EXPOSURE TO PERFLUORINATED COMPOUNDS THROUGH DRINKING WATER, AND FISH AND SEAFOOD BY THE POPULATION OF CATALONIA (SPAIN)

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Introduction

Perfluorinated chemicals (PFCs) form a group of environmental substances which have been widely used in consumer products. Due to their extensive applications, they may be released to the environment where they persist, and may bioaccumulate through the food chain[1]. Thus, PFCs may be ultimately taken up by the general population from a number of sources. Among these, water consumption has been identified as one of the most contributive routes of PFC exposure[2]. In addition, the diet, and particularly fish and seafood, are also major sources of PFCs exposure in humans[3].

Recently, a wide surveillance program aimed at analyzing the levels of a number of PFCs in environmental, biological, and food samples was initiated in Catalonia (Spain)[4-6]. Because of the potential implications on public health, a follow-up of the levels of PFCs in drinking water and certain foodstuffs is being performed since 2008. In this same line, in the present study we analyzed the concentrations of various PFCs (including perfluorooctanesulfonate (PFOS) and perfluorooctanoic acid (PFOA)) in drinking water from public fountains of Catalonia, as well as in samples of fish and seafood from the coastal areas of that country. The temporal trends of those levels were established, while the intake of PFCs through both pathways was estimated.

Materials and methods

In December 2009, water samples were collected in public fountains from 10 different locations of Catalonia (Figure 1). Sampling points belonged to five areas in which Catalonia is divided for health purposes: Barcelona, Girona, Lleida, Tarragona, and Terres de l'Ebre. Duplicate water samples were collected and kept refrigerated at 4ºC in 0.75 L polyethylene (PET) bottles. Moreover, 7 species of fish and seafood were acquired in 3 coastal areas. Samples of sardine, tuna, red mullet, hake, cuttlefish, mussel, and prawn caught in those areas were bought in local markets. For each species, 3 composite samples were prepared according to the following procedure: composites of small species (sardine, mussel and prawn) were prepared by using a minimum of 21 units, while those of larger species were formed by at least 12 units. Globally, 21 composite samples of fish and seafood were analyzed. Samples were freeze-dried at -80ºC with a Cryodos Telstar lyophilizer for 24 h, and then stored at -20 °C until analysis of PFCs[7]. The list of PFCs analyzed in this study is shown in Table 1.

Figure 1: Sampling points of drinking water (yellow), as well as fish and seafood (red).

Table 1: List of PFCs analyzed in drinking water, fish and seafood.

Compound	*Abbreviation*	*Molecular formula*
perfluorobutanoic acid*	PFBA	$C_4F_7O_2H$
perfluoropentanoic acid*	PFPeA	$C_5F_9O_2H$
perfluorobutanesulfonate	PFBuS	$C_4F_9SO_3^-$
perflurohexanesulfonate	PFHxS	$C_6F_{13}SO_3^-$
perfluorooctanesulfonate	PFOS	$C_8F_{17}SO_3^-$
perfluorodecanosulfonate	PFDS	$C_{10}F_{21}SO_3^-$
perfluorohexanoic acid	PFHxA	$C_5F_{11}CO_2H$
perfluoroheptanoic acid	PFHpA	$C_6F_{13}CO_2H$
perfluorooctanoic acid	PFOA	$C_7F_{15}CO_2H$
perfluorononanoic acid	PFNA	$C_8F_{17}CO_2H$
perfluorodecanoic acid	PFDA	$C_9F_{19}CO_2H$
perfluoroundecanoic acid	PFUnDA	$C_{10}F_{21}CO_2H$
perfluorododecanoic acid	PFDoDA	$C_{12}F_{25}CO_2H$
perfluorotridecanoic acid	PFTrDA	$C_{14}F_{29}CO_2H$
perfluorotetradecanoic acid	PFTDA	$C_{13}F_{27}CO_2H$

*The content was only determined in drinking water.

Thawed fish samples were grinded. From the homogenate, 1 g of sample was used for the analytical procedure. Internal standard mixture was added before 0.4 mL of a 0.2 M NaOH (in methanol) solution and the samples were left for 30 min. Extraction was performed using 4 mL of acrylonitrile, ultrasonication for 15 min, and shaking for 15 min. The samples were neutralized, centrifuged and the extraction was repeated once more, being the two extracts combined. Clean-up was performed with extraction three times with *n*-hexane (corresponding to a volume of 2:1 sample extract:hexane) and shaking with 50 mg of dispersive carbon (Supelclean ENVI-Carb (20/400 mesh), Supelco Bellefonte, PA, USA) and 100 µL of glacial acetic acid. After filtration and evaporation, the recovery standards (RS) *7H*-PFHpA, $^{13}C_8$PFOS and $^{13}C_8$-PFOA were added together with 2mM ammonium acetate. Blank samples (extraction blanks) and field blanks were performed in parallel with each batch of samples, and exactly treated as the other samples.

