Chemosphere 127 (2015) 117–126

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Levels of perfluorinated compounds in raw and cooked Mediterranean finfish and shellfish

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HIGHLIGHTS

- PFCs were measured for first time in 10 species of Mediterranean fish/shellfish.
- Almost all samples contained detectable levels of PFCs.
- The most prominent PFC was PFOS, found in levels up to 22 ng g^{-1}.
- PFC concentrations were generally higher after frying or grilling of fish samples.
- Human exposure by consumption of these fish species is below limit proposed by EFSA.

ARTICLE INFO

Article history:
Received 12 June 2014
Received in revised form 27 November 2014
Accepted 30 December 2014
Available online 9 February 2015

Handling Editor: I. Cousins

Keywords:
Seafood
Perfluorinated compounds
PFOS
PFOA
Cooking
Mediterranean diet

ABSTRACT

Perfluorinated compounds (PFCs) were analyzed in several species of small Mediterranean fish and shellfish, all of which are popular in Greek diet. Analysis was conducted in raw samples and in samples cooked by the two ways preferred in Greek cuisine, i.e. fried in olive oil and grilled. PFCs above the detection limit were found in all raw samples except sardine, mussel and squid. The predominant PFC was PFOS (perfluorooctane sulfonate), the highest concentration of which was measured in picarel (20.4 ng g^{-1} fresh weight). The PFOS values for the rest of the samples were between <LOD and 5.66 ng g^{-1} fw. The concentrations of the detected PFCs were in most cases higher after frying or grilling, this increase being statistically significant. Based on these results, the Tolerable Daily Intake for PFOS and PFOA (perfluorooctanoic acid) through consumption of fish and seafood was well below the values proposed by EFSA.

1. Introduction

Information that reaches the public about the benefits and risks from the consumption of fish and seafood is frequently vague and contradictory. On the one hand, fish is exalted as an essential part of a well-balanced diet containing high quality protein, important vitamins (D, B_{12} etc) and essential metals including copper, manganese, selenium and zinc (Olmedo et al., 2013). Fish and seafood traditionally form part of the so-called Mediterranean diet that has been associated with significant reduction in mortality. Additionally, fish constitutes a major source of omega-3 polyunsaturated fatty acids, which have been recognized as important agents for the prevention of coronary disease and the normal neurodevelopment and brain development of children. On the other hand, many undesirable substances have been detected in fish intended for human consumption, the most important of which are halogenated compounds (dioxins, and PCBs) and toxic heavy metals and their organometallic metabolites, especially methyl mercury (Mozaffarian and Rimm, 2006). Reports about fish contaminated by the above pollutants often reach the ears of the general public causing great concern to consumers, who demand scientifically based advice regarding fish consumption for a healthy and balanced diet, especially for sensitive population groups such as children and pregnant or breast-feeding women.

Perfluorinated compounds (PFCs) are a large group of compounds characterized by a completely fluorinated linear carbon chain with a hydrophilic head. Due to their chemical properties that

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http://dx.doi.org/10.1016/j.chemosphere.2014.12.081
0045-6535/

include thermal and chemical stability, amphiphilicity, non-flammability and surface-active properties, they have been used in many industrial and consumer applications including adhesives, cosmetics, cleaners, coatings, electronics and paper products (Lau et al., 2007). PFCs have been manufactured and released to the environment for more than 50 years, and are now acknowledged as widespread, persistent and bioaccumulative pollutants detected in water and sediments, biota and humans (Fromme et al., 2009). Animal studies have shown that they exhibit hepatotoxicity, neurotoxicity, immunotoxicity, developmental effects and possible carcinogenicity (Lau et al., 2004). Due to their moderate acute toxicity they were classified by EFSA in 2008 as "harmful if swallowed". PFOS (perfluorooctane sulfonate) and PFOA (perfluorooctanoic acid) are considered the most important PFCs. PFOS has been determined by OECD (Organisation for Economic Co-operation and Development) as a persistent, bioaccumulative and toxic (PBT) substance and has been included as a persistent organic pollutant (POP) in Annex B of the Stockholm Convention in 2009 (http://chm.pops.int/TheConvention/ThePOPs/ListingofPOPs/tabid/2509/Default.aspx). The EU has recommended monitoring of the presence of PFOS and PFOA and if possible, their precursors, in food (Recommendation 2010/161/EC, (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:068:0022:0023:EN:PDF).

Although sources of human exposure to PFCs include household dust (Haug et al., 2011) and drinking water (Domingo et al., 2012a), it has been established that food is the most important source of PFC intake for non-occupationally exposed humans (Domingo, 2012). Studies in many countries including Poland, Germany, Norway, Sweden, United Kingdom, China, and Canada, have shown that the most important contributor to PFC exposure through food is fish, and investigated the potential correlation between fish consumption and PFC levels in human serum. These studies were reviewed extensively by Domingo in 2012. A more recent study in Sweden also confirms the existence of a strong correlation between PFC levels in blood serum and fish consumption (Bjermo et al., 2013). The Scientific Panel on Contaminants in the Food Chain (CONTAM Panel) of the European Food Safety Authority (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.

