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Elimination of PCBs from Heavily Contaminated Carp (*Cyprinus carpio* L.) in Clean Water-Depuration Study

J. Hajšloviá,[1] R. Schoula,[1] V. Kocourek,[1] P. Gregor,[1] J. Kohoutková[1]
K. Holadová, J. Poustka,[1] Z. Svobodová,[2] B. Vykusová[2]

[1]Department of Food Chemistry and Analysis, Institute of Chemical Technology, Technická 3, 16628 Prague 6, Czech Republic
[2]Research Institute of Fish Culture and Hydrobiology, Vodnany, Czech Republic

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Crash-pollution of aquatic environment by hydrophobic organochlorine compounds is more or less immediately reflected by increased levels of these substances in fish, the use of which as a biomonitor was thoroughly discussed (de Boer et al. 1994a). Depending on many local natural parameters, successive partitioning of contaminants among all phases present, i.e. water, biota, sediments and suspended particulate matter takes place over the time. Bioaccumulation and biomagnification of PCBs in aquatic organisms as well as their elimination was investigated (Opperhuizen 1987, Sijm et al. 1992, Gobas et al. 1993). In general, the kinetics of these processes are determined by K_{ow} - the octanol/water partition coefficient - the value of which is related to the chlorine substitution pattern (Mackay 1982, Coristine et al. 1996). Contrary to the warm-blooded organisms, biotransformation in fish is relatively slow even for PCBs with lower chlorine content (Boon et al. 1989).

Some decrease of total PCBs in various fish species was recorded in several studies (Bruggeman et al., 1984, Boon et al., 1985) concerned with elimination of these contaminants after the termination of bioconcentration phase, nevertheless, significant differences (even orders of magnitude) exist in reported half-lives for particular congeners. This fact can be attributed to variances in experimental set-up e. g. duration of experiments, the ratio of gill area to body weight of studied fish species, their migratory behaviour, frequency of spawning etc. In general, a decrease of elimination rate is characteristic for increased hydrophobicity of particular congener. The contribution of biotransformation and physicochemical processes to elimination of PCBs was discussed in detail elsewhere (de Boer et al. 1994b).

In the present study, heavily contaminated carps living almost 3 years in Skalice river polluted by extensive leakage of Delor 103 (PCBs containing technical mixture with 48 %, w/w, chlorine) from local industry were transferred into clean water. Experiments conducted during subsequent months were aimed at investigation of concentration changes of 7 indicator PCBs (representing wide chlorination range) which are used for regulation purpose.

Correspondence to: J. Hajšloviá

MATERIALS AND METHODS

In April 1993, 43 carps *(Cyprinus carpio L.)*, almost 3 years old, were caught using electric fishing in Skalice river located in Central Bohemia. From this extensively polluted locality (breakdown contamination occurred in 1986) carps were transferred to an experimental pond close to Vodnany (South Bohemia) owned by Research Institute for Hydrobiology and Fisheries. The area of this reservoir was 1000 m^2, its depth 1.5 m; the levels of PCBs in water and sediments (only limited amount present) corresponded to the background levels typical for unpolluted area. Due to a limited availability of natural food (bentos) the carps were fed by barley for the period of 15 months and then the supply of this feed was ceased. Starting at the day of transfer, 5 fish subsamples, each consisting of 6 carps, were collected (always in April and November) during the experiment lasting 25 months, see **Table 1.** Filets (skin removed), gonads and livers were isolated from each fish and stored frozen at -18 °C until analysed. Leeches *(Erpobdella sp.)* were collected at the bottom of Skalice river in April 1993, at the same sampling place where fishes were caught.

Lipid content was determined gravimetrically: fish / leeches sample was thoroughly ground, mixed with anhydrous sodium sulphate and then lipids were extracted with acetone - hexane mixture, 1: 1, v/v (250 ml per 100 g).

Determination of indicator PCBs was carried out similarly as described in our previous study (Hajšlová et al. 1995). The method consisted of GPC clean-up of isolated fat and subsequent analysis of PCBs containing fraction by GC/ECD (two columns with different polarity in parallel).

RESULTS AND DISCUSSION

Prior to the transfer into the unpolluted experimental pond, the carps were living in extensively contaminated aquatic environment in Skalice river for the period of three years. The composition of PCBs-containing technical mixture Delor 103, the leakage of which polluted this locality, resembled that of Arochlor 1242 with major fraction of tri- and tetrachlorobiphenyls. Very similar PCBs pattern was proved in sediments, however, the composition of PCBs fraction isolated from fish was rather different : although indicator congeners 28 and 52 representing less chlorinated species were still dominating among other PCBs monitored in this study, see **Table 2.**, relatively high levels of hexachloro- (PCB 138 and 153) and heptachorobiphenyls (PCB 180) were present in analysed samples thus documenting extensive biomagnification of these highly hydrophobic congeners. The extent of this process in carps from “clean” water (control fish, background contamination only) was even more pronounced with dominating congeners 138, 153 and 180, see **Figure 1.** As regards reflecting sediments and bentos contamination in particular location, leeches *(Erpobdella sp.)* were shown to be very suitable. These non-migrating invertebrates were suggested as biomonitors for long-term monitoring programme concerned with rivers pollution.