Water samples (500 mL) were filtered through glass microfiber filters (GF/B, Whatman) before extraction using Oasis WAX (6cc/150mg, Waters, Milford, MA, USA). Before addition of the internal standard mixture, pH was adjusted to 4.0. The WAX cartridges were conditioned before vacuum was used to run through the water samples at a flow rate of approximately 1 drop per second. Sodium acetate buffer (4 mL, pH 4 25mM) and methanol/water (1:1, 4 mL) were added. Both eluates were discarded. After drying the cartridges using vacuum suction, the analytes were eluted with 2 mL of 2 % NH_4OH/methanol solution at a rate of one drop per second. The eluates were collected, filtrated and evaporated to suitable volume with a gentle stream of nitrogen gas. Recovery standards ($^{13}C_8$-PFOA, $^{13}C_8$PFOS, *7H*-PFHpA) and 2mM ammonium acetate were added to the final extract. Extraction and field blank samples were prepared with ultra-pure laboratory produced water and were exactly treated as the remaining samples.

Results and discussion:

PFOA and PFOS, the two most largely studied PFCs , showed the highest mean concentrations in drinking water (Table 2). In contrast, PFDS, PFDoDA and PFTDA were undetected in all samples. In general terms, the most polluted samples were those collected in municipalities belonging to the Barcelona area, the most industrialized zone of Catalonia. PFOS and PFOA levels were, at least, 3- and 4-fold higher, respectively, in Barcelona, while in the remaining four areas under evaluation, PFC levels were (on average) notably lower.

In order to study the temporal evolution of the concentrations of PFCs, the current results were compared with those obtained in our previous surveys. Despite the differential characteristics of water samples, some trends could be noted (Figure 2). In the last period (2008-2009), an important decrease in PFC levels was detected, with reductions of 48% and 47% for PFOS and PFOA, respectively. In recent years, the number of studies focused on determining levels of PFCs in the environment, and especially in water, has notably increased. Generally, the Catalan levels of PFCs are similar to those previously reported by other authors, although the range of reference

values is very high. Assuming an intake of 2 L of tap water per day, the mean exposure to PFOS for the adult population of Catalonia was estimated in 3.9 ng/day, while that of PFOA was estimated in 4.8 ng/day. These values are several times lower than the thresholds established by the European Food Safety Authority (EFSA).

Table 2: Concentration of PFCs in 10 drinking water samples from Catalonia.

ng/L	Mean	SD	Median	Min.	Max.
PFBA	0.84	1.28	0.29	<0.33	4.30
PFPeA	0.45	0.67	0.11	<0.10	1.70
PFBuS	1.53	3.16	0.13	<0.10	9.60
PFHxA	0.51	0.70	0.23	<0.10	2.20
PFHpA	0.78	1.14	0.21	<0.41	3.30
PFHxS	0.31	0.26	0.25	<0.10	0.73
PFOA	2.42	3.52	0.60	<0.40	9.60
PFNA	1.22	3.15	0.15	<0.10	9.60
PFOS	1.95	2.49	0.52	<0.05	6.20
PFDS	ND	-	-	-	-
PFDA	0.50	1.20	0.07	<0.10	3.70
PFUnDA	0.46	1.04	0.05	<0.10	3.20
PFDoDA	ND	-	-	-	-
PFTrDA	0.07	0.07	0.05	<0.10	0.25
PFTDA	ND	-	-	-	-

ND: Non-detected

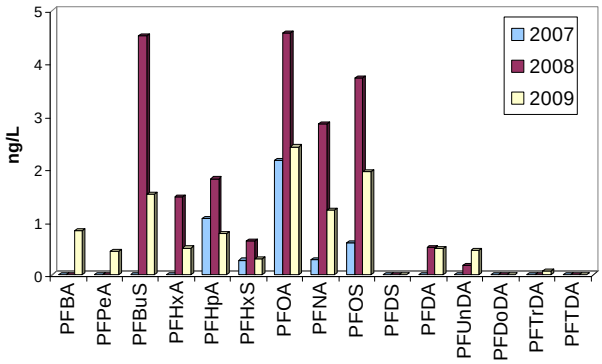

Figure 2: Temporal evolution of PFCs in drinking water.

