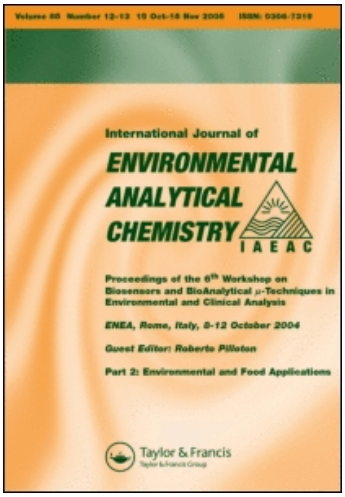

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Bioaccumulative Behaviour of Some PCB Congeners in Lake Geneva Brown Trout (*Salmo trutta lacustris* L.)

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Dedicated to Professor W. Haerdi on the occasion of his 60th birthday

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The bioaccumulation of PCBs has been studied for 60 Brown trout in Lake Geneva (Switzerland). The contamination level (1–3 ppm in wet weight, 10–30 ppm in lipid weight) is rather high.

Bioaccumulation curves (concentration as a function of the weight of the fish) have been established for 6 congeners and total PCBs. The total concentration of PCBs in wet weight increased with the weight of the fish; highly-chlorinated congeners (I.U.P.A.C. nr. 180, 2,3,4,5,2′,4′,5′-heptachlorobiphenyl) presented a faster, and lightly-chlorinated (I.U.P.A.C. nr. 28, 2,4,4′-trichlorobiphenyl) a slower, bioaccumulation tendency.

The total concentration of PCBs in lipid weight was constant, the concentration of congener 28 increasing and of congener 180 decreasing with the weight of the fish. This is attributed to the antagonistic effects of "growth and lipid dilution" and of the decrease of elimination kinetics as a function of the weight (and age) of the fish.

The partitioning theory and the pharmacokinetic approach are complementary methods for analysing this field data.

KEY WORDS: Bioaccumulation, PCBs, Salmonids, lipids, lake ecosystem, gas chromatography.

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1. INTRODUCTION

Polychlorobiphenyls (PCBs) are a family of very persistent pollutants which may have serious ecotoxicological consequences. They are bioaccumulated in aquatic environments and have been detected in many ecosystems near industrial regions and in particular in Lake Geneva (Switzerland).[1]

The bioaccumulative behaviour of a micropollutant greatly contributes to its potential ecotoxicity.[2] The contamination of fish as a function of their weight—or their age—(bioaccumulation curves) reflects an integral bioaccumulative behaviour and appears thus to be a useful tool for the study of global bioaccumulation phenomenon.

For this purpose, bioaccumulation of individual PCB congeners has received only limited attention. The application of modern analytical methods—capillary GC/ECD coupled with a suitable data treatment—shows the way in which the often considered total PCBs bioaccumulation reflects the behaviour of individual PCB congeners. Furthermore, bioaccumulation curves represent a particular interest for monitoring sampling strategies.[3]

2. MATERIAL AND METHODS

2.1 Sampling of fish

Analysed Brown trout (*Salmo trutta trutta* L.) have been caught in two different periods:

— in September 1984, 32 trout were trapped with nets in Lake Geneva at the prereproductive stage (gonads partially mature).

— in December 1984, 28 migrating trout were caught in the Aubonne River, a tributary of the Lake, with electric fishing at the beginning of the spawning season (gonads completely mature). Eggs of 10 females have been analysed separately.

Seven logarithmic weight classes were determined on the basis of the length–weight relationship of 83 fish caught in September 1984. Their limits are: 0.5, 1.0, 1.75, 2.75, 4.25, 6.25, 8.5 and 11.0 kg.

In the two first weight classes, trout were grouped by their sex and

analysed together. In September, fish have been selected according to their length so that each weight class averaged a total of about 7 to 8 kg.

