Non-dioxin-like polychlorinated biphenyls (NDL-PCBs) in eel, trout, and barbel from the River Roya, Northern Italy

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Non-dioxin-like polychlorinated biphenyls (NDL-PCBs) in eel, trout, and barbel from the River Roya, Northern Italy

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Running title : NDL-PCBs exposure through fish consumption in River Roya

Abstract

Non-dioxin-like polychlorinated biphenyls (NDL-PCBs) contribute to the dietary intake of total PCBs. They are consumed via ingestion of contaminated fish and fishery products. Thus, it is important to quantify their levels in aquatic organisms in order to evaluate the risks associated with fish consumption by humans. In 2013, an advisory against consumption of fish from the River Roya (IT) was disseminated after NDL PCBs were found to exceed maximum levels set by EU Regulations. We investigated the presence of NDL PCBs in in eel, trout and barbel from the River Roya. We found concentrations in the range of 9.2 to 27.6 ng g^{-1} ww in barbel, 9.2 to 97.0 ng g^{-1} ww in trout and 9.0 to 239.5 ng g^{-1} ww in eel. The distribution of congeners among different species did not show significant variations and was characterized by a higher proportion of 153, 138 and 180 congeners, according to their high resistance to metabolic degradation.

Keywords: NDL-PCBs; River Roya, freshwater fish

1. Introduction

Fish tissue has a distinct advantage in relation to monitoring inert environmental compartments. Sediment-bound organic contaminants are potentially resistant to chemical and biological transformation and release, unlike organic compounds in fish tissue that represents the bioavailable portion cycling through aquatic food webs.

In general, due to the high affinity of organic contaminants for fat tissues of animals, fish accumulate contaminant concentrations that may not be detectable in the water column (Pacini et al., 2013). Furthermore, concentrations in fish tissues represent a critical link connecting aquatic trophic chains to the human life cycle.

Persistent organic pollutants (POPs) include a group of pollutants that are semi-volatile, persistent in the environment, bioaccumulative and toxic for humans and wildlife. Two groups of POPs, PCDD/Fs and PCBs are produced by anthropogenic activities as well as by natural processes.

PCBs represent a particularly persistent group of chlorinated congeners, and are ubiquitous in the environment and exclusively of anthropological origin. Two classes of PCBs have been classified according to their toxicological properties, dioxin-like PCBs (DL-PCBs), which have an analogous toxicity to dioxins, and non-dioxin-like PCBs (NDL-PCBs). These compounds are related by structural features, such as the number and positions of chlorine atoms. Due to their stability, insulating and fire-retardant properties, PCBs were previously used in the manufacture of electrical equipment, heat exchangers, hydraulic systems, and other specialized applications. Despite a ban on their production during the 1980s, world-wide production has been estimated at 1,200,000 tons and approximately 30% of this production is then released into the environment, particularly confined to the oceans (Voltura and French, 2000).

Consumption of contaminated fish is one of the most relevant pathways for the transfer of PCBs, and represents a significant portion of the human health risk associated with the presence of PCBs in the environment (US EPA, 2007). A recent report by the European Food Safety Authority 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 emphasis for those fish that 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 1259/2011/EU regulation (in enforcement since January 1st 2012) has set 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. In its scientific opinion on the presence of NDL-PCBs in feed and food (2010), the EFSA Scientific Panel on Contaminants in the Food Chain (CONTAM Panel) noted the sum of six indicator PCBs represented about half the total NDL-PCB in foods. PCB-153 and PCB-138 contributed most to the sum of NDL-PCB congeners, followed by PCB-180, PCB-28, PCB-101 and PCB-52, but relatively high variations were observed for each congener throughout food and feed groups.