Table 1. Characteristics of particular fish subsamples analyzed during experiment (control : 3 year old carps living in clean experimental lake)

sampling period No.	1	2	3	4	5	control
months of depuration	**0**	7	**11**	**19**	**25**	-
No. of carps	6	6	6	6	6	6
(males)	*(2)*	*(2)*	(3)	*(2)*	*(3)*	*(3)*
mean weight of fish in gr.	3110	3870	3940	3080	1900	4330
range	*2200-5500*	*2400-5250*	*2100-5050*	*2200-3700*	*1700-2100*	*2700-6700*
mean lipid content in %						
filets	2.74	2.33	2.09	0.45	0.39	3.87
	0.80-7.50	*1.44-3.40*	*1.43-3.03*	*0.24-0.75*	*0.33-0.46*	*2.04-5.93*
livers	0.89	2.42	1.94	1.28	1.47	3.99
	0.23-1.28	*1.25-3.76*	*1.49-3.03*	*1.19-1.42*	*1.30-1.53*	*1.60-11.52*
gonads	0.83	2.88	1.90	1.35	1.69	1.12
	0.40-1.61	*1.78-3.86*	*1.06-2.58*	*0.98-2.48*	*1.05-2.48*	*0.57-1.70*

PCBs concentrations that were determined in fish samples at the first sampling period exceeded hygienic limits enforced in Czech Republic which state the value of MRL for freshwater fish 0.5 mg/kg (expressed as an equivalent of PCBs technical mixture, on fresh-weight basis). Compared to the value 1 mg/kg (sum of indicator congeners, on fat basis) proposed for updated hygienic decree, the concentration of investigated contaminants were higher even by one order of magnitude.

As can be seen from **Figure 2.**, successive decrease of lipid-normalised concentrations of the sum of indicator PCBs occurred between first and third sampling periods. The most distinct concentration drop was observed in all analysed tissues (for muscle see **Figure 3.**) for congener No. 28 (2,4,4' - trichlorobiphenyl) representing the group of readily metabolisable PCBs with free vicinal *meta* and *ortho* position and one chlorine atom in *ortho* position (Hutzinger et al. 1972, Boon et al. 1989). The relative ratio of other PCBs was not changed distinctly during these three sampling periods and small reduction of their concentration in lipids could be attributed to some growth-dilution of body burden. Under starvation conditions (fourth and fifth sampling), rapid increase of PCBs in fat occurred due to the significant decrease of lipids content esp. in muscle. Further noticeable reduction of congener No. 28 could be seen in fourth sampling, in fifth

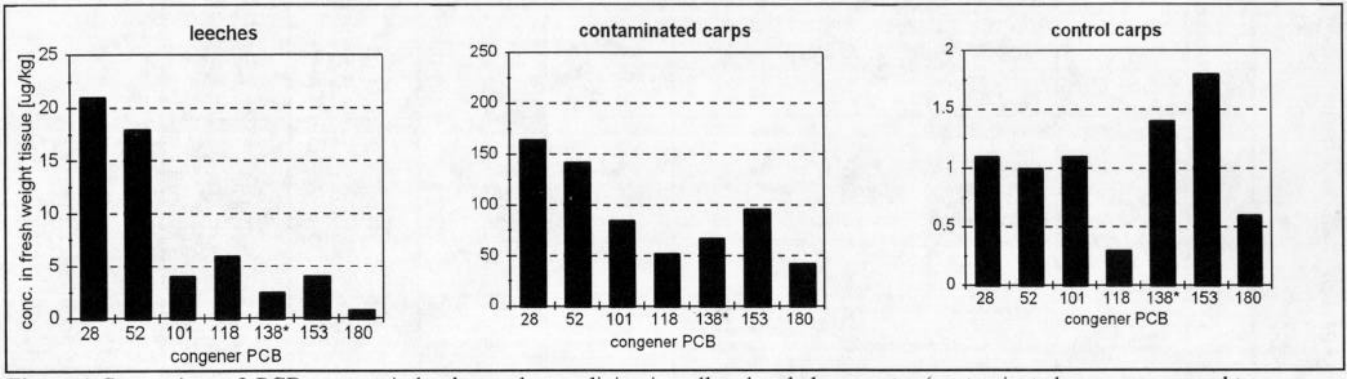

Figure 1 Comparison of PCBs pattern in leeches and carps living in polluted and clean water (contaminated carps were caught at Skalice river, April 1993)