2.2 Analysis

2.2.1 Sample preparation Whole fishes were cut and ground. A 40 g homogenized sample was digested overnight by a 2/3 (v/v) perchloric/acetic acid mixture[4] and extracted by 3×50 ml hexanes. A 5 g aliquot of extraction solvent was purified with 7% fuming sulfuric acid and injected in a gas chromatograph. All used reagents were Merck "*pro analysis*" and solvents Mallinkrodt "*for pesticide residue analysis*". The material was washed with a KOH-NaHCLO detergent solution (Contrad 90®, 5%) and rinsed with tested bidistilled water, acetone and hexanes. A blank was performed for every series of analyses.

Ten grams of the extraction solvent were evaporated for five days and weighed to determine the hexanes-extracted lipid content.[5]

2.2.2 Gas chromatography A Perkin–Elmer Sigma 2 gas chromatograph provided with a Supelco 30 m, i.d. 0.25 mm fused silica column coated by a SPB 5 bonded phase, and with an electron capture detector at 350 °C was used in injection *split* mode at 275 °C. The gas carrier was N_2. The oven heat temperature program began at 130 up to 265 °C with a ramp rate of 3.5 °C/min.

Sample injections were compared with a mixture [1:1:1] of Aroclor 1242, 1254 and 1260 (v:v:v) in the same range of concentration.

For peak confirmation, we used a fused silica capillary column (HP-5), crosslinked 5% phenyl methyl silicone, i.d. 0.20 mm—length 50 m—from Hewlett–Packard (Figure 1).

2.3 Data treatment

The calculation of total PCBs concentration was based on the computed peak surface of 33 congeners[6] (Perkin–Elmer LCl 100 integrator). To study the accumulation of individual PCB congeners, we selected six peaks which were assumed to contain mainly:

peak 1	congener	28	2,4,4′-trichlorobiphenyl
		31	2,5,4′-trichlorobiphenyl

peak 2	congener 52	2,5,2′,5′-tetrachlorobiphenyl
peak 3	congener 95	2,3,6,2′,5′-pentachlorobiphenyl
peak 4	congener 110	2,3,6,3′,4′-pentachlorobiphenyl
peak 5	congener 138	2,3,4,2′,4′,5′-hexachlorobiphenyl
peak 6	congener 180	2,3,4,5,2′,4′,5′-heptachlorobiphenyl

Peak identification was based on comparison with pure congeners. In the following text, peaks will be referred to by the I.U.P.A.C. number[7] of the main congeners that they represent (nr. 28, 52, 95, 110, 138, 180) (Figure 1).

