CONCENTRATIONS OF PERFLUORINATED COMPOUNDS IN SERUM ARE ASSOCIATED WITH SEAFOOD CONSUMPTION IN A NORWEGIAN COHORT

Haug, L S[1], Thomsen C[1], Meltzer H M[1], Becher G[1,2], Knutsen H K[1]

[1]Division of Environmental Medicine, Norwegian Institute of Public Health, P.O. Box 4404 Nydalen, NO-0403 Oslo, Norway
[2]Department of Chemistry, University of Oslo, P.O. Box 1033 Blindern, NO-0315 Oslo, Norway

Abstract
Perfluorinated compounds (PFCs) were determined in 175 serum samples from a group of Norwegians with a broad range of seafood consumption. The PFC concentrations measured were in close agreement with what has been found in other studies in Norway. Significantly higher concentrations of most PFCs were found in men compared to women and the concentrations increased with increasing age. Significant positive bivariate associations were found between consumption of lean fish and fish liver, and determined concentrations of PFCs in the serum samples. Significant correlations were also found between serum concentrations of PFCs and polychlorinated biphenyls, indicating similar exposure sources for these compounds.

Introduction
Perfluorinated compounds (PFCs) have been used in a multitude of industrial applications and consumer products for more than 50 years due to their unique physico-chemical characteristics. As a consequence, PFCs are found to be widespread in the environment and in humans. Several PFCs have long elimination half-lives in humans[1] and animal studies have demonstrated hepatotoxicity, developmental toxicity, immunotoxicity as well as hormonal effects[2]. Recently, more knowledge on the exposure of the general populations has become available, but the importance of different exposure pathways is still not completely understood[3]. Although dietary exposure is suggested to be the main exposure route in the general populations, the relative impact of the exposure routes may differ in different parts of the world, depending on the variation in use of PFCs and in dietary habits[3]. So far little is known about the dietary exposure to PFCs in the general Norwegian population or in subgroups with special dietary habits. It is suspected that fish may play an important role in exposure to PFCs in Norway. The PFC concentrations have generally been higher in fish than in other food groups[4], and Norwegians are among the highest reported consumers of fish in Europe[5].

The aim of the study was to investigate relationships between fish consumption and serum PFC concentrations in a group of Norwegians.

Materials and Methods
Study subjects
The subjects (195) included in this study were participants in part C of the "The Norwegian Fish and Game Study" which has been described elsewhere[6]. The participants provided serum samples and filled in a 12-page food frequency questionnaire in addition to a one-page query about background, providing data regarding personal variables and dietary habits the last 12 months. A sufficient amount of blood serum for PFC analysis was obtained from 185 of the subjects and of these, ten were excluded on the basis of unlikely energy intakes (less than 1000 or more than 4000 kcal/day). This left 175 participants for the statistical analysis.

Chemicals
Perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), PFOA, perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA), perfluorotridecanoic acid (PFTrDA), perfluorotetradecanoic acid (PFTeDA), perfluorobutane sulfonic acid (PFBS), PFHxS, perfluoroheptane sulfonic acid (PFHpS), PFOS, perfluorodecane sulfonic acid (PFDS), perfluorooctane sulfonamide (PFOSA), N-methylperfluorooctane sulfonamide (MeFOSA), N-ethylperfluorooctane sulfonamide (EtFOSA), perfluoro-n-[1,2,3,4-$^{13}C_4$]butanoic acid (MPFBA), perfluoro-n-[1,2-$^{13}C_2$]hexanoic acid (MPFHxA),

perfluoro-n-[1,2,3,4-$^{13}C_4$]octanoic acid (MPFOA), perfluoro-n-[1,2,3,4,5-$^{13}C_5$]nonanoic acid (MPFNA), perfluoro-n-[1,2-$^{13}C_2$]decanoic acid (MPFDA), perfluoro-n-[1,2-$^{13}C_2$]dodecanoic acid (MPFDoDA), sodium perfluoro-1-hexane [$^{18}O_2$] sulfonate (MPFHxS), sodium perfluoro-1-[1,2,3,4-$^{13}C_4$]octanesulfonate (MPFOS), n-methyl-d_3-perfluoro-1-octanesulfonamide (d-N-MeFOSA), n-ethyl-d_5-perfluoro-1-octanesulfonamide (d-N-EtFOSA) were purchased from Wellington Laboratories (Guelph, Ontario, Canada). The other chemicals used are described elsewhere[7].

