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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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# UNIVERSITÀ DEGLI STUDI DI TORINO

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1	Non-dioxin-like	polychlorinated	biphenyls	(NDL-PCBs)	in	eel,	trout,	and	barbel	from	the
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- 10

### 11 Abstract

12 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 13 important to quantify their levels in aquatic organisms in order to evaluate the risks associated with 14 15 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 16 17 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  $g^{-1}$ 18 ww in trout and 9.0 to 239.5 ng  $g^{-1}$  ww in eel. The distribution of congeners among different species 19 did not show significant variations and was characterized by a higher proportion of 153, 138 and 20 21 180 congeners, according to their high resistance to metabolic degradation.

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23 Keywords: NDL-PCBs; River Roya, freshwater fish

24

### 25 **1. Introduction**

26 Fish tissue has a distinct advantage in relation to monitoring inert environmental compartments.

27 Sediment-bound organic contaminants are potentially resistant to chemical and biological

28 transformation and release, unlike organic compounds in fish tissue that represents the bioavailable

29 portion cycling through aquatic food webs.

30 In general, due to the high affinity of organic contaminants for fat tissues of animals, fish

31 accumulate contaminant concentrations that may not be detectable in the water column (Pacini et

32 al., 2013). Furthermore, concentrations in fish tissues represent a critical link connecting aquatic33 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.

37 PCBs represent a particularly persistent group of chlorinated congeners, and are ubiquitous in the 38 environment and exclusively of anthropological origin. Two classes of PCBs have been classified 39 according to their toxicological properties, dioxin-like PCBs (DL-PCBs), which have an analogous 40 toxicity to dioxins, and non-dioxin-like PCBs (NDL-PCBs). These compounds are related by 41 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 42 43 equipment, heat exchangers, hydraulic systems, and other specialized applications. Despite a ban on 44 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 45 46 the oceans (Voltura and French, 2000).

47 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 48 49 in the environment (US EPA, 2007). A recent report by the European Food Safety Authority 50 showed that particularly high levels of non-dioxin-like PCBs (NDL-PCBs) can be found in fish and 51 fishery products (EFSA, 2010). Furthermore, a relevant number of national and international 52 regulatory bodies have established fish consumption guidelines, with a particular emphasis for those 53 fish that are known to accumulate a variety of chemicals. The European Union has also provided 54 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 55 56 tolerable levels (MLs) for the sum of the six "indicators" NDL-PCBs 28, 52, 101, 138, 153 and 180 57 ( $\Sigma_6$  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<sup>-1</sup> in muscle meat of eel, followed by 148 ng g<sup>-1</sup>in fish liver, and 23 ng g<sup>-1</sup>in muscle meat fish and fish products excluding eel (EFSA 2010).

69 The contamination of aquatic organisms depends on the chemical properties of each congener. The 70 bioaccumulation processes are influenced by exposure levels in the environment and various biotic 71 factors, such as the metabolic capacity (Hubaux and Perceval, 2011).

72 The European eel is considered a bottom-dwelling fish, showing high body lipid content, significant 73 longevity and carnivorous, and is exposed to lipophilic-persistent contaminants, such as PCBs, and 74 thus represents a species sensitive to bioaccumulation (Roche al., 2000). Moreover, eels constitute 75 an important economic value close to estuaries and rivers, and an essential food resource (Després, 76 2009). Significant levels of PCBs have already been reported in European eels in France, from the 77 Gironde and Adour estuaries (Tapie et al., 2011), in the Mondego estuary in Portugal (Nunes et al., 78 2011), and in Italian rivers, and have been suggested as being responsible for migration or 79 reproduction impairments (Mezzetta et al., 2011). Assessing PCB contamination of the European 80 eel is therefore of crucial interest as PCB levels are threatening public health beyond a maximum 81 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 82 83 due to the introduction of the Water Framework Directive 60/2000 UE (WFD). Since 2000, when 84 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., 85 2012). Smith and Darwall (2006) identified water pollution as the greatest threat to Mediterranean 86 87 endemic freshwater fish. In particular, the Common barbel has been previously used for ecotoxicological studies due to its reported sensitivity for particular biomarkers (Viganó et al., 2000). 88 89 Bottom-dwelling fish, such as barbel, tend to accumulate lipophilic contaminants in their tissues 90 directly from water and sediment as well as through their diet, enabling assessment of the transfer 91 of pollutants through the trophic web. Wild brown trout are predatory species at the top of the 92 trophic level; they are abundant in many freshwater ecosystems and are also feature as a part of the 93 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 94 95 fish populations is important, not only from an ecological point of view, but also as a matter of 96 concern for human health.