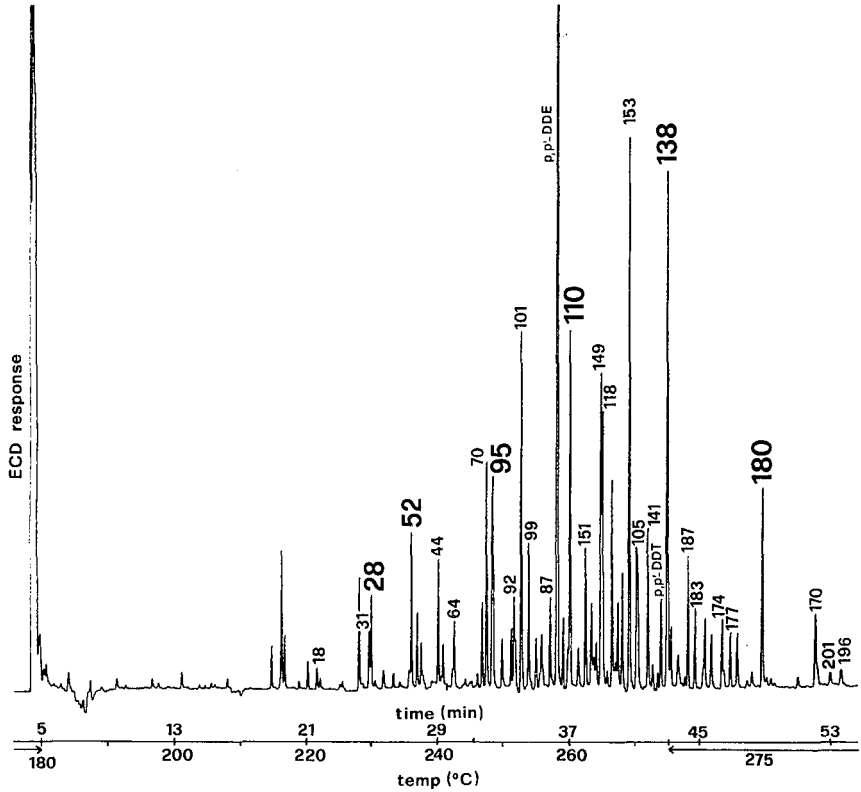

Figure 1 Chromatogram of PCBs extracted from a trout of 8.7 kg fished in December 1984 in Lake Geneva. PCB congeners corresponding to the main peak are identified by their I.U.P.A.C. number. Column: HP—5,50 m (see text).

3. RESULTS

3.1 Contamination of trout by total PCBs

3.1.1 In wet weight Total PCBs concentration in wet weight was higher in the trout group from December (1.2–2.9 ppm, mean $\pm$ s.e.: 2.2 ± 0.51 ppm, $N = 15$) than from September (0.37–2.32 ppm, mean $\pm$ s.e.: 1.45 ± 0.46 ppm, $N = 17$).

Bioaccumulation curves (concentrations in wet weight) were best fitted, among the exponent, exponential and logarithmic regressions, by the exponent method.

It was thus assumed:

$$Y = aX^b. \tag{1}$$

The regression revealed a relatively weak difference of total PCBs concentration between small (under 1 kg) and big (5–9 kg) trout (factor 2.5 in September and December). The weight-related increase in concentration came mainly from fish between 0.5 and 3 kg. From 3 to 9 kg, the concentration increase amounted only to about 25% (Figure 2). No contamination difference was observed between males and females.

3.1.2 In lipid weight The concentration of total PCBs in lipid weight was higher in the trout group from December (16.6–30.7 ppm, mean $\pm$ s.e. 23.6 ± 3.9, $N = 15$) than from September (6.2–16.8 ppm, mean $\pm$ s.e.: 10.7 ± 3.2 ppm, $N = 17$) (Figure 3). The dispersion of concentrations was higher than in wet weight.

We noted a remarkable constancy of lipid-weight concentration of total PCBs as a function of the weight of the fish. An increase of lipid content (4 to 18%) parallel to the increase of total PCBs concentration in wet weight was observed.

3.1.3 Contamination of the eggs The concentration of total PCBs in the eggs of 10 mature females caught in December is between 0.6 and 3.0 ppm in wet weight (mean $\pm$ s.e.: 1.21 ± 0.72 ppm), between 10 and 45 ppm in lipid weight (mean $\pm$ s.e.: 16.2 ± 10.5 ppm). The wet-weight concentration of the considered congeners was less in the eggs than in the remaining body (factor comprised between 2.0 for congener nr. 28 and 3.6 for congener nr. 180, 2.45 for total PCBs).

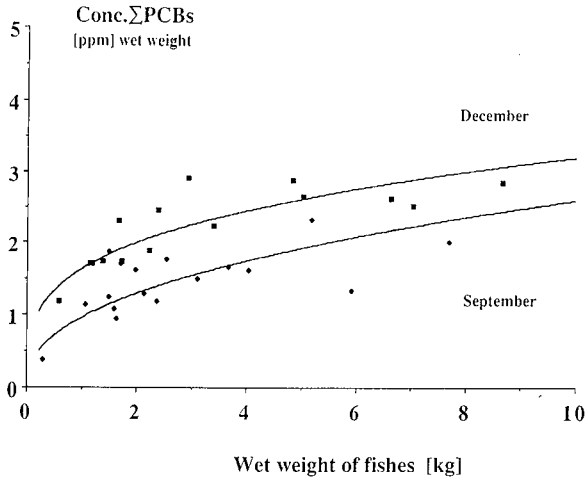

Figure 2 Wet-weight bioaccumulation curves of $\sum$PCBs (wet-weight concentration as a function of the weight of the fish). The equations of the adjusted curves are:

$$y = 0.96 \cdot X^{0.43} \qquad r = 0.79 \text{ in September}$$

$$y = 1.64 \cdot X^{0.29} \qquad r = 0.88 \text{ in December}$$

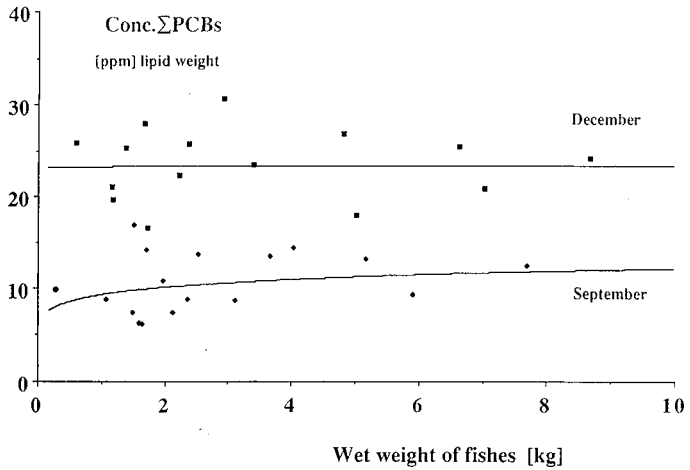

Figure 3 Lipid-weight bioaccumulation curves of $\sum$PCBs (lipid-weight concentration as a function of the weight of the fish).

This difference was smaller if the concentration is expressed in lipid-weight (corresponding factor: 1.5 for congener 28, 2.8 for congener 180 and 1.8 for total PCBs).

Spawning represented about a 9% loss of total PCBs for females, varying between 8% (congener 180) and 12% (congener 28).

PCBs were not distributed quantitatively with lipids, as the ratio

[(% PCBs eliminated by the eggs)/(% lipids eliminated by the eggs)]

was not equal to one. As this ratio was lower for highly-chlorinated biphenyls, these compounds appeared to be less mobile than lightly-chlorinated biphenyls (Figure 4). Nevertheless, spawning resulted only in a weak enrichment in highly-chlorinated biphenyls (5% for congener 180) for females.

Six regularly spaced samples of eggs of the same chain showed a constant concentration of the six considered congeners and of total PCBs.

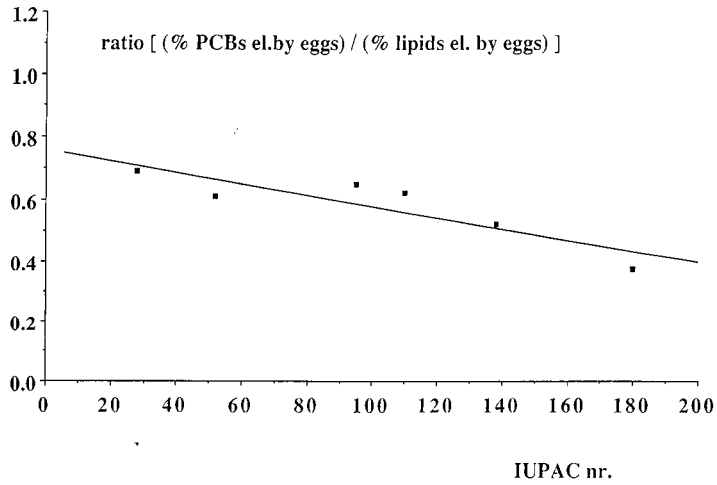

Figure 4 Role of lipids in the elimination of some PCB congeners by spawning: ratio [(% of PCB eliminated in the eggs)/(% of lipids eliminated in the eggs)] as a function of their I.U.P.A.C. nr.

