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Monitoring of perfluorinated compounds in edible fish from the Mediterranean Sea

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ABSTRACT

Perfluorooctanoic acid (PFOA) and perfluorooctane sulphonate (PFOS) are environmental contaminants belonging to a chemical group known as perfluorinated compounds (PCFs). The United States Environmental Protection Agency (US EPA) considers both compounds to be carcinogenic. The goal of the present study was to evaluate the contamination levels of PFOS and PFOA in edible fish of the Mediterranean Sea. Twenty six fish muscles, 17 fish livers, five series of cephalopods (each composed of ten specimens) and thirteen series of bivalves (each composed of about 50 specimens) were used for the investigation. A fast sample treatment, followed by an LC–ESI–MS/MS method is described for the identification, and quantification of PFOA and PFOS in fish. The method was *in-house*-validated through the determination of precision, accuracy, specificity, calibration curve, decision limit (CCα), and detection capability (CCβ). The results showed PFOA and PFOS levels in fishes and molluscs lower than those reported for analogue matrices in different geographic areas. Therefore, our biomonitoring results did not show that the Mediterranean Sea had any particularly alarming pollution by PFCs, although it is located in a semi-closed basin with scarce water change. Nonetheless, a worrying element is that a few fish showed extremely high contamination by PFOA and PFOS. This finding needs further clarification in order to assess whether such unusual contamination is linked to "dot-like" pollutant release, which could explain the anomaly.

1. Introduction

Perfluorinated compounds (PFCs) are a few hundreds of chemically and thermally stable compounds, mostly polymers, scarcely soluble in oil and water. Due to their peculiar characteristics, PFCs are used in several applications, such as emulsifiers, lubricants, fire-fighting foams, water-, grease- and soil-repellent coating materials in the textile industry (e.g. Gore-Tex, carpet protection, leather protection) and as processing aids in food paper containers, cooking tools, medical aids and plastics (e.g. Teflon), products for personal and domestic hygiene, electronics, photographic industry, inert components in pesticides and cement additives.

The USA produced about 3000 tons of PFCs in 2000 and, currently, several tons of PFCs per year are an input into the environment in The Netherlands (Hekster, Laane, & de Voogt, 2003). The Association of Plastic Manufacturers Europe (APME) estimated that, in PFCs production, only 16% of the polymerisation coadjutant is still present in the produced polymer, while 61% is emitted to water, air and land.

PFCs include perfluoroalkyl sulphonates (PFASs), such as perfluorooctane sulphonate (PFOS), and perfluorinated carboxylic acids (PFCAs), such as perfluorooctanoate (PFOA). Recent studies have revealed that, at present, PFOA and PFOS are ubiquitous environmental contaminants, bioaccumulating in animals and humans. Moreover, novel data have come to light, concerning the potential developmental, reproductive and systemic toxicity of PFOA and PFOS. The US EPA also considers both compounds to be carcinogenic. PFOS and PFOA are rarely used directly as materials or precursors, but they are intermediates in the synthesis of other PFCs and final metabolites or degradation products of several PFOS- or PFOA- related compounds (Dinglasan, Ye, Edwards, & Mabury, 2004).

In a 3M study (3M, 2001), PFOS and PFOA were the main PFCs detected in several media from six urban areas in the USA, at concentrations up to 2980 $\mu g\ l^{-1}$. The highest levels were found in sewage sludge, sewage treatment plant effluent and landfill leachate, particularly in cities were PFCs were manufactured or industrially used, but also in control cities.

PFOS and PFOA are the most important PFCs detected in groundwater (Moody & Field, 1999) and PFOS was detected at levels ranging from 36 ng/g to 1.7 μg/g in marine and estuarine biota from a Belgian river and the adjacent coastal zone of the North Sea, where a PFCs factory is located upstream of the river (Hoff et al., 2003).

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In Japanese (Moriwaki, Takata, & Arakawa, 2003) and American (Costner, Thorpe, & McPherson, 2005) domestic dust, PFOS and PFOA levels have been found in the range 11–2500 ng/g and 69–3700 ng/g, respectively, indicating a possible chronic exposure for the general population. A Canadian study found N-alkyl-perfluorooctane sulphonamide-ethanol (N-alkyl-FOSE) indoor levels 25 times higher than the corresponding outdoor levels (Shoeib, Harner, Wilford, Jones, & Zhu, 2004).

Six PFCs have been detected in the air of a highly urbanised size in Canada at levels ranging from 14 to 205 pg/m^3; five of the same PFCs were also found in a rural Canadian site at levels between 1.7- and 2.9-fold less of those found in the urbanised site (Hekster et al., 2003).