The contribution of PCB-153 and PCB-138 together consistently comprised at least 50% of the overall sum of the six congeners in each food group. The highest food contamination levels were observed in several fish and fish product categories, followed by products from terrestrial animals. The highest mean levels of NDL-PCBs in food when expressed on a whole weight basis were observed in fish and fish products, with 223 ng g^{-1} in muscle meat of eel, followed by 148 ng g^{-1}in fish liver, and 23 ng g^{-1}in muscle meat fish and fish products excluding eel (EFSA 2010).

The contamination of aquatic organisms depends on the chemical properties of each congener. The bioaccumulation processes are influenced by exposure levels in the environment and various biotic factors, such as the metabolic capacity (Hubaux and Perceval, 2011).

The European eel is considered a bottom-dwelling fish, showing high body lipid content, significant longevity and carnivorous, and is exposed to lipophilic-persistent contaminants, such as PCBs, and thus represents a species sensitive to bioaccumulation (Roche al., 2000). Moreover, eels constitute an important economic value close to estuaries and rivers, and an essential food resource (Després, 2009). Significant levels of PCBs have already been reported in European eels in France, from the Gironde and Adour estuaries (Tapie et al., 2011), in the Mondego estuary in Portugal (Nunes et al., 2011), and in Italian rivers, and have been suggested as being responsible for migration or reproduction impairments (Mezzetta et al., 2011). Assessing PCB contamination of the European eel is therefore of crucial interest as PCB levels are threatening public health beyond a maximum value, as well as posing a potential risk for the health of the eel itself (Geeraerts and Belpaire, 2010).The use of fish populations as indicators of river health is legislated and mandatory in Europe due to the introduction of the Water Framework Directive 60/2000 UE (WFD). Since 2000, when the WFD was established, many efforts in European countries have been focused on developing efficient tools to measure the ecological status of freshwater, based on fish studies (Ayllón et al., 2012). Smith and Darwall (2006) identified water pollution as the greatest threat to Mediterranean endemic freshwater fish. In particular, the Common barbel has been previously used for eco-toxicological studies due to its reported sensitivity for particular biomarkers (Viganó et al., 2000). Bottom-dwelling fish, such as barbel, tend to accumulate lipophilic contaminants in their tissues directly from water and sediment as well as through their diet, enabling assessment of the transfer of pollutants through the trophic web. Wild brown trout are predatory species at the top of the trophic level; they are abundant in many freshwater ecosystems and are also feature as a part of the human diet. The exposure of Wild brown trout to PCBs depends on the concentrations of these compounds in the surrounding environment. Therefore, the study of the impact of contaminants on fish populations is important, not only from an ecological point of view, but also as a matter of concern for human health.

The basin of the River Roya, mostly located in French territory, covers a total area of 662 km^2. The River Roya source is in France from the Colle di Tenda at about 1850 m above sea level and it flows for about 40 km, with numerous tributaries joining it. The river enters Italian territory at an altitude of 200 m above sea level, and flows into Ventimiglia.

The river is the most important water reserve of the entire territory and its water is subjected to intense use, especially for drinking water and hydroelectric power, with the presence of many large dams, both in the French and the Italian portion of the river. There is a significant lack of data on the POP contamination levels in fish in this ecosystem, as none has been analyzed in the Italian part of the river. Polychlorinated biphenyls can be accumulated in aquatic organisms through the food chain, and their dietary intake by the human population is already evident. Moreover, in 2012, the presence of NDL PCBs exceeding the legal limit of 300 ng g-1 in eel samples was reported by the French authorities and resulted in a fishing ban on the French side of the river. The present study focused on the residue levels of NDL PCBs in European eels, Common barbel and Brown trout caught in the River Roya (Italian branch), to achieve three objectives, namely to: i) obtain a representative assessment of NDL PCB contamination of freshwater fish in these ecosystems, analyzing the distribution of the six indicator congeners, and considering fish species and locations ii) verify the compliance with the maximum levels established by the European Commission Regulation (1259/2011) iii) increase the data about the potential human health risks associated with these contaminants due to fish consumption.

