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Organochlorine Residues in Fishes from Two Rivers in Cantabria, Spain: Implications for a Program of Otter (*Lutra lutra*) Reintroduction

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Persistent organochlorine compounds, like pesticides (including p,p'-DDE, the main metabolite of p,p'-DDT) and polychlorinated biphenyls (PCB), are known to be widely distributed in the environment. The capacity for bioconcentration (especially in aquatic environments) and accumulation along food chains (biomagnification) of these chemicals is well documented (Leonards et al. 1998; López-Martín and Ruiz-Olmo 1996; Yamaguchi et al. 2003), so the selection of organisms to monitor for levels of contamination has become a very common practice.

Nevertheless, some species are more suitable or scientifically interesting than others, for several reasons. Freshwater fishes are, in this sense, very useful, because their study can provide valuable information not only on pollution status of the riparian ecosystems, but also because data obtained can have implications in food toxicology and thus in the assessment of health for humans (edible species) (Binelli and Provini 2004) and for wild predators.

Among semi-aquatic predators, the European otter (*Lutra lutra*), whose populations have declined sharply in the last decades in Europe (Delibes 1990; López-Martín and Ruiz-Olmo 1996; Mazet et al. 2004), is one of the most emblematic and surveyed species. The diet of this mammal of the Mustelidae family is dominated by aquatic prey, consuming about 1 kg of food each day. This makes this riparian carnivore especially vulnerable to bioconcentration and biomagnification problems derived from the presence of some organochlorine compounds in fishes. Particularly of concern are PCB residues, that have been implicated in impairing survival and reproduction capacity of otters (Leonards et al. 1998; Mason and Madsen 1993; Roos et al. 2001; Yamaguchi et al. 2003).

That is why, after a similar study carried out in Catalonia (Northeast Spain, coast of the Mediterranean Sea) a few years ago (Mateo et al. 1999), apparently with good prediction power, we undertook another one in Cantabria (North of Spain, coast of the Atlantic Ocean) with similar objectives. In this case, we were asked to answer if organochlorine accumulation in fishes could be the reason that explains why in two close Cantabric rivers, the Asón and the Nansa, otters are now only present in the latter.

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MATERIALS AND METHODS

Fishes were captured, under license of the Autonomic Government of Cantabria, using electro fishing. Sampling was made in January 2001 by expert personnel, and took place in four different zones of the Asón basin (Regules, Negrillo, Valle and Coterillo) and two from the Nansa river (Bárcena and Rábago). Of the 42 fishes captured in the Asón and the 20 of the Nansa, 28 were brown trout (*Salmo trutta fario*, weighing on average 101±80.0 g), 17 Eurasian minnows (*Phoxinus phoxinus*, 5.41±1.55 g), 16 European eels (*Anguilla anguilla*, 95.4±137 g) and a gudgeon (*Gobio gobio*, 17.3 g). Each specimen was homogenized separately, and 5 g were dried in an oven at a temperature of 58ºC. All the samples were immediately stored frozen (-20ºC) until analysis.

Solvents and reagents used were pesticide residue analysis or equivalent high purity grade. Blanks were processed among samples to assure quality of analyses. Pesticide, PCB congeners and Aroclor^R 1254 and 1260 standards were purchased from Alltech (Deerfield, IL, USA), Chem Service (West Chester, PA, USA) and Sigma (St. Louis, MO, USA). Individual PCBs in Aroclor^R mixtures were identified with the corrected (Guitart et al. 1993) numbering of the Ballsmitter and Zell system.

Sample preparation, based on the sulfuric acid cleanup of an n-hexane extraction procedure, has been described and validated previously (Badía-Vila et al. 2000; Mateo et al. 1999). Approximately 0.4 g (accurately weighed) were processed per sample. Internal standards, PCBs #1 and #209, were added from the beginning. Chromatographic analyses were carried out on a Perkin-Elmer AutoSystem equipped with a 25 m L fused silica capillary column of 0.53 mm ID and 0.25 μm of film thickness (BPX5 from SGE, Ringwood, Australia), coupled to an electron capture detector (ECD), in conditions optimized for the analytes. The total PCB concentration (ΣPCBs) was calculated as the sum of individual congener concentrations, the ΣHCH (hexachlorocyclohexane) as the sum of the α, β and γ (lindane) isomers of HCH, and the Σcyclodienes as the sum of aldrin, heptachlor and heptachlor epoxide.

