifestyle variables were evaluated as possible predictors of PFC concentration (Table S1 and S2 in the Supporting Information). All contaminant data were right-skewed and log transformed to achieve normality before statistical analysis. Partial Least Square Regression (PLS-R) was used for evaluating the impact of the dietary and lifestyle-related variables on plasma concentrations of PFCs. Two different PLS models were used: PLS1 which handles one *y* variable and PLS2 which can handle several. To further investigate the results from the PLS regressions, the study group was divided into three equal sized groups according to their consumption of total marine food (lean fish, fatty fish, processed fish products, fish spread, other fish, fish roe, fish liver and shellfish). Analysis of variance (ANOVA) with Tukeys' honestly significant difference (HSD) post-hoc test was used for comparing differences between intake groups. To assess the relationship between the selected PFCs and plasma levels of omega-3, linear regression models were applied. Diagnostic plots of the residuals were evaluated to ensure that model assumptions were met. To avoid biased findings, only analytes that were detected in more than 90% of the samples were evaluated with multivariate data analysis. The p-values < 0.05 were considered to be significant.

Results

Plasma concentration of PFCs and marine derived omega-3 fatty acids are provided in Table 1. PFOS, PFNA and PFHxS co-varied and were positively associated to age and a cluster of marine food variables (shellfish, lean fish, other fish, fish raw, fish liver and fish spread – Figures 1 and S1 in the Supporting Information). A cluster of foodstuffs (ice cream, cheese, beef, water, white meat, rice, pasta, pastries and chocolate) and the number of people in the household were negatively associated with the same compounds. Individual PLS1 regressions of PFOS, PFHxS and PFNA (figures not shown) confirmed the results from the PLS2 regression. Of the variation in PFOS concentration in the study

group, 30% were explained by the investigated dietary and lifestyle-related variables. The corresponding numbers for PFNA and PFHxS were 26% and 21%, respectively. PFOA did not co-vary with the other investigated PFCs and were therefore evaluated individually. The dietary and lifestyle-associated variables explained 21% of the variation in PFOA concentration. No specific food cluster was found for PFOA, but intake of shellfish and chocolate and low consumption of coffee were positively associated with PFOA (Figure 2 and S2 in the Supporting Information).

When comparing differences in low, medium and high consumers of total marine food, it was found that high consumers were older and had significantly higher concentrations of PFOS and PFNA than the low consumers (Table 2). There was no significant difference in PFOA and PFHxS concentrations between the high and low consumers of total marine food. No association between plasma concentrations of the investigated PFCs and marine derived omega 3 fatty acids was found in the current study group. When adjusting for intake of fish liver oil and fish oil pills as food supplements, the results remained the same.

Discussion

By using multivariate data analysis, "fish eaters" (women who ate different kinds of fish and shellfish) were identified as having increased plasma concentrations of PFOS, PFNA and PFHxS (Figure 1). Younger women with a larger household and with a diet consisting of rice, pasta, water, white and red meat, chocolate, ice cream, cheese and pastries had lower concentrations of the same compounds (Figure 1). The latter food cluster was previously identified as "western eaters" in a study on dietary patterns within the NOWAC cohort [25]. The "fish eaters" were also identified as a food cluster in that same study. When comparing differences in PFC concentrations among low, medium and high consumers of total marine food, significantly higher concentrations of PFOS and PFNA were observed in the high consumers compared with the low consumers (Table 2). The magnitude of the difference between the intake groups was, however, marginal (median19 ng PFOS/ml in the low consumers of marine food compared to 21 ng/ml in the high consumers). It has to be emphasized that the current study group is characterized by a high intake of fish. This is especially evident when comparing the results with a similar study by Haldorsson et al. who identified red meat, animal fat and snacks as dietary predictors of PFOS and PFOA in a group of 1076 pregnant Danish women (the Danish National Birth Cohort). Only 19% of the women in the Danish National Birth Cohort (DBNC) consumed more than 30 g marine food/day, while 33% of the present study group consumed more than 100 g/day [26]. On the other hand, the Danish women consumed 69g red meat/day (not further defined) whereas the Norwegians ingested 42g of beef and processed meat products/day. There are in general differences in dietary habits between genders, age groups and both between and within countries. As PFCs seem to be ubiquitous in many kinds of food [13, 18, 27], but especially in animal products, it is likely that the identification of dietary predictors depends highly on the dietary habits within the population studied. Hence, fish will be identified as a significant predictor for PFCs in a fish-eating population, whereas meat will become a predictor in a group of high meat consumers. Among Canadian Inuit, traditional foodstuffs (mainly reindeer meat, arctic char and beluga skin and fat) were identified as major sources of

PFCs [28]. The levels of PFCs in reindeer and ringed seal meat were, however, comparable to levels detected in store-bought meat, suggesting that the intake amounts were more important than the actual levels in each foodstuff. Also cookies and processed cheese were identified as PFC sources, which again, could be a result of large consumption of these foodstuffs.

Interestingly, PFOA behaved differently from the other investigated PFCs and no specific food cluster was identified as having a strong correlation with increased PFOA concentrations. Intake of shellfish and chocolate and low consumption of coffee were, however, positively related to PFOA. Together with the fact that PFOA was not correlated to the other investigated PFCs (which co-varied strongly), this indicates that dietary sources are different for PFOA. Despite this, a large proportion of the variance was mainly explained by dietary variables (21%, Figure 2), suggesting that the total diet is an important contributor to human PFOA exposure. It also indicates that the concentrations of PFOA are uniform between women with different dietary habits. A large proportion of the variation in PFOS, PFNA and PFHxS concentration (30%, 26% and 21%, respectively) was mainly explained by the self-reported dietary variables. Thus, the diet seems to be a major contributor to the body burden of these PFCs, and in the current fish-eating study group, marine food specifically contributed to increased concentrations of PFOS, PFNA and PFHxS. Kärrman et al. measured PFCs in one-day composite food samples and related them to human plasma concentrations in two different regions of Japan [29]. They concluded that the diet accounted for 22- 110% of the plasma levels of PFOS and PFOA, but the importance of the diet varied between geographical regions even within a country.

In the current study there was no association between plasma concentrations of omega-3 fatty acids (as a biomarker for fatty fish intake) and the selected PFCs, indicating that, in contrast to the fat-soluble legacy persistent organic pollutants (POPs), the plasma levels of PFCs were not associated exclusively with fatty fish intake, but consumption of total marine food was important. This is shown in Figure 1. Fatty fish had previously been identified as a possible source of PFOS and PFHpS in a Norwegian coastal population (known for being high consumers of marine food, n=56), but the results were inconclusive [20]. Falandysz et al. also identified fish from the Baltic Sea as an important source of PFCs for Polish people by comparing PFC levels in a group of high consumers of Baltic fish (n=15) with a reference population (n=15) with unknown dietary habits [30]. Weihe et al. found a significant association between monthly numbers of pilot whale dinners and serum concentrations of PFOS, PFNA and perfluorodecanoic acid (PFDeA) and concluded that pilot whale was a more important source of PFCs than fish in the Faroe Islands [31]. It is important to emphasize that there are many advantages to investigating the dietary impact on the body burdens of PFCs in a large, representative, population based study (NOWAC, DNBC) rather than in specific groups of people, who are recruited to the study due to their special dietary habits. Results from the latter studies could easily be misleading in a public health perspective, because the dietary habits investigated do not represent those of the total population. Berger et al. [32] reported recently that fish from contaminated waters in Sweden may be a significant source of

PFOS, however, this is only valid for high consumers of fish from these waters and not the general population.

Some of the lifestyle associated variables (parity, smoking and total months of nursing) had no impact on the PFCs levels, which could result from the homogeneity of the study group. All participating women were around the same age (48-62 years), few were current smokers, most of them had two children and it was many years since they last gave birth and nursed. The similarities could therefore wipe out the possible effects of these lifestyle variables.

The concentrations of PFCs in the current study were slightly lower but comparable to levels previously reported from northern Norway [20] (20 ng/ml PFOS (blood drawn in 2004) versus 24 ng/ml (2005)). The PFOS concentrations were also in the same range or slightly lower than levels in women from the USA [33], Sweden [34], Denmark [19], Japan [12], Canada [35] and Australia [36], but considerable higher than in women from Vietnam [37], some parts of China [38], Brazil [12], India [12], and Malaysia [12]. Large within-country differences have been reported from China [38], Germany [39, 40] and Japan [29], indicating that a population based study gives the best estimate of the general contaminant load within a country. This is a major advantage with the current study because the participating women were randomly recruited from the whole country, representing Norwegian middle-aged women.

The current study group had a median percentage of linear PFOS isomers of 70% (range 45-87%), comparable to a previous study from northern Norway (69%) [20] and to a study group from Sweden (68%) [41], although considerable different from the percentage of linear PFOS in Vietnamese women (81%) [37], and in samples from UK and Australia (both 59%) [41]. In technical grade PFOS, the percentage of straight-chain PFOS is approximately 70-80%. Powley et al. [42] observed different isomer patterns of PFOS in seal and fish tissues from the western Arctic, suggesting that different species had various elimination rates for the branched-chain PFOS isomers. To the best of our knowledge there is no study investigating the isomer distribution of PFOS in food. Differences in elimination rate/bioaccumulation potential could result in different isomer compositions in food, which again will influence the proportion of linear PFOS in human blood samples. Good data on isomer distribution are thus essential to help identifying important sources of exposure.

There are many positive health effects related to high intake of fish, e.g. stable vitamin D status [43] and reduced risk of cardiovascular diseases and incident heart failure [44-46]. There are, in addition, only inconclusive epidemiological findings about adverse health effects from PFC exposure among occupationally exposed workers, who were exposed to higher concentrations than the general population [5-8]. There is therefore a need for a more thorough understanding of the effect of PFCs on public health. The most common cause of death in Norway during the period 1995-2006 was cardiovascular diseases [47]. With the current worldwide obesity epidemic, focus of concern for public health should be on the risks of a "no fish diet" rather than the marginally higher PFC concentrations a

fish-rich diet could imply for a fish eating population, especially as being a "fish eater" has only a marginal effect on PFC levels.

Acknowledgements

We gratefully acknowledge all participating women in NOWAC, the medical personnel helping out with data collection and Bente Augdal and Merete Albertsen for administrative work during the collection of data. Sandra Huber and Morten Moe has operated the LC-MS instrument and Mikael Harju has contributed with valuable knowledge in multivariate data analysis. This work was supported by grants from the Norwegian Research Council, University of Tromsø and The Royal Ministry of Fisheries and Coastal Affairs.

Supporting Information

Study group characteristics, weekly intake of the investigated foodstuffs and the score plots for the PLS regressions are provided in the Supporting Information.

References

1. Olsen, G.W.; Burris, J.M.; Mandel, J.H.; Zobel, L.R. Serum perfluorooctane sulfonate and hepatic and lipid clinical chemistry tests in fluorochemical production employees. *J. Occup. Environ. Med.* **1999**, *41*, 799-806.
2. Herzke, D.; Schlabach, M.; Mariussen, E.; Uggerud, H.; Heimstad, E., A literature survey on selected chemical compounds. Norwegian Pollution Control Authority, Oslo, Norway. Report no TA-2238/2007, **2007**; pp 8-65.
3. Haukas, M.; Berger, U.; Hop, H.; Gulliksen, B.; Gabrielsen, G.W. Bioaccumulation of per- and polyfluorinated alkyl substances (PFAS) in selected species from the Barents Sea food web. *Environ. Pollut.* **2007**, *148*, 360-371.
4. Jensen, A.A.; Leffers, H. Emerging endocrine disrupters: perfluoroalkylated substances. *Int. J. Androl.* **2008**, *31*, 161-169.
5. Alexander, B.H.; Olsen, G.W. Bladder Cancer in Perfluorooctanesulfonyl Fluoride Manufacturing Workers. *Ann. Epidemiol.* **2007**, *17*, 471-478.
6. Alexander, B.H.; Olsen, G.W.; Burris, J.M.; Mandel, J.H.; Mandel, J.S. Mortality of employees of a perfluorooctanesulphonyl fluoride manufacturing facility. *Occup. Environ. Med.* **2003**, *60*, 722-729.
7. Grice, M.M.; Alexander, B.H.; Hoffbeck, R.; Kampa, D.M. Self-reported medical conditions in perfluorooctanesulfonyl fluoride manufacturing workers. *J. Occup. Environ. Med.* **2007**, *49*, 722-729.
8. Lundin, J.I.; Alexander, B.H.; Olsen, G.W.; Church, T.R. Ammonium Perfluorooctanoate Production and Occupational Mortality. *Epidemiology.* **2009**, *20*, 921-928.
9. Lin, C.Y.; Chen, P.C.; Lin, Y.C.; Lin, L.Y. Association Among Serum Perfluoroalkyl Chemicals, Glucose Homeostasis, and Metabolic Syndrome in Adolescents and Adults. *Diabetes Care.* **2009**, *32*, 702-707.
10. US Environmental Protection Agency. EPA and 3M announce phase out of PFOS. **2000**. Available at: http://yosemite.epa.gov/opa/admpress.nsf/0/33aa946e6cb11f35852568e1005246b4 (accessed 12 January 2010)
11. DuPont. DuPont Progress Report on PFOA Phase Out. **2008.** Available at: http://www2.dupont.com/PFOA2/en_US/assets/downloads/pdf/PFOAProgressReport20081212.pdf (accessed 12 January 2010)
12. Kannan, K.; Corsolini, S.; Falandysz, J.; Fillmann, G.; Kumar, K.S.; Loganathan, B.G., et al. Perfluorooctanesulfonate and related fluorochemicals in human blood from several countries. *Environ. Sci. Techn.* **2004**, *38*, 4489-4495.
13. Ericson, I.; Marti-Cid, R.; Nadal, M.; Van Bavel, B.; Lindstrom, G.; Domingo, J.L. Human exposure to perfluorinated chemicals through the diet: Intake of perfluorinated compounds in foods from the Catalan (Spain) Market. *J. Agric. Food Chem.* **2008**, *56*, 1787-1794.
14. Kato, K.; Calafat, A.M.; Needham, L.L. Polyfluoroalkyl chemicals in house dust. *Environ. Res.* **2009**, *109*, 518-523.
15. Kannan, K. Perfluorinated compounds: From frying pans to polar bears. *Reprod. Toxicol.* **2009**, *27*, 413-413.

16. Hölzer, J.; Göen, T.; Rauchfuss, K.; Kraft, M.; Angerer, J.; Kleeschulte, P., et al. One-year follow-up of perfluorinated compounds in plasma of German residents from Arnsberg formerly exposed to PFOA-contaminated drinking water. *Int. J. Hygiene Environ. Health.* **2008**, *116*, 651-657,
17. Fromme, H.; Tittlemier, S.A.; Völkel, W.; Wilhelm, M.; Twardella, D. Perfluorinated compounds - Exposure assessment for the general population in western countries. *Int. J. Hygiene Environ. Health.* **2009**, *212*, 239-270.
18. Tittlemier, S.A.; Pepper, K.; Seymour, C.; Moisey, J.; Bronson, R.; Cao, X.L., et al. Dietary exposure of Canadians to perfluorinated carboxylates and perfluorooctane sulfonate via consumption of meat, fish, fast foods, and food items prepared in their packaging. *J. Agric. Food Chem.* **2007**, *55*, 3203-3210.
19. Halldorsson, T.I.; Fei, C.Y.; Olsen, J.; Lipworth, L.; McLaughlin, J.K.; Olsen, S.F. Dietary Predictors of Perfluorinated Chemicals: A Study from the Danish National Birth Cohort. *Environ. Sci. Techn..* **2008**, *42*, 8971-8977.
20. Rylander, C.; Brustad, M.; Falk, H.; Sandanger, T.M. Dietary predictors and plasma concentrations of perfluorinated compounds in a coastal population from northern Norway. *J. Environ. Public health.* **2009**, *2009*, Article ID 268219, DOI:10.1155/2009/268219
21. Lund, E.; Dumeaux, V.; Braaten, T.; Hjartaker, A.; Engeset, D.; Skeie, G., et al. Cohort profile: The Norwegian women and cancer study - NOWAC - Kvinner og kreft. *Int. J. Epidemiol.* **2008**, *37*, 36-41.
22. Waaseth, M.; Bakken, K.; Dumeaux, V.; Olsen, K.S.; Rylander, C.; Figenschau, Y., et al. Hormone replacement therapy use and plasma levels of sex hormones in the Norwegian Women and Cancer postgenome cohort - a cross-sectional analysis. *BMC Women Health.* **2008**, 8, DOI:10.1186/1472-6874-8-1
23. Araujo, P.; Nguyen, T.-T.; Frøyland, L.; Wang, J.; Kang, J.X. Evaluation of a rapid method for the quantitative analysis of fatty acids in various matrices. *J. Chromatogr.* **2008**, *1212*, 106-113.
24. Arsenault, G.; Chittim, B.; McAlees, A.; McCrindle, R.; Riddell, N.; Yeo, B. Some issues relating to the use of perfluorooctanesulfonate (PFOS) samples as reference standards. *Chemosphere.* **2008**, *70*, 616-625.
25. Engeset, D.; Alsaker, E.; Ciampi, A.; Lund, E. Dietary patterns and lifestyle factors in the Norwegian EPIC cohort: The Norwegian Women and Cancer (NOWAC) study. *Eur J Clin Nutr.* **2005**, *59*, 675-684.
26. Strom, M.; Mortensen, E.L.; Halldorsson, T.I.; Thorsdottir, I.; Olsen, S.F. Fish and long-chain n-3 polyunsaturated fatty acid intakes during pregnancy and risk of postpartum depression: a prospective study based on a large national birth cohort. *Am. J. Clin. Nutr.* **2009**, *90*, 149-155.
27. U.K. Food standards Agency. Fluorinated Chemicals: UK dietary intakes. Food Survey Information Sheet 11/06. **2006**, Available at: http://www.food.gov.uk/multimedia/pdfs/fsis1106.pdf (accessed 12 January 2010)
28. Ostertag, S.K.; Tague, B.A.; Humphries, M.M.; Tittlemier, S.A.; Chan, H.M. Estimated dietary exposure to fluorinated compounds from traditional foods among Inuit in Nunavut, Canada. *Chemosphere.* **2009**, *75*, 1165-1172.

29. Kärrman, A.; Harada, K.H.; Inoue, K.; Takasuga, T.; Ohi, E.; Koizumi, A. Relationship between dietary exposure and serum perfluorochemical (PFC) levels--A case study. *Environment Int.* **2009**, *35*, 712-717.
30. Falandysz, J.; Taniyasu, S.; Gulkowska, A.; Yamashita, N.; Schulte-Oehlmann, U. Is fish a major source of fluorinated surfactants and repellents in humans living on the Baltic coast? *Environ. Sci. Techn.* **2006**, *40*, 748-751.
31. Weihe, P.; Kato, K.; Calafat, A.M.; Nielsen, F.; Wanigatunga, A.A.; Needham, L.L., et al. Serum concentrations of polyfluoroalkyl compounds in Faroese whale meat consumers. *Environ. Sci. Techn.* **2008**, *42*, 6291-6295.
32. Berger, U.; Glynn, A.; Holmström, K.E.; Berglund, M.; Ankarberg, E.H.; Törnkvist, A. Fish consumption as a source of human exposure to perfluorinated alkyl substances in Sweden - Analysis of edible fish from Lake Vättern and the Baltic Sea. *Chemosphere.* **2009**, *76*, 799-804.
33. Calafat, A.M.; Wong, L.Y.; Kuklenyik, Z.; Reidy, J.A.; Needham, L.L. Polyfluoroalkyl chemicals in the US population: Data from the National Health and Nutrition Examination Survey (NHANES) 2003-2004 and comparisons with NHANES 1999-2000. *Environ. Health Perspect.* **2007**, *115*, 1596-1602.
34. Karrman, A.; Ericson, I.; van Bavel, B.; Darnerud, P.O.; Aune, M.; Glynn, A., et al. Exposure of perfluorinated chemicals through lactation: Levels of matched human milk and serum and a temporal trend, 1996-2004, in Sweden. *Environ. Health Perspect.* **2007**, *115*, 226-230.
35. Kubwabo, C.; Vais, N.; Benoit, F.M. A pilot study on the determination of perfluorooctanesulfonate and other perfluorinated compounds in blood of Canadians. *J. Environ. Monit.* **2004**, *6*, 540-545.
36. Karrman, A.; Mueller, J.F.; Van Bavel, B.; Harden, F.; Toms, L.M.L.; Lindstrom, G. Levels of 12 perfluorinated chemicals in pooled Australian serum, collected 2002-2003, in relation to age, gender, and region. *Environ. Sci. Techn.* **2006**, 40, 3742-3748.
37. Rylander, C.; Duong Trong, P.; Odland, J.O.; Sandanger, T.M. Perfluorinated compounds in delivering women from south central Vietnam. *J. Environ. Monit.* **2009**, *11*, 2002-2008
38. Yeung, L.W.Y.; So, M.K.; Jiang, G.; Taniyasu, S.; Yamashita, N.; Song, M., et al. Perfluorooctanesulfonate and Related Fluorochemicals in Human Blood Samples from China. *Environ. Sci. Techn.* **2006**, *40*, 715-720.
39. Midasch, O.; Schettgen, T.; Angerer, J. Pilot study on the perfluorooctanesulfonate and perfluorooctanoate exposure of the German general population. *Int. J. Hygiene Environ. Health.* **2006**, *209*, 489-496.
40. Holzer, J.; Midasch, O.; Rauchfuss, K.; Kraft, M.; Reupert, R.; Angerer, J., et al. Biomonitoring of perfluorinated compounds in children and adults exposed to perfluorooctanoate-contaminated drinking water. *Environ. Health Perspect.* **2008**, *116*, 651-657.
41. Karrman, A.; Langlois, I.; Bavel, B.v.; Lindstrom, G.; Oehme, M. Identification and pattern of perfluorooctane sulfonate (PFOS) isomers in human serum and plasma. *Environ. Int.* **2007**, *33*, 782-788.
42. Powley, C.R.; George, S.W.; Russell, M.H.; Hoke, R.A.; Buck, R.C. Polyfluorinated chemicals in a spatially and temporally integrated food web in the Western Arctic. *Chemosphere.* **2008**, *70*, 664-672.

