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PCBs in Fish of the Ardeche River: Potential Implications for the Survival of the Otter (*Lutra Lutra*)

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In the late 1950s and 1960s, a steep decline in otter (*Lutra lutra*) populations took place throughout most of Western Europe. Many different explanations for this decline have been presented; e.g. hunting, habitat destruction and indirect or direct influences of eutrophication; acidification; and toxic chemicals (Mason and Macdonald 1986). One factor most likely to have caused the widespread loss of range is contamination of aquatic food chains through bioaccumulation of organochlorine pesticides and polychlorinated biphenyls (PCBs) (Mason 1989).
In France, otter populations are extinct in the northern and eastern parts of the country but the species is still thriving on the Atlantic coast and in the Massif Central, where a population expansion has even been recorded recently (Bouchardy 1986, Fonderflick 1995, Rosoux 1995). There is currently no specific information on the actual toxicity of PCB's to otters.
The present study was carried out in Ardèche (France), at the junction between the Ardèche river and the Rhône. The Ardèche river and its tributaries were surveyed between 1998 and 2000. Spraint samples and footprints were observed (Bendelé, 2000).

The aims of the study are to determine PCB contaminant burdens in fish and to assess whether such burdens would be likely to have adverse impacts on the reproduction and expansion of the otter.

MATERIALS AND METHODS

The fish were captured in February 2002, using electric fishing powered by a 220V electric generator. A total of 80 specimens from 10 different species were captured, and classified by batches. Fish longer than 20 cm were beheaded. Each batch was ground and kept frozen (-20°C) until further analysis.

A sample of 1.0g was taken from each batch and 30 mL of hexane/acetone 75/25 mix was added. Each sample was blended with an Ultraturrax®. The supernatant was removed and filtered through a phase separator membrane. This extraction procedure was performed twice. The extract was evaporated at 60°C in a rotary evaporator. The dry extract was dissolved in 10 mL hexane.

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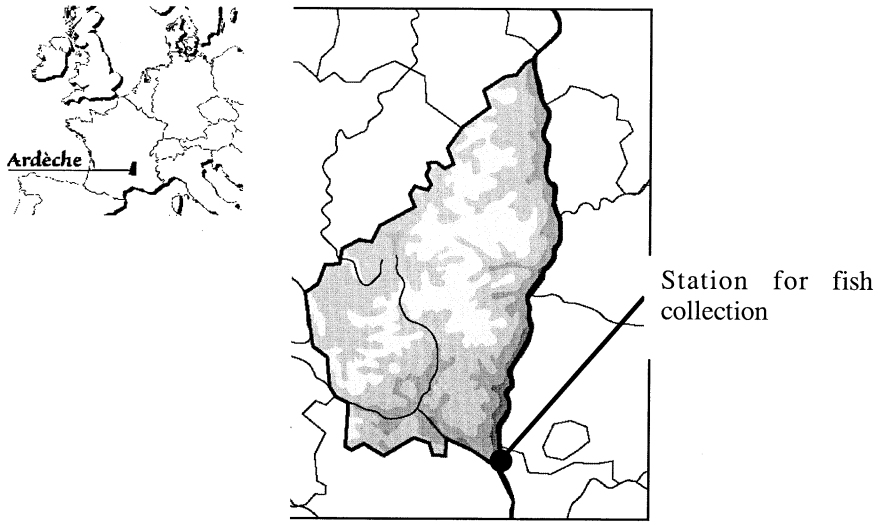

Figure 1. Study area in Ardèche showing sample localities.

Two millilitres of fuming sulphuric acid (SO_3 7%) were added, the test tube being closed and shaken immediately. After centrifugation at 3,000 rpm for 10 min, 1 mL of the supernatant was added to 1 mL of 2% potassium hydroxide in ethanol. The tubes were placed in a water bath at 50°C for 30 min. At the end of this period, 2 mL ultrapure water were added, the samples were vortexed and centrifugated once again for 10 min, they underwent another acid hydrolysis with 1 mL sulphuric acid. After a final centrifugation, the final supernatant was removed and kept frozen until further analysis.