PFOS meets the criteria for persistent organic pollutants (POPs) characteristics and restrictions for PFOS and other perfluoroalkylsulphonates (PFAS) commercialisation have been fixed from 2001 to 2004 by the US EPA (ENDS, 2004) and in 2006 by the 2006/122/CE Directive (EC, 2006). Despite these restrictions of PFOS use and commercialisation, as this compound is released by existing products and garbage dumps, its presence in the environment will likely continue to be an issue for several years.

In general, PFCs bioaccumulate in animals (Conder, Hoke, De Wolf, Russell, & Buck, 2008), although PFCAs with seven fluorinated carbons or less (including PFOA) have recently been defined as not bioaccumulative according to regulatory criteria (Conder et al., 2008). PFCs have been found in tissues of living organisms (Martin et al., 2004), including humans, with PFOS at the highest levels (Kannan et al., 2004). They have been found in exposed workers (Olsen, Burris, Burlew, & Mandel, 2003) and in non-occupationally exposed adults, the elderly and children (Harada et al., 2004; Kannan et al., 2004; Olsen et al., 2005), suggesting a widespread presence in the general population (Sakr et al., 2007). Generally, individuals living in urban and industrial areas show higher blood levels than do those living in rural and remote areas. In a study, PFOS levels in human blood from the general population of different countries have been found in the range of 1–200 ng/g, with the highest levels in the USA and Poland (Kannan et al., 2004). Differently from other POPs, PFAS bioaccumulate more in blood and drizzled tissues (liver, kidney, gallbladder) than in fat tissues, probably due to their chemical bond to haematic proteins (Han, Snow, Kemper, & Jepson, 2003). Differently from other POPs, PFAS bioaccumulation in humans is not found to be proportional to the age of the exposed individuals (Kannan et al., 2004). Unlike animals, humans seem to eliminate PFOA relatively slowly.

PFOS and PFOA hepatotoxicity has been evidenced for rodents (Kennedy et al., 2004) and monkeys (Butenhoff et al., 2002). In rodents, they promote liver carcinogenesis (Vanden Heuvel, Thompson, Frame, & Gillies, 2005), possibly due to PFCs interference with gap junction intercellular communication (Hu et al., 2002), which can also enhance permeability and toxicity of other xenobiotics. PFOA showed increased liver tumours, pancreatic acinar cell tumours, testicular Leydig cell adenomas (males), and mammary hyperplasia (females) compared with controls (Sibinski, 1987).

PFOS and PFOA have been found to exert endocrine disrupting effects in fish (Shi, Du, Lam, Wu, & Zhou, 2008), birds (Molina et al., 2006) and rats (Jensen & Leffers, 2008). PFOA is immunotoxic in mice (Peden-Adams et al., 2008) and PFOS can cross the haematoencephalic barrier in rats (Austin et al., 2003).

Few epidemiological studies have been carried out with significant populations of PFCs-exposed workers (Gilliland & Mandel, 1993). However, they have shown increased deaths by bladder and liver cancer in PFOS-exposed workers and by prostate cancer in PFOA-exposed workers. The general population, living near Dupont Teflon Industry in the USA, showed greater incidence of prostate and female reproduction organ tumours, lymphomas, leukaemia and multiple myeloma, although a study undertaken by Dupont itself showed no increased tumour incidence, but only increased cholesterol and triglycerides levels (10%) in individuals with blood PFOA levels higher than 1000 ng/g (DuPont., 2005).

Fish are bioindicators, as they are essential components of various ecosystems (sea, rivers, lakes, etc.) and they also represent an important human food source. A polish study has shown an association between consumed fish and PFOS/PFOA levels in human serum, independently of age and sex (Falandysz, Taniyasu, Gulkowska, Yamashita, & Schulte-Gehlman, 2006). Chronic exposure to high levels of chemical contaminants through fish consumption can represent a risk factor for human health.

The Mediterranean Sea, being a system with limited water exchange, is an ecosystem at risk and it can determine human PFCs exposure via sea fish consumed by the general population.

The aim of the present study was to monitor the current levels of PFOS and PFOA in some types of Mediterranean Sea fish which are most consumed in Italy. The resulting information is of primary importance for assessing the risk to human health arising from fish consumption in Italy. Moreover, since fish are good bioindicators of environmental contamination, this study can contribute to evaluating the pollution degree of the Mediterranean Sea by PFCs Finally, the development and validation of a sensitive and simple LC–ESI–MS/MS method for the determination of PFOS and PFOA in fish matrices has also been carried out.