43. Brustad, M.; Edvardsen, K.; Wilsgaard, T.; Engelsen, O.; Aksnes, L.; Lund, E. Seasonality of UV-radiation and vitamin D status at 69 degrees north. *Photochem. Photobiol. Sci.* **2007**, *6*, 903-908.
44. Mozaffarian, D. Fish and n-3 fatty acids for the prevention of fatal coronary heart disease and sudden cardiac death, *Am. J. Clin. Nutr*. **2008,** 1991S-1996S.
45. Mozaffarian, D.; Bryson, C.L.; Lemaitre, R.N.; Burke, G.L.; Siscovick, D.S. Fish Intake and Risk of Incident Heart Failure. *J. Am. Coll. of Cardiol.* **2005**, *45*, 2015-2021.
46. Kris-Etherton, P.M.; Harris, W.S.; Appel, L.J. Fish consumption, Fish oil, Omega-3 fatty acids and Cardiovascular Disease. *Circulation.* **2002**, *106*, 2747-2757.
47. Statistics Norway, Causes of death 1995-2006. **2009**. Official document D412. Available at: http://www.ssb.no/emner/03/01/10/nos_dodsarsak/nos_d411/nos_d411.pdf (accessed 12 January 2010)

Table 1. Plasma concentration of PFCs (ng/ml) and omega-3 fatty acids (mg/ml) in the study group (N=326)

	Total N=326				
Concentration	**Median**	**AM**	**Range**	**LOD**	**%>LOD**
PFOS (ng/ml)	20	22	5.43-84	N/A	
PFOS branched (ng/ml)	6.1	6.6	0.70-25	0.07-1.5	100
PFOS linear (ng/ml)	14	15	4.18-60	0.05-6.3	100
PFHxS (ng/ml)	1.0	1.4	<LOD-13	0.07-0.93	98
PFOA (ng/ml)	4.4	5.1	<LOD-21	0.28-4.7	94
PFNA (ng/ml)	0.81	0.87	<LOD-2.6	0.18-0.87	93
PFHpS (ng/ml)	0.32	0.34	<LOD-1.6	0.05-0.6	65
PFOSA (ng/ml)	0.02	0.06	<LOD-0.67	0.004-0.23	49
PFHpA (ng/ml)	NA	NA	<LOD-0.92	0.15-1.2	7
% linear PFOS	70	69	45-87	N/A	
Marine derived omega 3 fatty acids (mg/ml)[a]	0.23	0.25	0.09-0.82		

[a]Include eicosapentaenoic acid (EPA) C20:5n-3, ,docosapentaenoic acid (DPA) C22:5n-3 and docosahexaenoic acid (DHA) C22:6n-3

AM, Arithmetric mean; N/A, not available, LOD, method detection limit; %>LOD, Percentage of samples in which the analyte was detected; PFOSA, Perfluorooctanesulphonic acid; PFHpS, Perfluoroheptane sulphonate; PFHpA, Perfluoroheptanoate; PFOS branched, Sum of the branched isomers of perfluorooctane sulphonate; PFOS linear, The linear isomer of perfluorooctane sulphonate; PFOS, Sum of the branched and linear isomers of PFOS; PFOA, Perfluorooctanoate; PFHxS, Perfluorohexane sulphonate; PFNA, Perfluorononanoate

Table 2. PFC concentrations, fatty acids and age in three different intake groups of total marine food [a]

	0-60g/day (N=103)			61-100g/day (N=106)			>100 g/day (N=105)			F	p
	Median	Mean	Range	Median	Mean	Range	Median	Mean	Range		
PFOS (ng/ml)	19	19	5.8-45	20	22	5.4-49	21	24	6.5-84	4.43	0.01[b]
PFOA (ng/ml)	4.5	5.4	<LOD-21	4.4	5	<LOD-20	4.2	5.1	<LOD-16	0.47	0.62[b]
PFHxS (ng/ml)	0.88	1.3	0.28-13	1.0	1.4	<LOD-11.1	1.1	1.5	<LOD-9.5	1.46	0.23[b]
PFNA (ng/ml)	0.71	0.75	<LOD-1.5	0.85	0.86	<LOD-1.7	0.86	0.98	<LOD-2.64	8.14	<0.001[b]
Age (years)	55	55	49-62	56	56	48-62	57	56	49-62	3.24	0.04

[a]Total marine food includes intake of lean fish, fatty fish, other kinds of fish, fish roe, fish liver, processed fish products, fish spread and shellfish.

[b]Adjusted for age

PFOS, Sum of the linear and branched isomers of Perfluorooctane sulphonate; PFOA, Perfluorooctanoate; PFHxS, Perfluorohexane sulphonate, PFNA, Perfluorononanoate; LOD, method detection limit

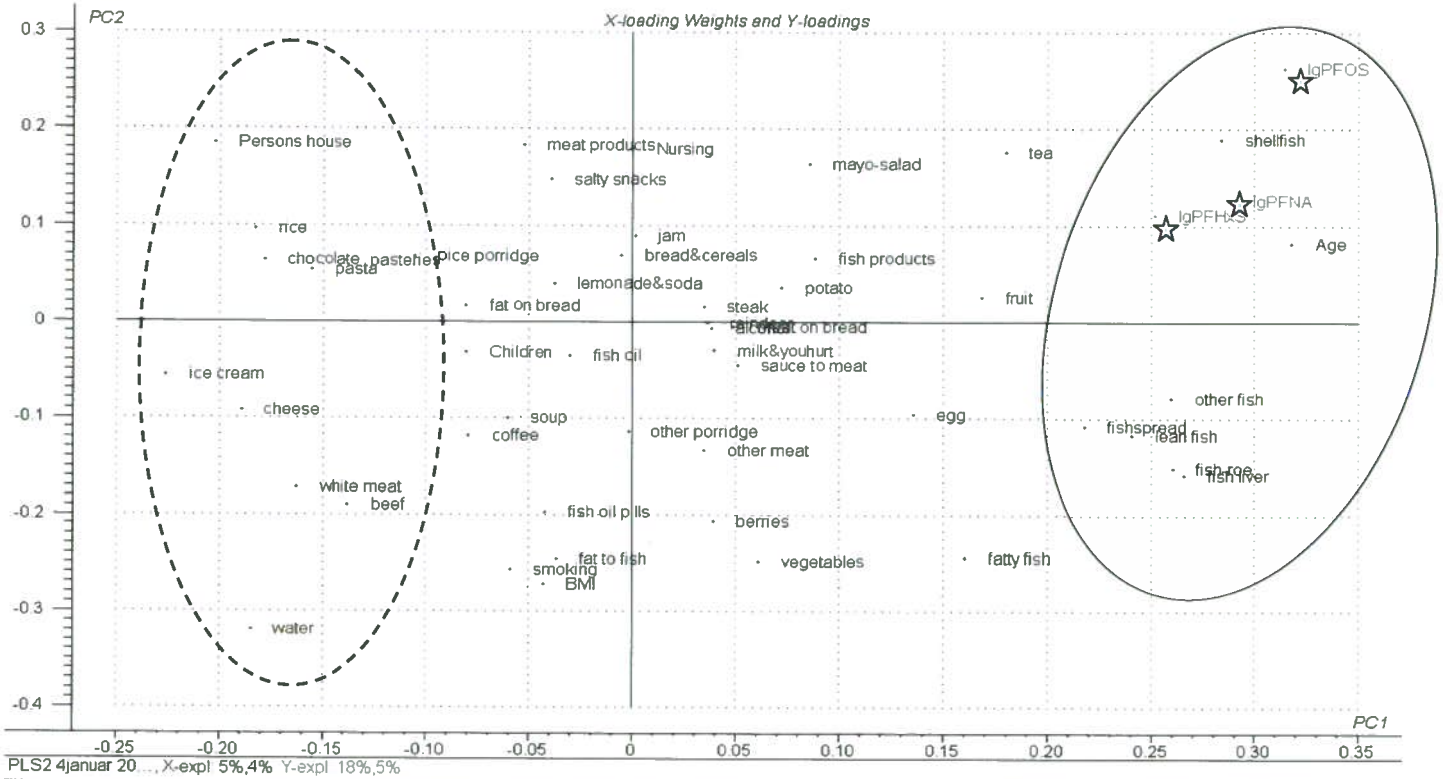

Figure 1. The loading plot for PFOS (perfluorooctane sulphonate), PFNA (perfluorononanoate) and PFHxS (perflurorhexane sulphonate). The loading plot describes the relationship between the self-reported dietary and lifestyle-related variables, and these investigated compounds. A cluster of marine variables (circled with a solid line) was positively associated with the investigated compounds whereas a cluster of western foodstuffs (dashed line) were negatively associated with the same compounds.

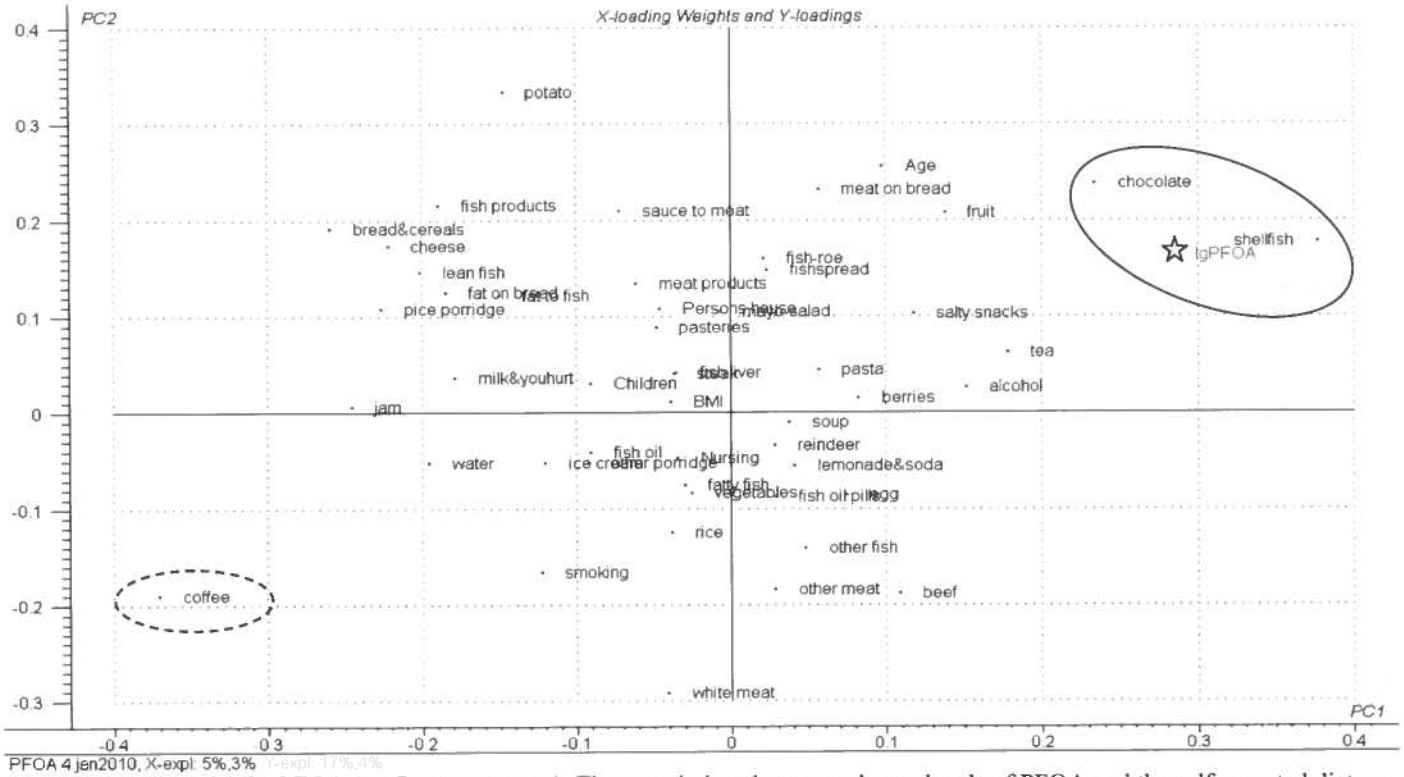

Figure 2. Loading plot for PFOA (perfluorooctanoate). The associations between plasma levels of PFOA and the self-reported dietary and lifestyle-related variables. Predictors circled with a solid line were positively associated with PFOA concentration while the predictors circled with a dashed line were negatively associated with PFOA

Supporting Information

Dietary pattern and plasma concentrations of perfluorinated compounds in 315 Norwegian women: the NOWAC postgenome study

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6 pages including front page

Table S1. Study group characteristics (N=326)

	Median	Mean	Range
Age (years)	56	56	48-62
Body mass index (kg/cm^2)	24.7	25.6	16.7-43.9
Number of persons in the household	2	2	1-7
Number of children	2	2	0-8
Number of months with nursing	10	13	0-96
	Frequency (Y/N)	Percentage (%)	
Current smoker	68/238	22	

Table S2. Dietary intake of the study group (N=315)

	Dietary intake (g/week)		
Foodstuff	**Median**	**Mean**	**Range**
Milk and yoghurt	812	1035	0–5687
Coffee	2100	2617	0–9240
Tea	1190	1968	0–21420
Water	2625	3887	0–8400
Lemonade and soda	601	955	0–9001
Bread and cereals	914	960	0–2363
Jam	40	72	0–560
Meat on bread	29	68	0–399
Mayo salad	0	26	0–525
Cheese	140	196	0–858
Fish spread for bread	59	78	0–483
Rice	70	109	0–450
Pasta	84	106	0–541
Porridge made of rice	81	102	0–2450
Other porridge	0	98	0–1715
Soup	235	248	0–2002
Fruit	1343	1615	0–5614
Vegetables	1174	1310	112–4857
Potatoes	441	556	0–1764
Fish roe	4	4	0–23
Fish liver	0	1	0–10
Processed fish products [a]	139	166	0–1021
Shellfish	25	29	0–107
Fat and sauce for fish dinners	41	59	0–521
Steak	24	33	0–350
White meat [b]	150	163	0–495
Beef	30	31	0–130
Reindeer meat	0	0	0–10
Other kind of meat [c]	66	114	0–568
Processed meat products [d]	274	287	0–1016
Sauce for meat	61	88	0–801
Eggs	118	111	0–411
Rice	25	48	0–400
Pastries	204	245	0–1817
Berries	12	24	0–325
Chocolate	23	47	0–460
Salty snacks	35	42	0–340
Alcohol	245	422	0–7143
Other kind of fish [e]	0	28	0–380
Lean fish [f]	143	183	0–1141
Fat fish [g]	100	129	0–1189
Fat on bread	67	82	0–432
Fish oil	0	9	0–77
	Frequency (Y/N)	**Percentage**	
Use of fish oil capsules	142/134	51	

[a]Include fish cakes, fish au gratin, and deep-fried fish.

[b]Include chicken and cutlets.
[c]Include meat that is not steak, beef, white meat, or reindeer meat.
[d]Include rissoles, sausages, pizza, lobscouse, and bacon/ham.
[e]Include fish that does not belong to lean fish or fatty fish.
[f]Include boiled and fried cod.
[g]Include wolfish, salmon, mackerel, and herring.

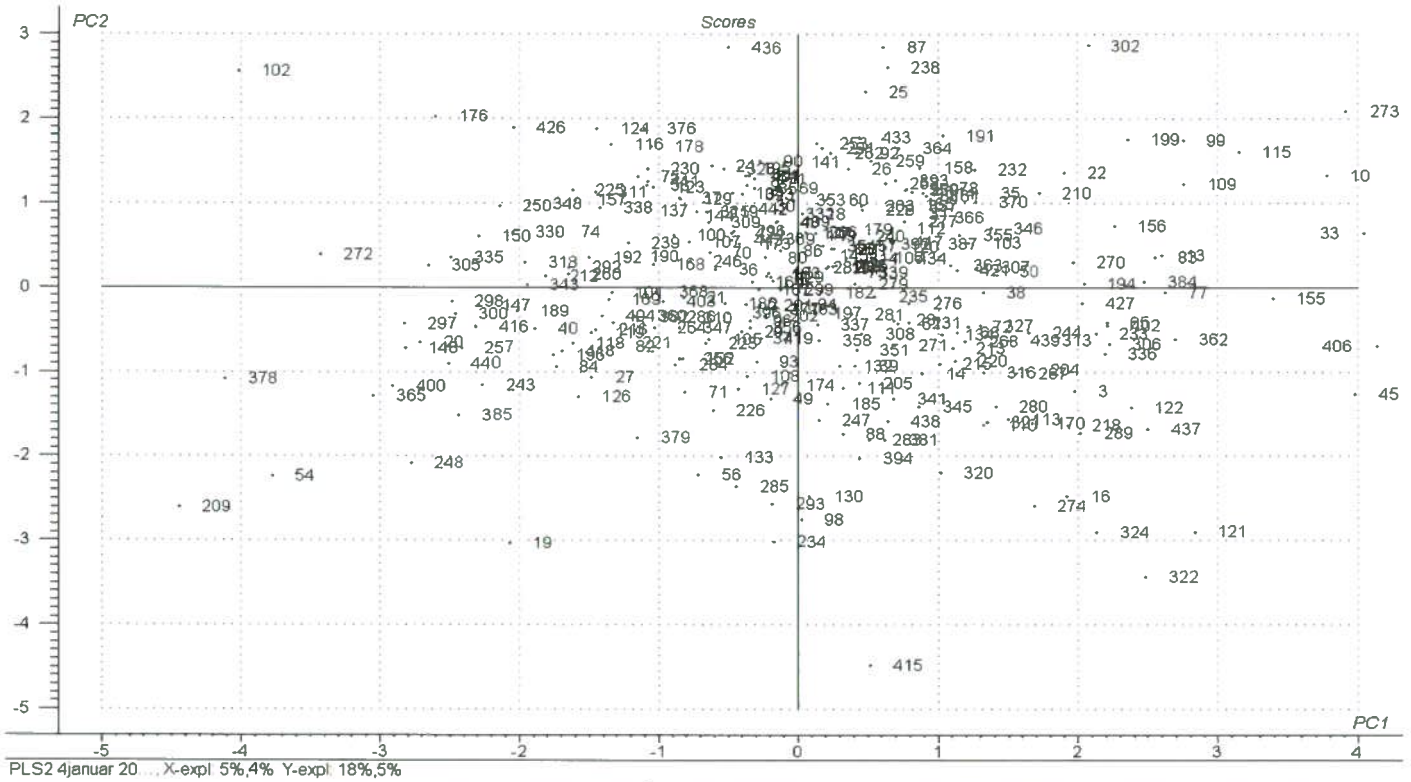

Figure S1. Score plot for the partial least square PLS2 regression of PFOS (perfluorooctanoate sulfonate), PFHxS (perfluorohexane sulfonate), and PFNA (perfluorononanoate) versus the lifestyle and dietary-related variables.

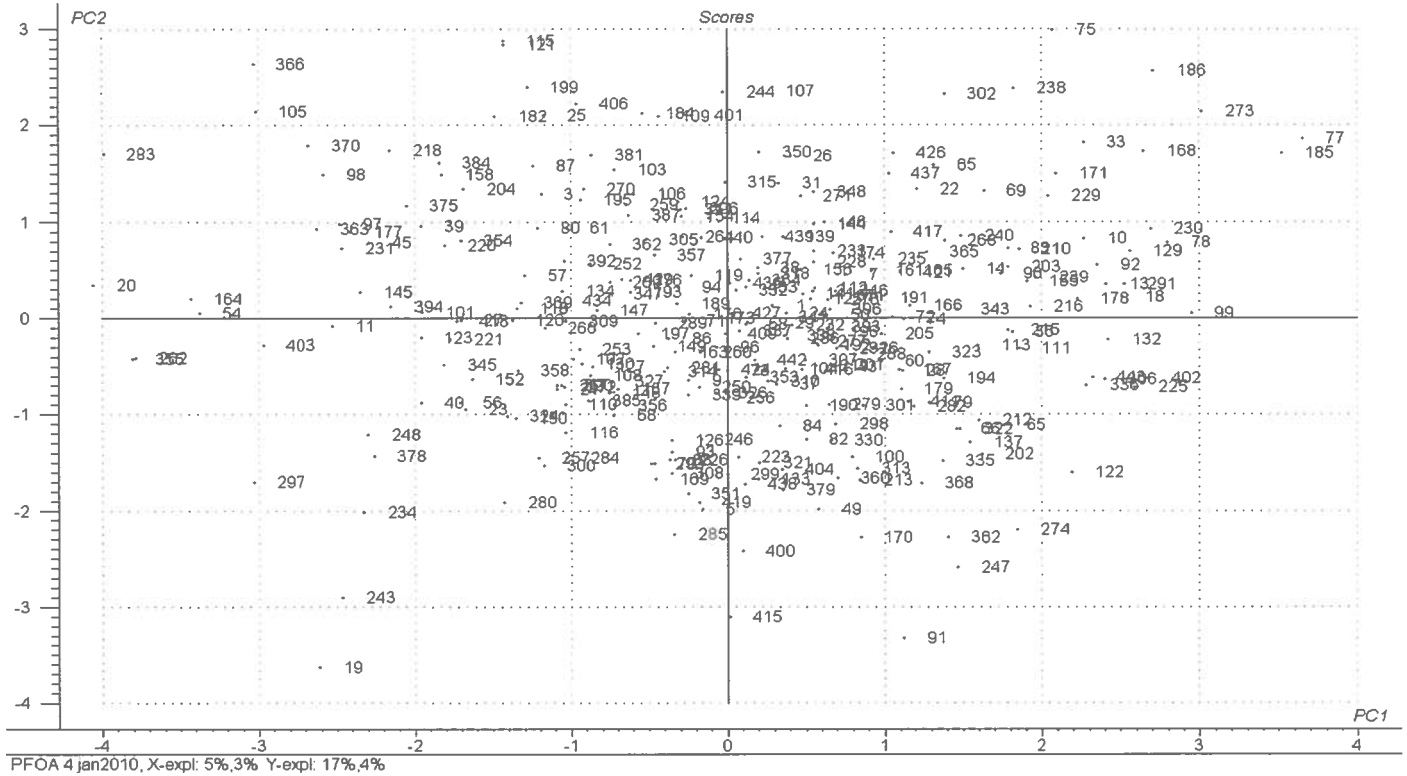

Figure S2. Score plot of the partial least square (PLS) regression of PFOA (perfluorooctanate) versus the versus the lifestyle and dietary-related variables.