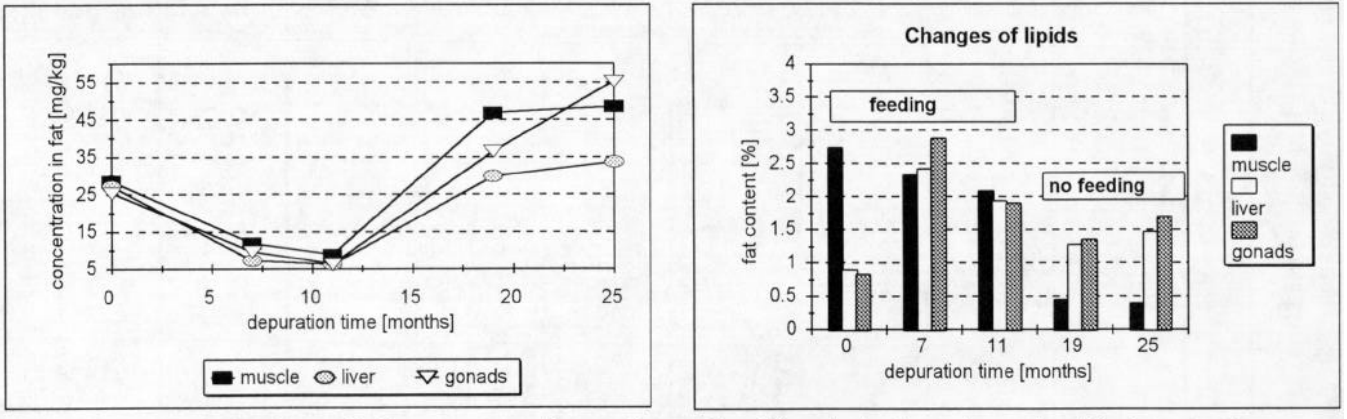

Figure 2 Changes of PCBs (sum of indicator congeners) in muscle, gonads and liver during depuration study - left; fat content in respective samples - right

Table 2. Concentration changes of indicator congeners in examined carp samples during depuration study (mg/kg fresh tissue)

	Months					
	0	7	11	19	25	Control
PCB 28						
muscle	0.164	0.037	0.019	0.004	>0.001	0.001
liver	0.057	0.023	0.012	0.008	0.001	0.001
gonads	0.056	0.032	0.013	0.013	0.001	>0.001
PCB 52						
muscle	0.142	0.089	0.039	0.039	0.014	0.001
liver	0.052	0.031	0.024	0.077	0.036	0.001
gonads	0.054	0.048	0.025	0.121	0.066	>0.001
PCB 101						
muscle	0.085	0.026	0.021	0.026	0.025	0.001
liver	0.034	0.019	0.013	0.055	0.056	0.001
gonads	0.017	0.034	0.014	0.073	0.139	>0.001
PCB 118						
muscle	0.052	0.016	0.022	0.020	0.025	>0.001
liver	0.022	0.014	0.014	0.043	0.080	>0.001
gonads	0.011	0.020	0.013	0.063	0.126	>0.001
PCB 138*						
muscle	0.067	0.041	0.025	0.033	0.040	0.001
liver	0.031	0.031	0.017	0.070	0.103	0.002
gonads	0.014	0.053	0.015	0.091	0.119	>0.001
PCB 153						
muscle	0.096	0.041	0.034	0.040	0.047	0.002
liver	0.043	0.031	0.023	0.084	0.130	0.002
gonads	0.020	0.053	0.022	0.107	0.258	>0.001
PCB 180						
muscle	0.042	0.025	0.020	0.026	0.032	0.001
liver	0.021	0.018	0.014	0.055	0.091	>0.001
gonads	0.008	0.030	0.012	0.071	0.195	>0.001
SUM PCBs						
muscle	0.648	0.275	0.180	0.188	0.183	0.008
liver	0.260	0.167	0.117	0.392	0.495	0.007
gonads	0.180	0.270	0.114	0.539	0.904	0.002

* expressed as a sum of PCB 138 and PCB 163

one only traces of this PCB were found, in addition, pronounced decrease of PCB 52 was found. This can be explained by increased availability of non-polar organic contaminants that are stored in the triglycerides during the mobilisation of energy reserves.