2. Materials and methods

2.1. Study area and sampling

The River Roya has its source in French territory near the Colle di Tenda and flows through the Mercantour National Park. The river enters Italy in the Olivetta San Michele district; the remainder of its course remains within the province of Imperia and, after crossing the Airole district, the river enters the sea at the city of Ventimiglia. The study area and sampling sites (Figure 1) were located in the Ligurian Region, North Italy. From north to south, two locations were chosen to represent the districts of Airole and Ventimiglia.

2.2. Sampled animals

For this study, 32 specimens of Wild brown trout (*Salmo trutta trutta* L), Common barbel (*Barbus barbus*) and European eel (*Anguilla Anguilla*) were collected. Fish were caught and killed by local fishermen according to fishing regulations.

Samples were immediately frozen and transported to the laboratory. The total length (cm) and weight (g) of each fish were measured before the analytical procedures. Dorsal muscle tissues were sampled from each fish, homogenized and stored at -20°C. The trout samples (no. 6) had a length ranging from 36 to 44 cm and a weight between 500 and 780 g; the barbel samples consisted of specimens (no.6) with a length ranging from 17 to 43 cm and a weight between 35 and 700 g. All trout and barbel samples were collected in the Airole location. Eel specimens (no. 20) had a length ranging from 25 to 110 cm and a weight between 35 and 1020 g. Nine samples were collected in Airole and 11 samples were from the Ventimiglia district.

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.

All 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 cleaning of the samples, PCB155 and PCB198 were added as internal standards. The purification step was performed using silica columns. The fat was removed on an Extrelut-NT3 column loaded with sulphuric acid. Final sample extracts were evaporated under a nitrogen stream to dryness and reconstituted by the addition of 100 μL of isooctane. 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 250°C and 270°C, respectively. 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 analyzed 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 controls and spiked samples in each round of analysis.

In line with European regulatory instructions (EU 1259/2011), the cumulative concentrations (Σ_6 NDLPCB) 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 from the analytical result when the UB was above the ML.

The Limit of Quantitation (LOQ) for the analyzed PCBs was 1.3 ng g^{-1}. As the validation is required for the analytical methods used in official food control, this method was validated according to the 2004/882/EC Regulation and ISO 17025 criteria.

2.4 Statistical analysis

Data were tested for normality by using the Kolmogorov–Smirnov test. As the assumptions for parametric analyses were not met, inference was made from non-parametric statistical techniques. In particular, pairwise correlations between PCB congeners and correlations between weight and Σ6 PCBs were examined using Spearman's rho test. All tests were performed in SPSS v. 20 (SPSS, Inc., Chicago, IL) for Macintosh.

3. Results and discussion

3.1 NDL PCB levels in fish

NDL PCBs were ubiquitous in all fish analyzed. No samples exceeded the limit for human consumption (125 ng g^{-1} ww in muscle meat of caught wild freshwater fish and 300 ng g^{-1} ww in eel muscle) established by the Commission Regulation (EU) 1259/2011. In fact, the average concentration of the sum of the six indicator PCBs was 24.5 ng g^{-1} ww in barbel, 40.0 ng g^{-1} ww in trout, and 131.5 ng g^{-1} in eel (Table 1). Considering Σ_6PCBs in *Barbus barbus*, our results are lower than those reported in common barbel fished in Rivers of Central Italy (Pacini et al., 2013). Otherwise, the mean value registered in trout was higher than those reported by other authors for the same species, collected in a remote high European mountain lake (Vives et al., 2005), and in the Marche rivers, central Italy (Piersanti et al., 2012). Pacini and coworkers found NDL PCBs in eel ranging from 8.97 to 39.2 ng g^{-1} in rivers of central Italy; an order of magnitude lower than our findings where we found a maximum value of 239.0 ng g^{-1} ww.