2. Materials and methods

2.1. Chemicals

Heptadecafluorooctane sulphonate (PFOS-H) (Fig. 1), purity 40% in water, pentadecafluorooctanoate (PFOA) (Fig. 2), purity 95%, internal standard perfluorododecanoic acid (PFDoA), purity 95% and tetrabutyl ammonium bisulphate (TBA), at 99% stated purity, were purchased from Sigma (Milan, Italy). Ammonium acetate, sodium carbonate, methyl tert-butyl ether (MTBE) and acetic acid were of analytical-reagent grade and were purchased from J.T. Baker (Florence, Italy). Acetonitrile and methanol (HPLC grade) were purchased from J.T. Baker (Florence, Italy). Ammonia was of analytical-reagent grade and was purchased from Carlo Erba (Milan, Italy). Water was purified in a Milli-Q system Millipore (Milan, Italy).

Individual standard stock solutions (1 mg ml^{-1}) were prepared in methanol and stored at −20 °C and they were stable for about one week. Individual and composite working standard solutions were prepared daily by appropriate dilution of the standard stock solutions with methanol.

2.2. Samples

Twenty six fish muscles, seventeen fish livers, five series of cephalopods (each composed of 10 specimens) and thirteen series of bivalves (each composed of about 50 specimens), supplied by Istituto Centrale per la Ricerca Scientifica e Tecnologica Applicata al Mare, ICRAM (Rome, Italy), were used for the investigation. Fish samples included pelagic fish such as grey mullet (*Mugil cephalus*), common Dentex (Dentex dentex), horse mackerel (*Trachurus mediterraneus*), porbeagle (*Lamna nasus*), common smooth-hound (*Mustelus mustelus*), sword fish (*Xiphias gladius*), tuna (*Thunnus thynnus*) and benthonic fish such as European conger (*Conger*

Fig. 1. Chemical structure of PFOS.

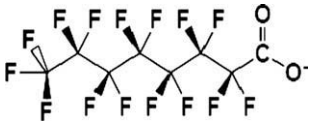

Fig. 2. Chemical structure of PFOA.

conger), small-spotted catshark (*Scyliorhinus canicula*), red mullet (*Mullus surmuletus*), common pandora (*Pagellus erythrinus*) and largescaled scorpionfish (*Scorpaena scrofa*). With cephalopod and bivalve molluscs, the samples consisted of three series of squids (*Loligo vulgaris*), two series of redshanks (*Tringa totanus*), eight series of blue mussels (*Mytilus edulis*) and five series of clams (*Venus*).

For fish, samples of muscle plus skin, in natural proportions, and liver were collected, whereas cephalopods and the flesh of bivalve molluscs were collected as a whole.

All samples were then homogenised and placed into polyethylene bags and stored at −80 °C prior to analysis.

2.3. Sample treatment

A rapid and simple liquid extraction and clean-up procedure, suitable for all the investigated tissues, was developed. The sample pre-treatment procedure was similar to that described by Tseng, Liu, Chen, and Ding (2006) except for some modifications. Briefly, 50 μl of internal standard solution (PFDoA at 10 mg kg^{-1}) and, after few minutes, 4 ml of distilled water, were added to the homogenised tissue sample (about 1 g). After homogenisation on an Ultra Turrax homogenizer, 1 ml of TBA (0.5 M, pH 10) and 2 ml of sodium carbonate solution (0.25 M) were added to the homogenised tissue sample (1 ml). The sample solution was agitated on a vortex mixer for 20 s and 4 ml MTBE were added. After agitation on a vortex mixer for 20 s and sonication for 15 min. at room temperature, the sample solution was centrifuged at 3000 rpm for 10 min (at 25 °C). The supernatant was then transferred into a 10 ml tube and the residue was again extracted twice with two fresh portions of MTBE solution (4 ml). The combined extracts were evaporated to dryness under nitrogen and redissolved with methanol (0.5 ml). After agitation on a vortex mixer for 20 s, the sample was filtered through 0.45-μm-pore-size nylon filter and the final solution was injected into the LC–MS/MS.

