A WIDE RANGE OF PERFLUORINATED COMPOUNDS DETECTED IN FOODSTUFF AND DRINKING WATER FROM NORWAY

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Abstract
Perfluorinated compounds (PFCs) were determined in 22 samples of food, drinking water and tea from Norway. A wide range of PFCs were detected in the samples, and both perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) were found in concentrations similar to what has been observed in other studies. Differences in the relative proportion of PFOA and PFOS between samples of animal origin and samples of non-animal origin were observed, and support findings that PFOS have a higher bioaccumulation factor in animals than PFOA.

Introduction
Perfluorinated compounds (PFCs) are a group of chemicals which are used in a wide variety of industrial applications and consumer products due to their unique physio-chemical properties. Due to large production volumes and widespread application of PFCs, there is a potential for contamination of the environment and thereby also food and drinking water. Food of animal origin has been found to be the major source of exposure to persistent organic pollutants such as polychlorinated dioxins and furans and polychlorinated biphenyls in general populations and is presently also suggested to being an important exposure source for PFCs[1]. In addition Ericson and coworkers reported that drinking water could contribute considerably to human exposure to PFCs[2]. Despite that, relatively few data on concentrations of PFCs in food and drinking water have been published world-wide[1].

The aim of the study was to determine concentrations of a wide range of PFCs in a variety of Norwegian foodstuffs as well as in drinking water and tea.

Materials and Methods
Samples
A total of 18 samples of food, 3 samples of drinking water as well as one sample of tea, were included in the study. Each sample was prepared of one kind of food (lettuce, carrot, potato, cheese, butter, milk, bread, strawberry jam, pork meat, lamb meat, beef, chicken meat, egg, fish sticks, canned mackerel, salmon, cod, and cod liver) by homogenizing and mixing equal amounts of three different brands or types, except for lamb meat (2 brands), cod (1 fish) and cod liver (1 liver). The three samples of drinking water were each of 1L of water collected from the tap in households receiving water from different water works. Tea was prepared from 1L of water boiled in an electric kettle (drinking water sample 3) and 3 kinds of tea bags (2 of each). All samples except cod liver, drinking water and tea were freeze dried by using a Heto CD 13-2 freeze drier (Thermo Scientific, San Jose, CA, USA).

Chemicals
Perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA), perfluorotridecanoic acid (PFTrDA), perfluorotetradecanoic acid (PFTeDA), perfluorohexadecanoic acid (PFHxDA), perfluorooctadecanoic acid (PFODA), perfluorobutane sulfonic acid (PFBS), perfluorohexan sulfonic acid (PFHxS) and perfluorooctane sulfonic acid (PFOS) were obtained from Wellington Laboratories

(Guelph, Ontario, Canada). Ammonium acetate (>99%, p.a. for HPLC), methanol (HPLC grade) and water (HPLC grade) were purchased from Fluka (Steinheim, Germany) and acetonitrile (HPLC) was purchased from Labscan (Dublin, Ireland). Formic acid (98-100%) was purchased from Scharlau (Barcelona, Spain), and Supelclean ENVI-carb (120/400 mesh) was purchased from Supleco (Bellafonte, PA, USA). Ammonium hydroxide (25% in water), glacial acetic acid (100%), and sodium acetate were purchased from E. Merck (Darmstadt, Germany). Labeled ^{13}C internal standard mix (perfluorocarboxylic acids, PFCA, and perfluorosulfonate acids, PFSA, in methanol) were obtained from Wellington Laboratories and the performance standard (7H-PFHpA, 98 % in methanol) was purchased from ABCR (Karlsruhe, Germany).

