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Chemical Contamination by PCBs in the Fishes of a French River: The Furan's (Jura)

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During recent years, PCBs have been found as major organochlorine micropollutants in France as well as in a great part of the world, in the environment and in animal outputs. This is due to their high stability and liposolubility.

In France, the studies that have been made show an important contamination by PCBs - at much higher levels than that by DDT and its derivatives - particularly in the aquatic ecosystems i.e. continental waters (MESTRES et al. 1971 a, BELAMIE 1971 , RICHOU-BAC et al. 1972) and coastal marine waters (MONOD et al. 1971 , HEURTAUX et al. 1973 , MARCHAND et al. 1976).

This work concerns the contamination by PCBs of the fishes in a running water ecosystem : the Furans river, which is located in the South of the Jura, in a non-industrial area, 80 km from Lyon (fig. 1). This stream is 27 km long and flows into the Rhone. From a general point of view, it is relatively little polluted, with the exception of a tributary, the Arene, which flows into the Furans, halt way down the course of the latter, and carries important amounts of domestic sewage (fig.2). This basin has been studied from an ecological point of view and with particular regard to the fish population.

MATERIALS AND METHODS

. *Sampling methods*. The most representative species were chosen after a general review of the population. These are : the brown trout (*Salmo trutta fario*), the grayling (*Thymallys thymallus*), the bullhead (*Cottus gobio*), the chub (*Leuciscus cephalus*), the barbel (*Barbus fluviatilis*), the nase (*Chondrostoma nasus*).

The fishes were captured by electro-fishing at the different stations (fig. 2). They were sexed, measured, weighed and underwent a rapid necropsic study (external and internal lesions, parasites, reproductive state...) ; they were sorted into size classes.

. *Analytical methods*.

The method we used collects the whole of the organochlorine residues. The organochlorine pesticides are afterwards degraded

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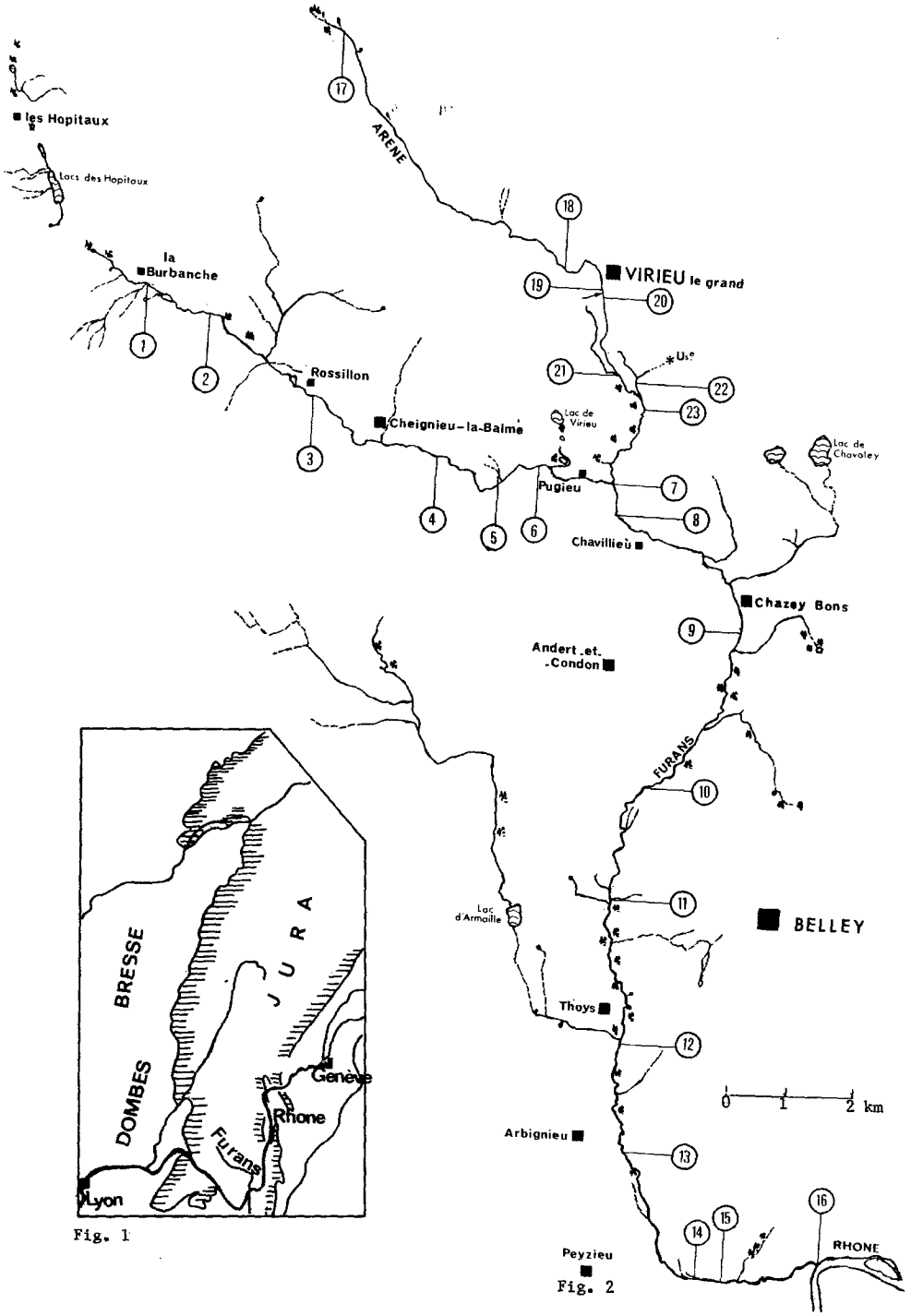