97 The basin of the River Roya, mostly located in French territory, covers a total area of 662 km<sup>2</sup>. The 98 River Roya source is in France from the Colle di Tenda at about 1850 m above sea level and it 99 flows for about 40 km, with numerous tributaries joining it. The river enters Italian territory at an 100 altitude of 200 m above sea level, and flows into Ventimiglia.

101 The river is the most important water reserve of the entire territory and its water is subjected to 102 intense use, especially for drinking water and hydroelectric power, with the presence of many large 103 dams, both in the French and the Italian portion of the river. There is a significant lack of data on 104 the POP contamination levels in fish in this ecosystem, as none has been analyzed in the Italian 105 part of the river. Polychlorinated biphenyls can be accumulated in aquatic organisms through the 106 food chain, and their dietary intake by the human population is already evident. Moreover, in 2012, 107 the presence of NDL PCBs exceeding the legal limit of 300 ng g-1 in eel samples was reported by 108 the French authorities and resulted in a fishing ban on the French side of the river. The present 109 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.

### 116 2. Materials and methods

### 117 *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.

### 124 *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 inAirole and 11 samples were from the Ventimiglia district.

136 2.3 Analytical methods

137 The quantification of NDL-PCBs was performed by adapting the method of Perugini (2004). The 138 quantified congeners were the six indicators 28, 52, 101,138, 153 and 180, and their cumulative 139 analytical concentration has been reported as  $\Sigma_6$  PCBs.

140 All samples were freeze-dried, powdered and transferred into Accelerated Solvent Extraction (ASE) 141 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 142 143 fat content. Before the dissolution of fat in hexane for cleaning of the samples, PCB155 and 144 PCB198 were added as internal standards. The purification step was performed using silica 145 columns. The fat was removed on an Extrelut-NT3 column loaded with sulphuric acid. Final sample 146 extracts were evaporated under a nitrogen stream to dryness and reconstituted by the addition of 147 100 µL of isooctane. GC/MS detection was performed on a Thermo Focus gas chromatographer, 148 equipped with a DB-5MS column (30 m x 0.25 mm, 0.25 µm film thickness), and coupled to a DSQ 149 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 150 151 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 152 and contamination, "blanks" was analyzed for each calibration run, using the same procedure. 153 154 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 155 round of analysis. 156

157 In line with European regulatory instructions (EU 1259/2011), the cumulative concentrations ( $\Sigma_6$ 158 NDLPCB) were expressed as "upper bound" (UB) concentrations, on the assumption that all the 159 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 analyticalresult when the UB was above the ML.

162 The Limit of Quantitation (LOQ) for the analyzed PCBs was 1.3 ng  $g^{-1}$ . As the validation is 163 required for the analytical methods used in official food control, this method was validated 164 according to the 2004/882/EC Regulation and ISO 17025 criteria.

165 *2.4 Statistical analysis* 

166 Data were tested for normality by using the Kolmogorov–Smirnov test. As the assumptions for 167 parametric analyses were not met, inference was made from non-parametric statistical techniques. 168 In particular, pairwise correlations between PCB congeners and correlations between weight and 169  $\Sigma$ 6 PCBs were examined using Spearman's rho test. All tests were performed in SPSS v. 20 (SPSS, 170 Inc., Chicago, IL) for Macintosh.

### 171 **3. Results and discussion**

### 172 *3.1 NDL PCB levels in fish*

173 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 174 175 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 176 trout, and 131.5 ng g<sup>-1</sup> in eel (Table 1). Considering  $\Sigma_6$  PCBs in *Barbus barbus*, our results are lower 177 178 than those reported in common barbel fished in Rivers of Central Italy (Pacini et al., 2013). 179 Otherwise, the mean value registered in trout was higher than those reported by other authors for 180 the same species, collected in a remote high European mountain lake (Vives et al., 2005), and in the 181 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 182 findings where we found a maximum value of  $239.0 \text{ ng g}^{-1}$  ww. 183

