

# Preliminary investigation by liquid chromatography-tandem mass spectrometry of perfluorinated compounds presence in bass reared and fished in Italy

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## Abstract

The term *perfluorinated compounds* (PFCs) defines synthetic fully-fluorinated compounds; since the 50s they have been largely employed in a wide range of industrial and consumer applications, due to their chemical and physical properties. Only recently, research on their potential adverse effects on both the environment and human health has been conducted, highlighting carcinogenic activity, toxic effects on reproduction, liver and kidney. Human exposure to PFCs is mainly through diet, and in particular fishery products are one of the most contaminated food. Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) are the two most important and investigated compounds of this group. The aim of this work was to carry out a preliminary monitoring on the presence of these two molecules in 50 bass muscle samples, of which 30 reared and 20 fished, collected from different Italian areas of the Mediterranean Sea. The method used was based on extraction with organic solvent followed by two purification steps: one with salts and one with dispersive solid phase. The extract was finally analysed by means of an ultra performance liquid chromatography-tandem mass spectrometric system. Data showed a relevant contamination of this species and evidenced a significant difference of concentrations between fished (PFOS 112.4->2000 ng/L and PFOA 3.3-487.0 ng/L) and reared basses (PFOS 11.1-104.5 ng/L and PFOA <3-51.4 ng/L). Even if these levels of contamination are sensibly lower than those observed in several studies, fished basses could represent a more relevant source of PFCs exposure in human diet.

## Introduction

The term *perfluorinated compounds* (PFCs) defines synthetic fully-fluorinated compounds; since the 50s they have been largely employed in a wide range of industrial and consumer applications (food packaging, fire fighting foams, detergents and non-stick cookware), due to their chemical and physical properties.

Only recently, research on their potential adverse effects on both the environment and human health has been conducted, highlighting carcinogenic activity, toxic effects on reproduction, liver and kidney (EFSA, 2012).

PFCs can be found in the environment, in fishes, in birds and mammals; however their fate along the food chain is not clear yet. Some information about PFOS levels in the population are available and evidence a uniform distribution. Since these substances are found in several organisms and tend to accumulate along the food chain, diet represents an important route of exposure for humans, but the relative contribution of each food category is not completely clear (Tittlemier *et al.*, 2007). However, it has been demonstrated that fishery products are one of the most contaminated food (EFSA, 2012). Moreover, the European Union issued Commission Recommendation 2010/161/EU (European Commission, 2010) on the monitoring of these contaminants in food in Member States.

Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) are the two most important and investigated compounds of this group, the first having also been added to the persistent organic pollutants (POPs) list on the 2009 Stockholm Convention (<http://chm.pops.int>). The aim of this work was to carry out a preliminary monitoring on the presence of these two molecules in both wild and farmed Italian basses.

**Table 1. Limits of detection and quantification.**

	PFOS (ng/L)	PFOA (ng/L)
LOD (ng/kg)	2	3
LOQ (ng/kg)	6	9

PFOS, perfluorooctane sulfonate; PFOA, perfluorooctanoic acid; LOD, limit of detection; LOQ, limit of quantification.

**Table 2. Perfluorooctane sulfonate and perfluorooctanoic acid presence in basses.**

Samples	PFOA (ng/kg)		PFOS (ng/kg)	
	Mean value	Range	Mean value	Range
Reared basses (n=30)	4.7	<3-51.4	33.4	11.1-104.5
Fished basses (n=20)	70.5	3.3-487.0	1233.8	112.4->2000

PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonate.

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## Materials and Methods

Fifty basses, of which 30 reared and 20 fished, were collected from different Italian areas of the Mediterranean Sea. The method used was described by Lacina *et al.* (2011).

Once internal standards have been added, 7.5 g of homogenised bass muscle underwent a protein precipitation with water, formic acid and acetonitrile, followed by vortex shaking. A first purification step was performed by the addition of MgSO<sub>4</sub> and NaCl and centrifugation. The supernatant was transferred into a new tube and a second purification was achieved using a C18 and Envi-Carb dispersive solid phases. After centrifugation the super-

nant was evaporated to dryness before being reconstituted in methanol. The extract was finally filtered with a 0.2 mm PVDF filter and injected in the LC system.

Analysis have been performed on Waters Acquity ultra performance liquid chromatography (UPLC) system (Waters Corporation, Milford, MA, USA) coupled to Waters Quattro Premier XE tandem mass spectrometry (MS/MS) (Waters Corporation). The chromatographic separation was achieved using a Waters Acquity UPLC HSS T3 column (Waters Corporation), with 5 mM ammonium acetate in water (A) and methanol (B) as mobile phase.

Limits of detection (LOD) and quantification (LOQ) are reported in Table 1.

## Results

PFOS have been detected in all the analysed samples, while PFOA was present in 72% of samples but only in 40% was above LOQ. Table 2 reports the mean values and the range of contamination.

These data evidence a significant difference of concentrations between reared and fished basses, in particular in these latter PFOS levels are 40 times greater.

## Discussion and Conclusions

This preliminary monitoring on basses reports for the first time a single species analysis on the presence of PFOS and PFOA in fish.

The study highlighted a relevant contamination of this species, in particular the wild ones. These levels of contamination could be mainly related to the different fish diet. Moreover, fished basses presented a mean weight three

times higher compared to the reared ones and were therefore older, meaning that they had a longer bioaccumulation time of these contaminants.

However, even if these levels of contamination are sensibly lower than the ones observed in several studies (Berger *et al.*, 2009; Domingo *et al.*, 2012; Gulkowaska *et al.*, 2006; Guo *et al.*, 2012; Hradkova *et al.*, 2010; Nania *et al.*, 2009; Schuetze *et al.*, 2010; Shi *et al.*, 2010) fished basses could represent a more significant source of PFCs exposure in human diet.

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