Fig. 1. Location of the Furans river

Fig. 2. The Furans basin with the sampling stations

by alkaline hydrolysis with boiling KOH, while the PCBs remain unchanged.

The extraction is made on 5 to 20 g of the sample, by means of bidistilled hexane; after pounding with Ultra-Turax and agitation, the hexane is filtered; the sample is extracted again by hexane. The hexanic phases are gathered and concentrated to about 10 ml. The extract is purified on Florisil, by eluting with a mixture of methylene chloride-petroleum ether (1 : 2) The eluate is dried off on Rotavapor and dissolved in 2 x 5 ml hexane. The final hexanic extract is analysed by GPC in the following conditions :

. apparatus Packard-Becker model 409;EC detector (Ni^{63} source); 2 m glass column, internal diameter 3 mm.
. stationary phases :
  - polar phase : SP 2250 (1,5 %) + SP 2401 (1,95 %)
  - non polar phase : SE 30 (4 %) + SP 2401 (6 %)
  on support Supelcon AW/DMCS 100-120 mesh.
. carrier-gas : Argon Methane 90-10.
. temperature : oven : 200°C; injection : 240°C; detector:280°C

The determination of organochlorine pesticide residues is made by treating the hexanic extract by boiling KOH, followed by a new GPC analysis in the same conditions.

As the pattern ofPCBs found is very near of that of DP5 (the French homonym for Aroclor 1254), the quantification of PCBs is made by reference to technical DP 5*, by comparing the height of the 9 main peaks of the sample-chromatogramm with that of the reference-peaks.

RESULTS

The levels of PCBs found in the fishes are relatively high, much higher than the levels of organochlorine pesticides :

- DDT and its metabolites : $\leq 0{,}5.10^{-2}$ ppm fresh weight
- Total HCH $< 10^{-2}$ ppm fresh weight
- HCB $< 10^{-2}$ ppm fresh weight

As to the contamination by PCBs, we have investigated :

1) if there exists a *longitudinal progress of the contamination* all along the river in the omnipresent species : brown trout, and bull head.
2) *the distribution of PCBs* in the body of the different species
3) the parameters that influence the bioaccumulation of PCBs (age, lipid content)

I.) Longitudinal progress of the contamination.

From the source down to the end on the stream, we analysed the levels of contamination in adult trout ($\geq$ 23 cm) and in

* provided by PRODELEC, France.

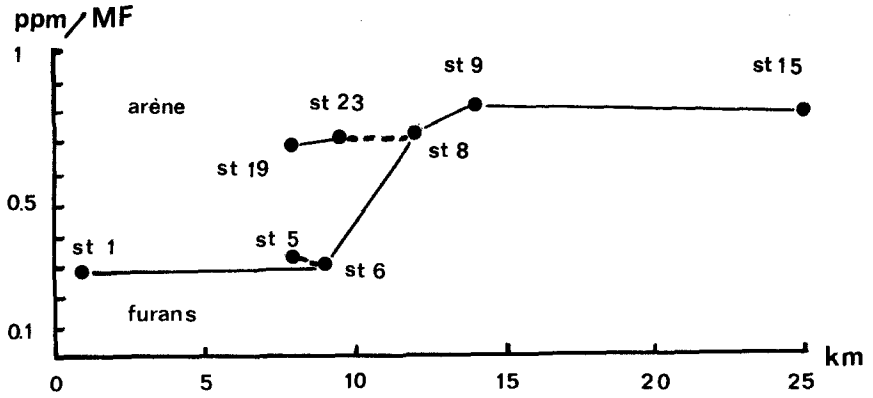

Fig. 3. Longitudinal progress of the global levels of PCB in adult brown trout from the Furans and the Arene (Aug. 1975).
Each value is the mean of the levels in liver, eggs, muscle and gills of more than 3 individuals and is expressed as ppm fresh weight (st = sampling station n°)