3.2 Bioaccumulative behaviour of PCB congeners

3.2.1 In wet weight The bioaccumulative behaviour of PCB congeners showed obvious differences. Lightly-chlorinated biphenyls concentration in wet weight regularly increased during the life of the trout (congener 28, Figure 5). For the young fish, the rate of increase of highly-chlorinated congeners (congener 180, Figure 5) was greater.

The four other considered congeners had an intermediate behaviour between the extreme tendencies of congeners nr. 28 and 180 (Figure 6).

Furthermore, the September bioaccumulation curve for total PCBs reflected a slower increase of wet-weight concentration as a function of the weight of the fish than in December (Figure 6).

3.2.2 In lipid weight For trout caught in September and in December, the lipid-weight bioaccumulation curves showed a constancy or a very slight increase in the concentration of medium-chlorinated congeners as a function of the weight of the fish. This increase was much more apparent for congener 28. However, the concentration of congener 180 in lipid weight decreased as a function of the weight of the fish (Figure 7).

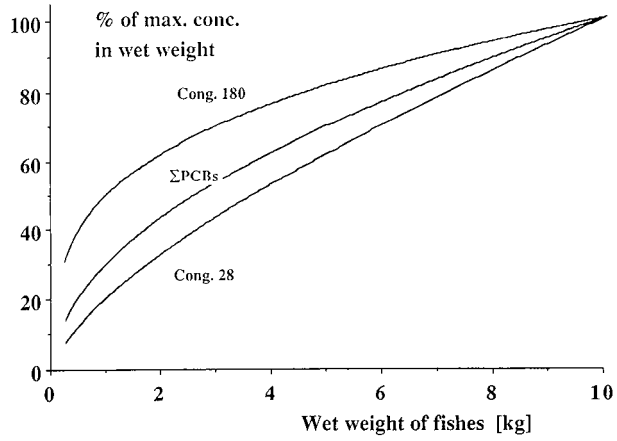

Figure 5 Wet-weight bioaccumulation curves of PCB congeners and of $\sum$PCBs for fish caught in December '84. To improve the comparison, concentrations are expressed as a percent of the maximum observed concentration.

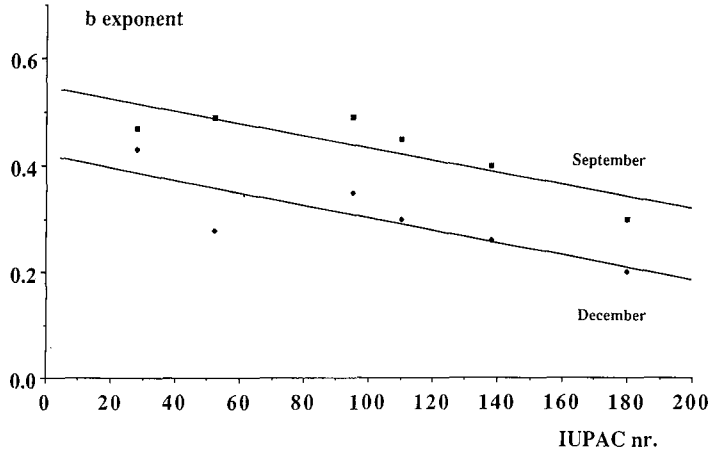

Figure 6 Computed *b* exponent of regression equation describing the shape of wet-weight bioaccumulation curves for some PCB congeners. The bioaccumulation is more progressive as a function of the weight of the fish when the value of the *b* exponent increases.