Chemical analysis
The samples and standards were prepared and analyzed according to a previously described method[7]. In brief, 150 µl of either serum from newborn calves (matrix matched calibration solutions) or human serum was transferred to a centrifugation tube, added internal standards, native PFCs (only calibration solutions) and methanol to make up a total volume of 150 µl methanol for precipitation of proteins, and then mixed using a whirl mixer. The samples were then centrifuged and the supernatant was transferred to a glass autosampler vial, added 0.1 M formic acid and mixed on a whirl mixer. The extracts were analysed by injection of 400 µl on a column switching liquid chromatography system coupled to a triple quadrupole mass spectrometer. The LOQ was limited by the lowest calibration point and set to 0.050 ng/ml for all PFCs except PFBA which was 0.10 ng/ml. In the statistical analyses, the concentration of all PFCs below the LOQ was set to LOQ divided by the square root of two. For quantification of PFOS, the total area of the linear and branched isomers was integrated.

The quality of the determinations in the study was controlled by analysing in-house quality control samples as well as human serum samples from an interlaboratory comparison study organized by Institute national de santé publique du Québec (Canada) for the Arctic Monitoring and Assessment Programme (AMAP) simultaneously to the samples. To assure that the sample containers as well as the other laboratory equipment used did not contain any traces of PFCs, method blanks were prepared. The method blanks did not contain any of the PFCs above LOQ.

The statistical analyses were carried out using SPSS (version 17.0), SPSS Inc. Chicago, IL, USA.

Results and Discussion
The participants in this study consisted of a total of 79 men and 96 women, their mean age being 56.7 and 52.5 years, respectively (median age 57 and 55 years). The concentrations of the 11 PFCs found in the serum samples are presented in Figure 1.

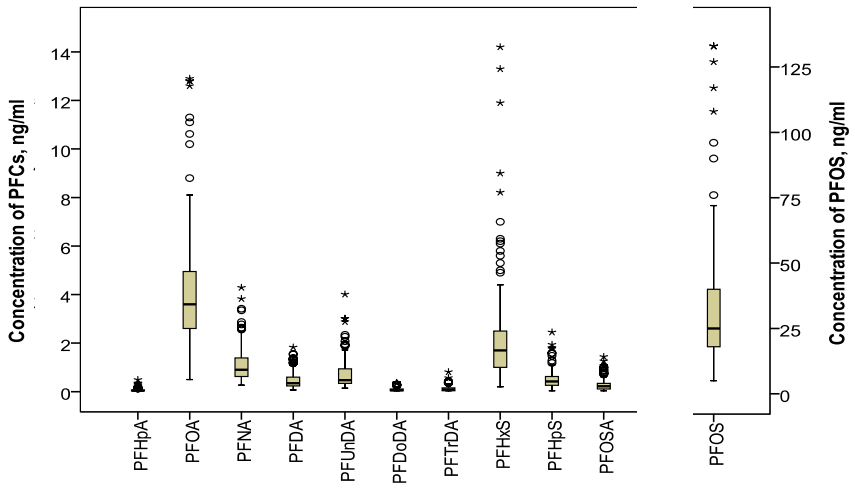

Figure 1: Box and wisker plot of concentrations of PFCs (ng/ml) in the 175 serum samples included in the study.

All samples contained PFOA, PFNA, PFDA, PFUnDA, PFHxS and PFOS, while PFHpS, PFOSA, PFTrDA, PFDoDA and PFHpA were found in 99, 91, 73, 58 and 36% of the samples, respectively. The remaining PFCs were not detected above LOQ in any of the samples. PFOS was found in highest amount in all samples followed by PFOA (94% of the subjects) or PFHxS (6% of the subjects). The median concentrations of PFOS, PFOA, PFHxS and PFNA in all 175 samples were 25, 3.6, 1.7 and 0.90 ng PFC/ml serum, respectively. This is in close agreement with what was found in a pool of 20 serum samples from Norwegian men (age 40-50 years) sampled in 2003[8] as well as in 35 high consumers of inland fish caught in a contaminated lake in Norway[9]. The PFC concentrations are also comparable to what has been reported world-wide[2].