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

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

188 Moreover, concentrations were influenced by locations, and the highest values for  $\Sigma_6$  NDL PCB in

189 Anguilla anguilla were found in the Airole district (Figure 2), which is close to the French territory.

190 *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 191 192 toxicity, but because they are easily quantified compared to the other NDL-PCBs and they represent 193 all relevant degrees of chlorination. Indeed, the EFSA Scientific Panel concerning Contaminants in 194 the Food Chain (CONTAM Panel) decided to use the sum of these six PCBs as the basis for the 195 evaluation because these congeners are appropriate indicators for different PCB patterns in various 196 sample matrices and are most suitable for a risk assessment of NDL-PCBs. In its scientific opinion 197 on the presence of non-dioxin-like PCBs in feed and food, the CONTAM Panel highlighted that the 198 sum of the six indicator PCBs represents about half the total NDL-PCB in food (EFSA, 2005).

199 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 > 200 201 PCB 101 in trout, PCB-153 > PCB-138 > PCB-180 > PCB-52 > PCB 101 > PCB-28 in barbel and 202 PCB-153 > PCB-138 > PCB-180 > PCB 101 > PCB-52 > PCB-28 in eel (Table 2, Figure 3). These 203 results are in line with findings reported in other studies, which have demonstrated that PCB-153 204 has an average contribution of roughly one third to the sum of the six indicator PCBs (EFSA, 2005; 205 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 206 207 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 212 7%; PCB-52 contributed between 2% and 12%, and PCB-28 contributed between 2% and 7% to the 213 sum of the indicator NDL-PCBs (Figure 3). The highest values for NDL-PCBs 153, 138 and 180 214 were all recorded in eel, while the highest values for NDL-PCBs 101, 52 and 28 were recorded in 215 trout and barbel.Moreover, there was a highly significant relationship (p < 0.001) between PCB153 216 and PCB138, as well as between PCB 180 and PCB153 (Table 4).

217 Pacini and co-workers (2013) examined fish from southern Italian rivers and found the commonest 218 congeners 153, 138, and 101, in decreasing amounts, while we also found the same pattern in 219 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 220 221 predators, such as the European catfish, with an average of 29% of  $\Sigma_6$  PCB, while the 138 congener accounted for approximately 24 % of  $\Sigma_6$  PCBs. These analytical NDL-PCB patterns were also 222 223 coherent with those observed in different fish species from the middle and lower stretches of the 224 River Po (Viganò et al., 2000), and from the Orbetello lagoon (Mariottini et al., 2006), where the 225 presence of PCBs is associated with residues from commercial technical mixtures.

226 Congeners 138 and 153 are characterized as less hydrophobic and less tightly bound to sediment 227 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 228 229 atoms in positions 2, 4, and 5 in one (PCB138) or both rings (PCB153) could have a greater 230 resistance to metabolism and elimination from fish organisms than the lower congeners, such as 28, 231 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 232 233 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.

### 238 *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*).

247 Consumption of fish from the River Roya is, therefore, a potential source of exposure to 248 environmentally persistent contaminants that may be associated with human health risks. An 249 association between fish consumption and PCBs in body tissues of sporting or professional 250 fishermen, and others consuming large quantities of contaminated fish, has been reported.

251 Toxicological data indicate that NDL-PCBs alter a number of physiological processes that are 252 important during the development of the species, in particular in the nervous and endocrine 253 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 254 255 incorporate NDL-PCBs. Our analyses of NDL-PCBs in the fish muscle of trout, barbel and eel in 256 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 257 fishery products are the major contributors of the dietary exposure to NDL-PCBs, and evaluating 258 259 the levels of these contaminants is necessary to protect consumers from NDL-PCB intake.

### 260 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

267 risks should be evaluated because fish consumption is an important route of exposure to NDL-

268 PCBs.

### 269

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# Table 1

Concentrations of NDL-PCBs (ng g<sup>-1</sup> 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

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**	
$\Sigma PCBs$	0 394*	0.537**	0 692**	0 957**	0 961**	0 922**

Pairwise Spearman's rank correlation coefficient for PCB congeners

 $\sum_{i=1}^{n} PCBs \qquad 0.394* \qquad 0.537** \qquad 0.692** \qquad 0.957** \qquad 0.961** \qquad 0.922**$ Note: \* = correlation significance p < 0.05 (two-tailed); \*\* = correlation significance p < 0.001 (two-tailed).