A gas chromatograph Hewlett-Packard HP5890 series 2 equipped with an electron-capture detector was used. A Restek Rtx-5 column 60 m-long with 0.25-mm internal diameter and 0.25-µm film thickness was used. The temperature program was: 2 min at 75°C, then 15°C/min up to 150°C, from 150 to 265°C at 1.2°C/min, then 25°C/min up to 300°C. Total duration of analysis was 110 min. Injection was performed automatically with an automatic injector (HP 6890).
For each run, 2 µL were injected and each run was followed by a 15-min rinse at 300°C. Each sample was run in duplicate.

Total PCB concentration was calculated as the sum of 16 individual peaks (IUPAC n°28, 52, 77, 101, 105, 118, 126, 128, 138, 149, 153, 156, 169, 170, 180 and 187). All standards were purchased from CIL (St Foy la Grande, France) and purity was > 99%. Linearity was determined between 5 and 100 ng/g (r^2>0.99 on standards and spiked samples). Limits of detection were between 0.5 and 1.0 ng/g for individual congeners.

Table 1. Recovery of selected individual PCB congeners from fortified samples.

PCB	28	52	77	101	105	118	126	128	138	153	169	180
% recovery	92	92	90	93	91	92	92	91	93	94	91	92
CV %	6.3	7.6	6.4	6.2	7.1	6.4	8.9	10.1	7.9	6.9	5.4	13.1

(n=6) and coefficient of variation of % recovery (CV%)

Contaminant concentrations between groups (species) were compared using non parametric multiple comparison tests. The Kruskal-Wallis test and the Mann-Whitney-Wilcoxon (Ott 1988) test were used for 2 X 2 and multiple group comparison, respectively.

RESULTS AND DISCUSSION

The means and ranges of PCB in fish are given in table 1, expressed in $\mu g.g^{-1}$ fresh weight. PCB concentrations showed a significant difference between species. The PCBs were significantly higher in eels (*Anguilla anguilla*) compared to perch (*Eupomotis gibbosus*), catfish (*Ictalarus melas*) and chubs (*Leuciscus cephalus*) (Mann-Whitney-Wilcoxon test, p=0.05). No difference between the three other species was detected.
Because of their lipophilic nature, PCBs tend to accumulate in fatty tissues. The highest concentrations of PCB were detected in the fattiest fish (*Anguilla anguilla*) with a concentration of 524.1 $\mu g.kg^{-1}$ wet weight.

To assess the significance of contaminant levels in fish, a hierarchy of concentrations is used (Weber 1990):

(i) *Critical levels* : concentrations in diet > 0.5 $mg.kg^{-1}$ (wet weight)
(ii) *Levels of concern* : concentration in diet > 0.05-0.5 mg. kg^{-1} (wet weight)
(iii) *Maximum allowable concentration*: concentration < 0.05 mg. Kg^{-1} (wet weight)

These levels are extrapolated to the otter from maximal tolerated residual limits in the American mink (*Mustela vison*), another semi-aquatic member of the family Mustelidae (Weber 1990).

The mean concentration of PCBs in the samples of fish was 135 $\mu g.kg^{-1}$ wet weight. This is considered as an average scenario, because otters eat any type of fish, without selecting them.

This concentration is considered problematic for Mustelidae, although there is no specific information for otters (Weber 1990). In long term experiments mink have been shown to exhibit a reduction in reproductive output on a diet with a concentration of 0.025 $mg.kg^{-1}$ (Den Boer 1984).

Table 2. Concentrations of PCBs ($\mu g.kg^{-1}$ wet weight) in fish of the Ardèche river.

Species	N	PCB	% of fat
Anguilla anguilla	5	524.1* (293.1-755.1)	19.59
Abramis brama	1	40.6	6.57
Leuciscus cephalus	13	90.1 (24.3-155.9)	1.02
Rutilus rutilus	2	69.1	0.6
Gobio gobio	2	95.7	0.63
Perca fluviatilis	2	59.2	0.75
Eupomotis gibbosus	9	75.8 (41.8-109.7)	0.56
Ictalurus melas	6	102.4 (22.7-181.9)	2.45
Pseudorasbora parva	1	60.3	4.34
Tinca tinca	1	35.0	0.24

* mean (range)

With a worst case scenario (diet only of eels), the mean concentration would be 524.1 $\mu g.kg^{-1}$ wet weight. This concentration is considered dangerous to the species, as a concentration of 0.25 $mg.kg^{-1}$ was shown to stop otter reproduction (Mac Donald *et al.* 1994).