The PFC concentrations in the serum samples were not normally distributed, hence Kruskal Wallis test and Spearman´s Rank Correlation were applied in the statistical evaluations. Significantly higher concentrations ($p \leq 0.05$) of all PFCs except PFHpA and PFOSA were found in men relative to women (see figure 2). Some publications in the literature have reported significant difference in PFC concentrations between sexes (e.g. ref. 10), while others have not (e.g. ref. 11). This inconsistency might be due to differences in the design of the studies, such as number of samples, selection of the populations, etc.

As shown in Figure 3 the PFOS concentration in serum increased with increasing age of the subjects ($p \leq 0.01$). Similar correlations were also found for all the other PFCs detected in the serum samples except for PFHpA. These results are comparable to another Norwegian study and an Australian study[8,12], while other investigations could not demonstrate any significant increases with increasing age (e.g. ref. 10).

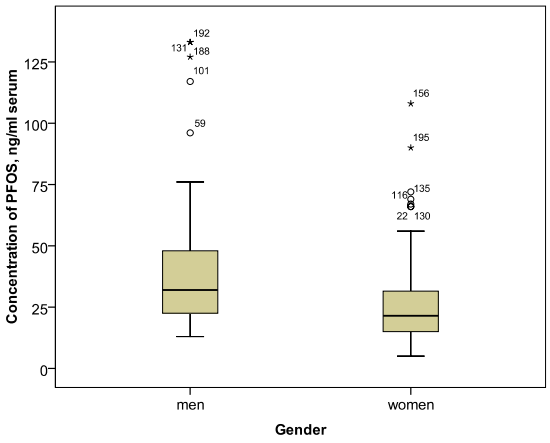

Figure 2: Box and wisker plot of concentrations of PFOS (ng/ml) in serum samples for men and women.

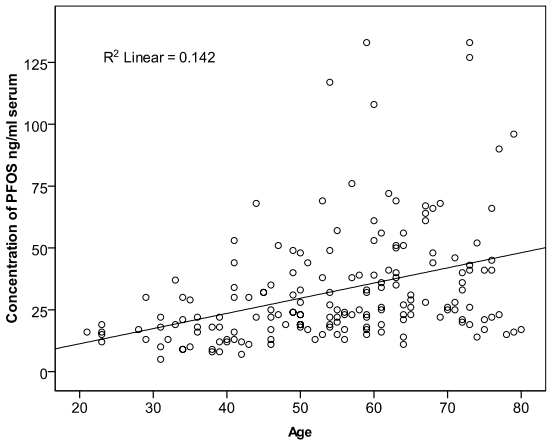

Figure 3: Relationship between age (year) and concentrations of PFOS (ng/ml) determined in the serum samples.

The subjects included in this study had a broad range of intake of seafood, e.g. the total consumption of fish ranged from 0 to 403 g per day. Significant correlations ($p \leq 0.01$) were found between consumption of lean fish (Fig. 4A) and fish liver (Fig. 4B) as well as total fish intake (results not shown), and the determined concentration of PFOS in serum. Similar associations were found for PFNA, PFDA, PFUnDA, PFHxS and PFHpS. Significant correlations were also found for PFOA, PFDoDA, PFTrDA as well as for PFOSA, though with lower correlation coeffecients. Significant, but weak correlations ($p \leq 0.05$) were also observed between most PFCs and reported intake of semi fatty fish, fatty fish, crab and roe. So far only a few studies have investigated the correlation between diet and concentrations of PFCs measured in human samples. Falandysz et al found that persons who reported to have a high consumption of fish, on average had higher PFC concentrations in their blood compared to other human subpopulations[13]. Further, Thomsen et al observed a positive association between reported intake of trout from Lake Mjøsa and PFC concentration in serum[9]. In

contrast results from the Danish National Birth Cohort showed no significant association between PFC concentrations in serum and fish consumption[14].