2.4. Liquid chromatography–tandem mass spectrometry analysis

Analysis were performed with an Agilent liquid chromatograph (Palo Alto, CA) assembled with a 1100 series LC quaternary pump, a micro vacuum degasser, an autosampler and a column oven. Chromatographic separation was obtained by means of a reversed-phase Gemini C18 column 50 mm× 2 mm, 110 Å (Phenomonex, Milford, MA) at 25 °C and two reversed-phase Hypercarb C18 pre-column cartridges, 4 mm × 10 mm, 110 Å (Keystone Scientific). These last were set between the HPLC pump and HPLC injector, in order to avoid contamination of the samples due to the release of PFOA from the polyterafluoroethylene (PTFE) components of the HPLC instrument (Flaherty et al., 2005). The eluents were 2 mM ammonium acetate (eluent A) and methanol (eluent B), under gradient conditions, at a flow-rate of 300 μl min^{-1}. The elution gradient consisted of the following steps: from 0 to 3 min, A at 40%; at 3.5 min, A at 0%; from 3.5 to 8 min, A at 0%; at 8.5 min, A at 40%; from 8.5 to 15 min, A at 40%. The injection volume was 5.0 μl and the run time was 15 min.

Mass spectral analyses were performed on an Applied Biosystems API 3000 triple-stage quadrupole mass spectrometer (Toronto, ON, Canada), equipped with a turbo ion spray interface operating in the negative ion selection mode and set at 400 °C, with the spray voltage set at −2 kV.

Detection and quantification of PFOA and PFOS were performed by multiple reaction monitoring (MRM) of the deprotonated precursor ions and the related product ions. For quantification, the internal standard (PFDoA) method with peak area ratio was used.

Solutions of 100 μg l^{-1} in methanol of each single analyte were directly infused at a flow-rate of 5 μl min^{-1}. The deprotonated precursor ions $(M–H)^-$, for PFOA and PFOS, were followed in negative ion mode at m/z 413.3 and m/z 499.4, respectively.

The instrument tuning was carried out using the automatic tuning tool of the Analyst 1.4 software (MDS-Sciex, Toronto, ON, Canada) to determine declustering, focusing and entrance potentials, fragmentation pattern, collision energy, and collision cell exit potential. Quadrupoles Q1 and Q3 were set on unit resolution. The analytical data were processed by Analyst 1.4 software.

The MS/MS fragmentation patterns obtained from PFOA and PFOS under the described conditions are shown in Fig. 3. The mass transition reactions used for PFOA identification and quantification were m/z 413.3 → 368.8 as quantifier, m/z 413.3 → 219.1 and m/z 413.3 → 168.8 as qualifiers. The mass transition reactions used for PFOS identification and quantification were m/z 499.4 → 80.0 as quantifier, m/z 499.4 → 129.9 and m/z 499.4 → 98.8 as qualifiers. The mass transition reaction used for the internal standard, PFDoA, was m/z 612.8 → 569.0. A collision energy = 5 eV and a dwell time = 500 ms were used for all of these mass transition reactions.

2.5. Analytical method validation

In order to achieve the *in-house* validation of the analytical method, the following parameters were considered: maximum permitted tolerances for relative ion intensities, recovery, repeatability, specificity, linearity, decision limit (CCα) and detection capability (CCβ).

For identification purposes, retention times of PFOA in the standards and in the samples were compared at a tolerance of ±2.5%. Moreover, in accordance with the 2002/657/EC Decision (EC, 2002), the relative ion intensities (each daughter ion area signal versus the base daughter ion area signal) of the spiked tissue samples were compared with the relative ion intensities of PFOA standard solutions, at the same concentration levels as used for the construction of the calibration curve. An analogous procedure was followed for identification purposes of PFOS.

In the absence of any certified reference material (CRM) for PFCs in fish or similar matrices, the method accuracy was evaluated on the basis of recoveries obtained from *in-house* standard materials (fortified samples). Briefly, a blank tuna tissue sample (previously analysed and found to be not contaminated) was fortified with PFOA and PFOS at four different levels (7.5, 15.0, 22.5 and 30.0 μg kg^{-1}). Precision, expressed as repeatability, was calculated by repeated analyses on the same sample sets as used for recovery tests, with the only difference that independent samples were re-extracted and analysed on two other occasions for calculating inter-day repeatability.

Specificity of the LC–MS/MS method was proved by following the mass transition reactions chosen to detect PFOA, PFOS and PFDoA in blank fish tissue samples (tuna).

Linearity was determined by means of standard and matrix calibration curves, obtained by LC–MS/MS analyses of standard solutions and the above-mentioned fortified samples, respectively. The response factor (r.f. = peak area − intercept/[analyte concentration]) test was applied for the purpose. The deviation of the r.f. of each point of the calibration curve must be within ±3% of the experimental slope.