Paper III

Perfluorinated compounds in delivering women from south central Vietnam†

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Received 30th April 2009, Accepted 28th August 2009
First published as an Advance Article on the web 28th September 2009
DOI: 10.1039/b908551c

The associations between age, body mass index (BMI), parity, place of residence (coastal or inland) and plasma concentrations of perfluorinated compounds (PFCs) were assessed in a study population from south central Vietnam. The study group consisted of 91 delivering women of varied age (18–40 years) from two different locations (37 urban, 36 rural and 18 with unknown residence). Perfluorooctane sulfonate (PFOS), perfluorooctanoate (PFOA) and perfluorohexane sulfonate (PFHxS) were detected in 98–100% of all samples. PFOS (median 3.2 ng/ml) was the most common compound followed by PFOA (median 1.6 ng/ml), PFHxS (median 0.7 ng/ml) and perfluorononanoate (PFNA) (median 0.7 ng/ml). The women from the coastal city Nha Trang had higher concentrations of all investigated compounds than women from the inland district Dien Khanh. The two study locations are situated only 10 km apart and the diet is considered somewhat similar, however, women in Dien Khanh are more self-sufficient with locally produced food. The family income in Nha Trang is also most likely higher than in Dien Khanh and this may affect living conditions, *e.g.* quality of housing, which in turn may influence the exposure to PFCs. There were no associations between age, parity or BMI and the investigated PFCs. Linear PFOS constituted 83% of the sum of PFOS. This is considerably higher than reported in other studies from Europe and Australia and may indicate differences in exposure sources between countries, or differences in exposure time and persistency of the different isomers.

Introduction

Perfluorinated compounds (PFCs) are a large group of chemicals with similar chemical structure; a hydrophobic fluorinated carbon tail, and a polar, hydrophilic head attached to one end of the carbon chain. PFCs act as surfactants and have been widely used in, among others, industrial products. As a consequence, they are also frequently found in the environment and have been detected in human blood all over the world.[1] Several PFCs have been shown to bioaccumulate and biomagnify in the food chain.[2,3] Concerns have been raised about these compounds since the concentrations found in the environment are relatively high, with the highest levels of PFOS among living organisms found in Polar bears from the Arctic.[4,5] Also, two of the most common contaminants in nature and in humans; perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) are potent peroxisome proliferators in rodents and in human hepatocytes.[6,7] As a result, alterations in fatty acid and lipid metabolism have been observed in rodents and chickens exposed to these compounds[8–11] which in turn may lead to a number of diseases. PFOS and PFOA have also been detected in cord blood at concentrations a little lower than in maternal blood, which clearly indicate a transfer of PFCs to the fetus.[12,13] Human levels of PFOS and PFOA increased from 1974 to 1989 and from 1977 to 2003 in southern industrial countries.[14,15] However, more recent data from the US provides strong evidence of levels starting to decline since the phase-out in 2000. A study on blood from American Red Cross donors showed that PFOS and PFOA concentrations were significantly lower (60 and 25%, respectively) in 2006

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† Electronic supplementary information (ESI) available: Further information on the compounds studied and a chromatogram of branched and linear PFOS. See DOI: 10.1039/b908551c

Environmental impact

This work contributes to improved knowledge about the environment and pollutants by:
- assessing human exposure to perfluorinated compounds (PFCs) in Vietnam. To our knowledge, this is the first study that investigates PFCs in the plasma from delivering women from South East Asia. This work contributes to increased knowledge about the global distribution of PFCs.
- investigating the impact of age, body mass index, parity and place of residence on blood concentrations of PFCs, which is important information when assessing human exposure routes to PFCs and the behavior of PFCs in the human body.
- adding more information about the percentage linear PFOS in human blood samples, which is essential knowledge when investigating the global fate of PFOS.

compared to 2000–2001.[16] Calafat *et al.* (2007) also reported a significant decline when comparing 1999–2000 samples with 2003–2004 samples from the US population.[17]

Both food and indoor dust[18] seem to be important human exposure routes to PFCs, even though some studies have recently concluded that the diet is the main pathway.[19] PFCs have also been detected in a range of consumer products,[20] amongst other food packaging, clearly indicating the complexity of exposure scenarios.

Previous studies indicate that there are worldwide geographical differences in human levels of PFCs, with people in the western world generally having higher concentrations than people in developing countries.[1,21] There are however large differences between countries within Asia, for example with elevated levels reported in Japan and low levels in India and Sri Lanka.[1,22,23] Also, large regional differences have been reported within China.[21] Differences in PFOS isomer ratios between countries have also been observed,[24] but there is currently limited information about the isomer distribution in humans. The only data presented from Vietnam is in breast milk where the levels were indicated to be moderate compared to other Asian countries.[25] Since Vietnam is a country of rapid development it is important to monitor background concentrations in the population to gain more information about exposure routes to humans and the global distribution of PFCs. In this study, we investigated the concentrations of eight different PFCs in 91 delivering women from south central Vietnam. Our main objectives were to provide more information about exposures to PFCs in South East Asia and evaluate differences in concentrations between an inland and a coastal population. This scope of work also adds more information about the isomer distribution of PFOS in human blood samples.

Materials and methods

Study participants

The women participating in this study were inhabitants of the Khanh Hoa province in south central Vietnam with place of residence in the city of Nha Trang or in the district Dien Khanh. Nha Trang is a coastal city with around 300 000 inhabitants while Dien Khanh is an inland district located approximately 10 km off the coast. All participating women were pregnant and recruited from three different hospitals during the year of 2005; the obstetric departments at the Provincial Hospital, the Public Delivery Clinic in Nha Trang or the obstetric clinic at the District Hospital in Dien Khanh. A standard form was filled out by the medical personnel at the time of delivery. The mothers name, age, weight, height, pregnancy and delivery data were obtained. Population characteristics are presented in Table 1. Criteria for being included in the study were that the women had lived in the community for at least five years and could provide a street address. In total 189 women fulfilled the criteria and were enrolled in the study. 91 samples were randomly selected from all the samples, for determination of eight PFCs. The budget did not allow PFC analysis in all samples. Of these 91 samples, 37 originated from delivering women from Nha Trang and 36 from Dien Khanh. For the remaining 18 it was, however, unclear if the mother had been residing at their local address for the sufficient amount of time and their residence was thus treated as unknown (Table 1).

Blood samples

Venous blood samples were drawn from the mothers within 3 hours after delivery in a standard vacutainer containing EDTA anticoagulant. The blood samples were centrifuged at the hospitals and plasma was transferred to pre-rinsed tubes before being stored at −20 °C and transported in the frozen state by courier to Norway for PFC determination.

Ethical considerations

This study was approved by the Regional Health Administration in Central Vietnam and the University of Tromsø, Norway. The women signed an informed consent form; the participation was voluntary.

Chemical analysis

Samples were extracted and cleaned-up using a modified method from Powley *et al.*[26] In short; 250 µl plasma was transferred to a 1.5 ml microcentrifuge tube (Brand GMBH, Wertheim, Germany) using an electronic pipette (Thermo Scientific, Waltham, MA, USA). 25 µl internal standard ($^{13}C_4$ PFOS and $^{13}C_4$ PFOA (0.1 ng/µl)) (Wellington laboratories, Guelph, ON, Canada) was added to the sample before the addition of 1.0 ml acetonitrile (Merck, Darmstadt, Germany). Samples were mixed thoroughly by a vortex mixer (VWR, West Chester, PN, USA) and then extracted for 3 × 10 min in a Branson 2210 ultrasonic bath (Branson Ultrasonics BV, Soest, the Netherlands). In between each extraction, samples were carefully mixed using the vortex mixer. Samples were then centrifuged at 2000 rpm for 5 min using a Jouan A14 centrifuge (Thermo Scientific Waltham, MA, USA). The supernatant was transferred to a 1.5 ml microcentrifuge tube (Brand GMBH, Wertheim, Germany), containing 25 mg ENVI-Carb 120/400 (Supelco, PN, USA) and 50 ul glacial acetic acid,

Table 1 Study population characteristics

	Total $N = 91$			Nha Trang $N = 37$			Dien Khanh $N = 36$		
Study population characteristics	Median	AM[a]	Range	Median	AM	Range	Median	AM	Range
Age (years)	26	27	18–40	27	27	19–38	26	27	19–40
BMI (kg/m^2)	23	23	17–28	24	24	18–28	23	23	19–28
Number of children	2	2	1–4	1	2	1–3	2	1.8	1–4

[a] AM: Arithmetic mean

mixed thoroughly and then centrifuged for 10 min at 10 000 rpm (Thermo Scientific Waltham, MA, USA). 500 µl supernatant was transferred to a 1.5 ml vial (VWR, West Chester, PN, USA) and mixed with 500 µl 2 mM aqueous NH_4OAc (BDH Laboratory Supplies, England). Finally, 40 µl of a methanolic 0.05 ng/µl solution of 3,7-dimethyl PFOA (Wellington laboratories, Guelph, ON, Canada) was added as recovery standard.

PFCs were analyzed using a quadrupole-time-of-flight (Q-TOF) mass spectrometer micro™ equipped with a 2777 autosampler (Waters, Milford, MA, USA) and a 1525 binary HPLC pump also from Waters (Milford, MA, USA). The method is slightly modified from Berger *et al.*[27] Mobile phases consisted of (A) 2 mM NH_4OAc in water and (B) 2 mM NH_4OAc in methanol (Merck, Darmstadt, Germany). Before analysis, mobile phases were degassed using an ultra sonic bath (Branson Ultrasonics BV, Soest, the Netherlands). A 50 µl sample was injected into an ACE 3 C18 reversed phase column, with particle size 3 µm, length 150 mm (ACT, Aberdeen, Scotland). Flow rate of mobile phase was 0.2 ml/min. The following gradient settings were applied for elution of the target analytes from the column: 0 min 50% B; 0–5 min linear gradient to 85% B; 5–10 min 85% B; 10–11 min linear gradient to 99% B; 11–20 min 99% B; 20–21 min linear gradient to 50% B; 21–28 min 50% B. The Q-TOF-MS was operated in negative electrospray ionization mode (ESI-) in the range *m/z* 100–725 and operated by the Mass Lynx 4.1 software. Settings were optimized before analysis and as follows: capillary voltage, −3 kV; sample cone voltage, 50 V (0.5–10.3 min), 35 V (0.5–20.0 min) and 20 V (10.3–20.0 min); desolvation and source temperature, 350 and 120 °C respectively; nitrogen was used as the cone gas at a flow of 20 L/min, as nebuliser gas maximum flow and as desolvation gas at 600 L/min. Target analytes and native analytical standards, their abbreviation, exact quantification mass and cone voltages are listed in Table S1 in the Supplementary information.†

The quantification was conducted by the QuanLynx software version 3.5 (Waters, Milford, MA, USA).

Quality control

The quality of the analysis was assured through regular analysis of blank samples and reference materials from previous rounds of international calibration programmes. For each batch of 30 samples, two blank samples and two reference samples were prepared. In addition, our laboratory participates regularly in an interlaboratory comparison program, the AMAP Ring Test for Persistent Organic Pollutants in Human Serum (three rounds per year) organized by Institut National de Santé Publique du Québec, Canada. Results from the interlaboratory comparison program indicate that the uncertainties of our analysis are well within +/−30% of assigned values. The recovery rates of the labelled internal standards varied between 100–180% due to ion suppression of the recovery standard. The method detection limit (LOD) was automatically calculated by the quantification software. No analytes at concentrations above LOD were detected in the blank samples. All analyte levels were well within the linear range of the instrument.

Linear PFOS was chromatographically separated from a co-elution of branched PFOS isomers and quantified both separately and as the sum of the two peaks, the latter referred to

Table 2 PFC concentrations in the study group

	Total $N = 91$[a]					Nha Trang $N = 37$				Dien Khan $N = 36$			
Concentration ng/ml plasma[g]	Median[b]	AM[c]	Range	LOD[d]	% > LOD[e]	Median	AM	Range	% > LOD	Median	AM	Range	% > LOD
PFOSA	NA[f]	NA	0.04–0.13	0.04	2	NA	NA	NA	0	NA	NA	0.04–0.07	3
PFHxS	0.70	4.0	0.19–69	0.15	100	1.00	4.2	0.22–30	100	0.68	3.1	0.26–48	100
PFHpS	0.05	0.07	0.06–0.65	0.06	45	0.07	0.10	0.06–0.4	65	NA	NA	0.06–0.35	28
Branched PFOS	0.51	0.79	0.20–7.4	0.20	98	0.61	0.89	0.31–5.1	100	0.47	0.58	0.2–3.0	97
Linear PFOS	2.6	2.9	0.99–8.9	0.20	100	2.9	3.3	1.4–8.3	100	2.1	2.6	0.99–6.5	100
PFOS	3.2	3.7	1.2–16			3.7	4.2	1.7–9.8		2.8	3.1	1.2–9.5	
PFHpA	NA	NA	NA	2.2	0	NA	NA	NA	0	NA	NA	NA	0
PFOA	1.6	5.6	0.55–94	0.55	98	2.1	5.8	0.55–53	97	1.2	4.8	0.55–6.5	97
PFNA	0.70	0.78	0.79–2.1	0.79	40	0.84	1.0	0.79–1.7	57	NA	NA	0.79–2.1	28
% Linear PFOS	83	81	28–93			83	80	28–90		83	83	50–93	

[a] 18 of the participants did not report their place of residence. [b] Median: Median value [c] AM: Arithmetic mean [d] LOD: method detection limit [e] % > LOD: Proportion above the method detection limit [f] NA: Not available [g] PFOSA: Perfluorooctanesulfonic acid; PFHxS: Perfluorohexane sulfonate; PFHpS: Perfluoroheptane sulfonate; PFOS: Perfluorooctane sulfonate; PFHpA: Perfluoroheptanoic acid; PFOA: Perfluorooctanoate; PFNA: Perfluorononanoate; PFOS: Sum of the branched and linear isomer of PFOS; %Linear PFOS: Percentage linear PFOS related to the sum of the linear and branched isomers.

as PFOS. The co-elution of branched PFOS isomers (one peak) was not structurally elucidated but rather identified as eluting earlier than the linear PFOS as indicated in Figure S1 in the Supplementary information.† Isomer specification was not performed for the other PFCs, where the linear isomer clearly dominated. On the basis of a study reporting similar response factors for branched and linear PFOS,[28] it was decided to use the labeled linear PFOS as the internal standard for the quantification of the branched isomers.

Statistical analysis

Statistical analysis was performed using the freely available software R, version 2.8.1 (http://www.cran.r-project.org). The impact of age, body mass index (BMI), parity and place of residence on PFOS, PFOA and PFHxS were investigated using linear models on log transformed variables or Wilcoxons' rank sum test. Statistics for perfluoroheptane sulfonate (PFHpS) and PFNA that had less than 80% > LOD were performed with the NADA package for R. Summary statistics for these compounds were calculated using Maximum Likelihood Estimates (MLE) for the total sample ($N = 91$) and the Kaplan–Meier method (KM) for the individual groups ($N = 37$ and $N = 36$). The non-parametric Peto-Prentice test as well as the non-parametric Akritas-Theil-Sen slope was used for evaluating the influence of age, BMI, parity and place of residence on the concentration of these compounds. All of the above methods are described by Helsel *et al.*[29] Perfluorooctanesulfonic acid (PFOSA) and perfluoroheptanoate (PFHpA) had less than 5% > LOD and were not evaluated statistically.

Model assumptions for the linear models, outliers and influential observations were evaluated using diagnostic plots of the residuals. Parameter estimates (β) with 95% confidence intervals, F-statistics, R^2-values, Wilcoxon's test estimator (W), Chi-square values (χ^2), degrees of freedom ($D.F.$) and the levels of significance (p-value) are reported in the text. The parameter estimates (β) are back-transformed log-results and should be interpreted as "the number of times the response variables increase/decrease by one unit increase in explanatory variable".

Results

Median, arithmetic mean, range, LOD and % > LOD of the eight monitored PFCs are reported in Table 2. PFOS, PFOA and PFHxS were detected in 98–100% of all samples. As for the percentage of linear PFOS in this study, there were large individual differences with a range of 17–93%, and an average value of 81%.

PFOA and PFHxS were highly correlated ($r = 0.94$) (Figure 1) and when removing the upper eight observations, the correlation remained strong ($r = 0.66$). PFOS and PFOA and PFOS and PFHxS showed a medium strong positive correlation, $r = 0.56$ and $r = 0.66$, respectively (Figure 1).

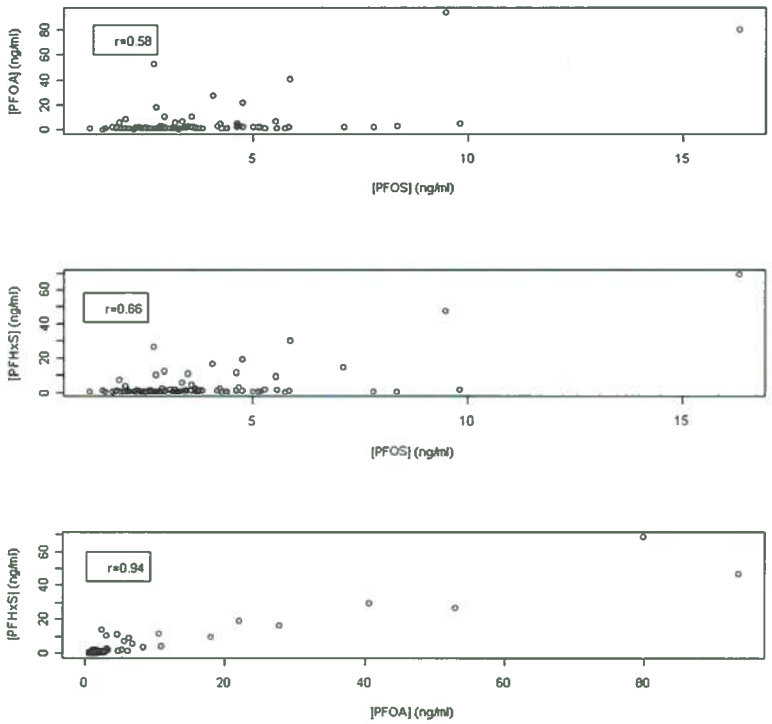

Fig. 1 Scatter plots between PFOS, PFOA and PFHxS. The correlation coefficients are reported in each graph.

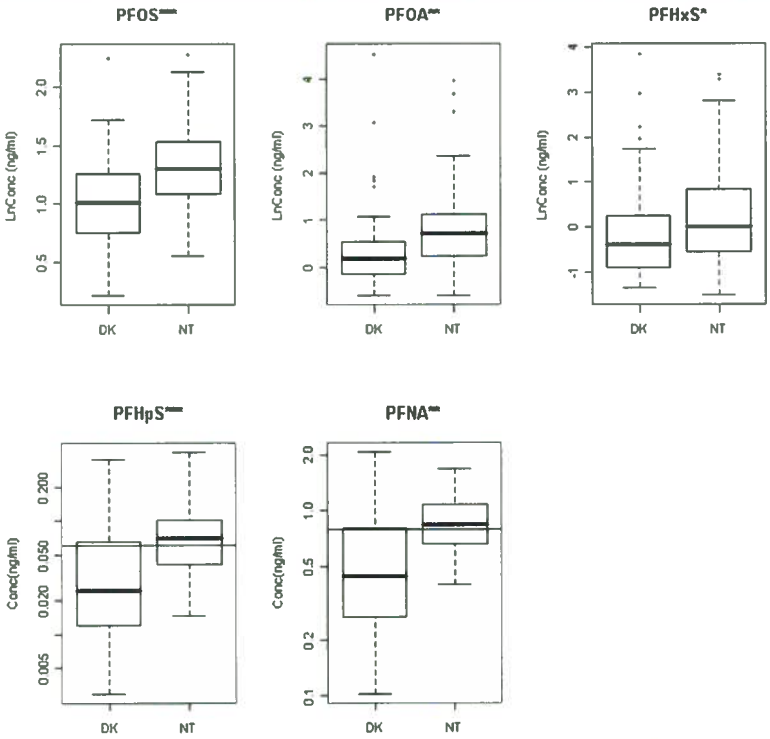

Fig. 2 Differences in PFOS, PFOA, PFHxS, PFHpS and PFNA concentration between Dien Khanh (DK) and Nha Trang (NT). Three asterisks (***) represents $p < 0.001$, two asterisks (**) $p < 0.01$ and one asterisk (*) $p < 0.05$. The bottom two plots (PFHpS and PFNA) are censored box plots. The horizontal line represents the LOD.

