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

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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.

Keywords: NDL-PCBs; Freshwaters; *Silurus glanis*

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 industrialized 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 recognized to possess a dioxin-like (DL-PCBs) or a non-dioxin-like (NDL-PCBs) activity. The DL-PCB group comprises 12 congeners characterized 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 (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 & 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 *Silurus 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 (*Silurus 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 & Mehner, 2005). *Silurus 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 thirty 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 4 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 to 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 (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 x 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 respectively 250°C and 270 °C. The oven temperature program was: 100°C for 1 min, ramp 20°C/min up to 190°C (isotherm for 2 min), ramp 3°C/min up to 250°C and ramp 50°C/min up to 300°C (isotherm for 20 min). All analyses were performed in duplicate. To check the purity of the reagents and contamination, "blanks" was analysed for each calibration run, using the same procedure. Moreover, the reference material for organo-chlorine compounds CARP-2 (Ground whole carp, NRC Canada) was utilized for quality control, together with control and spiked samples in each round of analysis.

In line with European regulatory instructions (EU 1259/2011) the cumulative concentrations (Σ_6 , Σ_{18}) were expressed as "upper bound" (UB) concentrations, on the assumption that all the values of the different congeners below the LOQ are equal to the LOQ. To establish the compliance of samples with the ML, the expanded measurement uncertainty was subtracted to the analytical result when the UB was above the ML.

The Limit of Quantitation (LOQ) for the analyzed PCBs was 6 ng g^{-1}. Cause the validation is required for the analytical methods used in food official control, this method was validated according to 2004/882/EC Regulation and ISO 17025 criteria.

2.3 Statistical analysis

Data were tested for normality by using Kolmogorov-Smirnov test. Since the assumptions for parametric analyses were not met, a Kruskal–Wallis analysis of variance by ranks, followed by Mann–Whitney U tests for pairwise comparisons, was performed to assess differences in the Σ_6 PCBs among fishes from different rivers. Significant differences were considered to occur if $p < 0.05$. Moreover, correlations between Σ_6 PCBs and Σ_{18} PCBs across samples, and between fish's weight and Σ_6 PCBs were examined using linear regression models.

3. Results and discussion

The six PCB congeners 28,52,101,138,153 and 180 were chosen as indicators not for their toxicity, but because they are easily quantified compared to the other NDL-PCBs, and they represent all relevant degrees of chlorination. Indeed, the EFSA (European Food Safety Authority) Scientific Panel concerning Contaminants in the Food Chain (CONTAM Panel) decided to use the sum of these six PCBs as the basis for the evaluation, because these congeners are appropriate indicators for different PCB patterns in various sample matrices and are most suitable for a risk assessment of NDL-PCBs. The CONTAM Panel underlines in its Scientific Opinion related to the presence of non-dioxin-like PCBs in feed and food that the sum of the six indicator PCBs represents about 50 % of the total NDL-PCB in food (EFSA, 2005). According to this, in our findings, a linear regression model showed an highly significant relationship ($R^2 = 0.98$; $p < 0.001$) between Σ_6 PCBs and Σ_{18} PCBs (Figure 1). For this reason, we can confirm that in our study the Σ_6PCBs well represents the environmental pollution of the study area. Our results show that 33.3% of the analysed fish samples had a NDL-PCBs content (Σ_6 PCBs) that exceeded the maximum levels of 125 ng g^{-1} fresh weight (fw) set by UE 1259/2011 (Figure 2). In particular, 50% of the specimens collected from Po River, 20% of the specimens collected from Tanaro River, 11% of the specimens collected from Bormida River and 27% of the specimens collected from Parma River were not compliant with EU ML (Figure 3). Σ_6 PCBs in total ranged from 19.7 ng g^{-1} to 1015.4 ng g^{-1}, with a mean concentration of 135.6 ng g^{-1}. Considering each location, the Po River registered the highest presence of NDL-PCB, with Σ_6 PCBs ranging from 19.7 ng g^{-1} to 1015.4 ng g^{-1}, and a mean concentration of 187.6 ng g^{-1} . In the other three sampling sites concentrations were similar: in the Tanaro River , the levels of Σ_6 PCBs ranging from 25.3 ng g^{-1} to 266.5 ng g^{-1}, mean concentration of 94.2 ng g^{-1} , in the Bormida River, Σ_6PCBs ranging from 36.6 ng g^{-1} to 195.6 ng g^{-1}, mean concentration of 95.6 ng g^{-1} ; in Parma River Σ_6 PCBs ranging from 26.1 ng g^{-1} to 240.1 ng g^{-1}, mean concentration of 92.4 ng g^{-1} . The statistical analysis confirmed significantly different mean concentrations in Σ_6 PCBs among the fishes from the four sampling sites (Kruskal–Wallis, $p < 0.05$, df 3). Multiple comparisons performed with the Mann-Whitney U tests showed that differences exist only between the Po and the other three rivers (Table 1).