In the 2002/657/EC Decision, CCα and CCβ replace the detection and quantification limits, respectively. However, in the absence of an established permitted limit fixed by the European Normative, as is in the case for PFOA and PFOS, CCα coincides with the limit of detection (LOD) and CCβ coincides with the limit of quantification

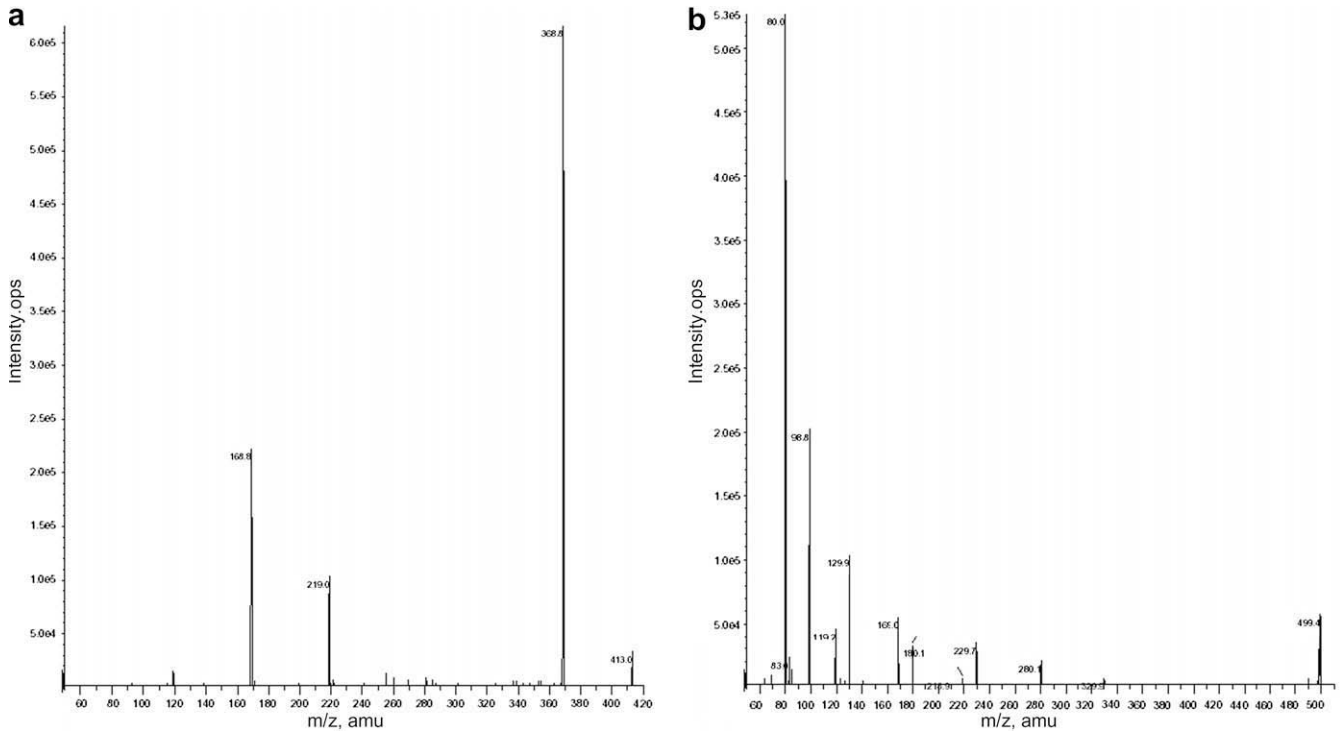

Fig. 3. (a–b) Full-scan of $[M–H]^-$ ions of PFOA (a) and PFOS (b) both at concentration of 100 μg l^{-1}.

(LOQ). LOD and LOQ for fish and molluscs were calculated according to the requirements of the International Conference on Harmonization (ICH, 1995), adopted by the EC, Japan and the USA. Ten blank tuna tissue samples were fortified with an amount of standard PFOA or PFOS able to produce signal-to-noise ratios ranging from 2.5 to 5. In our case, the suitable concentrations were 7.5 μg kg^{-1} for both PFOA and PFOS. LODs were determined by multiplying the standard deviations (SDs) calculated for the 10 blank tissue samples fortified with low PFCs concentrations by the Student *t*-test value ($n-1$, $1-\alpha = 0.95$). The exact equation of the calculation is as follows: LOD = SD x Student *t*-test value ($n-1$, $1-\alpha = 0.95$), where the SD is for the 10 blank tissue samples fortified with low PFCs concentrations. In our case, *n* was equal to 10; therefore, the Student *t*-test value was 3.250. The LOQs were estimated to be 10 times the same SD, divided by the slopes of the corresponding calibration curves (0.0062 for PFOA and 0.0024 for PFOS). The exact equation of the calculation is as follows: $LOQ = 10 \times SD/b$, where SD is the standard deviation for the 10 blank tissue samples fortified with low PFCs concentrations and *b* is the slope of the corresponding PFCs calibration curve.