Despite the significant toxicity of PFCs, the number of studies focusing on their concentrations in items intended for human consumption still remains limited. Most of the studies concerning the levels of PFCs in edible fish and seafood have been conducted in raw muscle tissue. However it is possible that these levels may be altered in a non-predictable way by cooking processes, as shown in a limited number of recent studies (Del Gobbo et al., 2008; Bhavsar et al., 2014). In the present study, the levels of PFCs were investigated in seven species of finfish and three species of shellfish, which are among the most commonly marketed species in the Aegean and Mediterranean Seas. To our knowledge, this is the first study reporting results from Greece, and the first one for most of these particular species of small Mediterranean fish and shellfish, which are quite popular in Mediterranean diet. The samples were analyzed raw as well as cooked following the most popular Greek culinary practices. Based on these results, the assessment of human exposure to PFCs through consumption of these fish species and the possible risk involved were attempted.

2. Materials and methods

2.1. Sample collection and preparation

The samples of the present study included finfish – anchovy, bogue, hake, picarel, sardine, sand smelt and striped mullet – and shellfish – Mediterranean mussel, shrimp and squid. The edible parts of these food items, which are all widely consumed in Greece, were analyzed raw, as well as cooked in the ways favored in Greek cuisine: pan-fried in olive oil (all samples), and grilled (anchovy, bogue, hake, sardine, striped mullet and squid).

All samples were obtained during the winter-early spring of 2011. Finfish, squids and shrimps were purchased from the local fish market in Kallithea, Athens, while mussels were obtained from a mariculture farm within the Saronikos Gulf, Attika. The fishing locations of the collected samples are shown in Fig. 1 and provided in Table 1 along with additional information about biometric data and sample cooking and treatment before analysis. The quantity of each sample was 2–4 kg, comprising individuals of similar size. Following immediate transport to the laboratory and recording of biometric data, the samples were washed with cold water, scales were removed from the finfish and they were subsequently prepared according to the traditional Greek culinary practice. Mussels were first put for 2–3 min in boiling water in a casserole until they were opened and then their flesh was removed from the shells to be used for cooking and analysis.

The washed fish and shellfish were pan-fried in Virgin Olive Oil (VOO), which was purchased in sealed plastic bottles from the local market. For this purpose, the samples were placed in a metal frying pan (30 cm diameter, 5 cm depth), which contained 300 mL VOO preheated at 170 °C and were fried until they were browned. To achieve uniform cooking the samples were turned and cooked in both sides by means of a wooden spatula, which was also used to remove the prepared food from the pan. The prepared fried seafood was placed in a clean plate covered with soft tissue paper to allow the excess of oil to drain. Both the frying oil and the food were weighed before and after frying to calculate water loss and oil uptake. After each frying operation, the used oil was discarded and the frying pan was thoroughly cleaned to be used for the next set of samples.

Five species of finfish as well as the squid were additionally grilled in a domestic electric oven at 180 °C. For this purpose, the food was placed on a grill and was heated from above by the oven's electric salamander. The metallic grill was covered with grease-proof paper on which small holes had been opened, to allow juices from the cooked food to drain. The paper had previously been analyzed and found to be PFC free. Food was weighed before and after cooking in order to calculate water loss.

Quadruplicate composite samples, consisting of 4–6 items of raw or cooked fish or shellfish, were transferred to clean screw-capped plastic containers and were freeze-dried for 48 h (Heto Lyolab 3000, Heto-Holten, Allerod, Denmark). Freeze-drying served also for moisture determination, as the water content of the freeze-dried samples was found to be less than 3%. The freeze dried samples were homogenized by means of a clean agate mortar and were subsequently analyzed.

2.2. Materials

The method of analysis used is suitable for quantitative determination of 12 perfluorinated compounds: 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 (PFDoA), perfluorobutane sulfonate (PFBS), perfluorohexane sulfonate (PFHxS) and perfluorooctane sulfonate (PFOS) and the qualitative detection of 5 more: perfluorotridecanoic acid (PFTrDA), perfluorotetradecanoic acid (PFTeDA), perfluorohexadecanoic acid (PFHxDA), perfluorooctadecanoic acid (PFODA) and perfluorodecane sulfonate (PFDS). Standard solutions of $^{13}C_4$-labelled PFBA, PFOA and PFOS, $^{13}C_2$-labelled PFHxA, PFDA, PFUnDA and PFDoA, $^{13}C_5$-labelled PFNA and $^{18}O_2$ PFHxS were purchased from Wellington