The recovery of the method was calculated with spiked samples, two with insecticides and two more with PCBs. Except for endrin and dieldrin, which were destroyed during the cleanup procedure, the values obtained for the rest of the pesticides and selected PCB congeners were between 82.2% (for α-HCH) and 117.9% (for p,p'-DDE). Thus, corrections based on recovery data were not taken into account in quantification.

The results were expressed as geometric mean in ng/g dry weight (DW) in order to satisfy conditions for normality of data, although for ΣPCBs and p,p'-DDE also the arithmetic mean and SD were calculated to facilitate comparisons. The differences between basins, among sampling points and among fish species, were studied by means of a generalized linear model (GLM), including the fish weight as co-variant, using the SPSS 9.0 statistical package.

Table 1. Concentrations (geometric mean and range, ng/g DW) of organochlorine compounds in fishes from two rivers in Cantabria.

	ASÓN n=42	NANSA n=20	TOTAL n=62
HCB	0.16 (nd-9.69)	0.04 (nd-2.85)	0.11 (nd-9.69)
α-HCH	0.48 (nd-4.23)	0.57 (nd-5.10)	0.51 (nd-5.10)
β-HCH	0.02 (nd-2.33)	0.14 (nd-13.19)	0.05 (nd-13.19)
γ-HCH	0.04 (nd-0.88)	0.08 (nd-4.91)	0.05 (nd-4.91)
ΣHCHs	0.63 (nd-4.93)	0.91 (nd-21.06)	0.71 (nd-21.06)
Heptachlor	0.04* (nd-0.64)	0.08* (nd-0.93)	0.05 (nd-0.93)
Heptachlor epoxide	nd (nd-1.79)	0.06 (nd-3.79)	0.02 (nd-3.79)
Aldrin	0.02 (nd-0.76)	0.05 (nd-2.54)	0.03 (nd-2.54)
ΣCyclodienes	0.06* (nd-2.12)	0.25* (nd-3.99)	0.11 (nd-3.99)
p,p'-DDE	27.25 (3.53-2012)	21.06 (3.00-44.31)	25.08 (3.00-2012)
ΣPCBs	89.57 (6.07-558.3)	55.56 (14.30-284.9)	76.79 (6.07-558.3)

(nd: not detected; asterisk: significant differences, see text)

RESULTS AND DISCUSSION

PCBs were, as a whole (ΣPCB), the main residues found in fish samples of both basins (Table 1). However, considered individually, p,p'-DDE was the organochlorine found at highest concentration. In terms of arithmetic mean and SD for the n=62 samples, they were present at 111.1±108.9 and 61.9±253.0 ng/g DW, respectively. No DDT family representatives other than p,p'-DDE (particularly p,p'-DDT and p,p'-DDD) were found in any of the analyzed samples.

Concentrations of organochlorine contaminants between both basins were very similar and not high (Table 1), and significant statistical differences were only found for heptachlor ($p=0.033$) and Σcyclodienes ($p=0.038$). Although the number of samples for statistical analysis is severely reduced when each sampling point is considered individually, it was also found that only for heptachlor ($p=0.009$) and between the Nansa zones Rábago (0.31 ng/g DW) and Bárcena (nd), a significant difference existed.

Table 2. Concentrations (geometric mean and range, ng/g DW) of organochlorine compounds in fish species from two rivers in Cantabria.

	Salmo trutta fario **(n=28)**	***Phoxinus phoxinus*** **(n=17)**	***Anguila anguila*** **(n=16)**	***Gobio gobio*** **(n=1)**
HCB	0.09 (nd-9.69)	0.09 (nd-2.85)	0.20 (nd-6.62)	nd
α-HCH	0.38 (nd-4.03)	0.70 (nd-5.10)	0.50 (nd-4.91)	3.86
β-HCH	0.03 (nd-2.33)	0.08 (nd-13.19)	0.03 (nd-1.24)	0.47
γ-HCH	0.03 (nd-1.00)	0.05 (nd-4.91)	0.08 (nd-0.70)	0.12
ΣHCHs	0.55 (nd-4.03)	1.04 (nd-21.06)	0.66 (nd-5.41)	4.45
Heptachlor	0.03 (nd-0.93)	0.08 (nd-0.93)	0.05 (nd-0.92)	0.51
Heptachlor epoxide	0.02 (nd-3.79)	0.01 (nd-2.99)	0.01 (nd-1.79)	1.05
Aldrin	0.02 (nd-0.37)	0.04 (nd-2.54)	0.03 (nd-0.76)	0.45
ΣCyclodienes	0.7 (nd-3.99)	0.14 (nd-3.12)	0.11 (nd-2.12)	2.00
p,p'-DDE	20.24 (8.69-171.3)	23.00 (3.00-79.08)	39.43 (12.22-2,012)	31.33
ΣPCBs	67.91 (14.19-558.3)	54.81 (6.07-187.1)	138.97 (57.91-464.0)	55.36