Women from Nha Trang had significantly higher concentrations of all the investigated PFCs than the women from Dien Khanh (Figure 2); PFOS ($\beta = 1.41$ (1.18, 1.68), $F_{1,70}$: 14.92, $R^2 = 0.1757$, $p = 0.000247$), PFOA ($W = 378.5$, $p = 0.00154$), PFHxS ($W = 486.5$, $p = 0.0482$), PFHpS ($\chi^2 = 11.2$, $D.F. = 1$, $p = 0.0004$) and PFNA ($\chi^2 = 6.5$, $D.F. = 1$, $p = 0.0053$). Age, BMI and parity (Figure 3) did not significantly influence the concentration of any of the investigated PFCs in the study group ($N = 91$).

Discussion

To our knowledge, this is the first study of PFCs in the plasma of delivering women from South East Asia. Moderate levels of PFCs were presented for one urban and one rural community in the Khanh Hoa province, with distinct and significant differences in concentration between the two communities that are only 10 km apart.

PFOS, PFOA and PFHxS were detected in nearly all women (>98%), clearly identifying these compounds as major contaminants also in pregnant women from Vietnam. Looking at the two communities together, PFOS dominated with a median level of 3.2 ng/ml plasma followed by PFOA (1.6 ng/ml plasma) and PFHxS and PFNA (both 0.70 ng/ml plasma). The PFOS concentration in this study group was approximately nine times lower than in delivering women from Denmark[30] and three times lower than in delivering women from North Carolina, USA and from Japan.[13,31] Furthermore, the PFOA concentrations were three times lower than in the Danish women, in the same range as the Americans and only detected in a few of the Japanese samples. The concentrations of PFHxS and PFNA in the Vietnamese women were comparable to the Americans.[31] The dominating PFCs were all correlated in the present study, with the strongest correlation between PFHxS and PFOA (Figure 1). Olsen *et al.*[32] observed a weak correlation between PFOA and PFHxS ($r = 0.32$) in American Red Cross blood donors and the strongest correlation between PFOS and PFOA ($r = 0.63$). Also Haug *et al.*[33] found the strongest correlation between PFOS and PFOA ($r = 0.95$) in pooled Norwegian samples. Since PFCs are lifestyle related pollutants, the impact of different exposure sources may vary between countries, especially between industrialized and developing countries, which may explain differences in covariance.

The proportion of linear PFOS was highly variable (17–93%) among the women in the present study, with a high median value of 83%. PFOS was mainly produced by electrochemical fluorination; a process that yielded approximately 70–80% straight chain isomers.[11,34] The observed linear percentage of 83% is comparable to technical mixtures but considerably higher than

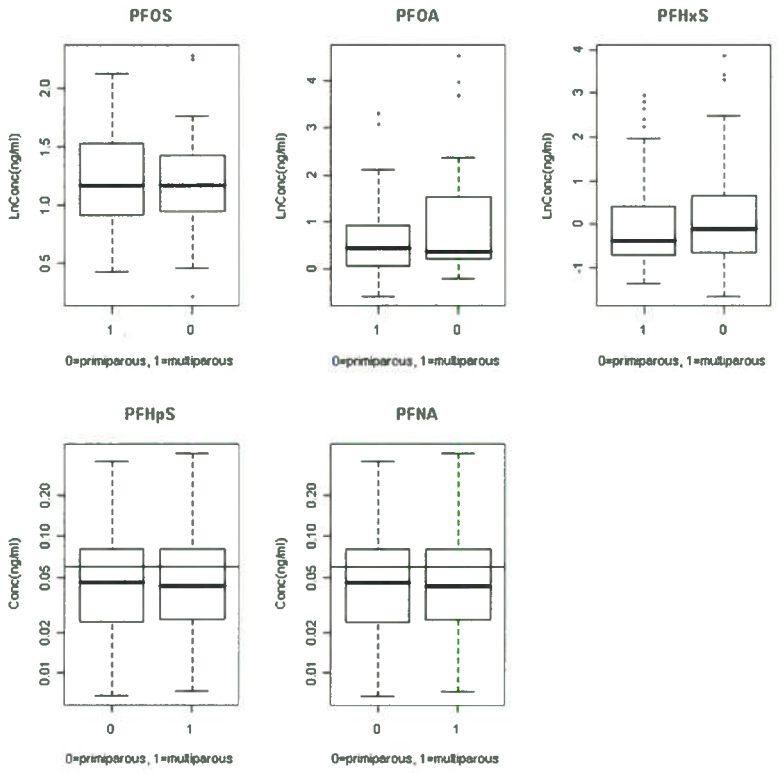

Fig. 3 The effect of parity on plasma concentrations of PFCs. In the present study there were no significant differences in plasma concentrations of the investigated compounds between primiparous and multiparous women. The bottom two plots (PFHpS and PFNA) are censored box plots. The horizontal line represents the LOD.

what was reported in previous studies with 68% in Sweden (range 50–70%) and 58% in Australia and England.[24] In a recent study from Norway, a range of 53–78% linear PFOS was reported.[33] The reported ranges are thus considerably narrower than what was observed in the present study, indicating clear differences between countries. This may be a result of either different exposure sources or a combination of exposure time and shorter half-life of the linear isomer. This has also been pointed out by others.[24,33] It seems likely that industrialized and developing countries, to some extent, are exposed through different pathways since the exposure scenarios of PFCs are complex and related to way of living. Thus, it is evident that data on isomer proportions are important in helping to identify sources of exposure and/or differences between countries, the influence of metabolism and differences in persistency.

The concentrations of PFCs did not vary significantly with age. A lack of trend has been observed in some studies[1,17,21] and other studies again have reported an increase or even a decrease with age.[22,33,35] The lack of age correlation in this study may partly be explained by the narrow age span of the participants (18–40 years) that in turn may affect the chances of detecting differences in levels with age. Nor did parity significantly affect the PFC concentrations (Figure 3). Considering these compounds are detected in both cord blood and breast milk one could expect that an increasing number of children would result in slower increase of PFCs. Especially since the majority of the women breastfed their children for a considerable period of time (on average 18 months). Decreasing plasma concentrations of PFOS and PFOA with increasing parity was observed by Fei *et al.* in a group of 1400 Danish women.[30] These contradictory findings may partly be explained by differences in sample size between the two studies.

The concentrations of all investigated PFCs were significantly higher in Nha Trang (urban coastal area) compared to Dien Khanh (rural inland area) indicating differences in exposure. Considering the diet, it is however similar in these two areas; Marine fish and shellfish are consumed in both Nha Trang and Dien Khanh and fresh water fish and meat from the countryside in Dien Khanh are sold in Nha Trang. The women from Dien Khanh are more self sufficient with locally produced food and this may constitute the largest difference regarding diet. There are also larger supplies of food from different geographical areas in Nha Trang since it is a large city. The family income in Nha Trang is most likely higher than in Dien Khanh and this may influence living conditions and the exposure to PFCs by other factors than the diet, *e.g.* the quality of housing. Three of the five

women with high PFHxS and PFOA concentrations were residents in Nha Trang. The other two were from Dien Khanh. Three were workers, one a farmer and the profession of the fifth woman was unknown. Unfortunately, the data is too limited to hypothesize about the reason for these high concentrations.

There is no doubt that PFCs are global pollutants. Large differences in exposure between countries and also minor but significant regional differences have been observed in many studies.[1,21,23] Our findings, with higher concentrations in urban areas compared to the rural district, support previous findings with higher PFC concentrations in industrialized areas, which in turn points at exposure routes other than the traditional diet. However the ratio of, for example, PFOS to PFOA seems to vary between countries and may reflect differences in use and/or differences in exposure scenarios.[1,30,31] For PFOS, the proportion of linear PFOS could reveal interesting information regarding exposure routes and biotransformation of these compounds.

Acknowledgements

We gratefully acknowledge all participating women and the medical personnel helping out with the data collection. The original study was funded by the national Norwegian aid organization (NORAD). Helse Nord (the North Norwegian Health Organization), AMAP (Arctic Monitoring and Assessment Program) as well as the Nordic Ministerial Council have contributed to the project.

References

1 K. Kannan, S. Corsolini, J. Falandysz, G. Fillmann, K. S. Kumar, B. G. Loganathan, M. A. Mohd, J. Olivero, N. Van Wouwe, J. H. Yang and K. M. Aldous, *Environ. Sci. Technol.*, 2004, **38**, 4489–4495.
2 M. Haukas, U. Berger, H. Hop, B. Gulliksen and G. W. Gabrielsen, *Environ. Pollut.*, 2007, **148**, 360–371.
3 C. R. Powley, S. W. George, M. H. Russell, R. A. Hoke and R. C. Buck, *Chemosphere*, 2008, **70**, 664–672.
4 R. Bossi, F. F. Riget, R. Dietz, C. Sonne, P. Fauser, M. Dam and K. Vorkamp, *Environ. Pollut.*, 2005, **136**, 323–329.
5 K. S. Betts, Perfluoroalkyl acids: What is the evidence telling us? (**115**, p. A250, 2007), Environ. Health Perspect., 2007, **115**, pp. A344–A344, ISSN:0091-6765.
6 A. A. Jensen and H. Leffers, *Int. J. Androl.*, 2008, **31**, 161–169.
7 C. J. Wolf, M. L. Takacs, J. E. Schmid, C. Lau and B. D. Abbott, *Toxicol. Sci.*, 2008, **106**, 162–171.
8 K. S. Guruge, L. W. Y. Yeung, N. Yamanaka, S. Miyazaki, P. K. S. Lam, J. P. Giesy, P. D. Jones and N. Yamashita, *Toxicol. Sci.*, 2006, **89**, 93–107.
9 J. A. Bjork, C. Lau, S. C. Chang, J. L. Butenhoff and K. B. Wallace, *Toxicology*, 2008, **251**, 8–20.
10 L. W. Y. Yeung, K. S. Guruge, N. Yamanaka, S. Miyazaki and P. K. S. Lam, *Toxicology*, 2007, **237**, 111–125.
11 W. Hu, P. D. Jones, T. Celius and J. P. Giesy, *Environ. Toxicol. Pharmacol.*, 2005, **19**, 57–70.
12 O. Midasch, H. Drexler, N. Hart, M. W. Beckmann and J. Angerer, *Int. Arch. Occup. Environ. Health*, 2007, **80**, 643–648.
13 K. Inoue, F. Okada, R. Ito, S. Kato, S. Sasaki, S. Nakajima, A. Uno, Y. Saijo, F. Sata, Y. Yoshimura, R. Kishi and H. Nakazawa, *Environ. Health Perspect.*, 2004, **112**, 1204–1207.
14 G. W. Olsen, H. Y. Huang, K. J. Helzlsouer, K. J. Hansen, J. L. Butenhoff and J. H. Mandel, *Environ. Health Perspect.*, 2005, **113**, 539–545.
15 K. Harada, N. Saito, K. Inoue, T. Yoshinaga, T. Watanabe, S. Sasaki, S. Kamiyama and A. Koizumi, *J. Occup. Health*, 2004, **46**, 141–147.
16 G. W. Olsen, D. C. Mair, T. R. Church, M. E. Ellefson, W. K. Reagen, T. M. Boyd, R. M. Herron, Z. Medhdizadehkashi, J. B. Nobilett, J. A. Rios, J. L. Butenhoff and L. R. Zobel, *Environ. Sci. Technol.*, 2008, **42**, 4989–4995.
17 A. M. Calafat, L. Y. Wong, Z. Kuklenyik, J. A. Reidy and L. L. Needham, *Environ. Health Perspect.*, 2007, **115**, 1596–1602.
18 K. Kato, A. M. Calafat and L. L. Needham, *Environ. Res.*, 2009, **109**, 518–523.
19 H. Fromme, S. A. Tittlemier, W. Völkel, M. Wilhelm and D. Twardella, *Int. J. Hyg. Environ. Health*, 2009, **212**, 239–270.
20 K. Kannan, *Reprod. Toxicol.*, 2009, **27**, 413–413.
21 L. W. Y. Yeung, M. K. So, G. Jiang, S. Taniyasu, N. Yamashita, M. Song, Y. Wu, J. Li, J. P. Giesy, K. S. Guruge and P. K. S. Lam, *Environ. Sci. Technol.*, 2006, **40**, 715–720.
22 A. Karrman, J. F. Mueller, B. Van Bavel, F. Harden, L. M. L. Toms and G. Lindstrom, *Environ. Sci. Technol.*, 2006, **40**, 3742–3748.
23 K. S. Guruge, S. Taniyasu, N. Yamashita, S. Wijeratna, K. M. Mohotti, H. R. Seneviratne, K. Kannan, N. Yamanaka and S. Miyazaki, *J. Environ. Monit.*, 2005, **7**, 371–377.
24 A. Karrman, I. Langlois, B. v. Bavel, G. Lindstrom and M. Oehme, *Environ. Int.*, 2007, **33**, 782–788.
25 L. Tao, J. Ma, T. Kunisue, E. L. Libelo, S. Tanabe and K. Kannan, *Environ. Sci. Technol.*, 2008, **42**, 8597–8602.
26 C. R. Powley, S. W. George, T. W. Ryan and R. C. Buck, *Anal. Chem.*, 2005, **77**, 6353–6358.
27 U. Berger and M. Haukås, *J. Chromatogr., A*, 2005, **1081**, 210–217.
28 G. Arsenault, B. Chittim, A. McAlees, R. McCrindle, N. Riddell and B. Yeo, *Chemosphere*, 2008, **70**, 616–625.
29 R. D. Helsel, *Nondetects and Data Analysis. Statistics for censored Environmental Data.*, John Wiley& Sons, Inc. Hoboken, New Jersey, USA, 2005.
30 C. Y. Fei, J. K. McLaughlin, R. E. Tarone and J. Olsen, *Environ. Health Perspect.*, 2007, **115**, 1677–1682.
31 O. S. von Ehrenstein, S. E. Fenton, K. Kato, Z. Kuklenyik, A. M. Calafat and E. P. Hines, *Reprod. Toxicol.*, 2009, **27**, 239–245.
32 G. W. Olsen, T. R. Church, J. P. Miller, J. M. Burris, K. J. Hansen, J. K. Lundberg, J. B. Armitage, R. M. Herron, Z. Medhdizadehkashi, J. B. Nobiletti, E. M. O'Neill, J. H. Mandel and L. R. Zobel, *Environ. Health Perspect.*, 2003, **111**, 1892–1901.
33 L. S. Haug, C. Thomsen and G. Becher, *Environ. Sci. Technol.*, 2009, **43**, 2131–2136.
34 A. O. De Silva and S. A. Mabury, *Environ. Sci. Technol.*, 2006, **40**, 2903–2909.
35 J. Holzer, O. Midasch, K. Rauchfuss, M. Kraft, R. Reupert, J. Angerer, P. Kleeschulte, N. Marschall and M. Wilhelm, *Environ. Health Perspect.*, 2008, **116**, 651–657.

Supplementary Material (ESI) for Journal of Environmental Monitoring

Supplementary information for

"Perfluorinated compounds in delivering women from south central Vietnam"

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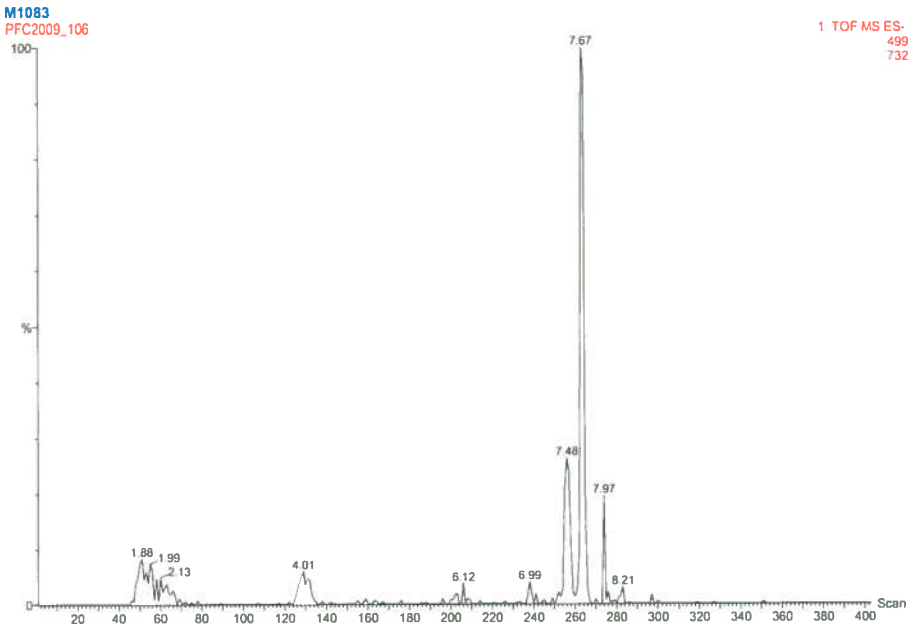
Table 1. Target analytes and native analytical standards, their abbreviation, exact quantification mass and cone voltages

Compound	Abbreviation	Quantification mass (m/z) (Cone voltage (V))
3,5-Bis(trifluoromethyl)phenyl acetic acid[1]	BTPA	227.03 (35)
Mass labelled Perfluorooctanoate[2]	13 C-PFOA	372.98 (35)
Mass labelled Perfluorooctane sulfonate[2]	13C-PFOS	502.93 (50)
Perfluorooctanesulfonic acid[3]	PFOSA	497.95 (35)
Perfluorohexane sulfonate[3]	PFHxS	398.94 (50)
Perfluoroheptane sulfonate[3]	PFHpS	449.12 (50)
Perfluorooctane sulfonate branched isomers	PFOS branched	498.93 (50)
Perfluorooctane sulfonate linear isomer[3]	PFOS linear	498.93 (50)
Perfluoroheptanoate[3]	PFHpA	318.98 (35)
Perfluorooctanoate[3]	PFOA	368.98 (35)
Perfluorononanoate[3]	PFNA	418.97 (35)

1.Used as recovery standard. 2. Used as internal standard. 3. Native standard.

Figure 1. Chromatogram of branched (RT7.48) and linear isomers (RT 7.67) of PFOS.



Paper IV

Perfluorinated compounds and blood gene signatures in postmenopausal women: the NOWAC postgenome study

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Running title

Perfluorinated compounds and blood gene signatures

Key words

Gene expression, PFCs, PFOS, peripheral blood cells, microarray

Acknowledgements

We gratefully acknowledge all NOWAC participants and Bente Augdal and Merete Albertsen for administration during data collection. Livar Frøyland (NIFES) has measured fatty acid levels. Sandra Huber and Marten Moe have operated the LC-MS instrument. This work was supported by grants from the Norwegian Research Council, the University of Tromsø and the Royal Ministry of Fisheries and Coastal Affairs.

Competing interest declaration

No competing interest

List of abbreviations

AM: Arithmetic mean

BMI: Body Mass Index

DAVID: Database for Annotation, Visulization and Integrated Discovery

FDR: False Discovery Rate

HPLC-QTOF-MS: High Performance Liquid Chromatography- Quadrupole Time Of Flight- Mass Spectrometer

HT: Hormone therapy

KEGG: The Kyoto Encyclopedia for Genes and Genomes

LOD: Method limit of detection

NIFES: National Institute of Nutrition and Seafood Research

NOWAC: The Norwegian Women and Cancer Study

PFCs: Perfluorinated compounds

PFCAs: Perfluoroalkyl carboxylic acids

PFHxS: Perfluorohexane sulphonate

PFOA: Perfluoroocatnoate

PFOS: Perfluorooctane sulphonate

PFNA: Perfluorononanoate

PPAR: Peroxisome proliferator activated receptor

S/N: Signal to Noise ratio

Abstract

Objective: This study was undertaken to assess the impact of plasma concentrations of perfluorooctane sulphonate (PFOS), perfluorooctanoate (PFOA) and perfluorohexane sulphonate (PFHxS) on blood gene signatures in a group of 270 healthy, postmenopausal women from Norway.

Methods: Gene expression analysis was performed using Applied Biosystems microarrays. Forty-eight gene sets, all previously linked to PFC exposure were evaluated.

Results: Genes encoding for enzymes within the citric acid cycle were differentially expressed between the "PFOS high" (>30ng/ml, n=42) and the "PFOS low" (<30ng/ml, n=228) group.

Conclusions: To the best of our knowledge, this is the first population based study assessing the impact of PFCs on human blood gene expressions. Our results suggest that PFOS interfere with the glucose metabolism in the general population. Several important catabolic pathways, e.g. carbohydrate, fat and protein catabolism, converge in the citric acid cycle, emphasizing the need for comprehensive understanding of the impact of PFOS on human health. Our findings advocate that gene signatures in peripheral blood cells is a promising tool for evaluating the effects of pollutants on human health.

Introduction

Over the last decade, perfluorinated compounds (PFCs) have received increased attention due to their ubiquitous presence in the environment (Powley et al. 2008) and in human blood (Calafat et al. 2007; Rylander et al. 2009a). PFCs have unique properties of repelling both water and oil and have been widely used in industrial applications, e.g. as constituents of surface treatment products and as processing aids in the production of fluoropolymers (Fromme et al. 2009). Except for being produced as industrial chemicals and being present in consumer products, some perfluoroalkyl carboxylic acids (PFCAs), e.g., perfluorononanoate (PFNA), are also degradation products of more volatile precursor PFCs (Ellis et al. 2004).

