DIETARY EXPOSURE TO THE PERSISTENT PERFLUORINATED COMPOUNDS PFOS AND PFOA IN GREECE - PRELIMINARY RESULTS

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Introduction

Perfluorinated compounds (PFCs) represent a large group of chemicals that have been used in a wide variety of industrial and consumer applications including adhesives, cosmetics, cleaners, coatings, and electronics.

Owing to their persistence in the environment and bioaccumulative potential several PFCs are present in nearly all areas of ecosystems and have been detected worldwide in environmental samples. Biomonitoring studies have shown extensive human exposure to these compounds. The routes of human exposure to perfluorinated compounds have not been well characterized, but food, food packaging, household dust, and drinking water are all likely to contribute[1]. Among these compounds, research focuses on perfluoroocanesulfonate (PFOS) and perfluorooctanoic acid (PFOA) since these have been produced in highest amounts for several decades in the past and have been recognized by the European Food Safety Authority (EFSA) as emerging contaminants in the food chain. EFSA has established tolerable daily intakes (TDI) of 150 ng kg $^{-1}$ b.w. day^{-1} for PFOS and 1500 ng kg $^{-1}$ b.w. day^{-1} for PFOA. As has been shown in many studies, they have moderate acute toxicity and have been classified as "harmful if swallowed"[2,3].

PFOS and PFOA accumulate in the liver and blood where they are mainly attached to proteins[4]. Animal studies have shown several toxic effects, including induction of enzymes involved in lipid metabolism and peroxisome proliferation in liver cells[5]. Possible carcinogenicity, immunotoxicity, reproductive and/or developmental toxicity have been suggested in rodents[6,7,8]. Findings from human studies suggest that PFCs are able to cross the placenta[9] and might be related to decreased fecundity and impaired sperm quality[10,11]. PFOS, its salts and PFOS-F (perfluorooctane sulfonyl fluoride) have been added to the list of persistent organic pollutants (POPs) of the Stockholm Convention on Persistent Organic Pollutants, in an amendment of May 2009 that included nine new chemicals as persistent organic pollutants. PFOA, like PFOS, is a very stable chemical compound, however due to its relatively short half-life it has not been characterized as a POP. According to EFSA, collection of data for concentration levels in the food chain is of critical importance. Studies on PFOS and PFOA levels in food samples of different types have been performed in many countries: USA[12], UK[13], Spain[14], Norway[15] and Canada[16].

The main objective of this study was to perform a first survey of perfluorinated compound levels in food samples from markets in Greece. The study included the analyses of meat products (beef, pork, chicken, lamp), fish (wild and aquaculture), eggs, vegetables (carrots), cereals and milk (cow and sheep).

Methods and materials

Materials

Standards of $^{13}C_4$-labelled solutions of PFOS and PFOA were purchased from Wellington. Ammonium acetate, methanol, acetonitrile, isopropanol and sea sand were purchased from Merck (Darmstadt, Germany). Ultrapure water was provided by a Nanopure apparatus, Barnsted/Thermolyne, USA. For solid phase extraction, Octadecyl (C18) cartridges (500 mg/8 ml) from Altech (Deerfield, Illinois, USA) were used.

Collection of samples

Food samples were collected through the services of the Hellenic Food Authority and were appropriately transported to the laboratory. The samples were kept at -20 ^{0}C until they were processed. Food items sampled were typical of the Mediterranean diet.

Sample preparation

Extraction

Meat, fish and vegetable samples were extracted by accelerated solvent extraction, using an ASE Dionex 300 apparatus, as previously described[17]. Briefly, 5 g of sample (fresh weight) were weighed, internal standards were added (200 μL of a solution containing 100 ng/mL $^{13}C_4$-labelled PFOS and 100 ng/mL $^{13}C_4$-labelled PFOA in methanol) and homogenization with 25 g sea sand was performed using a mortar and pestle. In a 34-mL ASE extraction cell a glass fiber filter was placed at the bottom, the homogenized mixture was added and the cell was filled up with sand and capped. Extraction cells were loaded on the ASE Dionex 300 apparatus. Extraction program included heating to 110 ^{0}C, 7 min static period, 3 cycles of extraction with ultrapure water, 100% flush volume, pressure at 1500 psi and purge to 1 min. The final extract was further cleaned up by solid phase extraction on C18 cartridges as described bellow.

For milk samples, a modification of a method developed by Powley et al[18] was applied. Briefly, 5 mL milk were pipetted into a 50-mL polypropylene centrifuge tube. Two hundred microliters of the internal standard working solution (containing 100 ng/mL $^{13}C_4$-labelled PFOS and 100 ng/mL $^{13}C_4$-labelled PFOA in methanol) were added. Twenty milliliters of acetonitrile were added and the sample was vortexed for 1 min and subsequently shaken at 300 rpm on an orbital shaker for 20 min. Finally, the sample was centrifuged at approximately 5000 rpm for 5 min to clarify the supernatant. The organic phase was evaporated till dryness in a flash evaporator and the residue obtained was dissolved in 5 mL of phosphate buffer solution (PB) 0.05 M, pH = 7.8.

