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NDL-PCBs in muscle of the European catfish (*Silurus glanis*): An alert from Italian rivers

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HIGHLIGHTS

- The top predator *Silurus glanis* reflects the severe pollution by organic matter of Italian Rivers (Northern Italy).
- 33.3% Of the analysed samples exceeded the maximum levels of 125 ng g^{-1} fresh weight (fw) set by Regulation in fish muscle.
- Po River registered the highest presence of NDL-PCB, with 50% of samples not compliant with EU ML.
- The Σ_6PCBs well represented total NDL-PCBs levels.
- The contribution of PCB-153 and PCB-138 together was about 70% of the overall sum of the six congeners.

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ABSTRACT

The non-dioxin-like polychlorinated biphenyls (NDL-PCBs) highly contribute to the PCB dietary intake of total PCBs. Most of the NDL-PCBs are assumed through ingestion of contaminated fish and fishery products. Therefore, it is important to quantify their presence in aquatic organisms to evaluate human risks associated with fish consumption. The European catfish is a top food-chain predator and is considered a reliable bio-monitoring tool reflecting the state of the environmental organic pollution. From 2006 to 2009, 54 European catfish were captured in four sites covering the area of the Po River (North Italy), and their muscles were analysed to determine the levels of 18 PCBs congeners. All samples presented detectable levels of 18 congeners and, on average, results showed an important presence of NDL-PCBs. The sum of the six congeners (28, 52, 101, 138, 153, 180 IUPAC) was used as indicator of the total PCBs concentration. The 33% of the samples analysed exceeded the maximum levels of 125 ng g^{-1} set by European regulations in fish. The values measured ranged from 19.7 to 1015.4 ng g^{-1} (mean 135.6 ± 149.8 ng g^{-1}).

The concentrations of NDL-PCBs were not related to fish weight or sex, while a significant variability was found among sites ($p < 0.05$), according to the geographical location of many industrial activities in the catchment area of the Po River. PCB 153 and 138 were present in higher concentrations (40% and 30% respectively). We hypothesise that this is due to their high resistance to metabolic degradation.

1. Introduction

Freshwater fish are considered reliable indicators for the presence of persistent bio accumulative and toxic lipophilic compounds in river basins (Roche et al., 2000; Patrolecco et al., 2010; Pacini et al., 2013). Monitoring fish tissues has a distinctive advantage in relation to monitoring inert environmental compartments. Sediment bound organic contaminants rendering the latter refractory to chemical and biological transformation and release, while the fraction of organic compounds detected in fish tissues represents a bioavailable portion that cycles through aquatic food webs. Since organic contaminants have a great affinity for the lipids in animal tissues, fish are able to accumulate the contaminant concentrations not detectable in the water column.

Polychlorobiphenyls (PCBs) are a group of persistent organic contaminants including 209 compounds (congeners), exhibiting different degrees and patterns of chlorination (WHO, 1993). During the 1930s and for approximately 50 years, these chemicals were commercially produced in different industrialised countries as technical mixtures (e.g., Aroclors®, Clophens®, Fenclors®, Kanechlors®, Pyralenes®) to be used mainly as dielectric fluids, organic diluents, plasticizers, adhesives, and flame retardants. Although banned in the 1970s and 1980s in the United States and Europe, respectively, PCBs are still present in the environmental and can be traced in animal tissues. Due to their high persistence and bioaccumulation and toxic potential, PCBs can occur at levels of concern. Despite the large number of theoretical congeners, only about 130 are likely to occur in the technical mixtures and, among them, fewer were and are environmentally relevant.

According to their toxicological properties, PCBs are usually recognised to possess a dioxin-like (DL-PCBs) or a non-dioxin-like

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(NDL-PCBs) activity. The DL-PCB group comprises 12 congeners characterised by non- or mono-*ortho* chlorosubstitution. These congeners exert their toxicity primarily through the binding of the aryl hydrocarbon receptor (*Ah*R), similarly to polychlorodibenzodioxins (PCDDs) and polychlorodibenzofurans (PCDFs) (van den Berg et al., 2006). The NDL-PCB group includes the remaining congeners, analytically predominant in environmental matrices and animal tissues. These congeners appear to act *via* different modes and some direct effects on neuronal cells – such as the reduction of dopamine neurotransmitter levels or the interference with calcium homeostasis (Brown et al., 1998; Tilson et al., 1998) – may be peculiar for those chemicals.

Several evidences suggest that even low DL- and NDL-PCB doses can cause subtle effects when exposure is prolonged over time, and particularly, if it occurs during the prenatal and postnatal development in mice (Haave et al., 2011). For these reasons, a more specific concern has been raised as to the effects on children's neurological development (http://www.sciencedirect.com/science/article/pii/S0045653508003184, Walkowiak et al., 2001; Vreugdenhil et al., 2004). Moreover, possible relations with specific neurobehavioral changes in human adults, such as the attention-deficit/hyperactivity disorders, have been reported (Schoeters and Birnbaum, 2004).