Sample clean up and extraction

The method employed is based on methods by Powley et al[3] and Taniyasu et al[4]. Extraction and clean up was performed by alkaline digestion and liquid extraction, followed by solid phase extraction (SPE) using weak anion exchange (Waters Oasis® WAX, Waters, Mildford, MA, USA) and additional clean up with ENVI-carb. Briefly, 10 mL of 200 mM sodium hydroxide in methanol was added to 10 g of freeze-dried food sample in polypropylene tubes. Labeled ^{13}C internal standards were added and after 30 minutes, 40 mL of methanol was added. Sample was vortex mixed before shaking at 1100 rpm for 30 min. Hydrochloric acid, HCl (1 mL, 1 M) was added, followed by centrifugation at 5000 x *g* for 20 min. The supernatant, was transferred to a 100 mL glass flask, and a second extraction with 40 mL methanol was carried out. The two supernatants were combined and rotary evaporated to 10 mL and mixed with 15 mL of water. After centrifugation at 3000 x g for 20 minutes solid phase extraction was performed by loading the supernatant on WAX single use cartridges (6 cm^3/150 mg), previously conditioned with 4 mL of methanol and 4 mL of water. The cartridges were eluted with 4 mL of acetate buffer solution pH 4, 4 mL 40% methanol in water and 8 mL of methanol (all discarded). To collect target compounds the cartridge was eluted with 2 mL of 2% NH4 in methanol. Further clean up was performed using 50 mg of ENVI-carb and 100 µL of glacial acetic acid. After vortex mixing thoroughly, this fraction was filtered (0,2 µm) before the final volume was set to 500 µL including 7H-PFHpA added as performance standard and 300 µL of 2 mM sodium acetate in water. Food samples with high lipid and protein content (meat, fish and dairy products) required extraction with acetonitrile as described by Kärrman et al[5] before loading onto the WAX cartridge as described. Extraction of drinking water was performed using 500 mL of water, filtered with glass microfiber (1 µm) (Whatman®, Maidstone, UK) and set to pH 4 by using HCl (1 mL, 1 M). After spiking with ^{13}C-labeled internal standards samples were loaded onto a WAX cartridge and treated as the food samples. For extraction of one tea sample a hydrophilic-lipophilic-balanced (Waters Oasis® HLB, Waters, Mildford, MA, USA) column was used instead of the WAX cartridge.

Instrumental analysis

Extracts were separated on an Acquity UPLC® BEH C18 (1.7 µm particles, 2.1 mm i.d., 50 mm length), and a PFC Isolator column (Waters, USA) was used to retard PFCs originated from the system. Analytes were analyzed on a Quattro Premier XE MS/MS system (Waters, Mildford, MA, USA) run in electrospray ionization mode (ESI). Quantitative analysis of the 16 PFC was performed using the internal standard method monitoring the transitions giving the two most abundant peaks in multiple reaction monitoring, using the most abundant as quantifier and the second transition as qualifier. Instrumental blanks were routinely run as well as procedural blank samples incorporated in each batch of food and water sample and treated as a real sample.

Results and Discussion

Sixteen different PFCs were determined in 22 samples of food, drinking water and tea. The concentrations of 10 PFCs found in the samples are presented in Table 1. As can be seen, the results have been divided into three different categories; concentrations above limit of detection (LOD) (category 1: in bold), results where the signal was above the signal/noise ratio=3 (s/n=3) but below LOD (category 2: in italics) and cases where the signal was below s/n=3 (category 3: < LOD). LOD was calculated as three times the standard deviation of the method blanks, which is a relatively conservative approach. For the following interpretations both category 1 and category 2 results have been considered detected concentrations and are thus treated equally. One should however keep in mind that the category 2 results have higher uncertainties, but are considered good estimates of actual amounts in the samples. In addition to the results presented in Table 1, PFBA and PFPeA were found in butter (51pg/g fresh weight) and potato (15pg/g fresh weight), respectively.

Table 1: Concentrations of PFCs in samples of food (pg/g fresh weight), drinking water (pg/L) and tea (pg/L)