Leonards *et al.* (1994) proposed less severe thresholds:

(iv) *Critical levels* : concentrations in diet > 371 $\mu g.kg^{-1}$ (wet weight)

(v) *Concentration without effect:* concentration in diet < 145 $\mu g.\ kg^{-1}$ (wet weight)

With Leonards et *al*'s values, our average scenario of exposure is considered to be without effect. These extrapolations result from the hypothesis that otters are equally sensitive to the effects of PCBs as are minks. In fact, minks are known to be particularly sensitive to PCBs (Aulerich *et al.* 1985, Bleavins *et al.* 1980). These threshold values are still discussed and questioned (Kruuk and Conroy 1996).

Another interesting point is the percentage of each congener as shown in figure 2. The most abundant congeners are in decreasing order 153 > 138 > 77 > 187 > 180. This result is not surprising, since congeners 153 and 138 have a long half-life (Liem *et al.* 1994).

Coplanar congeners have an action and a mechanism of action known on the reproduction and on the growth in many species: rats, guinea-pigs, minks, human (Aulerich *et al.* 1985, Kihlström *et al.* 1992, Lucier *et al.* 1987, Carter *et al.* 1983).

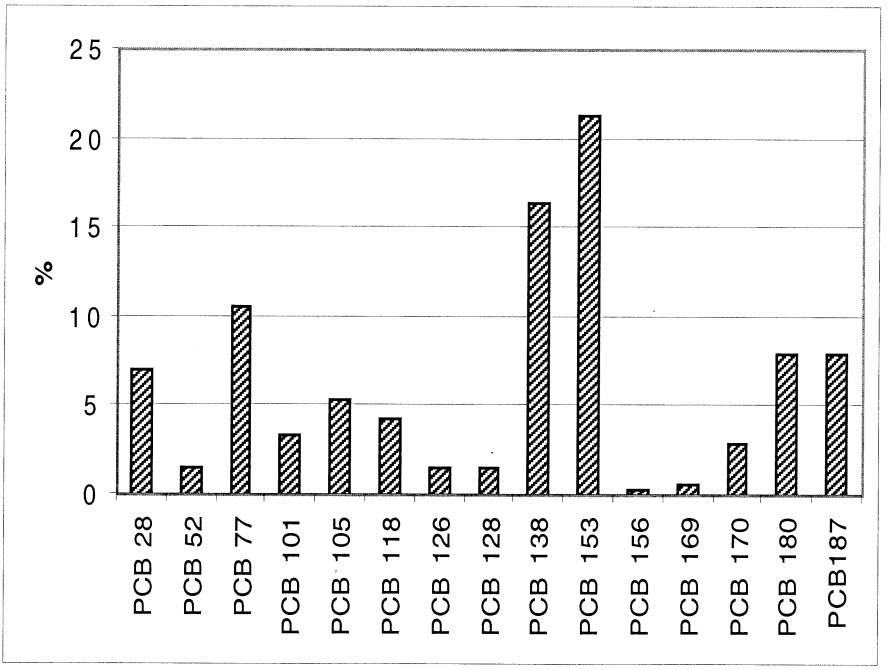

Figure 2. Mean percentage of selected PCB congeners in fish sample.

In our study, two thirds of congeners are non-coplanar ones. Serum thyroxine (T4) and serum triiodothyronine (T3) levels were reduced in rats exposed to PCBs (Brucker-Davis 1998, Kato 1998). The effects in the otter are unknown. This strong proportion of non-coplanar congeners would be interesting to take into account in order to investigate long term effects of PCBs in otters.

Our present study showed that PCB contamination of fish may pose a problem for the reproduction of otters. Otter populations should be monitored for PCB's and their potential effect on reproduction. PCBs however, are not the only problem faced by the otters. Otters are exposed to other micropollutants (e.g., copper, mercury, cadmium...), and also to habitat degradation. The potential effects of PCBs or other contaminants should be weighed against other environmental degradation factors.

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