# Occurrence of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in perch from Lake Varese (North Italy)

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**Introduction**

Perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) are environmental contaminants belonging to a chemical group known as perfluorinated compounds. PFOS and PFOA are very persistent in the environment and bio accumulate in humans. The European Food Safety Authority (EFSA) recently pointed out that they are associated with adverse health effects. Diet is considered the main source of exposure to PFCs, which have been found more frequently in fish and other seafood, compared to other food groups. In fact, aquatic ecosystems represent the final reservoir for PFCs due to their great affinity for sedimentary and living organic matter. In these systems, measured levels of persistent organic pollutants (POPs) could increase along the trophic web, ultimately affecting humans that consume aquatic species. In this study, PFOS and PFOA was detected by LC-MS/MS in muscle samples of *Perca fluviatilis* from Lake Varese (North Italy).

**Materials and methods**

Sampling area.

Lake Varese is a smallish lake in the Lombardy region (North Italy) fed by underground springs. The lake is spread over an area of 14.5 square kilometers and has an average depth of 11 meters.

Study specie.

The European perch is a predatory freshwater fish species feeding on invertebrates and fish. Perch specimens were captured by gillnetting in 2012, in agreement with the animal welfare legislation procedure. Length ranged from 16.5 to 29.5 ± 0.5 cm and weight ranged from 53 – 371 ± 1 g. Fish were transported to the laboratory where samples were dissected to obtain muscle tissues.

*Reagents and analytical method.* Fish muscle (2.5 g) was homogenized with 2.5 mL of sodium hydroxide using Ultraturrax homogenizer (IKA, Staufen, Germany). The extract was purified and conditioned with 4 mL of methanol and 4 mL of water. The cartridge-purified extract was washed with 4 ml of 25 mM acetate buffer (pH 4-5), followed by 8 mL of methanol. Analytes were eluted with 1 mL of 2% ammonium hydroxide in methanol. The solvent was dried by evaporation by means of a nitrogen stream. The residue was reconstituted in the mobile phase and subjected to LC-MS/MS analysis, performed by an Agilent HPLC 1100 procedure (Agilent Technologies, Palo Alto, CA, USA). Mass spectral analyses were performed using an Applied Biosystems API 4000 triple quadruple mass spectrometer (Applied Biosystems Sciex, Ontario, Canada) operating in electrospray ionization (ESI) negative ion mode. Detection and

quantification of the two molecules were performed by selected reaction monitoring (SRM), as shown in Table 1.

| Analyte | Parent ion | Product Ion | Decluring potential (V) | Collision Energy (V) |
|---|---|---|---|---|
| PFOA | 412.7 | 368.9 | -61 | -14 |
| | | 169.0 | | -26 |
| | | 219.0 | | -22 |
| PFOS | 498.5 | 80.1 | -50 | -94 |
| | | 99.0 | | -72 |
| | | 169.0 | | -51 |
| Internal standard | 420.7 | 376.0 | -32 | -14 |

**Table 1.** MS experimental condition of PFOA and PFOS and internal standard

The method limit of quantification (LOQ) was 0.50 ng $g^{-1}$ for PFOA and 0.70 ng $g^{-1}$ for PFOS, with a recovery rate of 99-102% and 96-108%, respectively.

**Results**

PFOA was not found in any of the investigated samples above the limit of quantitation of 0.50 ng $g^{-1}$ fresh weight (fw), whereas PFOS was detected in all samples with concentrations ranging from 5.4 to 17.2 ng $g^{-1}$ fw (mean 9.6 ng $g^{-1}$ fw). The Scientific Panel on Contaminants in the Food Chain (CONTAM) set a provisional TDI of 150 ng $kg^{-1}$ b.w. per day (EFSA, 2008). In an adult consumer with a body weight of 60 kg, this value is reached consuming fish that contains 30 ng $g^{-1}$ of PFOS, considering a consumption of 300 g fish per day. We estimated human exposure from fish consumption by calculating the Estimated Human Daily Intake (EHDI), as follows: EHDI = (C X DC)/BW, where C is the contaminant mean concentration, DC indicates the daily fish consumption for the Italian population, as reported by the National Research Institute for Food and Nutrition (Leclercq et al., 2009), and BW is the human body weight (60 kg). The consumption figures used were the 50th and 95th percentile intakes for the total population in consumers of all ages; we obtained EHDI values of 5.15 ng $kg^{-1}$ bw day and 23.55 ng $kg^{-1}$ bw day.

**Discussion**

PFCs concentrations are usually higher in fish caught from fresh water compared to fish from open oceans (Berger et al., 2009). In the 2011 EFSA Opinion a constantly higher mean PCFs concentration in fish from fresh water was demonstrated. Among PFCs, PFOS had the highest mean concentrations that in fish meat ranged from 0.04 to 211 ng $g^{-1}$. In the present study, PFOS mean values were 9.6 ng $g^{-1}$ fw in *Perca fluviatilis*, while PFOA values were less than the limit of quantitation in all samples. This is the first study that document the presence of PFCs in Lake Varese. Once in the environment PCFs are extremely persistent and don't undergo significant further abiotic or biotic degradation. However, PFOS exhibits a higher tendency to bind to organic matter and bio accumulate compared to PFOA, due to its longer perfluoroalkyl

chain length (Conder et al. 2010). Monitoring data from top predators at various locations show highly elevated levels of PFOS and demonstrate the substantial bioaccumulation and bio magnification properties of PFOS (Bossi et al., 2005a,b). Consumption of fish and fishery products is known to be a source of exposure to PFOS, PFOA, and other PFCs (Nania et al., 2009). Several studies indicated that PFOS and PFOA are present in the environment, including within the human body (EFSA 2012). Adverse health effects, e.g. hepatotoxicity, developmental toxicity, neurobehavioral toxicity, reproductive toxicity, hormonal effects, as well as a weak genotoxic and carcinogenic potential have been demonstrated in experimental studies in animals (Zhang et al., 2009; Pinkas et al., 2010). Comparing our EHDI with the TDI established for PFOS we can conclude that the intake related to fish consumption from Lake Varese is well below the tolerable daily intake (TDI). Even if our results did not show a particularly alarming level of pollution by PFCs, other food and sources other than food may contribute to the total human exposure. Particularly for high fish consumers the intake from fish may constitute a considerable contribute to the TDI of PFOS. Then, measures should be taken to reduce the consumption of these damaging substances by humans.

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