Perfluorooctane sulphonate (PFOS) is usually the most abundant PFC in human blood samples, but perfluorooctanoate (PFOA) and perfluorohexane sulphonate (PFHxS) are also frequently detected (Kannan et al. 2004). PFOS and PFOA act as peroxisome proliferators in rodents (Wolf et al. 2008), and changes in blood lipid levels and gene expressions related to the fatty acid metabolism have been observed in rats and chickens exposed to these compounds (Berthiaume and Wallace 2002; Curran et al. 2008; Yeung et al. 2007). Alterations in cell membrane fluidity, increased liver weight and increased mortality among newborn rats have also been associated with PFOS and PFOA exposure (Hu et al. 2003; Lau et al. 2007). The toxicity of PFHxS has not been evaluated thoroughly, but reduced serum cholesterol levels and increased liver weight was, among others, recently observed in PFHxS exposed rodents (Butenhoff et al. 2009).

Occupationally exposed workers in fluoropolymer industries have been studied in relation to morbidity, self-reported medical conditions and bladder cancer (Alexander and Olsen 2007; Alexander et al. 2003; Grice et al. 2007). No association between place of work (as an exposure marker) and these endpoints was found. However, there was a positive association between PFOA exposure, prostate cancer and diabetes mortality even though the results were inconsistent (Lundin et al. 2009). A positive relationship between serum PFOS concentrations, blood insulin levels, β-cell function and insulin resistance status was also recently reported in a general population, suggesting that some PFCs are associated with the metabolic syndrome (Lin et al. 2009). Several PFCs are found in human cord blood and some have also been detected in human breast milk, clearly indicating that the growing fetus and newborn child are exposed to PFCs (Midasch et al. 2007; Tao et al. 2008). However, results from epidemiological studies investigating the impact of PFOS and PFOA on birth outcomes, such as birth weight, are so far conflicting (Fei et al. 2007; Monroy et al. 2008; Olsen et al. 2009).

Gene expression signatures of human blood or tissues may have large implications in epidemiology as early biomarkers of disease (Lund and Dumeaux 2008) or through investigations of the etiology of diseases (Wild 2009). Expression profiles in peripheral human blood cells have been successfully used to assess the impact of environmental exposures, such as smoking (Lampe et al. 2004), metal fumes (Wang et al. 2005), ionizing radiation (Amundson et al. 2000), dioxin and benzene exposure (McHale et al. 2007; McHale et al. 2009). Despite that, using blood for gene expression analysis is not a straight forward procedure due to inter-individual variations in blood cell distributions

and the risk of gene expression changes due to technical variables such as batch number, amplification date, collection, storage time (Eady et al. 2005; Radich et al. 2004; Tanner et al. 2002). However, Dumeaux et al. (2010) showed recently that body mass index (BMI), smoking, fasting status, hormone therapy (HT) and other medication use were mirrored in blood of the women included in the current analysis after adjustment for the significant effect of technical variables. To the best of our knowledge, there is no previous study investigating gene expression profiles in the general population in relation to plasma levels of PFCs. This method could be a promising tool for evaluating the impact of pollutants on public health, based on human data and not derived from *in-vitro* or animal model studies. Thus, this study was undertaken to assess blood gene signatures in relation to plasma concentrations of PFOS, PFOA and PFHxS in a random group of 270 postmenopausal, healthy Norwegian women.

Materials and Methods

Study participants and collection of blood samples

The women taking part of the current study are all participants in the Norwegian Women And Cancer Study (NOWAC) (Lund et al. 2008) which consists of more than 150 000 women who have answered one to three detailed questionnaires regarding diet and lifestyle. From the original cohort, more than 50 000 women (born between 1943 and 1957) were randomly recruited in batches of 500 to the NOWAC postgenome study (Dumeaux et al. 2008). In addition to answer a two-page questionnaire regarding lifestyle and defined exposures, these women also donated a blood sample. Of a randomly

selected batch of 500 women, 270 (blood drawn in 2005) fulfilled the inclusion criteria for the current analysis. Criteria for being included were defined as having postmenopausal status, successful blood delivery in one PAX gene Blood RNA tube (Preanalytix, Qiagen, Hilden, Germany) and in one blood collection tube containing citrate buffer. The blood sample had also to be frozen within three days after collection. In addition, sufficient RNA quantity, integrity or purity was demanded, at least 40% of the microarray probes had to have signal to noise ratio (S/N) $\geq$3, no use of diabetes medication were allowed and the PFC analysis had to be successful.

Mean age among the 270 women included in the current study were 56 years, 26% were current smokers, 18% used HT and 57% used some other medication. Mean body mass index (BMI) was 25.5 kg/m^2 and 9% of the women were fasting before blood delivery.

HT use, sex hormone levels and detailed information about the blood collection procedures have been reported by Waaseth et al. (2008).

Chemical analysis

The plasma analyses of PFCs have previously been described in detail by Rylander et al. (2009b). In brief, plasma concentrations of perfluorinated compounds were determined using sonication facilitated liquid-liquid extraction, activated charcoal clean-up and analysis on HPLC-QTOF-MS. The quality of the analysis was assured through repetitive analyses of blank samples and reference samples obtained from previous international comparison programs. For each batch of 30 samples, one reference material and two

blank samples were prepared. Three times each year, our laboratory also participates in the AMAP Ringtest for Persistent Organic Pollutants in Human Serum, an international comparison program, organized by Institut National de Santé Publique du Québec, Canada. Results from interlaboratory comparisons indicate that the uncertainties of our analysis are well within +/- 30% of the assigned values. Recovery rates of the internal standard varied between 60% and 150%, with the values above 100% as a result of matrix induced ion suppression of the recovery standard signal.

The method limit of detection (LOD) was automatically calculated by the quantification software and accounted for individual matrix effects. PFOS, PFOA and PFHxS were detected in a few blank samples. If the concentration of these compounds in the blank samples were larger than the software determined LOD for that batch of samples, LOD was determined from three times the concentration of analytes in the blanks.

Data defined as PFOS, PFOA and PFHxS are the sum of the linear and the dominating branched isomers.

The plasma concentrations of fatty acids were analyzed at the National Institute of Nutrition and Seafood Research in Bergen (NIFES), Norway. The method used has been described elsewhere (Rylander C. et al. 2010, submitted).

RNA isolation, data capturing and preprocessing of data

All methods for RNA analysis, data capturing and preprocessing of data is described in detail by Dumeaux et al. (2010). Microarray analysis was performed on the 270 samples using the Applied Biosystems expression array system (Foster City, Louisiana, USA). Briefly, 500 ng of total RNA was amplified and labeled using the NanoAmp RT-IVT Labeling Kit for one round of amplification. 10 µg of DIG-labeled cRNA was fragmented and hybridized to AB Human Genome Survey Microarray V2.0, in accordance with the Chemiluminescence Detection Kit Protocol. The AB Expression System software was used to export signal intensities, signal to noise ratios (S/N), and flagging values. Gene-wise intensities were adjusted for technical variability i.e. batch number, RNA extraction date, time between blood collection and storage.

Statistical analysis

The freely available software R version 2.8.1 (http://www.cran.r-project.org) with the Bioconductor packages was used for the statistical analysis. Study participants were divided into two groups ("high" and "low") according to their concentrations of PFOS, PFOA and PFHxS. All contaminant data were right-skewed and samples following the normal quantile-quantile plot (normal qq-plot) were defined as the "low" group while samples with high, non-normal values were defined as the "high" group. These cut-off values were determined to 30 ng/ml, 7.2 ng/ml and 1.7 ng/ml for PFOS, PFOA and PFHxS, respectively. Gene-wise linear models were used for evaluating differences between groups. Enrichment of 48 gene sets were evaluated for PFOS, PFOA and PFHxS, respectively, using the global test (Goeman et al. 2004). All tested gene sets had

previously been linked to PFC exposure and were curated from the literature, the Kyoto Encyclopedia of Genes and Genomes (KEGG) (Kanehisa and Goto 2000) and Gene Ontology (GO) (Table S1 in the Supplemental material). The global test was adjusted for multiple testing using false discovery rates (FDR) (Benjamini and Hochberg 1995). Comparative p-values were calculated for each gene set and indicate the proportion of random gene sets of the same size as the tested gene lists being significant by chance.

Differences in age, BMI, fasting status, HT use, use of other medication, smoking and the ratio of n-6/n-3 fatty acids between the "high" and "low" groups were evaluated using linear models and chi square tests. Variables that were significantly different between the two groups were adjusted for in the gene set enrichment analysis. The gene plot from the global test was used to select core genes that were most important for explaining the differences between the groups. Core genes were defined as genes with a standard deviation > 1.5 above the expected value under the null hypothesis of no association between gene set expression and exposure group. Genes that were strongly correlated to the core genes ($r > 0.75$) were further evaluated using functional clustering in the Database for Annotation, Visualization and Integrated Discovery (DAVID) (Dennis et al. 2003) to investigate groups of molecular pathways or processes related to PFC exposure.

Results

In the current study group, the dominating PFC was PFOS (median 19 ng/ml), followed by PFOA (4.4 ng/ml) and PFHxS (0.97 ng/ml) (Table 1). Two single genes- cytochrome C oxidase subunit VIb polypeptide 2 (*COX6B2*) (p=1.1e-5, FDR=0.14) and chromosome

17 open reading frame 74 (*MGC17624*) (p=1.7e-5, FDR=0.14) - were differentially expressed when comparing the "PFOS high" to the "PFOS low" group using gene-wise linear models. No significant single genes were differentially expressed according to PFOA and PFHxS concentrations.

Two gene sets (gene set 39 & 46 in Table S1 in the Supplemental material), both related to the citric acid cycle were differentially expressed between the "PFOS high" and "PFOS low" group (Table 2). Figures 1 and 2 show the gene plot for each of the significant pathways. There were eight core genes (*NNT, PDHB, SDHD, SDHC, SUCLA2, IDH3A, MDH1, SUCLG2*) that were down-regulated in the "PFOS high" group and one gene (*ACO2*) that was up-regulated (Table 2, Figure 3). The core genes expressed in the citric acid cycle pathway were strongly correlated to 58 identified single genes (Table S2 in the Supplemental material). Within these 58 genes, a functional cluster of three metabolic processes was identified (median FDR=2.3%, Table 3).

The women in the "PFOS high" group were significantly older and had lower ratio of n-6/n-3 fatty acids than the women in the "PFOS low" group (Table 4). There were no differences in smoking status, BMI, fasting, medication use or HT use between the two groups (Table 4). When adjusting for age and the ratio of n-6/n-3 fatty acids, the genes within the citric acid cycle pathway remained differentially expressed between the "PFOS high" and "PFOS low" group. None of the tested gene sets were significantly enriched in the PFOA or PFHxS groups.

Discussion

To the best of our knowledge, this is the first population based study investigating the impact of organic pollutants on blood gene signatures in humans. We have successfully used gene expressions in peripheral blood cells for assessing the effects of PFCs in the general population. Our results suggest that gene profiles in human blood are a promising tool for exploring which biological pathways that are being affected by contaminants. Blood gene signatures may therefore serve as complements to already established toxicological methods. It has to be emphasized that technical noise were present and adjusted for in the current microarray analysis (Dumeaux et al. 2010), however, rapid improvements in this field are expected and improved technical performance will ascertain enhanced future gene expression results. Hence, high through put assays open new research avenues to investigate the effects of pollutants on human health.

Our finding regarding the citric acid cycle pathway being affected by PFOS is consistent with a previous study reporting a positive association between serum PFOS concentrations (mean 24 ng/ml), blood insulin levels, β-cell function and insulin resistance status (i.e. indicators of metabolic syndrome) in 969 adults from the general population (Lin et al. 2009). The authors suggested that PFCs may have an effect on the glucose metabolism in the general population, which is in line with our result. There are in addition some toxicological indications that PFCs may interfere with the carbohydrate metabolism (Guruge et al. 2006) in rodents, which also converges in the citric acid cycle. A set of 58 genes were identified as co-varying with the core genes in the citric acid cycle pathway. A cluster of three metabolic processes were identified within that gene list,

indicating that genes involved in the metabolism were differentially expressed in the "PFOS high" group. Eight of the nine core genes in the citric acid cycle gene sets encode central enzymes within that pathway. All, except for *ACO2*, were down-regulated in the "high" group. This could be a result of several different processes. PFOS may interfere with the citric acid cycle itself, it could be a result of a feedback mechanism induced by PFOS, or the mitochondrion could be affected and in turn the citric acid cycle. A large number of diseases are associated with citric acid cycle disorders, e.g. diabetes and Alzheimer's disease (Brooks et al. 2007), suggesting that the impact of PFOS on public health needs further investigations. Increased mortality of diabetes has also been observed in occupational exposed workers that were medium exposed to PFOA (Lundin et al. 2009). Although their results were inconsistent, they emphasize the need for more thorough understanding of the effects of PFCs on humans.

As PFCs were present in the blood of all participants in the current study group, it was not possible to divide them into sub-groups of "exposed" and "non-exposed" individuals. Differences between the "high" and "low" groups will therefore be small and there will be low chances of detecting differentially expressed single genes. Our analyses were therefore focused on gene set enrichment instead of single genes analysis. Surprisingly, two single genes (COX6B2 and MGC17624) were differentially expressed between the "PFOS high" and the "PFOS low" group from the gene-wise linear analysis. This is an interesting finding, but could also be a result of chance.

The significantly affected pathways had false discovery rates of 37% and 25%. Breitling et al. recommended that gene sets with FDR ≤ 10% should be considered interesting (Breitling 2006). In the current study, we accepted higher FDR values since our gene sets were curated from previous publications and thereby supported by toxicological/ epidemiological findings. In addition, none of the tested gene sets had comparative p-values high enough to raise concerns for false positive results.

We found no significantly enriched pathways for PFOA or PFHxS in this study group, which could be a result of low and uniform concentrations of the analytes within the population studied. Although PFOA and PFOS have been identified as potent peroxisome proliferators in rodents (Lau et al. 2007), the PPAR pathway was not enriched for any of the investigated PFCs. A number of factors, including species-specific differences, could be the reason for the lack of differentially expressed genes within that biological pathway. Additionally, effects of PFOS and PFOA are mainly seen in the liver among test animals, and less pronounced effects in blood cells are therefore expected.

Conclusion

In this unique study of a representative group of Norwegian middle-aged women, environmental PFOS exposure induced changes in genes encoding for enzymes involved in the citric acid cycle. These findings suggest that PFOS interfere with the glucose metabolism in the general population. Several important catabolic pathways (e.g. carbohydrate, protein and fat catabolism) converge in the citric acid cycle, emphasizing the need for comprehensive understanding of the impact of PFOS on human health.

References

Alexander BH, Olsen GW. 2007. Bladder Cancer in Perfluorooctanesulfonyl Fluoride Manufacturing Workers. Ann Epidemiol. 17: 471-478.

Alexander BH, Olsen GW, Burris JM, Mandel JH, Mandel JS. 2003. Mortality of employees of a perfluorooctanesulphonyl fluoride manufacturing facility. Occup Environ Med. 60: 722-729.

Amundson SA, Do KT, Shahab S, Bittner M, Meltzer P, Trent J, et al. 2000. Identification of potential mRNA biomarkers in peripheral blood lymphocytes for human exposure to ionizing radiation. Radiat Res 154: 342-346.

Benjamini Y, Hochberg Y. 1995. Controlling the false discovery rate – a practical and powerful approach to multiple testing. J R Stat Soc Ser B-Methodol 57: 289-300.

Berthiaume J, Wallace KB. 2002. Perfluorooctanoate, perflourooctanesulfonate, and N-ethyl perfluorooctanesulfonamido ethanol; peroxisome proliferation and mitochondrial biogenesis. Toxicol Lett. 129: 23-32.

Breitling R. 2006. Biological microarray interpretation: The rules of engagement. Biochim Biophys Acta 1759: 319-327.

Brooks WM, Lynch PJ, Ingle CC, Hatton A, Emson PC, Faull RLM, et al. 2007. Gene expression profiles of metabolic enzyme transcripts in Alzheimer's disease. Brain Res. 1127: 127-135.

Butenhoff JL, Chang S-C, Ehresman DJ, York RG. 2009. Evaluation of potential reproductive and developmental toxicity of potassium perfluorohexanesulfonate in Sprague Dawley rats. Reprod Toxicol. 27: 331-341.

Calafat AM, Wong LY, Kuklenyik Z, Reidy JA, Needham LL. 2007. Polyfluoroalkyl chemicals in the US population: Data from the National Health and Nutrition Examination Survey (NHANES) 2003-2004 and comparisons with NHANES 1999-2000. Environ Health Perspect. 115: 1596-1602.

Curran I, Hierlihy SL, Liston V, Pantazopoulos P, Nunnikhoven Ae, Tittlemier S, et al. 2008. Altered Fatty Acid Homeostasis and Related Toxicologic Sequelae in Rats Exposed to Dietary Potassium Perfluorooctanesulfonate (PFOS). J Toxicol Environ Health 71: 1526 - 1541.

Dennis G, Sherman BT, Hosack DA, Yang J, Gao W, Lane HC, et al. 2003. DAVID: Database for annotation, visualization, and integrated discovery. Genome Biol 4: 11.

Dumeaux V, Borresen-Dale AL, Frantzen JO, Kumle M, Kristensen VN, Lund E. 2008. Gene expression analyses in breast cancer epidemiology: the Norwegian Women and

Cancer postgenome cohort study. Breast Cancer Res; doi: 10.1186/bcr1859 [Online 13 February 2008]

Dumeaux V, Olsen KS, Nuel G, Paulssen RH, Børresen-Dale A-L, Lund E. In press. Deciphering normal blood gene expression - The NOWAC postgenome study. PLoS Genet.

Eady JJ, Wortley GM, Wormstone YM, Hughes JC, Astley SB, Foxall RJ, et al. 2005. Variation in gene expression profiles of peripheral blood mononuclear cells from healthy volunteers. Physiol Genomics 22: 402-411.

Ellis DA, Martin JW, De Silva AO, Mabury SA, Hurley MD, Sulbaek Andersen MP, et al. 2004. Degradation of Fluorotelomer Alcohols: A Likely Atmospheric Source of Perfluorinated Carboxylic Acids. Environ Sci Technol 38: 3316-3321.

Fei CY, McLaughlin JK, Tarone RE, Olsen J. 2007. Perfluorinated chemicals and fetal growth: A study within the Danish National Birth Cohort. Environ Health Perspect 115: 1677-1682.

Fromme H, Tittlemier SA, Völkel W, Wilhelm M, Twardella D. 2009. Perfluorinated compounds - Exposure assessment for the general population in western countries. Int J Hyg Environ Health 212: 239-270.

Goeman JJ, van de Geer SA, de Kort F, van Houwelingen HC. 2004. A global test for groups of genes: testing association with a clinical outcome. Bioinformatics 20: 93-99.

Grice MM, Alexander BH, Hoffbeck R, Kampa DM. 2007. Self-reported medical conditions in perfluorooctanesulfonyl fluoride manufacturing workers. J Occup Environ Med 49: 722-729.

Guruge KS, Yeung LWY, Yamanaka N, Miyazaki S, Lam PKS, Giesy JP, et al. 2006. Gene expression profiles in rat liver treated with perfluorooctanoic acid (PFOA). Toxicol Sci 89: 93-107.

Hu Wy, Jones PD, DeCoen W, King L, Fraker P, Newsted J, et al. 2003. Alterations in cell membrane properties caused by perfluorinated compounds. Comp Biochem Physiol C Toxicol Pharmacol 135: 77-88.

Kanehisa M, Goto S. 2000. KEGG: Kyoto Encyclopedia of Genes and Genomes. Nucleic Acids Res 28: 27-30.

Kannan K, Corsolini S, Falandysz J, Fillmann G, Kumar KS, Loganathan BG, et al. 2004. Perfluorooctanesulfonate and related fluorochemicals in human blood from several countries. Environ Sci Technol 38: 4489-4495.

Lampe JW, Stepaniants SB, Mao M, Radich JP, Dai HY, Linsley PS, et al. 2004. Signatures of environmental exposures using peripheral leukocyte gene expression: Tobacco smoke. Cancer Epidemiol Biomarkers Prev 13: 445-453.

Lau C, Anitole K, Hodes C, Lai D, Pfahles-Hutchens A, Seed J. 2007. Perfluoroalkyl acids: A review of monitoring and toxicological findings. Toxicol Sci 99: 366-394.

Lin CY, Chen PC, Lin YC, Lin LY. 2009. Association Among Serum Perfluoroalkyl Chemicals, Glucose Homeostasis, and Metabolic Syndrome in Adolescents and Adults. Diabetes Care 32: 702-707.

Lund E, Dumeaux V. 2008. Systems Epidemiology in Cancer. Cancer Epidemiol Biomarkers Prev 17: 2954-2957.

Lund E, Dumeaux V, Braaten T, Hjartaker A, Engeset D, Skeie G, et al. 2008. Cohort profile: The Norwegian women and cancer study - NOWAC - Kvinner og kreft. Int J Epidemiol 37: 36-41.

Lundin JI, Alexander BH, Olsen GW, Church TR. 2009. Ammonium Perfluorooctanoate Production and Occupational Mortality. Epidemiology 20: 921-928.