		PFBuS	PFHxS	PFOS	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnDA	PFDoDA
pg/g fresh weight	**Lettuce**	<0.12	<0.06	*0.17*	**0.98**	*0.43*	**1.8**	<1.0	**0.78**	<1.3	**1.3**
	Carrot	<0.25	<0.11	**0.67**	<1.3	<0.89	**2.0**	<2.1	<1.4	<2.5	<2.4
	Potato	<0.48	<0.22	**1.0**	*3.1*	**1.1**	**5.3**	<4.1	**3.0**	**2.2**	<4.8
	Cheese	<1.5	<0.65	**12**	<7.7	**7.4**	*13*	**16**	*6.6*	*4.1*	*<15*
	Butter	<1.6	**1.3**	**2.3**	**2.5**	<5.6	**12**	<13	<8.6	<16	<16
	Milk	<0.24	<0.11	**7.0**	**1.5**	<0.87	**4.7**	<2.1	**4.0**	<2.5	<2.4
	Bread	<1.5	**1.7**	**17**	**14**	**11**	**51**	**9.5**	**17**	<15	<15
	Strawberry jam	<1.3	<0.59	**3.0**	<7.0	<4.7	**14**	**3.7**	**8.70**	<13	<13
	Pork meat	<0.81	**1.2**	**17**	<4.3	**2.8**	**15**	*5.5*	**16**	<8.2	<8.0
	Lamb meat	<0.77	**16**	<1.49	<4.1	**5.3**	**12**	**10**	<4.2	<7.8	<7.7
	Beef	<0.63	<0.28	**60**	<3.3	*7.6*	**12**	**15**	*23*	<6.4	<6.2
	Chicken meat	*3.2*	<2.3	**21**	<13	**20**	**52**	*6.8*	<23	**13**	<9.2
	Egg	*2.0*	**3.50**	**39**	**13**	<16	*30*	<7.4	*12*	**9.9**	<8.1
	Fish sticks	*5.0*	*1.6*	**13**	<18	*21*	*49*	<11	*17*	**18**	<13
	Canned mackerel	<5.5	<3.0	**43**	<18	<24	*24*	<11	<31	**19**	<12
	Salmon	*2.2*	**5.5**	**55**	*11*	*16*	*46*	*10*	*26*	*4.5*	*<12*
	Cod	<3.4	**2.8**	**100**	<11	<15	*30*	*5.9*	*13*	**21**	<7.5
	Cod liver	<15	<8.2	**310**	<48	<66	*51*	*14*	*39*	**230**	<33
pg/L	**Drinking water sample 1**	<45	**150**	**230**	**780**	**760**	**2500**	<220	**1000**	**350**	<220
	Drinking water sample 2	<45	**120**	**310**	**310**	**320**	**1200**	<220	**520**	**200**	**430**
	Drinking water sample 3	<45	**45**	**71**	<110	<120	**650**	<220	*220*	*65*	*130*
	Tea	<45	<57	<30	<110	**470**	**9500**	<220	<330	**170**	**740**

Bold: concentrations above LOD (category 1)
Italic: signal above s/n=3 but below LOD (category 2)
< LOD: signal below s/n=3 (category 3)

In contrast to what has previously been published in the literature, a wide range of PFCs were observed in the samples. PFOA was observed in all samples and the PFOS concentrations were above s/n=3 in all samples except in lamb meat and tea. The remaining PFCs were detected less frequent. PFDA was found in 15 of the 22 samples followed by PFHpA (n=13), PFUnDA (n=12), PFHxS (n=11), PFNA (n=10), PFHxA (n=9), PFBuS (n=4) and PFDoDA (n=4). PFTrDA, PFTeDA, PFHxDA and PFODA were not observed above s/n=3 in any of the samples. The median detection limit (see Table 2) in the food samples in the present study was 7.6 pg/g fresh weight (0.1 – 650pg/g fresh weight). This is similar to what was reported by Ericson et al.[6] (1-650pg/g fresh weight), but lower than found by Fromme et al.[7] (50-200pg/g fresh weight) and Tittlemier et al.[8] (500-1000pg/g fresh weight).

The recoveries of the internal standards were in general satisfactory, however recoveries below 20% were observed for all internal standards for the analysis of bread. Low recoveries were also observed for some internal standards in a few of the other samples especially for the long-chained perfluorinated acids, $^{13}C_2$ PFUnDA and $^{13}C_2$ PFDoDA.