The results of the monitoring of NDL PCBs in food and feed (EFSA 2010) reported a mean level of 23.4 ng g^{-1} for muscle meat fish and fish products excluding eel (on a total of 2384 samples from

European countries) and 223 ng g^{-1} for muscle meat eel (on a total of 182 samples from European countries). Our findings are in complete agreement with EFSA data.

Moreover, concentrations were influenced by locations, and the highest values for Σ_6 NDL PCB in *Anguilla anguilla* were found in the Airole district (Figure 2), which is close to the French territory.

3.2 Contribution of single NDL-PCB congeners to the sum of the six indicators

The six PCB congeners 28, 52, 101, 138, 153 and 180 were chosen as indicators not due to 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 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. In its scientific opinion on the presence of non-dioxin-like PCBs in feed and food, the CONTAM Panel highlighted that the sum of the six indicator PCBs represents about half the total NDL-PCB in food (EFSA, 2005).

In our study, the overall mean contribution of the individual NDL-PCBs (upper-bound) to the sum of the six indicators followed the pattern: PCB-153 > PCB-138 > PCB-180 > PCB-52 > PCB-28 > PCB 101 in trout, PCB-153 > PCB-138 > PCB-180 > PCB-52 > PCB 101 > PCB-28 in barbel and PCB-153 > PCB-138 > PCB-180 > PCB 101> PCB-52 > PCB-28 in eel (Table 2, Figure 3). These results are in line with findings reported in other studies, which have 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, Squadrone et al., 2013). Considering the contribution of single NDL-PCB congeners to the sum of the six indicators, NDL-PCBs 153, 138 and 180 were analytically predominant in all fish, followed by the remaining congeners 101, 52 and 28.

The mean contribution of PCB-153 ranged from 30% in trout to 42% in eel (EFSA 23% in freshwater fish to 44% in eel), while PCB-138 ranged from 27% in trout to 29% in eel (EFSA 19% to 32%) and, together, their contribution was at least half in both food groups (freshwater fish and eel). PCB-180 contributed between 17% and 22%, while PCB-101 contributed between 3% and

7%; PCB-52 contributed between 2% and 12%, and PCB-28 contributed between 2% and 7% to the sum of the indicator NDL-PCBs (Figure 3). The highest values for NDL-PCBs 153, 138 and 180 were all recorded in eel, while the highest values for NDL-PCBs 101, 52 and 28 were recorded in trout and barbel.Moreover, there was a highly significant relationship ($p < 0.001$) between PCB153 and PCB138, as well as between PCB 180 and PCB153 (Table 4).

Pacini and co-workers (2013) examined fish from southern Italian rivers and found the commonest congeners 153, 138, and 101, in decreasing amounts, while we also found the same pattern in *Silurus glanis* from north Italian rivers (Squadrone et al., 2013). Similarly, Brazova et al. (2012) found that PCB153 was present in higher concentrations than other congeners in the muscle of top predators, such as the European catfish, with an average of 29% of Σ_6PCB, while the 138 congener accounted for approximately 24 % of Σ_6PCBs.These analytical NDL-PCB patterns were also coherent with those observed in different fish species from the middle and lower stretches of the River Po (Viganò et al., 2000), and from the Orbetello lagoon (Mariottini et al., 2006), where the presence of PCBs is associated with residues from commercial technical mixtures.

Congeners 138 and 153 are characterized as less hydrophobic and less tightly bound to sediment than the higher chlorinated octa-, nona-, and deca-PCBs, which is 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 (PCB138) or both rings (PCB153) could have a greater resistance to metabolism and elimination from fish organisms 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 is due to their slow rate of biotransformation and elimination (Dip et al., 2003; Hoekstra et al., 2003).

PCB138 and PCB153 cause histological damage in organs including the liver and behave as neurological and neurochemical toxic agents (Sa´nchez- Alonso et al., 2003; Duffy and Zelikoff, 2006). A high incidence of necrosis in the liver can occur due to these congeners (Oliveira Ribeiro et al., 2005), and thus they pose a risk to human health.