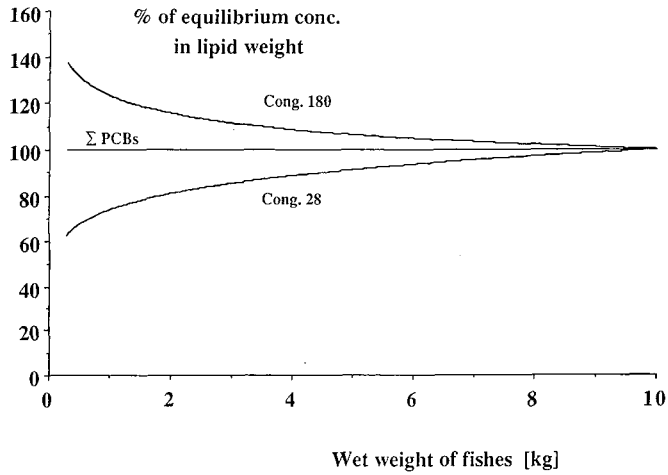

Figure 7 Lipid-weight bioaccumulation curves of two congeners and of $\sum$PCBs for fish caught in December '84. To improve the comparison, concentrations are expressed in percent of the maximum observed concentration. An exponent method of regression is applied to the three curves[1] (see text).

4. DISCUSSION

4.1 Level of contamination and trend

The observed PCB concentrations in Brown trout from Lake Geneva can be considered as rather high. Although clearly lower than the concentration in Salmonids from the US Great Lakes in the '70s,[8,9] it is higher than in many ecosystems in industrial regions.[10,11]

Furthermore, despite a strict limitation on the use of PCBs in Switzerland since 1972, our data do not show in Lake Geneva, as observed elsewhere,[11,12] a clear decrease of the contamination of trout.[1,13,14] Its contamination must be considered at best as stable.

The maximum legal concentration in Switzerland in the edible portion of fish is 1 ppm in wet weight.[15]

Reproduction inhibition appears at very low concentration by Salmonids: *in natura*, egg mortality is highly correlated to PCB concentrations of 10 to 78 ppm (lipid weight) in Charr eggs (*Salvelinus alpinus*)[16] and of 1.0 to 1.9 ppm (wet weight) in Atlantic Salmon eggs (Johansen, in Ref. 16). The concentration measured in the trout of Lake Geneva must thus be considered as serious from an alimentary and an ecotoxicological point of view.

4.2 Bioaccumulation curves of total PCBs

4.2.1 In wet weight The concentration of total PCBs in Brown trout slowly increases during their life. This implies that their body burden of total PCBs is increasing more rapidly than they grow, which compensates the dilution effect resulting from growth.[17,18,19,20] Such an increase of contamination during the life of the fish has been reported by many authors for PCBs[5, 9, 20, 21, 22, 23, 24], DDT[25, 26, 27] and mercury.[20,28]

Nevertheless the shape of the bioaccumulation curve varies greatly according to the species and to the ecological conditions of the study. For example, Bache *et al.*[9] observed an important difference of concentrations (1.5 to 26 ppm) of total PCBs for Lake trout (1 to 12 years), but Ernst *et al.*[21] did not report any significant correlation between age and wet weight in Yellow gurnard (*Trigla lucerna*).

The shape of the bioaccumulation curve is very important from an ecotoxicological point of view,[20,23] since it determines the dif-

ference of concentration levels between young (=small and immature) and old (=big and mature) fish.

Sex and initial PCB burden of eggs did not influence the shape of bioaccumulation curves.

4.2.2 In lipid weight We observed a remarkable constancy of lipid-weight concentration of total PCBs as a function of the weight of fish. Many authors made a similar observation for PCBs[3,12,26,29] and DDT,[27] although Burgermeister *et al.*[5] reported a decrease of the lipid-weight concentration as a function of the weight for Burbot (*Lota lota*).

The increase of concentration in wet weight thus reflects an increase of the lipid content as a function of the weight of the fish. So Lieb *et al.*,[30] in contamination experiment with Rainbow trout, observed an apparent PCB plateau level in lipid weight, but an increasing concentration in wet weight.

As fish consume their lipids during the prespawning season, their concentration in lipid weight is higher in December (spawning) than in September. This difference is much more apparent in lipid weight than in wet weight. This clearly points out the importance of fish metabolic processes for the ecotoxicological risks of lipophilic chemicals.