For the assessment of all the mentioned parameters, the analyte response was always related to the internal standard response (30.0 μg kg^{-1} of PFDoA).

Finally, in order to comply with internal quality control (IQC) procedures, two control samples (house reference materials) were inserted into each analytical batch made up of six samples. The individual values obtained for control samples were plotted on a Shewhart control chart during the entire duration of the study.

3. Results and discussion

3.1. Method validation parameters

The developed analytical method proved to be very adequate within the scope of the present investigation. The performance limits satisfy the criteria fixed by various international organisations for the analytical methods applicable to the determination of residues and contaminants in biological matrices. Moreover, the peculiar assembling of the two pre-column cartridges eliminated risks from exogenous contamination of the samples due to the release of PFOA from the PTFE components of the HPLC instrument.

The tolerance criteria requested by the 2002/657/EC European Decision for identification purposes of the analytes were satisfied.

Under the chromatographic conditions described above, the retention time of PFOA and PFOS were 1.90 min and 2.70 min, respectively.

The accuracy and the precision, expressed as intra- and interday repeatability of the method, are listed in Table 1. Recovery data were satisfactory for PFOS, with values ranging from 90% to 113%,

Table 1
Performance of the analytical method for the determination of PFOA and PFOS in fish.

Fortification level (μg kg^{-1})	Measured content[a] (μg kg^{-1})	Recovery (%)	Intraday repeatability[b] CV (%)	Interday repeatability[c] CV (%)
PFOA				
7.5	6.3 ± 1.4	83	12	22
15.0	10.1 ± 2.4	67	17	24
22.5	13.6 ± 2.3	60	3	17
30.0	16.3 ± 4.6	54	19	28
PFOS				
7.5	8.5 ± 1.2	113	5	14
15.0	16.0 ± 2.2	107	20	14
22.5	20.3 ± 5.7	90	20	28
30.0	27.1 ± 8.2	90	17	30

[a] Values are means ± SD for nine samples.
[b] Values are referred to three independent samples analysed on one day.
[c] Values are referred to nine independent samples analysed on three different days (three samples analysed each day).

whereas, for PFOA, the recoveries were lower (from 54% to 83%) but nevertheless acceptable.

The coefficients of variation (CV) values for intraday repeatability ranged from 3% to 19% and from 5% to 25% for PFOA and PFOS, respectively. As far as interday repeatability is concerned, the CV values varied from 17% to 28% and from 14% to 30% for PFOA and PFOS, respectively. These data indicate that both intraday and interday repeatability are good, since all CV values are below the recommended limits based on the Horwitz curve modified by (Thompson & Lowthian (1997).

The LOD and LOQ values were 1.5 μg kg^{-1} and 5 μg kg^{-1} for PFOA and 2 μg kg^{-1} and 6 μg kg^{-1} for PFOS, respectively.

The calculated matrix calibration curves showed a fair linearity over the whole range of tested concentrations (7.5–30.0 μg kg^{-1}) for both PFOA and PFOS. The resulting equations were $y = 0.0062x - 0.0015$ and $y = 0.0024x - 0.0024$ (where y is the analyte/IS peak area ratio and x is the analyte concentration) with the squared correlation coefficients (r^2) are equal to 0.9882 and 0.9587, for PFOA and PFOS, respectively.

3.2. Biomonitoring results

Table 2 and Figs. 4 and 5 show PFOA and PFOS levels found in fish, cephalopods and bivalve tissues, aggregated for type of habitat (pelagic fishes or benthonic fishes, predator cephalopod molluscs and filter-feeders, bivalve molluscs).

Analyzing the biomonitoring results presented in this study, a few general conclusions can be drawn. The first is that the measured PFOA and PFOS levels were relatively low compared to those found in the same matrices in previous studies carried out in different geographic areas (Olivero-Verbel et al., 2006; Tseng et al., 2006). Regarding PFOA, the measured levels were lower than the LOD of the analytical method (1.5 μg kg^{-1}) in 14 fish muscles, three fish livers and four out of the thirteen series of filter-feeders, bivalve molluscs. PFOA measured levels were higher than the LOD, but lower than the LOQ (5 μg kg^{-1}), in three fish muscles, six fish livers, in the five series of predator cephalopod molluscs and in four series of filter-feeders, bivalve molluscs. Summarizing, 62% of the total PFOA sample levels were under the LOQ value of the analytical method.