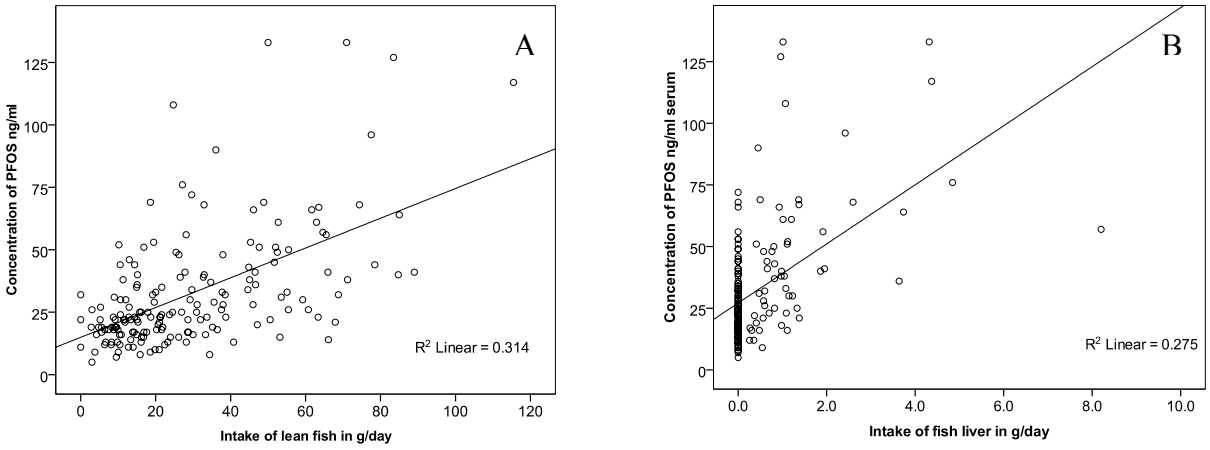

Figure 4. The relationship between the serum concentration of PFOS in ng/mL (Y-axis) in the 175 samples and the intake of lean fish (A) and fish liver(B) in g/day (X-axis).

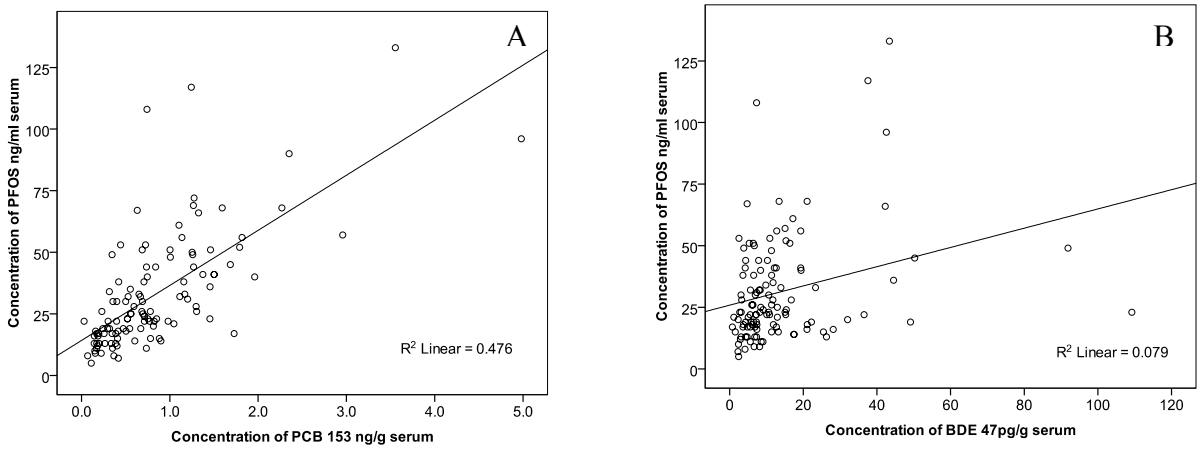

Figure 5. The relationship between the concentration of PFOS in ng/mL (Y-axis) and the concentration of PCB 153(A) or BDE 47(B) in ng/g (X-axis) in the 175 serum samples.

As shown by others, the serum PFC concentrations were strongly intercorrelated (results not shown). Previously, the concentrations of polybrominated diphenylethers (PBDEs) and polychlorinated biphenyls (PCBs) have been determined in the same serum samples[6,15]. PFCs were significantly correlated to the PCBs ($p \leq 0.01$), as is seen in Figure 5A showing PFOS versus PCB 153 as an example. However, the relationship between the PFCs and PBDEs (see Figure 5B) was not as pronounced. This indicates common exposure sources of PCBs and PFCs, while the exposure to PBDEs seems in part to come from other sources, dust ingestion or inhalation of air being likely sources.

Multivariate statistical methods will be applied to further identify factors that influence the observed variation in concentrations of PFCs in the serum and to clarify to which extent consumption of seafood affects the body burden of PFCs.

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