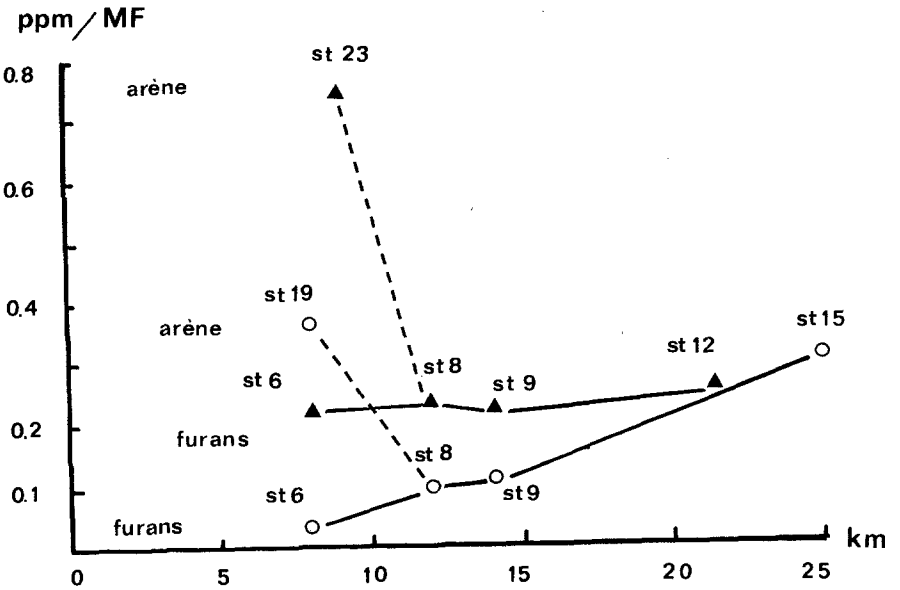

Fig. 4. Longitudinal progress of the PCB levels in one year old trout(▲) and bullhead (●) (Aug. 1975)
(PCB concentrations of the whole fish, expressed as ppm fresh weight)

one year old trout as well as in adult bullheads (>9 cm). Samples were collected in Aug. 1975.

The results are illustrated in figs. 3 and 4 which show the variations of the levels all along the Furans and stress the influence of the Arene, which causes a marked increase of the PCB levels in the fishes.

2) Variations of the levels according to the species and the organ.

The mean levels found in stations 9-12-13-15 (sampling in Aug. 1975) are shown in table 1.

Table 1. PCB accumulation by the fishes of the Furans (as ppm fresh weight). Each value is the mean of 3 or more values (the number is indicated between parenthesis).

	SALMONIDS		CYPRINIDS		
Species / Organs	Trout (4)	Grayling (3)	Barbel (3)	Chub (3)	Nase (5)
Liver	0,57	0,77	8,12	4,53	1,34
Gills	0,97	0,76	1,24	3,98	
Muscle	0,36	1,03	1,26	2,71	0,11
Kidney	0,33	0,40	5,67	2,58	0,49
Abdom. fat		3,68			
Eggs	0,70	0,56	3,85	2,83	1,10

The most striking fact is the high degree of accumulation by the barbel and the chub (Cyprinids) in comparison with the Salmonids, trout and grayling. The nase accumulates much less PCB. The grayling seems to have slightly higher concentrations than the trout ; the important PCB content in its abdominal fat may be noted.

The distribution of PCBs between the organs varies according to the species ; the liver always concentrates most ; the gills and the eggs also contain high concentrations ; the levels in the kidney and the muscle vary with the species.

3) Relation between age/size and PCB content.

For the samples of trout from the Furans and the Arene, we determined the coefficient of correlation between the size of the fish and the PCB content in the main accumulation organs : liver, eggs, gills, muscle, with 19 replications and 17 degrees

of freedom for each organ (α 0,05 = 0,455). It appears that for the liver (α = 0,648), the eggs (α = 0,617) and the gills (α = 0,458), a clear relationship exists between the size of the trout and their contamination levels. For the muscle, no such correlation appears.