SPE clean up

Solid-phase extraction was performed as follows. After conditioning a C18 cartridge with 2.5 mL of methanol and 5 mL of water, the fraction collected from ASE or the liquid extraction residue dissolved in phosphate buffer was passed through the cartridge. The cartridge was then washed with 5 mL of water and PFOS and PFOA were eluted from the cartridge with 5 mL of methanol. The flow rate of the cartridge was approximately 1–2 drops per second. The organic phase was evaporated till dryness in a flash evaporator. The dry residue was dissolved in 200 μL of the HPLC mobile phase.

Instrumental analysis. All sample extracts were analysed by liquid chromatography-tandem mass spectrometry (LC-MS-MS) with electrospray ionization (ESI) operating in negative mode. The extracts (35 μL injection volume) were chromatographed on a HyPurity Advance C18 column (5 μm, 50 mm × 2.1 mm i.d, Thermo) using a Surveyor MS Pump Plus (Thermo). The gradient operated at a flow rate of 0.25 mL/min starting from 20% isopropanol in 5mM ammonium acetate:methanol 80:20 (A) to isopropamol:methanol 50:50 (B) in 3 min. The HPLC was interfaced to a triple quadrupole TSQ QUANTUM ULTRA (Thermo) equipped with an Ion MAX-S thermoelectrospray source operating in negative ion mode. The source temperature was maintained at 350 ^{0}C and the spray voltage at −3500 V. The analyses were performed with a multiple reaction monitoring (MRM) method that monitored two mass transitions (parent ion/product ion) for each analyte:

PFOS : 498.9 → 80, 498.9 → 98.9 $^{13}C_4$PFOS : 502.9 → 80, 502.9 → 98.9

PFOA : 413 → 219, 413 → 369 $^{13}C_4$PFOA : 417 → 168.9, 417 → 372

The values of the voltages applied to the tube lense offset and the collision cell were optimized by direct infusion of a solution containing the analytes. Confirmation of analyte identity was based on retention time and on the relative response of the secondary mass transition to the primary mass transition. Quantification of the target compounds was calculated by the sum of areas of the two product ions using a response factor calibration curve vs the $^{13}C_4$-labelled standard. The LOD and LOQ were determined as signal/noise (S/N) ratio of 3 and 10, respectively. More specifically, LOD was calculated at 0.15 ng/g and LOQ at 0.50 ng/g for both PFOS and PFOA.

Results and discussion

This is a preliminary study of dietary exposure to perfluorinated compounds in Greece. In our previous study[19], we applied extraction of fish tissue by ethyl acetate using a probe homogenizer, centrifugation of the extract to obtain the organic phase containing PFOS and PFOA and evaporation of the solvent before SPE clean up. Accelerated solvent extraction applied in this study is a modern extraction method that allows automated and rapid extraction of larger number of samples. Moreover, the use of water as extraction solvent makes feasible the direct application of the extract to SPE clean-up column. The absence of the solvent evaporation step is advantageous for analyte recovery, and also shortens the time required for analysis.

Forty samples of several food types were analysed. The results are presented in Table 1. PFOS and PFOA concentrations were below the LOD (0.15 ng/g) in most (32) samples.

PFOS was detected in eight samples, at concentrations ranging from 0.89 ng/g to 5.80 ng/g wet weight. PFOS was not detected in any sample of milk, pork, chicken and aquaculture fish. PFOA was detected in six samples at concentrations ranging from 0.62 ng/g to 6.19 ng/g wet weight. PFOA was not detected in any sample of milk, lamp and aquaculture fish.

Maximum concentration of both chemicals (5.80 ng/g wet weight PFOS and 6.19 ng/g wet weight PFOA) was measured in the same sample of wild fish. Generally, the concentrations measured are in the same range as or lower than those reported in studies from Canada[16], Norway[15], Spain[14], UK[13] and USA[12].

The low levels of PFOS and PFOA in Greek food samples (below the limit of detection in most samples), are probably due to very limited industrial production of products containing perfluorinated compounds.

Table 1. PFOS and PFOA concentrations (ng/g, wet weight) in food samples analysed

Food (No of samples)	PFOS	PFOA
Beef (7)	<0.15 – 1.71	<0.15 – 0.72
Pork (4)	<0.15	<0.15 – 0.83
Chicken (5)	<0.15	<0.15 – 1.00
Lamp (3)	<0.15 – 1.74	<0.15
Wild fish (5)	<0.15 – 5.80	<0.15 – 6.19
Aquaculture fish (4)	<0.15	<0.15
Cow milk (7)	<0.15	<0.15
Sheep milk (3)	<0.15	<0.15
Egg (1)	2.42	0.62
Carrot (1)	1.22	<0.15
Cereal (1)	<0.15	<0.15

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