3.3 Human health implications

As part of the human diet, fish represents a good source of high quality protein (providing 17% of the total animal protein and 6% of all protein consumed by humans), vitamins and other essential nutrients. However, they are also considered as the main source of human exposure to contaminants such as PCBs and dioxins (Domingo and Bocio, 2007).

The response of wildlife to PCB exposure and congeners varies widely, possibly reflecting not only variation in susceptibility, but also different mechanisms of action or selective metabolism of individual congeners. PCBs tend to biomagnify in the food chain, reaching greater toxicity at higher trophic levels, such as in piscivorous and fatty fish (e.g. *A. anguilla*).

Consumption of fish from the River Roya is, therefore, a potential source of exposure to environmentally persistent contaminants that may be associated with human health risks. An association between fish consumption and PCBs in body tissues of sporting or professional fishermen, and others consuming large quantities of contaminated fish, has been reported.

Toxicological data indicate that NDL-PCBs alter a number of physiological processes that are important during the development of the species, in particular in the nervous and endocrine systems. The European Union has undertaken short- and long-term actions, aimed at reducing environmental contamination and human exposure, which have recently been extended to incorporate NDL-PCBs. Our analyses of NDL-PCBs in the fish muscle of trout, barbel and eel in the River Roya, assess the presence of these organic compounds in this area. Moreover, our studies represent a way to improve the understanding of the environmental and human risks. Fish and fishery products are the major contributors of the dietary exposure to NDL-PCBs, and evaluating the levels of these contaminants is necessary to protect consumers from NDL-PCB intake.

4. **Conclusions**

In the present work, all fish samples caught from the River Roya were below the maximum acceptable level set by European Regulations for the sum of the six indicators of NDL-PCB. However, as the Roya basin is an area devoted to tourism and recreational fishing, lacking in industrial activities that can cause river pollution, the presence of these organic compounds in some samples which reach levels close to the maximum limit, should be explored further. In addition, local people and sporting fishermen consume fish from this river, and, therefore, the possible health risks should be evaluated because fish consumption is an important route of exposure to NDL-PCBs.

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Table 1

Concentrations of NDL-PCBs (ng g^{-1} ww) in fish from the River Roya

species	***no.***	***Min***	***Max***	***Mean***	**±** ***SD***
Brown trout	6	9.2	97.0	40.0	4.2
Barbus barbus	6	9.2	27.6	24.5	7.5
Anguilla anguilla	20	9.0	239.5	131.5	61.8

Table 2

NDL-PCB levels in fish and the contribution of the indicator PCBs to the sum.