The consumption of contaminated fish is one of the most relevant pathways for transfer PCBs from the environment to humans (US EPA, 2007). A recent report of the European Food Safety Authority (EFSA) showed that particularly high levels of non dioxin-like PCBs (NDL-PCBs) can be found in fish and fishery products (EFSA, 2010). Furthermore, a relevant number of national and international regulatory bodies have established fish consumption guidelines with a particular respect for those fish who are known to accumulate a variety of chemicals. The European Union has also provided recommendations of alternative diets in order to avoid consumption of contaminated products. Moreover, the regulation 1259/2011/EU (enforced since January 1th 2012) has set *de novo* a maximum tolerable levels (MLs) for the sum of the six "indicators" NDL-PCBs 28, 52, 101, 138, 153 and 180 (Σ_6 NDL-PCBs) in fish flesh.

Fish can be considered a valid bio indicator for the level of pollution in freshwater environment. European catfish (*Silurus glanis*) is a top food-chain predator in the freshwater ecosystem, and can reflect the environmental contamination. This species is nowadays popular among European anglers and, for this reason, it has been introduced in many European countries, including France, the Netherlands, Spain, and the UK (Elvira, 2001). In Italy this species has received an increasing interest also for commercial purposes, as it is the case for the Eastern European market, where its flesh is greatly appreciated. In a previous study, Squadrone et al. (2012) estimated the concentrations of mercury, cadmium, lead, arsenic and chromium in several organs of this predator within the area of the Po river basin (Northern Italy). They found levels of mercury exceeding the Maximum Levels (MLs).

The aim of this study is to evaluate, in the same area, the levels of NDL-PCBs in *S. glanis*, in order to evaluate the reliability of this fish species as a bio-indicator of organic and chemical pollution.

In particular, the compliance with the maximum levels established by the European Commission Regulation (1259/2011) were verified, and the distribution of the six indicators congeners, their variations with sampling sites, gender, age and size were discussed.

2. Materials and methods

2.1. Study species

The European catfish (*S. glanis*), also known as wels catfish, is one of the largest European freshwater fish. This species is native in Eastern Europe and Western Asia and is abundant in the Danube and Volga basins. The European catfish inhabits the lower reaches of large rivers and muddy lakes, tends to prey on fish smaller than could be expected for its size and mouth gape (Adámek et al., 1999; Wysujack and Mehner, 2005). *S. glanis* is a bottom dwelling nocturnal predator, feeding in the whole water column. Fry and juveniles are benthic, feeding on a wide variety of invertebrates and fish, while adults prey on fish and other aquatic vertebrates. The sexual maturity is reached at 2–3 years, and this catfish species can live for over 30 years.

Only the flesh of young specimens is valued as food, and is palatable when the catfish weighs less than 15 kg (33 lb). Larger than this size, the fish is highly fatty and not recommended for consumption.

2.2. Field sampling

Fifty-four specimens (28 males and 26 females) of European catfish were collected from late spring to early fall 2009–2011 in the following four sites:

1. Po River (Lat. 45.138098, Long. 8.558135).
2. Tanaro River (Lat. 44.919446, Long. 8.6099719).
3. Bormida River (Lat. 44.906940, Long. 8.646197).
4. Parma River (Lat. 44.832150, Long. 10.314585).

All the sites belong to the hydrographical basin of the Po River – the largest river in Italy – and were selected according to accessibility and fish abundance. 24 animals (12 males and 12 females) were collected from Po River, Alessandria district, 10 (6 males and 4 females) from Tanaro River, Alessandria district, 9 (6 males and 3 females) from Bromida River, Alessandria district, and 11 (4 males and 7 females) from Parma River, Parma district.

Fish were captured using an electro-fishing boat, providing up to 100 Hz, in agreement with the animal welfare legislation prescription. Specimens were preserved on ice and transported to the laboratory. Animals were dissected to obtain muscle samples, which were immediately frozen and stored at −20 °C. Fish age was estimated by growth bands in vertebrae. The overall sample consisted of specimens ranging from a length of 60–120 cm and a weight between 1.5 and 10.5 kg (males: 86.80 ± 22.12 cm, 5.10 ± 4.07 kg; females: 83.55 ± 17.05 cm, 4.59 ± 2.24 kg; mean ± SD).

2.3. Analytical methods

The quantification of NDL-PCBs was performed by adapting the method of Perugini et al. (2004). The quantified congeners were the six indicators 28, 52, 101,138, 153 and 180, and their cumulative analytical concentration has been reported as Σ_6 PCBs. Other 18 NDL PCB congeners (95, 105, 110, 118, 146, 149, 151, 155, 170, 177, 183, and 187) were detected and their cumulative analytical concentration has been reported as Σ_{18} PCBs.

All the samples were freeze-dried, powdered and transferred into Accelerated Solvent Extraction (ASE) cells (102, 1 atm and 100 °C). The extraction solvent was a mixture of *n*-hexane/acetone 1:1 (v/v). The extract was filtered and evaporated to dryness, permitting the gravimetric determination of the fat content. Before the dissolution of fat in hexane for sample cleaned up, PCB 155 and PCB 198 were added as internal standards. The purification step was performed using silica columns. The fat was removed on a Extrelut-NT3 column loaded with sulphuric acid. The final sample extract was evaporated under a nitrogen stream to dryness and reconstituted by addition of 100 μL of isooctane. The GC/MS detection was performed on a Thermo Focus gas chromatographer, equipped with a DB-5MS column (30 m × 0.25 mm, 0.25 μm film thickness), and coupled to a DSQ single quadruple mass spectrometer. The GC injector and transfer line temperatures were