Table 2: LOD in samples of food (pg/g fresh weight) and liquid (pg/L)

	Food, pg/g fresh weight				Liquid, pg/L
	Mean	Median	Min	Max	
PFBuS	2.8	1.5	0.1	15	45
PFHxS	1.5	0.7	0.1	8.2	57
PFOS	2.2	2.0	0.2	6.3	30
PFBA	3.5	2.3	0.5	8.9	110
PFPeA	100	65	15	310	120
PFHxA	10	8.1	0.7	48	360
PFHpA	12	5.5	0.4	66	220
PFOA	38	22	1.8	200	330
PFNA	10	9.8	1.0	31	160
PFDA	16	8.4	0.7	84	220
PFUnDA	9.3	6.2	1.3	23	57
PFDoDA	12	11	1.2	33	94
PFTrDA	5.0	3.3	0.7	14	36
PFTDA	22	15	3.3	62	59
PFHxDA	200	100	1.4	650	660
PFODA	73	52	11	220	160

The highest concentrations of PFOS were found in cod liver followed by cod, beef (recovery <10%), salmon and canned mackerel, supporting the findings of Ericson et al.[6] where the highest concentrations were found in composite samples of white fish and blue fish. Tittlemier et al.[8] detected PFOS in only 9 of 54 Canadian composite samples and the highest amounts were observed in beef and fish. A significant correlation (Spearman´s Rank Correlation, $p \leq 0.01$) was observed between concentrations of PFOS and PFOA found in the food samples, though several non-fish products were among the samples with the highest amounts of PFOA. So far few studies have been able to detect other PFCs than PFOS and PFOA, likely do to high detection limits. However the concentrations of PFOS and PFOA found in the food samples in the present study are in the same range as found in other studies[1,6,8].

In the three samples of drinking water PFOA was the dominating PFC (see Figure 1). The concentrations determined were similar to what has been reported in the literature[1,2]. Interestingly, a relatively large difference in concentrations between the samples was observed for the perfluorinated carboxylic acids, although all samples were collected in the Oslo area. This illustrates a need for further analyses to sort out whether the PFC contamination originates from the raw water, the water works, the water pipes or a combination of these.

The concentrations of PFHpA, PFOA, PFUnDA and PFDoDA were 3-15 times higher in the sample of tea than in the drinking water it was prepared of (no. 3). However, we have not investigated whether the additional amount of these perfluorinated carboxylic acids came from the tea or from the electric kettle.

The relative amounts of PFOA and PFOS in the investigated samples are illustrated in Figure 2. In the samples of animal origin PFOS was generally the dominating PFC, while in the non-animal samples the concentrations of PFOA were 3-10 times higher than the concentrations of PFOS. This is in accordance with the observed higher bioaccumulation potential of PFOS in animal biota compared to PFOA[9].