(nd: not detected)

Comparing among fish species, there were no differences in the content of organochlorine chemicals (Table 2), although eels (and also the only gudgeon individual analyzed) showed a higher (but non-significant) concentration of some of the residues. Eels are known to have a higher content of lipids than other species (Mateo et al. 1999; Mazet et al. 2004; Yamaguchi et al. 2003), which could account for the results obtained here with lipophilic contaminants, but in our case it can be explained more easily by their lower content in water (mean of 75%) than the other three species (ranging 79-82%). On the other hand, weight of the fishes (and, thus, the size and age) were found to be not statistically related to the concentration of these environmentally persistent chemicals.

The profile of pooled PCB data for all the fishes (n=62) is very close to the highly chlorinated mixture Aroclor^{R} 1260, which agrees with previous data published for the south of Europe (Badia-Vila et al. 2000; Mateo et al. 1999; Mazet et al. 2004). The most abundant peaks in our chromatograms (see Figure 1) correspond to the congeners #105+132+153, followed by #138+158+160 and #180+193.

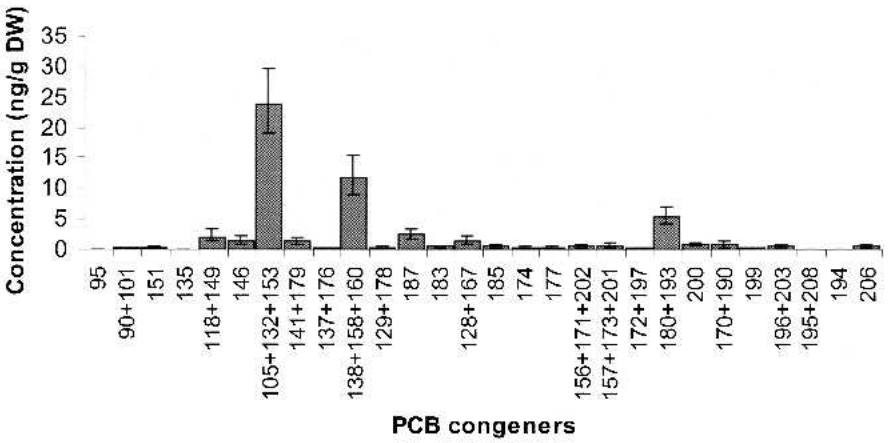

Figure 1. Concentration (geometric mean with 95% CI, ng/g DW) of PCBs in fishes (n=62) from the Asón and Nansa basins.

For the ΣPCBs, if the geometric mean level for all samples (n=62), 76.79 ng/g DW, is recalculated to wet weight (WW), the result obtained is 16.26 ng/g WW (range 10.65-23.34 ng/g WW). This value is clearly below the threshold of 110 ng/g WW in the otter diet that has been considered critical for survival of this species (Ruiz-Olmo 1995), and that we used for the previous work in Catalonia (Mateo et al. 1999). It is also below the values of 50-150 ng/g WW that Mazet et al. (2004) used in their assessment in France, or the more conservative level of 26 ng/g WW suggested by Macdonald and Mason (1994) for long-term protection of otter populations. Mason (1998) and Mason and Madsen (1993) also considered that PCB levels no longer pose a threat to otter populations in England and Wales, and in Denmark.

In conclusion, levels of organochlorine residues found in fishes in these two Cantabric rivers can be considered lower or similar to previously reported data, either from Spain (López-Martín et al. 1995; López-Martín and Ruiz-Olmo 1996; Mateo et al. 1999) or from other European countries (Mazet et al. 2004; Roos et al. 2001; Yamaguchi et al. 2003). Therefore, none of both riparian ecosystems are heavily polluted with these persistent compounds. The concentrations of organochlorines can also be considered harmless for humans, at least if consumption of fishes is moderate (for example, in many countries consumption restrictions of ΣPCB usually begin at levels above 500 ng/g WW) (Binelli and Provini 2004), and are also safe for wild top predators. Thus, the absence of otters in the Asón basin since the middle of the 20th century cannot be due, with the present data, just to a higher uptake of PCBs and organochlorine pesticides through the diet in this river. Consequently, additional factors, including other micropollutants or even external factors (hunting, drowning in fish traps, habitat disturbances, traffic accidents, availability of water and food, etc.), need to be considered in future studies on the feasibility of the otter reintroduction project.

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