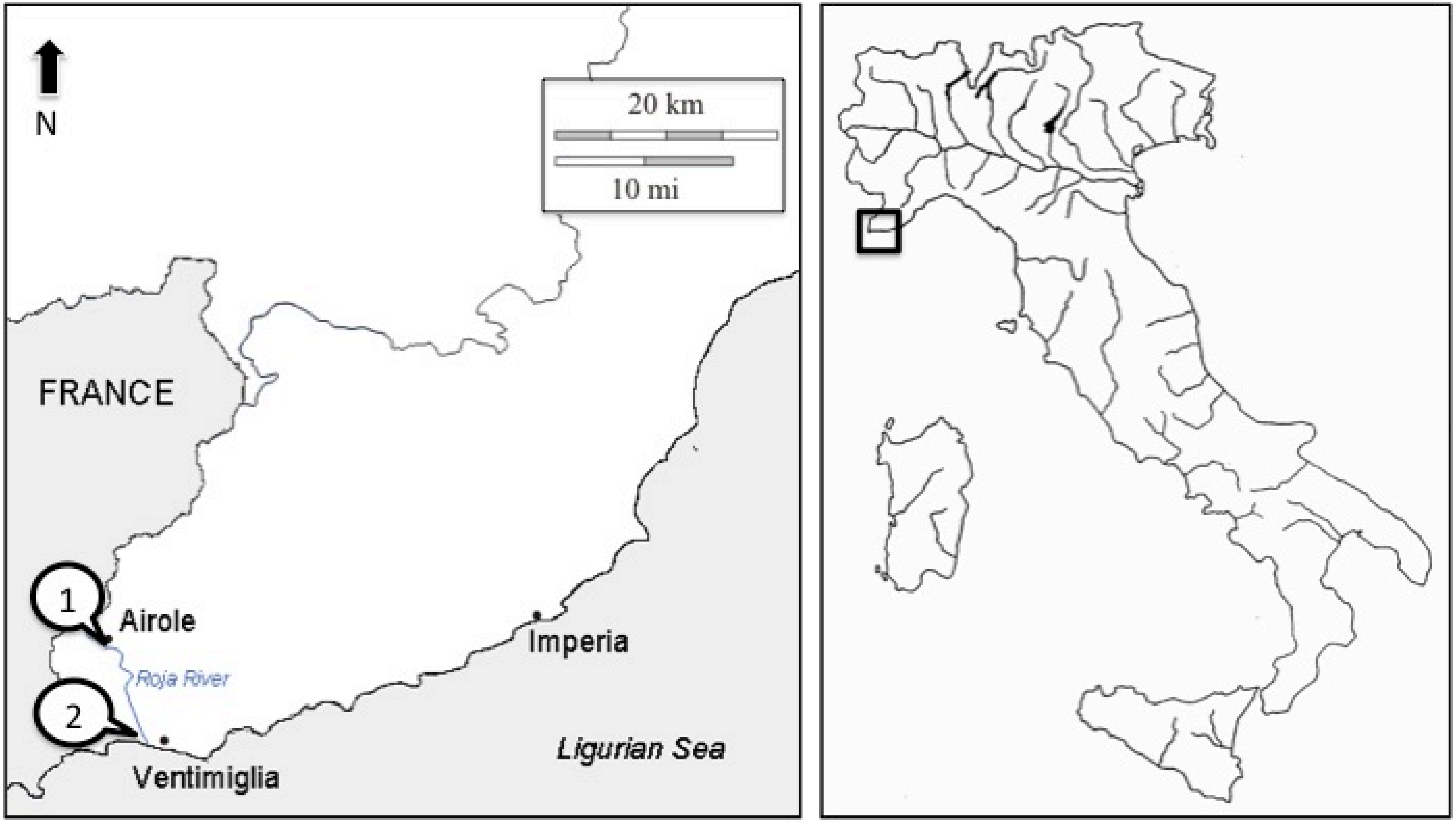
Indicator PCBs	*Fish*	*Min*	*Max*	*Mean*	*Contribution to the sum*
28	Trout	1.3	6.9	2.98	7%
	Barbel	1.2	1.3	1.22	5%
	Eel	1.3	8.2	2.06	2%
52	Trout	1.6	12.5	4.65	12%
	Barbel	1.2	1.6	1.27	5%
	Eel	1.3	15.9	3.3	2%
101	Trout	1.1	7.0	2.73	7%
	Barbel	1.2	1.3	1.22	5%
	Eel	1.4	8.0	3.7	3%
138	Trout	1.8	27.4	10.93	27%
	Barbel	1.8	8.0	6.97	28%
	Eel	1.8	84.9	37.91	29%
153	Trout	1.9	29.8	11.93	30%
	Barbel	1.9	9.9	8.57	35%
	Eel	1.9	95.0	55.11	42%
180	Trout	1.3	17.6	6.77	17%
	Barbell	1.3	6.1	5.30	22%
	Eel	1.3	61.9	29.37	22%