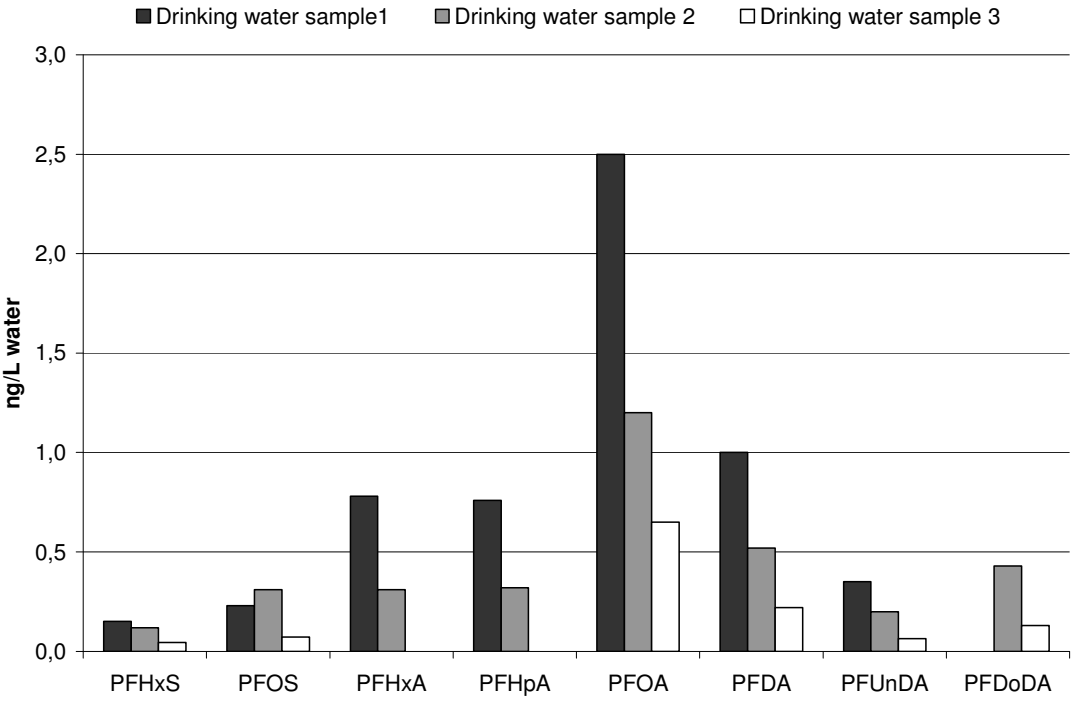

Figure 1: Concentrations of PFCs in drinking water (ng/L).

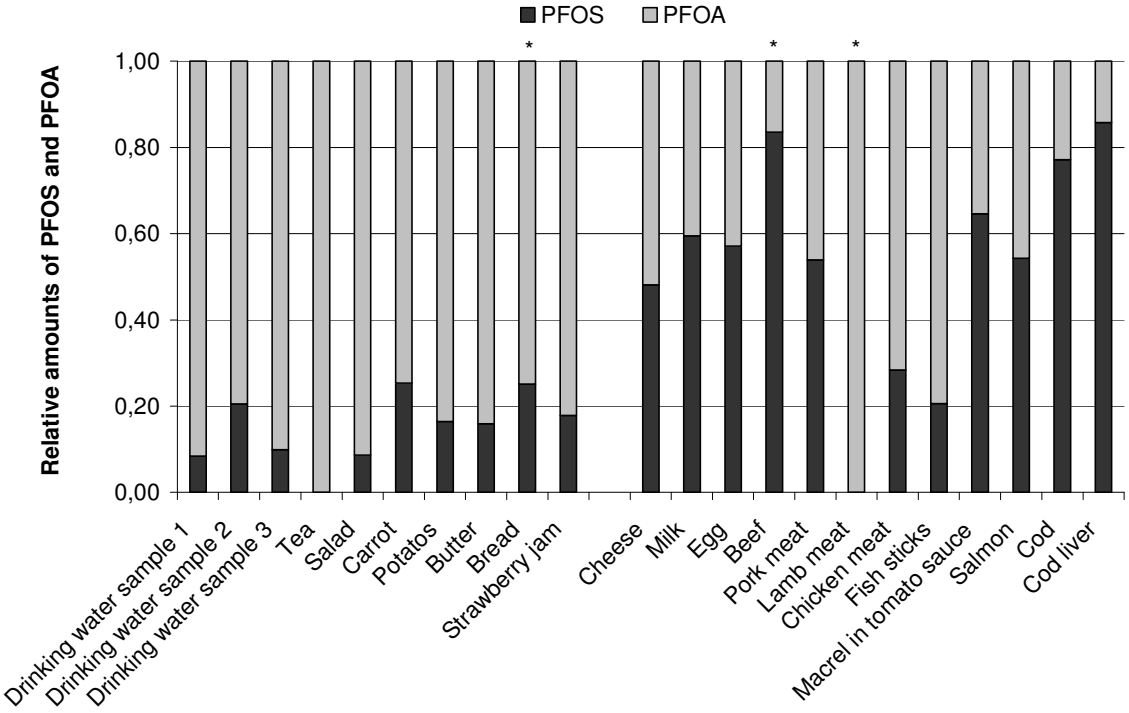

Figure 2: Relative amounts of PFOS and PFOA in samples of food, drinking water and tea. * Low recovery of $^{13}C_4$ PFOS.

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