4.3 Bioaccumulation curves of individual PCB congeners

PCBs are a mixture of numerous congeners that differ in their chlorination degree and stereochemical configuration.

We observed in our study a significant difference in the bioaccumulation behaviour of some individual congeners. Lightly-chlorinated congeners present an apparent "slow bioaccumulation" as highly-chlorinated show an apparent "fast bioaccumulation" during the life of the fish. Few data are available on this subject in the literature. Bache *et al.*,[9] working with a packed column, did not observe any difference between groups of PCBs. Weigelt[31] reported no decisive influence of the age of marine fish on the qualitative composition of PCB compounds (on capillary column).

The pollutant pressure and biology of fish determine the exposure of fish to pollutants at a given time. This may influence the shape of the bioaccumulation curves.[32] In our case, such an influence is improbable, all the analysed fish being mature and thus having spent at least one year in the lake. This allows an equilibration and

minimizes the effect of their contamination during the first years of their life in the river (2 to 3 years[33,34]), which represents only 20 to 30 percent of their PCB body burden. Furthermore, the representation of PCB congeners accumulated by River trout (*Salmo trutta fario*) in a tributary does not allow us to explain the differential bioaccumulative tendencies.

Lipids play an important part as a storage and exchange medium.[35] According to the partitioning theory, bioaccumulation of lipophilic chemicals by fish results from a passive process of thermodynamic equilibrium between water and lipids of the biota.[36] This theory allows us to explain the often observed constancy of PCBs mixture concentration in lipid weight as a function of the weight of the fish.

Nevertheless, our data for individual congeners are inconsistent with this theory. We expected the concentration of individual congeners in lipid weight to be constant as a function of the weight of the fish, but this was not the case (Figure 7). The equilibrium process for lipophilic compounds requires a long time,[18] longer for highly-chlorinated than for lightly-chlorinated congeners.[37] But this long equilibration time does not allow us to explain the opposite tendencies of congeners nr. 28 and 180.

We believe that physiological and pharmacokinetic elements have to be considered. The difference between the shape of bioaccumulation curves of both congeners is mainly visible for young mature trout. These are characterized by a marked increase of their lipid content and by a high but decreasing specific metabolism.[20,33,38]

For congener 28, which is more rapidly eliminated than congener 180,[19,39] the high level of specific metabolism of young trout induces an important elimination. The influence of age on elimination kinetics has been demonstrated for methylmercury.[40] Elimination is then predominant on the dilution effect and results in an increasing concentration in lipid weight as a function of the weight of the fish.

On the contrary, dilution of congener 180 in the fast growing lipid pool is predominant on its elimination and results in a decreasing concentration in lipid weight as a function of the weight of the fish.

Our field data show the complementarity of both partitioning and pharmacokinetic theories. The equilibrium concept appears to be valid but must be complemented by a kinetic approach.[41] Observed concentrations thus reflect a pseudo-equilibrium. The theoretical equi-

librium appears to correspond to a concentration that is approached at a velocity influenced by biological and physico-chemical factors.

Thus, in elimination experiments, although metabolisation might take place according to the nature of the chemicals involved,[42,43] elimination may reflect a reequilibration of the lipophilic pollutants between the fish and the newly uncontaminated medium. This is supported by some studies showing a strong correlation between elimination and absorption kinetics and on the one hand the partition coefficient of chemicals,[43,44] on the other hand the lipid pool size of the fish.[45]

The importance of the composition of the PCBs mixtures on the shape of their bioaccumulation curves is underlined. The lipid-weight bioaccumulation curve of total PCBs reflects the respective contributions of individual congeners that have a specific bioaccumulative behaviour. Its marked constancy results from a well balanced mixture of congeners in the ecosystem.

This study shows that from an ecotoxicological point of view the impact of a multicomponent family of pollutants, like PCBs, has to be studied not as a whole but through some selected significant individual congeners. For this purpose, recent developments in analytical chemistry and commercial availability of pure individual congeners of these types of pollutants are useful and appropriate tools.

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