The levels of PFCs in samples of fish and seafood from Catalonia are summarized in Table 3. Among the analyzed PFCs, 7 compounds showed detected values in at least one composite samples, while PFBuS, PFHxA, PFHpA, PFDS, PFDA, and PFTDA were not detected. PFOS was, by large, the compound showing the highest concentration in fish and seafood (6.38 ng/g dry weight –dw-), being detected in all samples, excepting mussel. High PFOS levels were found in red mullet and sardine (21.8 and 16.5 ng/g dw, respectively). Regarding PFOA (mean level: 0.13 ng/g dw), the most important concentrations corresponded to red mullet and prawn (0.21 and 0.20 ng/g dw, respectively).

No significant differences in PFC concentrations were observed according to the area of collection. However, notable higher levels of PFOS, PFOA and PFUnDA were found in the south- and mid-coastal areas, in comparison with the northern area. In a previous study, the levels of some PFCs were already determined in a few food samples acquired in Catalan markets and supermarkets[6]. Among the studied food items, white fish, seafood, tinned fish, and blue fish were separately selected. In that study, PFOS, PFOA and PFHpA were the only detected PFCs in foodstuffs. In general terms, the PFC concentrations in the species of fish and seafood here analyzed are similar to those reported in the scientific literature[8].

Table 3: Concentrations of PFCs (in ng/g dw) de PFCs in several samples of fish and shellfish.[a]

Species	*PFBuS*	*PFHxA*	*PFHpA*	*PFHxS*	*PFOA*	*PFNA*	*PFOS*	*PFDS*	*PFDA*	*PFUnDA*	*PFDoDA*	*PFTrDA*	*PFTDA*
Sardine	<0.15	<0.15	<0.15	0.07	<0.15	0.22	16.50	<0.10	*	1.40	0.48	0.91	<0.25
Tuna	<0.15	<0.15	<0.15	<0.075	0.09	0.07	1.20	<0.10	<0.10	0.69	*	*	*
Hake	<0.15	<0.15	<0.15	0.06	0.17	0.14	3.13	<0.10	<0.10	0.72	<0.20	0.28	<0.25
Red mullet	<0.15	<0.15	<0.15	0.09	0.21	1.55	21.80	<0.10	<0.10	1.1	0.32	0.46	<0.25
Cuttlefish	<0.15	<0.15	<0.15	<0.075	<0.15	0.51	1.03	<0.10	<0.10	*	*	*	*
Mussel	<0.15	<0.15	<0.15	<0.075	<0.15	0.07	<0.10	<0.10	<0.10	<0.10	*	*	*
Prawn	<0.15	<0.15	<0.15	<0.075	0.20	0.35	0.98	<0.10	<0.10	0.45	0.41	0.71	<0.25
MEAN	<0.15	<0.15	<0.15	0.05	0.13	0.41	6.38	<0.10	<0.10	0.74	0.33	0.59	<0.25

[a]Three composite samples were analyzed for each foodstuff. *Samples with recoveries of <20% or >150%

Based on the current data, we estimated the intake of PFCs through the consumption of fish and seafood. For this purpose, consumption data of the analyzed foodstuffs were obtained from the ENCAT survey[9]. Considering the whole set of 13 PFCs (and accounting a value of one-half of the detection limit for undetected compounds, ND=1/2LOD), the mean dietary intake of PFCs (exclusively due to fish and seafood consumption) for the adult population of Catalonia was estimated in 97.0 ng/day. PFOS showed the largest contribution, with a mean level of 71.3 ng/day (73% of the total). It would derive from the high consumption of PFCs through the intake of sardine and red mullet (31.4 and 27.4 ng/day, respectively). In our previous survey, PFC intake was 34.1 ng/day, when we consider only fish and seafood. However, important procedural differences exist between both surveys, which make difficult the comparison. Anyhow, these data confirm that PFOS is the PFC with higher concentrations in marine species, mainly in blue fish, the species presenting the major lipid content.

The current PFC concentrations in samples of drinking water, fish and seafood from Catalonia are of similar ranges to those reported in other international studies. Concerning PFOS only, water consumption seems to be a minor route of exposure in comparison to dietary intake.

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