McHale CM, Zhang L, Hubbard AE, Zhao X, Baccarelli A, Pesatori AC, et al. 2007. Microarray analysis of gene expression in peripheral blood mononuclear cells from dioxin-exposed human subjects. Toxicology 229: 101-113.

McHale CM, Zhang LP, Lan Q, Li GL, Hubbard AE, Forrest MS, et al. 2009. Changes in the peripheral blood transcriptome associated with occupational benzene exposure identified by cross-comparison on two microarray platforms. Genomics 93: 343-349.

Midasch O, Drexler H, Hart N, Beckmann MW, Angerer J. 2007. Transplacental exposure of neonates to perfluorooctanesulfonate and perfluorooctanoate: a pilot study. Int Arch Occup Environ Health 80: 643-648.

Monroy R, Morrison K, Teo K, Atkinson S, Kubwabo C, Stewart B, et al. 2008. Serum levels of perfluoroalkyl compounds in human maternal and umbilical cord blood samples. Environ Res 108: 56-62.

Olsen GW, Butenhoff JL, Zobel LR. 2009. Perfluoroalkyl chemicals and human fetal development: An epidemiologic review with clinical and toxicological perspectives. Reprod Toxicol 27: 212-230.

Powley CR, George SW, Russell MH, Hoke RA, Buck RC. 2008. Polyfluorinated chemicals in a spatially and temporally integrated food web in the Western Arctic. Chemosphere 70: 664-672.

Radich JP, Mao M, Stepaniants B, Biery M, Castle J, Ward T, et al. 2004. Individual-specific variation of gene expression in peripheral blood leukocytes. Genomics 83: 980-988.

Rylander C, Duong TP, Odland JO, Sandanger TM. 2009a. Perfluorinated compounds in delivering women from south central Vietnam. J Environ Monit 11: 2002-2008.

Rylander C, Brustad M, Falk H, Sandanger TM. 2009b. Dietary predictors and plasma concentrations of perfluorinated compounds in a coastal population from northern Norway. J Environ Public health; doi: 10.1039/b908551c [Online 5 October 2009]

Tanner MA, Berk LS, Felten DL, Blidy AD, Bit SL, Ruff DW. 2002. Substantial changes in gene expression level due to the storage temperature and storage duration of human whole blood. Clin Lab Haematol 24: 337-341.

Tao L, Ma J, Kunisue T, Libelo EL, Tanabe S, Kannan K. 2008. Perfluorinated Compounds in Human Breast Milk from Several Asian Countries, and in Infant Formula and Dairy Milk from the United States. Environ Sci Technol 42: 8597-8602.

Wang ZX, Neuburg D, Li C, Su L, Kim JY, Chen JC, et al. 2005. Global gene expression profiling in whole-blood samples from individuals exposed to metal fumes. Environ Health Perspect 113: 233-241.

Wild CP. 2009. Environmental exposure measurement in cancer epidemiology. Mutagenesis 24: 117-125.

Wolf CJ, Takacs ML, Schmid JE, Lau C, Abbott BD. 2008. Activation of mouse and human peroxisome proliferator-activated receptor alpha by perfluoroalkyl acids of different functional groups and chain lengths. Toxicol Sci 106: 162-171.

Waaseth M, Bakken K, Dumeaux V, Olsen KS, Rylander C, Figenschau Y, et al. 2008. Hormone replacement therapy use and plasma levels of sex hormones in the Norwegian Women and Cancer postgenome cohort - a cross-sectional analysis. BMC Women Health; doi: 10.1186/1472-6874-8-1 [Online 14 January 2008]

Yeung LWY, Guruge KS, Yamanaka N, Miyazaki S, Lam PKS. 2007. Differential expression of chicken hepatic genes responsive to PFOA and PFOS. Toxicology 237: 111-125.

Table 1. Plasma concentrations of the selected PFCs in the study group

	Concentration (ng/ml)				
	Median	AM	Range	LOD	%>LOD
PFOS					
Total sample N=270	19	21	5.7-84	0.01-2.10	100
High group N=42	37	40	31-84		
Low group N=228	18	18	5.4-30		
PFOA					
Total sample N=270	4.4	5.1	0.79-21	0.11-1.56	100
High group N=32	9.2	11	7.3-21		
Low group N=238	2.9	2.8	0.79-3.5		
PFHxS					
Total sample N=270	0.97	1.3	0.15-13	0.01-1.23	94
High group N=46	2.7	3.4	1.7-13		
Low group N=224	0.87	0.9	0.15-1.7		

PFOS, Perfluorooctane sulphonate; PFOA, perfluorooctanoate; PFHxS, perfluorohexane sulphonate; AM, Arithmetic mean; LOD, Method limit of detection; %>LOD, Proportion of samples with concentrations > LOD

Table2. Significant gene sets associated with PFOS exposure.

	No. probes tested	p-value	FDR adjusted	Comparative p-value	Core genes (official gene symbols) up-regulated in the PFOS high group	Core genes (official gene symbols) down-regulated in the PFOS high group
Citric acid cycle	27	0.0393	0.37	0.18	ACO2	SUCLA2, IDH3A, MDH1, PDHB, SUCLG2, SDHD, SDHC
Citric acid cycle	10	0.0504	0.30	0.30	ACO2	NNT, PDHB, SDND, SDHC

PFOS, perfluorooctane sulphonate; FDR, false discovery rate

Table 3. Functional cluster of three metabolic processes, related to the 58 genes highly correlated to the core genes from the citric acid cycle pathway.

Process (no genes in process)	p-value	Fold enrichment	FDR (%)
Macromolecule metabolic process (29)	0.000064	1.7	0.1
Cellular metabolic process (29)	0.0012	1.5	2.3
Primary metabolic process (29)	0.0013	1.5	2.4

FDR, false discovery rate

Table 4. Characteristics of the two PFOS groups.

	PFOS high (N=42)	PFOS low (n=228)	p-value
Age (years)	57	55.5	0.01
Body mass index (kg/m$^{2)}$	25.4	25.5	0.92
Smoking (Y/N)	7/35	63/164	0.19
Hormone therapy use (Y/N)	8/32	40/185	0.91
Medication use (Y/N)	21/20	130/94	0.52
Fasting (Y/N)	5/35	19/196	0.66
n-6/n-3 fatty acid ratio (mg/ml)[a]	5.2	6.2	0.006

PFOS, perfluorooctane sulphonate

[a]Include linolenic acid (LA)18:2n-6; eicosadienoic acid 20:2n-6; arachidonic acid (AA) 20:4n-6; dihomo-gamma-linolenic acid (DGLA) 20:3n-6; 16:3n-3; 16:4n-3; alpha-linolenic acid (ALA) 18:3n-3; stearidonic acid 18:4n-3; eicosatrienoic acid (ETE) 20:3n-3; eicosatetraenoic acid (ETA) 20:4n-3; eicosapentaenoic acid (EPA) C20:5n-3; docosapentaenoic acid (DPA) C22:5n-3 and docosahexaenoic acid (DHA) C22:6n-3

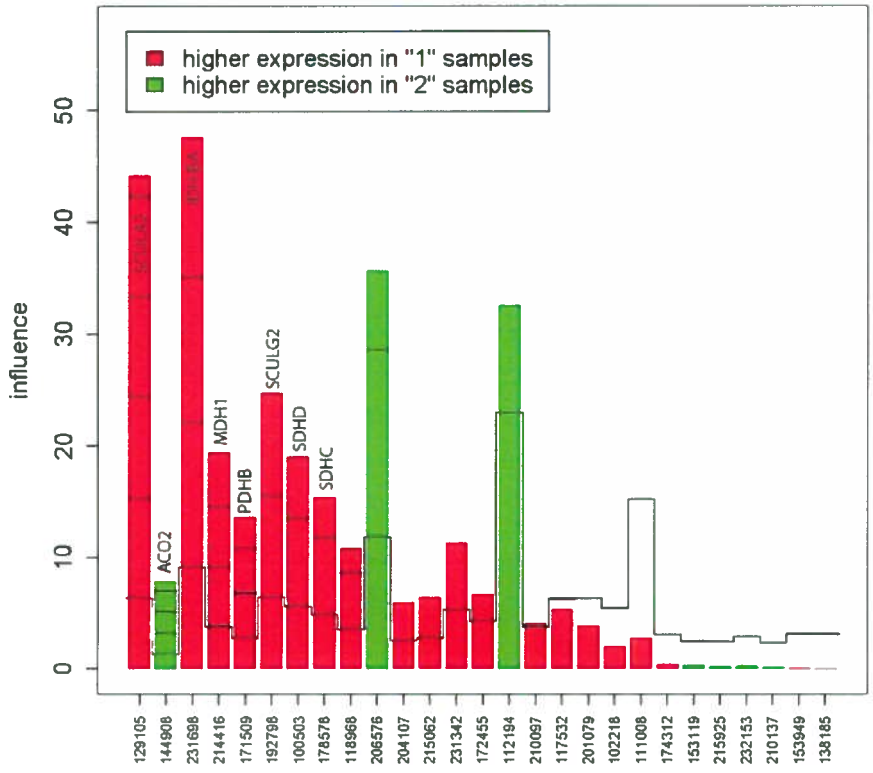

Figure1. Gene plot of the influence of "high" vs. "low" PFOS on the citric acid cycle (gene set 39). Core genes are identified by official gene symbols. The x-axis shows the probe IDs and the y-axis shows the influence of each gene on the test result.

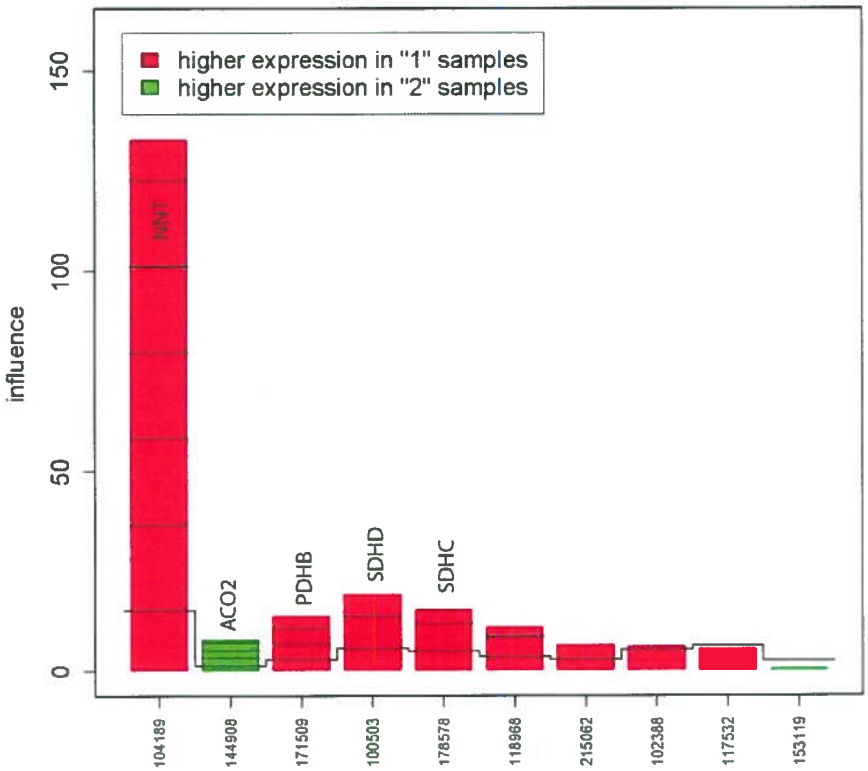

Figure 2. Gene plot of the influence of "high" vs. "low" PFOS on the citric acid cycle (gene set 46). Core genes are identified by official gene symbols. The x-axis shows the probe IDs and the y-axis shows the influence of each gene on the test result.

Supplemental material for

Perfluorinated compounds and blood gene signatures in postmenopausal women: the NOWAC postgenome study

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Table S1. Gene sets tested in gene set enrichment analysis

Gene set ID number	Description	Number of genes	Reference
1	Fatty acid beta oxidation	46	KEGG
2	Fatty acid beta oxidation	24	GO
3	Genes linked to PFOS and PFOA exposure in chickens	36	(Yeung et al. 2007)
4	Gap junction intracellular communication	96	KEGG
5	Genes linked to oxidative stress in hepatocytes from freshwater tilapia after PFOS and PFOA exposure	17	(Liu et al. 2007)
6	Oxidative stress	94	GO
7	Regulation of fatty acid oxidation	18	GO
8	Steroid metabolism	11	KEGG
9	Cholesterol biosynthesis	9	KEGG
10	Cholesterol metabolism	44	GO
11	Steroid biosynthesis	17	KEGG
12	Cholesterol biosynthesis	9	GO
13	Genes linked to PFOS exposure in rat	19	(Guruge et al. 2006)
14	Genes linked to PFOS and PFOA exposure in rat	13	(Guruge et al. 2006)
15	Xenobiotic metabolic process	13	GO
16	Xenobiotic metabolism	70	KEGG
17	Synthesis and degradation of ketone bodies	9	KEGG
18	Fatty acid elongation	10	KEGG
19	Fatty acid metabolic process	134	GO
20	Unsaturated fatty acid biosynthesis	22	KEGG
21	Fatty acid biosynthesis	7	KEGG
22	Fatty acid biosynthesis and regulation	19	GO
23	Apoptosis	88	KEGG
24	Genes linked to PFOS exposure in zebrafish	6	(Shi et al. 2008)
25	Bile acid biosynthesis	16	KEGG
26	Bile acid metabolic process	11	GO
27	Genes linked to PFOS exposure in rat liver	24	(Bjork et al. 2008)
28	Genes linked to PFOS exposure in hepatocytes from Atlantic salmon	27	(Krøvel et al. 2008)
29	Genes linked to PFOS exposure in carp	20	(Hagenaars et al. 2008)

30	Genes linked to PFOS exposure in chicken embryo hepatocytes	6	(Cwinn et al. 2008)
31	Genes linked to PFOS exposure in bottlenose dolphin	8	(Mollenhauer et al. 2009)
32	Genes linked to PFOS exposure in rats	4	(Chang et al.)
33	Genes linked to PFOS exposure in mouse	8	(Rosen et al. 2007)
34	Glycolysis	63	KEGG
35	Glucose metabolic process	57	GO
36	Diabetes 2	44	KEGG
37	Genes linked to PFOA exposure in rat	76	(Guruge et al. 2006)
38	Leukocyte transendothelial migration	116	KEGG
39	Citric acid cycle	32	KEGG
40	Genes linked to PFOA exposure in mouse liver	4	(Ren et al. 2009)
41	Retinol metabolism	64	KEGG
42	PPAR	69	KEGG
43	Insulin signaling	138	KEGG
44	Glucose homeostasis	22	GO
45	Retinol metabolic process	7	GO
46	Citric acid cycle	7	GO
47	Insulin receptor signaling pathway	33	GO
48	Lipid transport	95	GO

Table S2. Genes highly correlated ($r > 0.75$) to the core genes from gene set 39 and 46 (citric acid cycle).

Official gene symbol
CYCS, PNRC2, HMGN1, ARPC5, GRPEL1, SLBP, SLTM, HNRPH3, SMT3, SPCS2, C9orf156, DDX1, CRLF3, CDKN1B, RAB1A, CALM2, HNRPA1, ACTR1B, CCAR1, FLJ20647, RBBP7, PPP1CC, PPCS, PSMC2, HMGN1, COX5A, GTF2A2, HMGN3, LMBRD1, MORF4L2, NDNL2, MGC12981, VDAC2, SELT, ARL2BP, MIS12, CEBPZ, XPA, GOLGA7, BXDC5, MGC4767, NIF3L1, SFRS10, CNOT8, C1orf108, OCIAD1, IGBP1, RPP38, PHGDHL1, SSB, PMPCB, PDHA1, TMED10, ZNF9, YWHAZ, C2orf25, MORF4L1, HNRPA1P4

References

Bjork JA, Lau C, Chang SC, Butenhoff JL, Wallace KB. 2008. Perfluorooctane sulfonate-induced changes in fetal rat liver gene expression. Toxicology 251: 8-20.

Chang S-C, Ehresman DJ, Bjork JA, Wallace KB, Parker GA, Stump DG, et al. 2009. Gestational and lactational exposure to potassium perfluorooctanesulfonate (K+PFOS) in rats: Toxicokinetics, thyroid hormone status, and related gene expression. Reprod Toxicol 27:387-399

Cwinn MA, Jones SP, Kennedy SW. 2008. Exposure to perfluorooctane sulfonate or fenofibrate causes PPAR-[alpha] dependent transcriptional responses in chicken embryo hepatocytes. Comp Biochem Physiol C Toxicol Pharmacol 148: 165-171.

Guruge KS, Yeung LWY, Yamanaka N, Miyazaki S, Lam PKS, Giesy JP, et al. 2006. Gene expression profiles in rat liver treated with perfluorooctanoic acid (PFOA). Toxicol Sci 89: 93-107.

Hagenaars A, Knapen D, Meyer IJ, van der Ven K, Hoff P, De Coen W. 2008. Toxicity evaluation of perfluorooctane sulfonate (PFOS) in the liver of common carp (Cyprinus carpio). Aquat Toxicol 88: 155-163.

Krøvel AV, Søfteland L, Torstensen B, Olsvik PA. 2008. Transcriptional effects of PFOS in isolated hepatocytes from Atlantic salmon Salmo salar L. Comp Biochem Physiol C Toxicol Pharmacol 148: 14-22.

Liu C, Yu K, Shi X, Wang J, Lam PKS, Wu RSS, et al. 2007. Induction of oxidative stress and apoptosis by PFOS and PFOA in primary cultured hepatocytes of freshwater tilapia (Oreochromis niloticus). Aquat Toxicol 82: 135-143.

Mollenhauer MAM, Carter BJ, Peden-Adams MM, Bossart GD, Fair PA. 2009. Gene expression changes in bottlenose dolphin, Tursiops truncatus, skin cells following exposure to methylmercury (MeHg) or perfluorooctane sulfonate (PFOS). Aquat Toxicol 91: 10-18.

Ren HZ, Vallanat B, Nelson DM, Yeung LWY, Guruge KS, Lam PKS, et al. 2009. Evidence for the involvement of xenobiotic-responsive nuclear receptors in transcriptional effects upon perfluoroalkyl acid exposure in diverse species. Reproduc Toxicol 27: 266-277.

Rosen MB, Schmid JE, Das KP, Wood CR, Zehr RD, Lau C. 2009. Gene expression profiling in the liver and lung of perfluorooctane sulfonate-exposed mouse fetuses: Comparison to changes induced by exposure to perfluorooctanoic acid. Reproduct Toxicol 27: 278-288

Shi X, Du Y, Lam PKS, Wu RSS, Zhou B. 2008. Developmental toxicity and alteration of gene expression in zebrafish embryos exposed to PFOS. Toxicol Appl Pharmacol230: 23-32.

Yeung LWY, Guruge KS, Yamanaka N, Miyazaki S, Lam PKS. 2007. Differential expression of chicken hepatic genes responsive to PFOA and PFOS. Toxicology 237: 111-125.

Appendix I

Høst 2004

KVINNER OG KREFT

KONFIDENSIELT

Hvis du samtykker i å være med, sett kryss for JA i ruten ved siden av.
Dersom du ikke ønsker å delta kan du unngå purring ved å sette kryss for NEI og returnere skjemaet i vedlagte svarkonvolutt.
Vi ber deg fylle ut spørreskjemaet så nøye som mulig.

Skjemaet skal leses optisk. Vennligst bruk blå eller sort penn.
Du kan ikke bruke komma, forhøy 0,5 til 1. Bruk blokkbokstaver.

Med vennlig hilsen
Eiliv Lund

Jeg samtykker i å delta i spørreskjemaundersøkelsen JA ☐ NEI ☐

Overgangsalder

Har du regelmessig menstruasjon fremdeles?

- ☐ Ja
- ☐ Har uregelmessig menstruasjon
- ☐ Vet ikke (menstruasjon uteblitt pga. sykdom o.l.)
- ☐ Vet ikke (bruker hormonpreparat med østrogen)
- ☐ Nei

Hvis Nei;

- har den stoppet av seg selv? ☐
- har du operert vekk eggstokkene? ☐
- har du operert vekk livmoren? ☐
- annet? ☐

Alder da menstruasjonen opphørte ☐

Graviditeter, fødsler og amming

Har du noen gang vært gravid? Ja ☐ Nei ☐

Hvis Ja; hvor mange barn har du født i alt ☐

Hvor gammel var du ved siste fødsel? ☐

P-pillebruk

Har du brukt p-piller eller minipiller? Ja ☐ Nei ☐

Hvis ja, hvor mange år har du brukt p-piller i alt? ☐

Bruker du p-piller nå? Ja ☐ Nei ☐

Bruk av hormonpreparater med østrogen i overgangsalderen

Har du noen gang brukt østrogentabletter/plaster? Ja ☐ Nei ☐

Hvis Ja; hvor mange år har du brukt østrogentabletter/plaster i alt? ☐

Hvor gammel var du første gang du brukte østrogentabletter/plaster? ☐

Bruker du tabletter/plaster nå? Ja ☐ Nei ☐

UTFYLLENDE SPØRSMÅL TIL ALLE SOM HAR BRUKT PREPARATER MED ØSTROGEN I FORM AV TABLETTER ELLER PLASTER FRA 1998 OG FREM TIL I DAG.