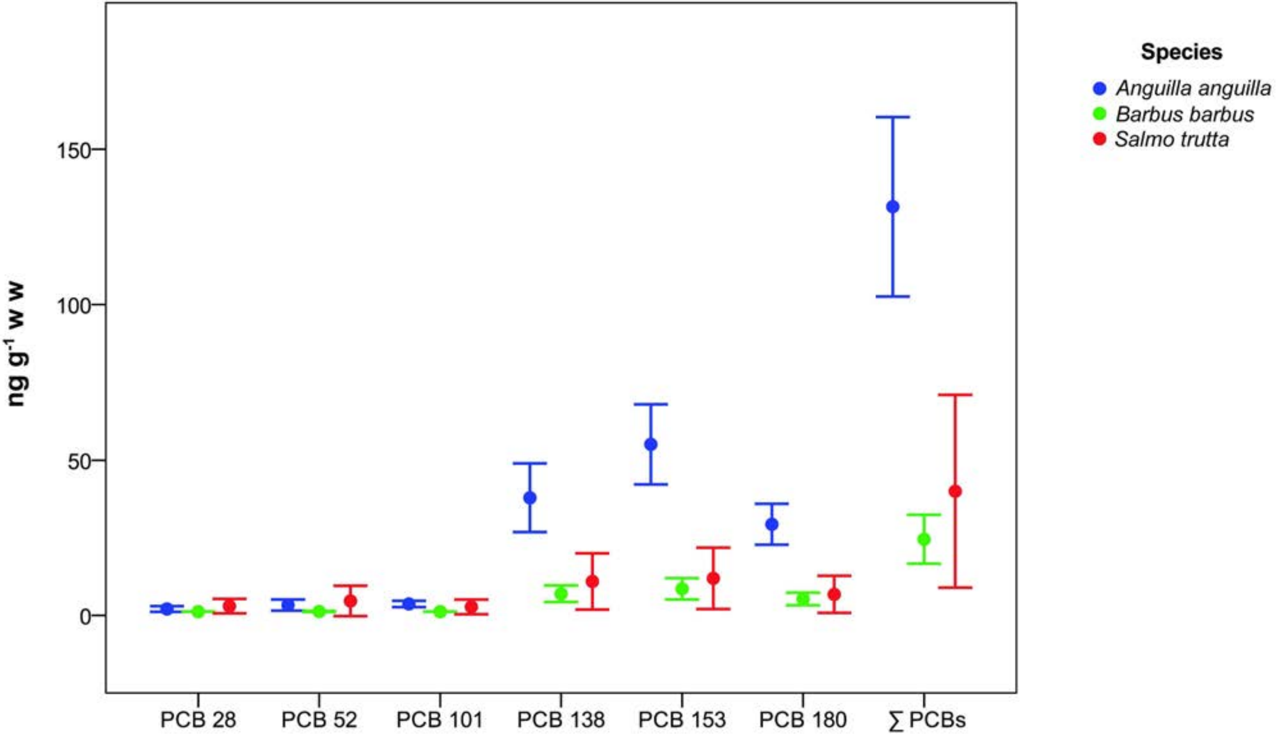
Table 3

Pairwise Spearman's rank correlation coefficient for PCB congeners

PCB congeners	PCB 28	PCB 52	PCB 101	PCB 138	PCB 153	PCB 180
PCB 52	0.665**					
PCB 101	0.407*	0.682**				
PCB 138	0.444*	0.491**	0.643**			
PCB 153	0.280	0.458**	0.570**	0.933**		
PCB 180	0.210	0.386*	0.586**	0.876**	0.932**	
∑ PCBs	0.394*	0.537**	0.692**	0.957**	0.961**	0.922**

Note: * = correlation significance $p < 0.05$ (two-tailed); ** = correlation significance $p < 0.001$ (two-tailed).


N
20 km
10 mi
FRANCE
1
Airole
Roja River
2
Ventimiglia
Imperia
Ligurian Sea



ng g-1 w w
150
100
50
0
PCB 28
PCB 52
PCB 101
PCB 138
PCB 153
PCB 180
∑ PCBs
Species
Anguilla anguilla
Barbus barbus
Salmo trutta