Regarding PFOS, the measured levels were lower than the LOD of the analytical method (2 μg kg^{-1}) in six fish muscles, two fish livers and twelve out of the thirteen series of filter-feeders, bivalve molluscs. PFOS measured levels were higher than the LOD, but lower than the LOQ (6 μg kg^{-1}), in eleven fish muscles, four fish livers, in the five series of predator cephalopod molluscs and in one series of filter-feeders, bivalve molluscs. Summarizing, 67% of the total PFOS sample levels were under the LOQ value of the analytical method.

A second conclusion concerns the PFOA and PFOS measured levels in big predator fishes (sword fish, tuna, porbeagle, small-spotted catshark and common smooth-hound). In contrast to what was expected, these concentrations were always lower than the LOD of the analytical method. The only exceptions were a sample of porbeagle muscle and a sample of small-spotted catshark liver, where anyway the measured concentrations were quite low (7 and 16 μg kg^{-1}, respectively). The observed trend is not consistent

Table 2
PFOA and PFOS concentrations in some marine organisms of the Mediterranean Sea.

Sample type	Sample number	PFOA[a] (μg kg^{-1})			PFOS[a] (μg kg^{-1})		
		Mean	Median	Range	Mean	Median	Range
Pelagic fishes							
Muscle	16	3	<1.5	<1.5–12	4	3	<2–14
Liver	9	6	6	<1.5–13	13	7	<2–40
Benthonic fishes							
Muscle	10	14	<1.5	<1.5–40	13	3	<2–43
Liver	8	9	2.5	<1.5–37	53	78	3–83
Cephalopod molluscs							
Squids	30[b]	2.5	2.5	<1.5–2.5	3	3	<2–3
Redshanks	20[c]	2.5	2.5	<1.5–2.5	3	3	<2–3
Bivalve molluscs							
Mussels	400[d]	<1.5	<1.5	<1.5–2.5	<2	<2	<2–3
Clams	250[e]	15	16	12–16	<2	<2	<2–3

[a] When PFOA and PFOS concentrations were <LOQ but >LOD, for mean, median and range calculation, they were given as half of the LOQ (2.5 and 3 μg kg^{-1} for PFOA and PFOS, respectively).
[b] Three series composed of 10 specimens.
[c] Two series composed of 10 specimens.
[d] Eight series composed of 50 specimens.
[e] Five series composed of 50 specimens.

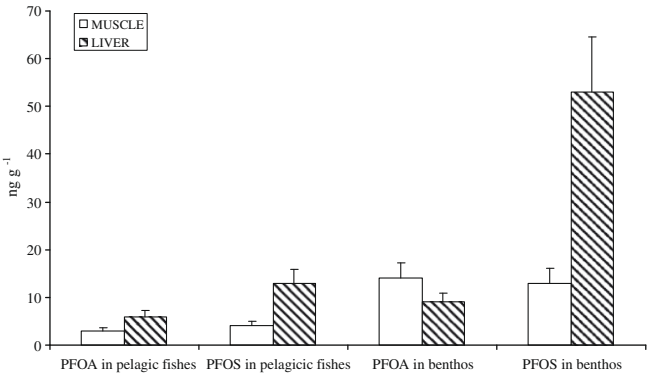

Fig. 4. Histogram of PFOA and PFOS concentration averages found in liver and muscle of pelagic and benthonic fish.