Considering the contribution of single NDL-PCB congeners to the sum of the six indicators, NDL-PCBs 153 and 138 were analytically predominant (40% and 30% respectively) followed by the other congeners 101 (9.7%), 180 (9.3%), 52 (6.9%) and 28 (3.5%) has shown in Table 2. Our results are in line with findings reported in other studies which demonstrated that PCB-153 has an average contribution of roughly one third to the sum of the six indicator PCBs (EFSA, 2005; BFR, 2006; Jursa et al., 2006). Indeed, the mean contribution of PCB-153 and PCB-138 across food groups ranged from 23% to 44% and from 19% to 32%, respectively (EFSA, 2010). Together their contribution was at least 50% in each food group. PCB-180 and PCB-101 contributed between 10% and 29%, and 4% and 19%, respectively. PCB-52 and PCB-28 contributed both between 1% and 17% to the sum of the indicator NDL-PCBs (EFSA, 2010). In fish species from Danube River in Serbia (Jankoviç et al., 2010) congeners 138 and 153 were similarly the most abundant, and the same results were obtained by Pacini et al. (2013) in different fish species from southern Italy. In this latter study, the commonest congeners were 153, 138, and 101, in decreasing priority, and the concentrations they found for the Σ_6PCBs ranged from 1.30 to 195 ng g^{-}1 fw. Similarly, Brazova et al. (2012) found that PCB 153 was present in higher concentrations than other congeners in muscle of top predators as the European catfish, with an average of 29% of Σ_6PCB, while the 138 congener accounted for approximately 24 % of Σ_6PCBs.

Our analytical NDL-PCB patterns were coherent also with those observed in different fish specie from the middle and lower stretches of the Po river (Viganò et al., 2000), from the Orbetello lagoon (Mariottini et al., 2006), and from several Campania rivers (Pacini et al., 2013) where the presence of PCBs was associated with residues of commercial technical mixtures.

Congeners 138 and 153 are characterized as less hydrophobic and not so tightly bound to sediment than higher chlorinated octa-, nona-, and deca-PCBs, reason why they are more readily available to water organisms (McFarland & Clarke, 1989). Moreover, these congeners with chlorine atoms in positions 2, 4, and 5 in one (PCB 138) or both rings (PCB 153) could have a greater resistance to metabolism and elimination from fish organism than the lower congeners such as 28, 52, and 101 (Jacob & Boer, 1994; Nie et al., 2006). The high proportion of 138 and 153 PCB compounds found in our samples of fish muscle could explain their low rates of biotransformation and inability to be metabolized (Brazova et al., 2012).

In the previous study performed in the same area, in order to detect the presence of toxic metals in muscle of *Silurus glanis*, we found that length, weight and age were significantly related to Hg content. This suggested an increasing bio-accumulation with the increasing size of this fish species (Squadrone et al., 2012). In this investigation no significant relationship was found between Σ_6 PCBs concentration and fish weight both considering sex together ($R^2 = 0.02$; $p > 0.05$) and separately (Male: $R^2 = 0.08$, $p > 0.05$; Female: $R^2 = 0.01$, $p > 0.05$). We can assess that several factors including the metabolic activity of individual organs, fish species, age, size, feeding habits, or the complex PCB transport in an organism may control PCB accumulation (Ashley et al., 2000; Brázová et al., 2012).

4. Conclusions

Toxicological data indicate that NDL-PCBs alter a number of physiological processes important during the development of the species, in particular in the nervous and endocrine systems (Vreugdenhil et al., 2004). The European Union has undertaken short and long term actions, aimed to reduce environmental contamination and human exposure, that have recently been extended to incorporate NDL-PCBs. Analysis of NDL-PCBs in fish muscle of a top predator as *Silurus glanis* in northern Italy water bodies reflects the severe pollution by organic compounds in this area. Moreover, it represents a way to assess the presence of these substances in rivers and to improve the understanding of the environmental and human risks. Fish and fishery products are the major contributors of the dietary exposure and evaluating the levels of these contaminants is necessary to protect consumers from NDL-PCBs intake. The Σ_6 PCBs well represented total NDL-PCBs levels, and the contribution of both PCB-153 and PCB-138 was about 70% of the overall sum of the six congeners, higher than previous reported in literature. The concentration of NDL-PCBs in a top food-chain predator well reflects the level of the aquatic environment pollution. Despite the fact that commercial production of PCBs has been previously banned or severely restricted, fish continue to accumulate these chemicals from sediments polluted during the previous decades. It is of great importance to consider this persisting contamination and additional investigations in the interested water bodies should be performed.

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