Har du svart «ja», ber vi deg utdype dette nærmere ved å svare på spørsmålene nedenfor. For hver periode med sammenhengende bruk av samme hormonpreparat håper vi du kan si oss hvor gammel du var da du startet, hvor lenge du brukte det samme hormonpreparatet og navnet på dette. Dersom du har hatt opphold eller skiftet merke skal du besvare spørsmålene for en ny periode. Dersom du ikke husker navnet på hormonpreparatet, sett «usikker». For å hjelpe deg til å huske navnet på hormonpreparatene ber vi deg bruke den vedlagte brosjyre som viser bilder av hormonpreparater som har vært solgt i Norge. Vennligst oppgi også nummer på hormontabletten/plasteret som står i brosjyren.

Periode	Alder ved start	Brukt samme hormontablett/ plaster/ sammenhengende fra 1998 år	måned	Nr.	Navn på hormontablett/plaster/ (se brosjyre)
1.					
2.					
3.					
4.					
5.					

Østrogenpreparat til lokal bruk i skjeden

Har du noen gang brukt østrogen-krem/stikkpille? Ja ☐ Nei ☐

Hvis Ja;
bruker du krem/stikkpille nå? Ja ☐ Nei ☐

Hormonspiral

Har du noen gang brukt hormonspiral (Levonova)? Ja ☐ Nei ☐

Hvis Ja; hvor mange hele år har du brukt hormonspiral i alt? ☐

Hvor gammel var du første gang du fikk innsatt hormonspiral? ☐

Bruker du hormonspiral nå? Ja ☐ Nei ☐

Selvopplevd helse

Oppfatter du din egen helse som; (Sett ett kryss)

Meget god ☐ God ☐ Dårlig ☐ Meget dårlig ☐

Sykdom

Har du eller har du hatt noen av følgende sykdommer? (sett ett eller flere kryss)

	Ja	Nei	Hvis ja: Alder ved start
Kreft	☐	☐	
Høyt blodtrykk	☐	☐	
Hjertesvikt/hjertekrampe	☐	☐	
Hjerteinfarkt	☐	☐	
Slag	☐	☐	
Sukkersyke (diabetes)	☐	☐	
Depresjon (oppsøkt lege)	☐	☐	
Hypothyreose/lavt stoffskifte	☐	☐	

For følgende tilstander ber vi deg krysse av for hvilket år tilstanden oppsto første gang.

	for 98	98	99	00	01	02	03
Muskelsmerter (myalgi)	☐	☐	☐	☐	☐	☐	☐
Fibromyalgi/Fibrositt	☐	☐	☐	☐	☐	☐	☐
Kronisk tretthetssyndrom	☐	☐	☐	☐	☐	☐	☐
Ryggsmerter ukjent årsak	☐	☐	☐	☐	☐	☐	☐
Nakkeslengskade	☐	☐	☐	☐	☐	☐	☐
Osteoporose (b.skjørhet)	☐	☐	☐	☐	☐	☐	☐
Brudd							
Underarmen (håndledd)	☐	☐	☐	☐	☐	☐	☐
Lårhalsen	☐	☐	☐	☐	☐	☐	☐
Ryggvirvel (kompresjon)	☐	☐	☐	☐	☐	☐	☐

Andre legemidler

Bruker du noen av disse legemidlene daglig nå?

Fontex, Fluoxetin	Ja ☐	Nei ☐
Cipramil, Citalopram, Desital	Ja ☐	Nei ☐
Seroxat, Paroxetin	Ja ☐	Nei ☐
Zoloft	Ja ☐	Nei ☐
Fevarin	Ja ☐	Nei ☐
Cipralex	Ja ☐	Nei ☐

Hvis Ja; hvor lenge har du brukt dette legemidlet sammenhengede? Måneder ☐ År ☐

Har du benyttet noen av disse legemidlene tidligere? Ja ☐ Nei ☐

Hvis Ja; hvor lenge har du benyttet disse legemidlene i alt? År ☐

Høyde og vekt

Hvor høy er du? (i hele cm) ☐

Hvor mye veier du i dag? (i hele kg) ☐

Hvor mye veide du da du var 18 år? (i hele kg) ☐

Kroppstype i 1. klasse. (Sett ett kryss)

Veldig tynn ☐ Tynn ☐ Normal ☐ Tykk ☐ Veldig tykk ☐

Røykevaner

Har du i løpet av livet røykt mer enn 100 sigaretter til sammen? Ja ☐ Nei ☐

Hvis Ja, ber vi deg fylle ut for de siste fem årene hvor mange sigaretter du i gjennomsnitt røykte pr. dag i denne perioden.

Antall sigaretter pr. dag

0	1-4	5-9	10-14	15-19	20-24	25+
☐	☐	☐	☐	☐	☐	☐

Hvor gammel var du da du tok din første sigarett? ☐

Røyker du daglig nå? Ja ☐ Nei ☐

Hvis Nei, hvor gammel var du da du sluttet? ☐

Røykte noen av dine foreldre da du var barn? Ja ☐ Nei ☐

Hvis Ja, hvor mange sigaretter røykte de til sammen pr. dag? (antall) ☐

Brystkreft i nærmeste familie

Har noen nære slektninger hatt brystkreft?

	Ja	Nei	Vet ikke	Alder ved start
Datter	☐	☐	☐	
Mor	☐	☐	☐	
Søster	☐	☐	☐	

Mammografiundersøkelse

Har du vært til undersøkelse av brystene med mammografi Ja ☐ Nei ☐

Hvis Ja;
hvor gammel var du første gangen? (hele år)

Hvor mange ganger har du vært undersøkt?

-etter invitasjon fra Mammografiprogrammet

-etter henvisning fra lege

-uten henvisning fra lege

Fysisk aktivitet

Vi ber deg angi din fysiske aktivitet etter en skala fra svært liten til svært mye ved 14 års alder, ved 30 års alder og i dag. Skalaen nedenfor går fra 1-10. Med fysisk aktivitet mener vi både arbeid i hjemmet og i yrkeslivet samt trening og annen fysisk aktivitet som turgåing ol.

Alder	Svært lite									Svært mye
14 år	1	2	3	4	5	6	7	8	9	10
30 år	1	2	3	4	5	6	7	8	9	10
I dag	1	2	3	4	5	6	7	8	9	10

Hvor mange timer pr. dag i gjennomsnitt går eller spaserer du utendørs?

	sjelden/ aldri	mindre enn 1/2 time	1/2-1 time	1-2 timer	mer enn 2 timer
Vinter	☐	☐	☐	☐	☐
Vår	☐	☐	☐	☐	☐
Sommer	☐	☐	☐	☐	☐
Høst	☐	☐	☐	☐	☐

Hvor mange trapper (hele etasjer) går du i gjennomsnitt pr. dag

For hver av følgende aktiviteter du deltar i, ber vi deg oppgi hvor mange minutter pr. dag du bruker i gjennomsnitt til hver av aktivitetene.

Aktivitet	Minutter: Vinter	Vår	Sommer	Høst
Se på TV				
Lesing				
Håndarbeid				
Hagearbeid				
Dusj/bad/egenpleie				
Trening/jogging				
Sykling				

Hvor mange hele timer pr. dag bruker du på arbeidsplassen i gjennomsnitt til å

	Timer:
Sitte	
Stå	
Gå	
Løfte	
Tunge løft/pleie	

Kosthold

Påvirker noen av følgende forhold kostholdet ditt? (sett gjerne flere kryss)

- ☐ Er vegetarianer/veganer
- ☐ Spiser ikke norsk kost til daglig
- ☐ Har allergi/intoleranse
- ☐ Kronisk sykdom
- ☐ Har anoreksi
- ☐ Har bulimi
- ☐ Prøver å gå ned i vekt
- ☐ Lav glykemisk mat

Vi er interessert i å få kjennskap til hvordan kostholdet ditt er vanligvis. Kryss av for hvert spørsmål om hvor ofte du i gjennomsnitt siste året har brukt den aktuelle matvaren, og hvor mye du pleier å spise/drikke hver gang.

Drikke

Hvor mange glass melk drikker du vanligvis av hver type? (Sett ett kryss pr. linje)

	aldri/ sjelden	1-4 pr. uke	5-6 pr. uke	1 pr. dag	2-3 pr. dag	4+ pr.dag
Helmelk (søt, sur)	☐	☐	☐	☐	☐	☐
Lettmelk (søt, sur)	☐	☐	☐	☐	☐	☐
Ekstra lettmelk	☐	☐	☐	☐	☐	☐
Skummet (søt, sur)	☐	☐	☐	☐	☐	☐

Hvor mange kopper kaffe/te drikker du vanligvis av hver sort? (Sett ett kryss for hver linje)

	aldri/ sjelden	1-6 pr. uke	1 pr. dag	2-3 pr. dag	4-5 pr. dag	6-7 pr. dag	8+ pr.dag
Kokekaffe	☐	☐	☐	☐	☐	☐	☐
Traktekaffe	☐	☐	☐	☐	☐	☐	☐
Pulverkaffe	☐	☐	☐	☐	☐	☐	☐
Svart te	☐	☐	☐	☐	☐	☐	☐
Grønn te	☐	☐	☐	☐	☐	☐	☐

Bruker du følgende i kaffe eller te:

	Kaffe		Te	
Sukker (ikke kunstig søtstoff)	☐ Ja	☐ Nei	☐ Ja	☐ Nei
Melk eller fløte	☐ Ja	☐ Nei	☐ Ja	☐ Nei

Hvor mange glass vann drikker du vanligvis?
(Sett ett kryss for hver linje)

	aldri/ sjelden	1-6 pr. uke	1 pr. dag	2-3 pr. dag	4-5 pr. dag	6-7 pr. dag	8+ pr.dag
Springvann/ flaskevann	☐	☐	☐	☐	☐	☐	☐

Hvor mange glass appelsinjuice, saft og brus drikker du vanligvis? (Sett ett kryss for hver linje)

	aldri/ sjelden	1-3 pr. uke	4-6 pr. uke	1 pr. dag	2-3 pr. dag	4+ pr. dag
Appelsinjuice	☐	☐	☐	☐	☐	☐
Annen juice	☐	☐	☐	☐	☐	☐
Saft/brus med sukker	☐	☐	☐	☐	☐	☐
Saft/brus sukkerfri	☐	☐	☐	☐	☐	☐

Yoghurt/kornblanding

Hvor ofte spiser du yoghurt (1 beger)? (Sett ett kryss)

☐ Aldri/sjelden ☐ 1 pr. uke
☐ 2-3 pr. uke ☐ 4+ pr. uke

Hvor ofte spiser du kornblanding, havregryn eller müsli? (Sett ett kryss)

☐ Aldri/sjelden ☐ 1-3 pr. uke
☐ 4-6 pr. uke ☐ 1 pr. dag

Brødmat

Hvor mange skiver brød/rundstykker og knekkebrød/ skonrokker spiser du vanligvis?
(1/2 rundstykke = 1 brødskive) (Sett ett kryss for hver linje)

	aldri/ sjelden	1-4 pr. uke	5-7 pr. uke	2-3 pr. dag	4-5 pr. dag	6+ pr. dag
Grovt brød	☐	☐	☐	☐	☐	☐
Knelpp/halvfint	☐	☐	☐	☐	☐	☐
Fint brød/baguett	☐	☐	☐	☐	☐	☐
Knekkebrød o.l.	☐	☐	☐	☐	☐	☐

I neste spalte er det spørsmål om bruk av ulike påleggstyper. Vi spør om hvor mange brødskiver med det aktuelle pålegget du pleier å spise. Dersom du også bruker matvarene i andre sammenhenger enn til brød (f. eks. til vafler, frokostblandinger, grøt), ber vi om at du tar med dette når du besvarer spørsmålene.

På hvor mange brødskiver bruker du? (Sett ett kryss pr. linje)

	Aldri/ sjelden	1-3 pr. uke	4-6 pr. uke	1 pr. dag	2-3 pr. dag	4+ pr.dag
Syltetøy	☐	☐	☐	☐	☐	☐
Brunost, helfet	☐	☐	☐	☐	☐	☐
Brunost, halvfet/mager	☐	☐	☐	☐	☐	☐
Hvitost, helfet	☐	☐	☐	☐	☐	☐
Hvitost, halvfet/mager	☐	☐	☐	☐	☐	☐
Kjøttpålegg, Leverpostei	☐	☐	☐	☐	☐	☐
Rekesalat, italiensk o.l.	☐	☐	☐	☐	☐	☐

På hvor mange brødskiver pr. uke har du i gjennomsnitt siste året spist? (Sett ett kryss pr. linje)

	Aldri/ sjelden	1 pr.uke	2-3 pr.uke	4-6 pr.uke	7-9 pr.uke	10+ pr.uke
Makrell i tomat, røkt makrell	☐	☐	☐	☐	☐	☐
Kaviar	☐	☐	☐	☐	☐	☐
Sild/Ansjos	☐	☐	☐	☐	☐	☐
Laks (gravet/røkt)	☐	☐	☐	☐	☐	☐
Annet fiskepålegg	☐	☐	☐	☐	☐	☐

Hva slags fett bruker du vanligvis på brødet?
(Sett gjerne flere kryss)

☐ Bruker ikke fett på brødet
☐ Smør
☐ Hard margarin (f. eks. Per, Melange)
☐ Myk margarin (f. eks. Soft, Vita, Solsikke)
☐ Smørblandet margarin (f.eks. Bremyk)
☐ Brelett
☐ Lettmargarin (f. eks. Soft light, Letta, Vita Lett)
☐ Middels lett margarin (f. eks. Olivero, Omega)

Dersom du bruker fett på brødet, hvor tykt lag pleier du å smøre på? (En kuvertpakke med margarin veier 12 gram).
(Sett ett kryss)

☐ Skrapet (3 g) ☐ Tynt lag (5 g)
☐ Godt dekket (8 g) ☐ Tykt lag (12 g)

Frukt og grønnsaker

Hvor ofte spiser du frukt? (Sett ett kryss pr. linje)

	aldri/ sjelden	1-3 pr.mnd.	1 pr.uke	2-4 pr.uke	5-6 pr.uke	1 pr.dag	2+ pr. dag
Epler/pærer	☐	☐	☐	☐	☐	☐	☐
Appelsiner o.l.	☐	☐	☐	☐	☐	☐	☐
Bananer	☐	☐	☐	☐	☐	☐	☐
Annen frukt	☐	☐	☐	☐	☐	☐	☐

Hvor ofte spiser du ulike typer grønnsaker?
(Sett ett kryss pr. linje)

	aldri/ sjelden	1-3 pr.mnd.	1 pr.uke	2 pr.uke	3 pr.uke	4-5 pr.uke	6-7 pr. uke
Gulrøtter	☐	☐	☐	☐	☐	☐	☐
Kål	☐	☐	☐	☐	☐	☐	☐
Kålrot	☐	☐	☐	☐	☐	☐	☐
Brokkoli/blomkål	☐	☐	☐	☐	☐	☐	☐
Blandet salat	☐	☐	☐	☐	☐	☐	☐
Tomat	☐	☐	☐	☐	☐	☐	☐
Grønnsak-blanding (frossen)	☐	☐	☐	☐	☐	☐	☐
Løk	☐	☐	☐	☐	☐	☐	☐
Andre grønn-saker	☐	☐	☐	☐	☐	☐	☐

For de grønnsakene du spiser, kryss av for hvor mye du spiser hver gang. (Sett ett kryss for hver sort)

Gulrøtter	☐ 1/2 stk	☐ 1 stk	☐ 1 1/2 stk	☐ 2+ stk.
Kål	☐ 1/2 dl	☐ 1 dl	☐ 1 1/2 dl	☐ 2+ dl
Kålrot	☐ 1/2 dl	☐ 1 dl	☐ 1 1/2 dl	☐ 2+ dl
Brokkoli/ blomkål	☐ 1-2 buketter	☐ 3-4 buketter	☐ 5+ buketter	
Blandet salat	☐ 1 dl	☐ 2 dl	☐ 3 dl	☐ 4+ dl
Tomat	☐ 1/4 stk	☐ 1/2 stk	☐ 1stk	☐ 2+ stk
Grønnsak-blanding	☐ 1/2 dl	☐ 1 dl	☐ 2 dl	☐ 3+ dl

Hvor mange poteter spiser du vanligvis (kokte, stekte, mos)? (Sett ett kryss)

☐ Spiser ikke/spiser sjelden poteter ☐ 1-4 pr. uke
☐ 5-6 pr. uke ☐ 1 pr. dag ☐ 2 pr. dag
☐ 3 pr. dag ☐ 4+ pr. dag

Ris, spaghetti, grøt, suppe

Hvor ofte bruker du ris og spaghetti/makaroni?
(Sett ett kryss pr. linje)

	aldri/ sjelden	1-3 pr. mnd.	1 pr. uke	2 pr. uke	3+ pr.uke
Ris	☐	☐	☐	☐	☐
Spagetti, makaroni, nudler	☐	☐	☐	☐	☐

Hvor ofte spiser du grøt ? (Sett ett kryss pr. linje)

	aldri/ sjelden	1 pr. mnd.	2-3 pr. mnd.	1 pr. uke	2-6 pr. uke	1+ pr. dag
Risengrynsgrøt	☐	☐	☐	☐	☐	☐
Annen grøt (havre o.l.)	☐	☐	☐	☐	☐	☐

Hvor ofte spiser du suppe?
(Sett ett kryss pr. linje)

	aldri/ sjelden	1-3 pr. mnd.	1 pr. uke	2 pr. uke	3+ pr.uke
Som hovedrett	☐	☐	☐	☐	☐
Som forrett, lunsj eller kveldsmat	☐	☐	☐	☐	☐

Fisk

Vi vil gjerne vite hvor ofte du pleier å spise fisk, og ber deg fylle ut spørsmålene om fiskeforbruk så godt du kan. Tilgangen på fisk kan variere gjennom året. Vær vennlig å markere i hvilke årstider du spiser de ulike fiskeslagene.

	aldri/ sjelden	like mye hele året	vinter	vår	sommer	høst
Torsk, sei, hyse, lyr	☐	☐	☐	☐	☐	☐
Steinbit, flyndre, uer	☐	☐	☐	☐	☐	☐
Laks, ørret	☐	☐	☐	☐	☐	☐
Makrell	☐	☐	☐	☐	☐	☐
Sild	☐	☐	☐	☐	☐	☐
Annen fisk	☐	☐	☐	☐	☐	☐

Med tanke på de periodene av året der du spiser fisk, hvor ofte pleier du å spise følgende til middag?
(Sett ett kryss pr. linje)

	aldri/ sjelden	1 pr. mnd.	2-3 pr. mnd.	1 pr. uke	2+ pr. uke
Kokt torsk, sei, hyse, lyr	☐	☐	☐	☐	☐
Stekt torsk, sei, hyse, lyr	☐	☐	☐	☐	☐
Steinbit, flyndre, uer	☐	☐	☐	☐	☐
Laks, ørret	☐	☐	☐	☐	☐
Makrell	☐	☐	☐	☐	☐
Sild	☐	☐	☐	☐	☐
Annen fisk	☐	☐	☐	☐	☐

Dersom du spiser fisk, hvor mye spiser du vanligvis pr. gang? (1 skive/stykke = 150 gram)

Kokt fisk (skive)	☐ 1	☐ 1,5	☐ 2	☐ 3+
Stekt fisk (stykke)	☐ 1	☐ 1,5	☐ 2	☐ 3+

Hvor mange ganger pr. år spiser du fiskeinnmat?
(Sett ett kryss pr. linje)

	0	1-3	4-6	7-9	10+
Rogn	☐	☐	☐	☐	☐
Fiskelever	☐	☐	☐	☐	☐

Dersom du spiser fiskelever, hvor mange spiseskjeer pleier du å spise hver gang? (Sett ett kryss)

☐ 1 ☐ 2 ☐ 3-4 ☐ 5-6 ☐ 7+

Hvor ofte bruker du følgende typer fiskemat?
(Sett ett kryss pr. linje)

	aldri/ sjelden	1 pr. mnd.	2-3 pr. mnd.	1 pr. uke	2+ pr. uke
Fiskekaker/pudding/boller	☐	☐	☐	☐	☐
Plukkfisk/fiskegrateng	☐	☐	☐	☐	☐
Frityrfisk/fiskepinner	☐	☐	☐	☐	☐

Hvor stor mengde pleier du vanligvis å spise av de ulike rettene? (Sett ett kryss for hver linje)

Fiskekaker/pudding/boller (stk.) (2 fiskeboller=1 fiskekake)	☐ 1	☐ 2	☐ 3	☐ 4+
Plukkfisk, fiskegrateng (dl)	☐ 1-2	☐ 3-4	☐ 5+	
Frityrfisk, fiskepinner (stk.)	☐ 1-2	☐ 3-4	☐ 5-6	☐ 7+

I tillegg til informasjon om fiskeforbruk er det viktig å få kartlagt hvilket tilbehør som blir servert til fisk. Hvor ofte bruker du følgende til fisk?

	aldri/ sjelden	1 pr. mnd.	2-3 pr. mnd.	1 pr. uke	2+ pr. uke
Smeltet/fast smør	☐	☐	☐	☐	☐
Smeltet/fast margarin/fett	☐	☐	☐	☐	☐
Seterrømme (35%)	☐	☐	☐	☐	☐
Lettrømme (20%)	☐	☐	☐	☐	☐
Saus med fett (hvit/brun)	☐	☐	☐	☐	☐
Saus uten fett (hvit/brun)	☐	☐	☐	☐	☐

For de ulike typene tilbehør du bruker til fisk, vær vennlig å kryss av for hvor mye du vanligvis pleier å spise.