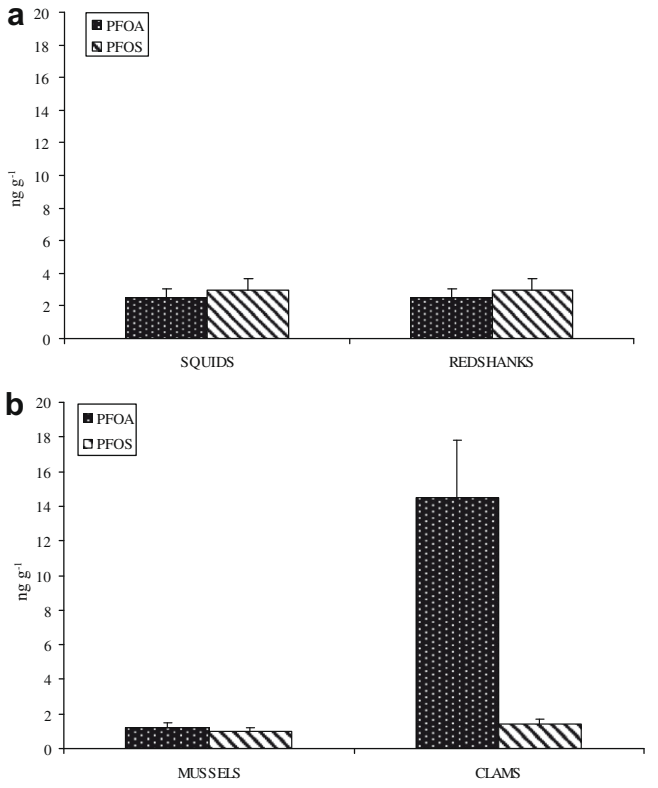

Fig. 5. (a–b) Histogram of PFOA and PFOS concentration averages found in predator cephalopod molluscs (a) and filtering feeders, bivalve molluscs (b).

with the process of biomagnification expected for these two contaminants. However, our results confirm what was already found in a previous study on sword fish (17 muscle and 12 liver samples) caught in the Mediterranean Sea, where PFOS and PFOA concentrations were always lower than the LOQ of the analytical method (5 μg kg^{-1}) (Alessi et al., 2006).

A relationship between fish habitats and PFOA and PFOS levels was pointed out. Indeed, benthonic fish showed PFOA and PFOS levels, on average, higher than pelagic fish (Table 2 and Fig. 3). This could be explained by the fact that benthonic fish can absorb contaminants both from seawater and from sandy and muddy sediments, as reported by various environmental studies that revealed the presence of PFOA and PFOS in river and marine sediments (Berger, Järnberg, & Kallenborn, 2004; Nakata et al., 2006).

Our results confirm accumulation in the liver of both PFOS and PFOA, particularly the former, where the levels showed a muscle: liver ratio equal to approximately 1:10. Bioaccumulation was less evident for PFOA levels in both pelagic and benthonic fishes. In these last, indeed, a slight inverse trend was found, although not statistically significant.

A third relevant conclusion concerns filter-feeders, bivalve molluscs, whose PFOS concentrations were all very low and comparable between mussels and clams, while PFOA levels were very different between the two species. In mussels, PFOA levels were low and comparable to PFOS levels while, in clams, they were higher, i.e. in the range between 12 and 16 μg kg^{-1}. This different distribution could be related to the diverse habitat of the two mollusc species: in fact, mussels generally live in tidal waters, stuck through the byssus to rocks or to hard substrates suspended in the seawater (in the case of mussel culture); by contrast, clams generally live in brackish waters, buried in the sand or in the muddy seabed. This hypothesis is confirmed by a recent study carried out in the Ariake Sea (Japan), where PFOA levels were found to be an order of magnitude higher than PFOS concentrations in the sea sediments (Nakata et al., 2006).

A final conclusion can be drawn about the measured levels of the two PFCs in horse mackerel muscle samples (PFOA equal to 172 μg kg^{-1}), in large scaled scorpion fish muscle samples (PFOA equal to 110 μg kg^{-1}) and in a European conger liver sample (PFOS equal to 431 μg kg^{-1}), which were unusually high compared to the average measured values. This anomaly is difficult to interpret considering that other fish belonging to the same species did not show such high PFCs levels. We can suggest a "dot-like" contamination, related to municipal and/or industrial discharges, that affect specific and limited areas of the Mediterranean Sea.

4. Conclusions

This study contributed to knowledge of the levels of PFOA and PFOS in fish of the Mediterranean Sea. First, we found PFOA and PFOS levels in fish and molluscs lower than those reported for analogue matrices in different geographic areas. Therefore, our biomonitoring results did not show that the Mediterranean Sea had a particularly alarming pollution by PFCs, although it is located in a semi-closed basin with scarce water change. Nonetheless, a worrying element emerges from our study in relation to those few fish that showed an extremely high contamination by PFOA and PFOS. This finding needs further clarification in order to assess whether the unusual high contamination is linked to "dot-like" pollutants release, which could explain the anomaly. The monitoring should therefore continue, mapping the sampling areas with a tighter grid, and also by means of GPS devices, in order to be able to identify and correlate pollution sources with the most polluted marine zones. This would be of great relevance, as fish and molluscs are an important food resource in the Mediterranean area.

Finally, fish and molluscs selected for our investigation proved to be good indicators of environmental contamination, being able to discriminate the contamination degree of different marine environmental compartments (sediment, sea water) on the basis of different fish habitats.

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