4) *Relation between total lipid content of the tissues and PCB content.*

a) For a same tissue from a same species.
We compared these 2 parameters for 6 samples of muscle from brown trout (collected in Dec. 1975 in the Arene - station 23). As shown in fig. 5, the relation of the PCB levels in the muscles the lipid content is linear ; the equation of the regression curve is y = 0,34 x + 0,02 with a coefficient of correlation of 0,93 (significant at the 0,01 level).

b) For different tissues from a same species.
One always observes more important levels in fatty tissues (fat, liver, eggs, gills) than in unfatty tissues (muscle, kidney) ; none the less, there does not seem to exist any proportion between the lipid content of different tissues and the levels of PCB. The muscle shows higher concentrations of PCB levels than the fatty tissues if expressed according to lipid content.

c) For a same tissue from different species.
There does not seem to exist any relation between the PCB levels and the dorso lumbar muscle of different species (barbel, chub, trout, grayling).
The chub, e.g. has an unfatty flesh (0,60 % fat content) and high muscular levels of PCBs (1-3 ppm), whereas the Salmonids, from the same location, though they have a more fatty flesh (1-3 % fat content), show much lower muscular levels of PCBs (0,4 - 0,6 ppm).

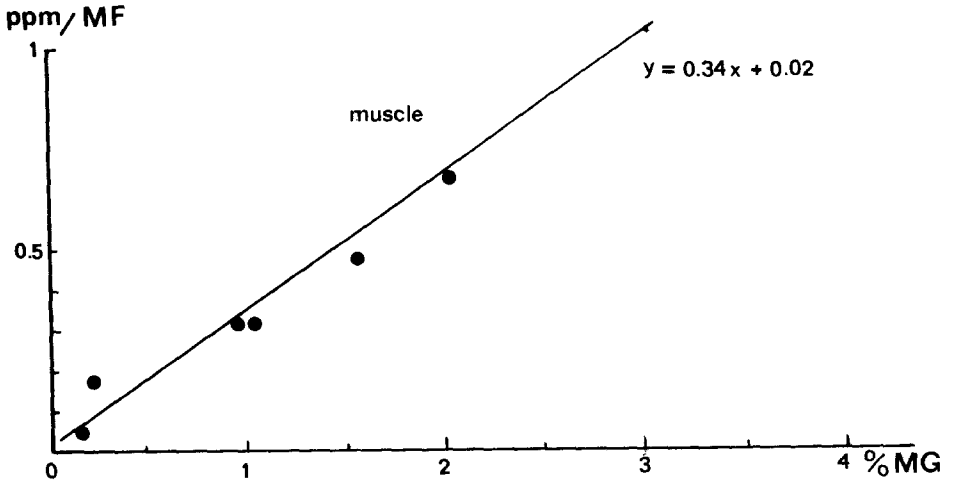

Fig. 5. Relation between PCB levels and lipid content in brown trout muscle (Déc. 1975 sample- Arene st. 23)

DISCUSSION

1) *Longitudinal progress of the contamination and origin of PCBs.*

The influence of the Arene on the contamination in the Furans (figs 3 and 4) shows that an important source of PCB is located in this area. This could be explained by important outflows directly into the Arene, in the neighbourhood of the town of Virieu. These consist in domestic wastes, particularly plastic objects (bags, bottles) which sometimes form real obstructions across this brook. The progressive release of PCBs from these materials has been demonstrated by several studies (MESTRES et al. 1971, JENSEN 1972).Contributions from atmospheric and phreatic sources certainly exist.

2) *Bioaccumulation of PCBs and possible effects in the fishes.*

The differences observed between the Salmonids (trout and grayling), on the one hand, and chub and barbel, on the other hand, could be due to different feeding habits since the former are invertebrate predators and the latter are mainly algivorous (but in fact with a mixed diet). Nevertheless, the nase is a strict algivorous fish and contains lower levels of PCBs than the Salmonids. Therefore it seems that the notable differences in bioaccumulation are more likely due to metabolic changes.

. A correlation between lipid content of the tissues and PCB levels has been demonstrated by several studies. Certain authors found a constant lipids to PCB ratio when considering different organs, which was not the case for the fishes we analysed.

. The relatively high PCB levels in the fishes of the Furans is to be stressed since this stream is remote from all industry. Other studies have shown that, in other rivers or lakes of the Rhone-Alpes region, the levels of PCBs are of the same order of magnitude. Still higher levels have been found in fishes from industrial areas (KECK, 1977 unpublished). This raises the question of the impact of this contamination on sensitive species such as the grayling. During recent years, this fish has occasionally failed to reproduce and its numbers in French rivers are progressively decreasing. PCBs may also be involved in the initiation of certain epidemics, especially a recent disease called " *mycosic syndrome* " that strikes the Salmonids at the spawning season. The diseased trout that we analysed in 1974-75 showed the same levels as healthy trout ; as to grayling, out of 3 diseased fishes caught in April 1976, 2 contained abnormally high PCB levels in their abdominal fat (29,6 and 46 ppm fresh weight). Although it is difficult to be affirmative in this controver-

sial subject, it appears that the long term effects of PCBs on reproduction or on the initiation of diseases in fishes, especially in sensitive species such as grayling, must be seriously considered and carefully studied.

ACKNOWLEDGEMENT

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