Smeltet/ fast smør (ss)	☐ 1/2	☐ 1	☐ 2	☐ 3	☐ 4+
Smeltet/ fast margarin (ss)	☐ 1/2	☐ 1	☐ 2	☐ 3	☐ 4+
Seterrømme (ss)	☐ 1/2	☐ 1	☐ 2	☐ 3	☐ 4+
Lettrømme (ss)	☐ 1/2	☐ 1	☐ 2	☐ 3	☐ 4+
Saus med fett (dl)	☐ 1/4	☐ 1/2	☐ 3/4	☐ 1	☐ 2+
Saus uten fett (dl)	☐ 1/4	☐ 1/2	☐ 3/4	☐ 1	☐ 2+

Hvor ofte spiser du skalldyr (f. eks. reker, krabbe og skjell)? (Sett ett kryss)

☐ Aldri/sjelden ☐ 1 pr. mnd ☐ 2-3 pr. mnd ☐ 1+ pr. uke

Kjøtt

Hvor ofte spiser du reinkjøtt?

☐ Aldri/sjelden ☐ 1 pr. mnd. ☐ 2-3 pr. mnd. ☐ 1 pr. uke
☐ 2-3 pr. uke ☐ 4+ pr. uke

Hvor ofte spiser du følgende kjøtt- og fjærkreretter? (Sett ett kryss for hver rett)

	aldri/ sjelden	1 pr.mnd.	2-3 pr.mnd.	1 pr.uke	2+ pr.uke
Steik (okse, svin, får)	☐	☐	☐	☐	☐
Koteletter	☐	☐	☐	☐	☐
Biff	☐	☐	☐	☐	☐
Kjøttkaker, karbonader	☐	☐	☐	☐	☐
Pølser	☐	☐	☐	☐	☐
Gryterett, lapskaus	☐	☐	☐	☐	☐
Pizza med kjøtt	☐	☐	☐	☐	☐
Kylling	☐	☐	☐	☐	☐
Bacon, flesk	☐	☐	☐	☐	☐
Andre kjøttretter	☐	☐	☐	☐	☐

Dersom du spiser følgende retter, oppgi mengden du vanligvis spiser: (Sett ett kryss for hver linje)

Steik (skiver)	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5+
Koteletter (stk.)	☐ 1/2	☐ 1	☐ 1,5	☐ 2+	
Kjøttkaker, karbonader (stk.)	☐ 1	☐ 2	☐ 3	☐ 4+	
Pølser (stk. à 150g)	☐ 1/2	☐ 1	☐ 1,5	☐ 2+	
Gryterett, lapskaus (dl)	☐ 1-2	☐ 3	☐ 4	☐ 5+	
Pizza m/kjøtt (stykke à 100 g)	☐ 1	☐ 2	☐ 3	☐ 4+	

Hvilke sauser bruker du til kjøttretter og pastaretter? (Sett ett kryss pr. linje)

	aldri/ sjelden	1 pr. mnd.	2-3 pr. mnd.	1 pr. uke	2+ pr. uke
Brun saus	☐	☐	☐	☐	☐
Sjysaus	☐	☐	☐	☐	☐
Tomatsaus	☐	☐	☐	☐	☐
Saus med fløte/rømme	☐	☐	☐	☐	☐

Hvor mye bruker du vanligvis av disse sausene?

Brun saus (dl)	☐ 1/4	☐ 1/2	☐ 3/4	☐ 1	☐ 2+
Sjysaus (dl)	☐ 1/4	☐ 1/2	☐ 3/4	☐ 1	☐ 2+
Tomatsaus (dl)	☐ 1/4	☐ 1/2	☐ 3/4	☐ 1	☐ 2+
Saus med fløte/ rømme (dl)	☐ 1/4	☐ 1/2	☐ 3/4	☐ 1	☐ 2+

Andre matvarer

Hvor mange egg spiser du vanligvis i løpet av en uke? (stekte, kokte, eggerøre, omelett) (Sett ett kryss)

☐ 0 ☐ 1 ☐ 2 ☐ 3-4
☐ 5-6 ☐ 7+

Hvor ofte spiser du iskrem? (til dessert, Krone-is osv.) Sett ett kryss for hvor ofte du spiser iskrem om sommeren, og ett kryss for resten av året

	aldri/ sjelden	1 pr. mnd.	2-3 pr. mnd.	1 pr. uke	2+ pr.uke
Om sommeren	☐	☐	☐	☐	☐
Resten av året	☐	☐	☐	☐	☐

Hvor mye is spiser du vanligvis pr. gang? (Sett ett kryss)

☐ 1dl ☐ 2 dl ☐ 3 dl ☐ 4+ dl

Hvor ofte spiser du bakevarer som boller, kaker, wienerbrød eller småkaker (Sett ett kryss pr. linje)

	aldri/ sjelden	1-3 pr. mnd.	1 pr. uke	2-3 pr uke	4-6 pr. uke	1+ pr.dag
Gjærbakst (boller o.l.)	☐	☐	☐	☐	☐	☐
Wienerbrød, kringle	☐	☐	☐	☐	☐	☐
Kaker	☐	☐	☐	☐	☐	☐
Pannekaker	☐	☐	☐	☐	☐	☐
Vafler	☐	☐	☐	☐	☐	☐
Småkaker, kjeks	☐	☐	☐	☐	☐	☐
Lefser, lomper	☐	☐	☐	☐	☐	☐

Hvor ofte spiser du dessert? (Sett ett kryss pr. linje)

	aldri/ sjelden	1 pr. mnd.	2-3 pr mnd.	1 pr uke	2-3 pr. uke	4+ pr. uke
Pudding sjokolade/karamell	☐	☐	☐	☐	☐	☐
Riskrem, fromasj	☐	☐	☐	☐	☐	☐
Kompott, fruktgrøt, hermetisk frukt	☐	☐	☐	☐	☐	☐
Jordbær (friske, frosne)	☐	☐	☐	☐	☐	☐
Andre bær (friske, frosne)	☐	☐	☐	☐	☐	☐

Hvor ofte spiser du sjokolade? (Sett ett kryss)

	aldri/ sjelden	1-3 pr. mnd.	1 pr. uke	2-3 pr uke	4-6 pr. uke	1+ pr.dag
Mørk sjokolade	☐	☐	☐	☐	☐	☐
Lys sjokolade	☐	☐	☐	☐	☐	☐

Dersom du spiser sjokolade, hvor mye pleier du vanligvis å spise hver gang? Tenk deg størrelsen på en Kvikk-Lunsj sjokolade, og oppgi hvor mye du spiser i forhold til den.

☐ 1/4 ☐ 1/2 ☐ 3/4 ☐ 1 ☐ 1,5 ☐ 2+

Hvor ofte spiser du snacks? (Sett ett kryss)

	aldri/ sjelden	1-3 pr. mnd.	1 pr. uke	2-3 pr. uke	4-6 pr. uke	1+ pr. dag
Potetchips	☐	☐	☐	☐	☐	☐
Peanøtter	☐	☐	☐	☐	☐	☐
Andre nøtter	☐	☐	☐	☐	☐	☐
Annen snacks	☐	☐	☐	☐	☐	☐

Tran og fiskeoljekapsler

Bruker du tran (flytende)? Ja ☐ Nei ☐

Hvis ja; hvor ofte tar du tran?
Sett ett kryss for hver linje.

	aldri/ sjelden	1-3 pr. mnd.	1 pr. uke	2-6 pr. uke	daglig
Om vinteren	☐	☐	☐	☐	☐
Resten av året	☐	☐	☐	☐	☐

Hvor mye tran pleier du å ta hver gang?

☐ 1 ts. ☐ 1/2 ss. ☐ 1+ ss.

Bruker du tranpiller/fiskeoljekapsler? Ja ☐ Nei ☐

Hvis ja; hvor ofte tar du tranpiller/fiskeoljekapsler?
Sett ett kryss for hver linje.

	aldri/ sjelden	1-3 pr. mnd.	1 pr. uke	2-6 pr. uke	daglig
Om vinteren	☐	☐	☐	☐	☐
Resten av året	☐	☐	☐	☐	☐

Hvilken type tranpiller/fiskeoljekapsler bruker du vanligvis, og hvor mange pleier du å ta hver gang?

Navn _____ Antall ☐

Kosttilskudd

Bruker du kosttilskudd? Ja ☐ Nei ☐

Hvis ja, hvor ofte bruker du kosttilskudd?
(Sett ett kryss pr. linje)

Navn på kosttilskudd	aldri/ sjelden	1-3 pr. mnd.	1 pr. uke	2-6 pr. uke	daglig
_____	☐	☐	☐	☐	☐
_____	☐	☐	☐	☐	☐
_____	☐	☐	☐	☐	☐
_____	☐	☐	☐	☐	☐

Bruker du soyapreparater mot plager i overgangsalderen? Ja ☐ Nei ☐

Varm mat

Hvor mange ganger i løpet av en måned spiser du varm mat?

Til frokost ☐ Til middag ☐

Til lunsj ☐ Til kvelds ☐

Kosthold som barn

Hvor mye melk drakk du som barn hver dag? (sett ett kryss)

☐ drakk ikke melk ☐ 1-3 glass ☐ 4-6 glass ☐ 7 glass eller me

Hvor ofte spiste du grønnsaker til middag som barn?
(sett ett kryss)

☐ aldri ☐ 1 gang i uken eller mer sjelden
☐ 2-3 ganger i uken ☐ 4 eller flere ganger pr. uke

Hvor ofte spiste du fisk til middag som barn? (sett ett kryss)

☐ aldri ☐ 1 gang i uken eller mer sjelden
☐ 2-3 ganger i uken ☐ 4 eller flere ganger pr. uke

Alkohol

Er du totalavholdskvinne? Ja ☐ Nei ☐

Hvis Nei; hvor ofte og hvor mye drakk du i gjennomsnitt siste året? (Sett ett kryss for hver linje)

	aldri/ sjelden	1 pr. mnd.	2-3 pr. mnd.	1 pr. uke	2-4 pr. uke	5-6 pr. uke	1 pr. dag	2+ pr. dag
Øl (1/2 l.)	☐	☐	☐	☐	☐	☐	☐	☐
Vin (glass)	☐	☐	☐	☐	☐	☐	☐	☐
Brennevin (drink)	☐	☐	☐	☐	☐	☐	☐	☐
Likør/Hetvin (glass)	☐	☐	☐	☐	☐	☐	☐	☐

Sosiale forhold

Er du idag: (Sett ett kryss)

☐ gift ☐ samboer ☐ ugift ☐ skilt ☐ enke

Hvor mange personer er det i ditt hushold? ☐

Hvor høy er bruttoinntekten i husholdet pr. år?

☐ inntil 150.000 kr. ☐ 151.000-300.000 kr.
☐ 301.000-450.000 kr. ☐ 451.000-600.000 kr.
☐ 601.000-750.000 kr. ☐ over 750.000 kr.

Hva er din arbeidssituasjon? (sett ett eller flere kryss)

☐ Arbeider heltid ☐ Arbeider deltid ☐ Pensjonist
☐ Hjemmearbeidende ☐ Under utdanning ☐ Uføretrygdet
☐ Under attføring ☐ Arbeidssøkende

Arbeider du utendørs i yrkessammenheng? Ja ☐ Nei ☐

Hvis Ja;
hvor mange timer pr. uke? ☐ Sommer ☐ Vinter

Solvaner

Får du fregner når du soler deg? Ja ☐ Nei ☐

For å kunne studere effekten av soling på risiko for hudkreft, ber vi deg gi opplysninger om hudfarge. Sett ett kryss på det tallet under fargen som best passer din naturlige hudfarge (uten soling).

1	2	3	4	5	6	7	8	9	10

Hvor mange ganger pr. år er du blitt forbrent av solen slik at du har fått svie eller blemmer med avflassing etterpå? (ett kryss for hver aldersgruppe)

Alder	Aldri	Høyst 1 gang pr. år	2-3 g. pr. år	4-5 g. pr. år	6 eller flere ganger pr. år
40-49 år	☐	☐	☐	☐	☐
50 + år	☐	☐	☐	☐	☐

Hvor mange uker i gjennomsnitt pr. år har du vært på badeferie i syden eller i Norge?

Alder	Aldri	1 uke	2-3 uker	4-5 uker	7 uker eller mer
40-49 år	☐	☐	☐	☐	☐
50 + år	☐	☐	☐	☐	☐
Siste 12 mnd.	☐	☐	☐	☐	☐

Hvor ofte har du solt deg i solarium?

Alder	Aldri	Sjelden	1 gang pr. mnd.	2 ganger pr. mnd.	3-4 ganger pr. mnd	oftere enn 1 gang pr. uke
40-49 år	☐	☐	☐	☐	☐	☐
50+ år	☐	☐	☐	☐	☐	☐
Siste 12 mnd.	☐	☐	☐	☐	☐	☐

Hvor ofte dusjer eller bader du?

	mer enn 1 g. dagl.	1 g. dagl.	4-6 g. pr. uke	2-3 g. pr. uke	1 g. pr. uke	2-3 g. pr. mnd	sjelden/ aldri
Med såpe/ shampo	☐	☐	☐	☐	☐	☐	☐
Uten såpe/ shampo	☐	☐	☐	☐	☐	☐	☐

Når bruker du krem med solfaktor? (sett evt. flere kryss):

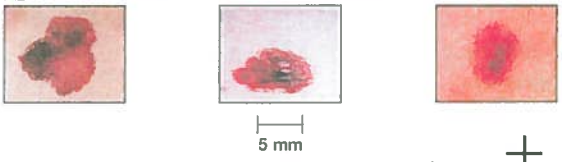
☐ i påsken ☐ i Norge eller utenfor syden
☐ solferie i syden ☐ aldri

Hvilken solfaktor bruker du i disse periodene?

	Ingen	1-4	5-9	10-14	15+
Påsken	☐	☐	☐	☐	☐
I Norge eller utenfor syden	☐	☐	☐	☐	☐
Solferie i syden	☐	☐	☐	☐	☐

Hvor mange uregelmessige føflekker større enn 5 mm har du sammenlagt på begge bena (fra tærne til lysken)? Tre eksempler på føflekker større enn 5 mm med uregelmessig form er vist nedenfor.

☐ 0 ☐ 1 ☐ 2-3 ☐ 4-6 ☐ 7-12 ☐ 13-24 ☐ 25+



Hvor ofte bruker du følgende hudpleiemidler?
(Sett ett kryss pr. linje)

	aldri/ sjelden	1-3 pr.mnd.	1 pr.uke	2-4 pr.uke	5-6 pr.uke	1 pr.dag	2+ pr. dag
Ansiktskrem	☐	☐	☐	☐	☐	☐	☐
Håndkrem	☐	☐	☐	☐	☐	☐	☐
Body lotion	☐	☐	☐	☐	☐	☐	☐
Parfyme	☐	☐	☐	☐	☐	☐	☐

Til slutt vil vi spørre deg om ditt samtykke til å kontakte deg på nytt pr. post. Vi vil hente adressen fra det sentrale personregister.

Ja ☐ Nei ☐

Er du villig til å avgi en blodprøve?

Ja ☐ Nei ☐

Takk for at du ville delta i undersøkelsen

ISM SKRIFTSERIE - FØR UTGITT:

1. Bidrag til belysning av medisinske og sosiale forhold i Finnmark fylke, med særlig vekt på forholdene blant finskættede i Sør-Varanger kommune.
Av Anders Forsdahl, 1976. (nytt opplag 1990)

2. Sunnhetstilstanden, hygieniske og sosiale forhold i Sør-Varanger kommune 1869-1975 belyst ved medisinal-beretningene.
Av Anders Forsdahl, 1977.

3. Hjerte-karundersøkelsen i Finnmark - et eksempel på en populasjonsundersøkelse rettet mot cardiovasculære sykdommer. Beskrivelse og analyse av etterundersøkelsesgruppen.
Av Jan-Ivar Kvamme og Trond Haider, 1979.

4. D. The Tromsø Heart Study: Population studies of coronary risk factors with special emphasis on high density lipoprotein and the family occurrence of myocardial infarction.
Av Olav Helge Førde og Dag Steinar Thelle, 1979.

5. D. Reformer i distriktshelsetjenesten III: Hypertensjon i distriktshelsetjenesten.
Av Jan-Ivar Kvamme, 1980.

6. Til professor Knut Westlund på hans 60-års dag, 1983.

7.* Blodtrykksovervåkning og blodtrykksmåling.
Av Jan-Ivar Kvamme, Bernt Nesje og Anders Forsdahl, 1983.

8.* Merkesteiner i norsk medisin reist av allmennpraktikere - og enkelte utdrag av medisinalberetninger av kulturhistorisk verdi.
Av Anders Forsdahl, 1984.

9. "Balsfjordsystemet." EDB-basert journal, arkiv og statistikksystem for primærhelsetjenesten.
Av Toralf Hasvold, 1984.

10. D. Tvunget psykisk helsevern i Norge. Rettsikkerheten ved slikt helsevern med særlig vurdering av kontrollkommisjonsordningen.
Av Georg Høyer, 1986.

11. D. The use of self-administered questionnaires about food habits. Relationships with risk factors for coronary heart disease and associations between coffee drinking and mortality and cancer incidence.
Av Bjarne Koster Jacobsen, 1988.

12.* Helse og ulikhet. Vi trenger et handlingsprogram for Finnmark.
Av Anders Forsdahl, Atle Svendal, Aslak Syse og Dag Thelle, 1989.

13. D. Health education and self-care in dentistry - surveys and interventions.
Av Anne Johanne Søgaard, 1989.

14. Helsekontroller i praksis. Erfaringer fra prosjektet helsekontroller i Troms 1983-1985.
Av Harald Siem og Arild Johansen, 1989.

15. Til Anders Forsdahls 60-års dag, 1990.

16. D. Diagnosis of cancer in general practice. A study of delay problems and warning signals of cancer, with implications for public cancer information and for cancer diagnostic strategies in general practice.
Av Knut Holtedahl, 1991.

17. D. The Tromsø Survey. The family intervention study. Feasibility of using a family approach to intervention on coronary heart disease. The effect of lifestyle intervention of coronary risk factors.
Av Synnøve Fønnebø Knutsen, 1991.

18. Helhetsforståelse og kommunikasjon. Filosofi for klinikere.
Av Åge Wifstad, 1991.

19. D. Factors affecting self-evaluated general health status - and the use of professional health care services.
Av Knut Fylkesnes, 1991.

20. D. Serum gamma-glutamyltransferase: Population determinants and diagnostic characteristics in relation to intervention on risk drinkers.
Av Odd Nilssen, 1992.

21. D. The Healthy Faith. Pregnancy outcome, risk of disease, cancer morbidity and mortality in Norwegian Seventh-Day-Adventists.
Av Vinjar Fønnebø, 1992.

22. D. Aspects of breast and cervical cancer screening.
Av Inger Torhild Gram, 1992.

23. D. Population studies on dyspepsia and peptic ulcer disease: Occurrence, aetiology, and diagnosis. From The Tromsø Heart Study and The Sørreisa Gastrointestinal Disorder Studie.
Av Roar Johnsen, 1992.

24. D. Diagnosis of pneumonia in adults in general practice.
Av Hasse Melbye, 1992.

25. D. Relationship between hemodynamics and blood lipids in population surveys, and effects of n-3 fatty acids.
Av Kaare Bønaa, 1992.

26. D. Risk factors for, and 13-year mortality from cardiovascular disease by socioeconomic status. A study of 44690 men and 17540 women, ages 40-49. **Av Hanne Thürmer, 1993.**

27. Utdrag av medisinalberetninger fra Sulitjelma 1891-1990. **Av Anders Forsdahl, 1993.**

28. Helse, livsstil og levekår i Finnmark. Resultater fra Hjerte-karundersøkelsen i 1987-88. Finnmark III. **Av Knut Westlund og Anne Johanne Søgaard, 1993.**

29. D. Patterns and predictors of drug use. A pharmacoepidemiologic study, linking the analgesic drug prescriptions to a population health survey in Tromsø, Norway. **Av Anne Elise Eggen, 1994.**

30. D. ECG in health and disease. ECG findings in relation to CHD risk factors, constitutional variables and 16-year mortality in 2990 asymptomatic Oslo men aged 40-49 years in 1972. **Av Per G. Lund-Larsen, 1994.**

31. D. Arrhythmia, electrocardiographic signs, and physical activity in relation to coronary heart risk factors and disease. The Tromsø Study. **Av Maja-Lisa Løchen, 1995.**

32. D. The Military service: mental distress and changes in health behaviours among Norwegian army conscript. **Av Edvin Schei, 1995.**

33. D. The Harstad injury prevention study: Hospital-based injury recording and community-based intervention. **Av Børge Ytterstad, 1995.**

34.* D. Vilkår for begrepsdannelse og praksis i psykiatri. En filosofisk undersøkelse. **Av Åge Wifstad, 1996.** (utgitt Tano Aschehoug forlag 1997)

35. Dialog og refleksjon. Festskrift til professor Tom Andersen på hans 60-års dag, 1996.

36. D. Factors affecting doctors´ decision making. **Av Ivar Sønbø Kristiansen, 1996.**

37. D. The Sørreisa gastrointestinal disorder study. Dyspepsia, peptic ulcer and endoscopic findings in a population. **Av Bjørn Bernersen, 1996.**

38. D. Headache and neck or shoulder pain. An analysis of musculoskeletal problems in three comprehensive population studies in Northern Norway. **Av Toralf Hasvold, 1996.**

39. Senfølger av kjernefysiske prøvespreninger på øygruppen Novaya Semlya i perioden 1955 til 1962. Rapport etter programmet "Liv". Arkangelsk 1994.
Av A.V. Tkatchev, L.K. Dobrodeeva, A.I. Isaev, T.S. Podjakova, 1996.

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ISBN 978-82-90263-22-0