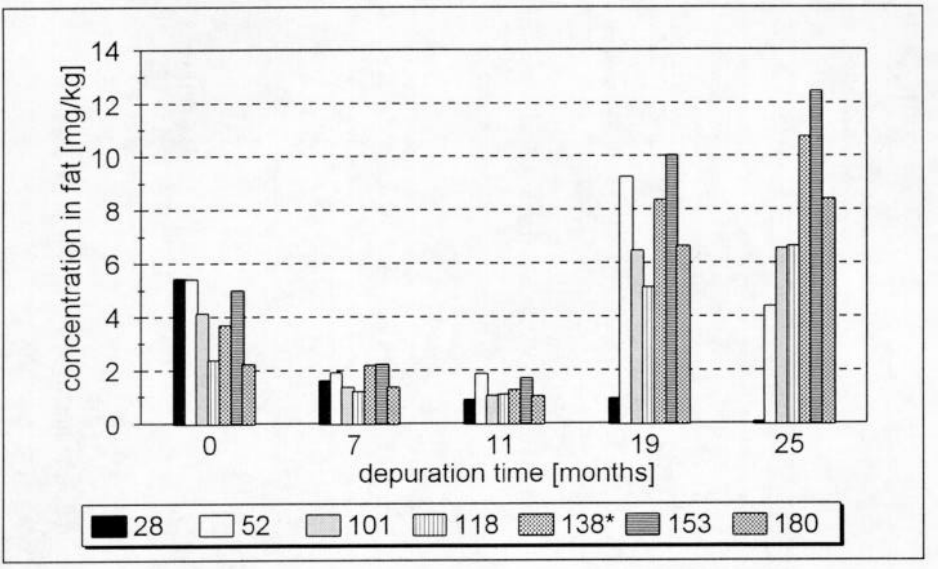

Figure 3. Changes of indicator congeners in carps muscle during depuration study

In **Figures 4.** and **5.** there are shown relative changes of indicator PCBs in muscle, liver and gonads during this depuration study. While these values, when calculated on lipid basis did not change in a wide range, successive decrease of muscle/liver and muscle/gonads PCBs concentration ratios when expressed on the basis of fresh tissue was documented. To interpret this phenomenon, several aspects have to be considered. For gonads, fat content increase that was observed in the second sampling was reflected in dilution of PCBs content and thus drop of respective ratio. As regards spawning, it should be noticed that some clearance of female fish may occur on its account; this fact could be reflected in slight increase of muscle/gonads ratio in the third sampling. Surprisingly, identical distribution of all congeners among analysed tissues was recorded in last two samplings.

It can be attributed to changes of lipids composition that occurred as a result of starvation. Neutral lipids (triglycerides) were preferably metabolised under these conditions thus leaving more polar fraction in muscle. The importance for determining congeners distribution was pointed out by Kammann (1990). Based on these considerations, similar composition of lipid fraction in muscle, liver and gonads and, consequently, similar solubility of PCBs in this fraction may explain our results.

In any case, although apparent “decontamination” of filets took place (the decrease of total PCBs in this edible tissue occurred), in accordance with other studies (de Boer, 1994a), no decrease of total fish body burden for highly chlorinated PCBs was recorded.

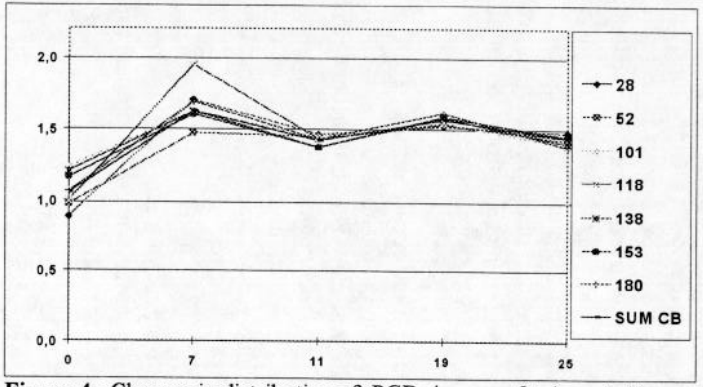

Figure 4a Changes in distribution of PCBs in carps fat (muscle/liver ratio)

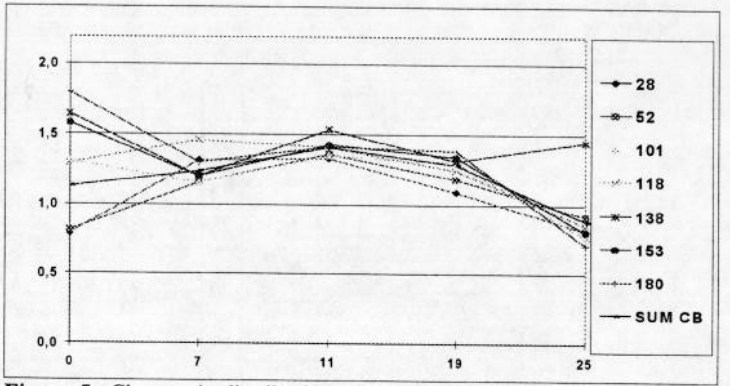

Figure 5a Changes in distribution of PCBs in carps fat (muscle/gonads ratio)

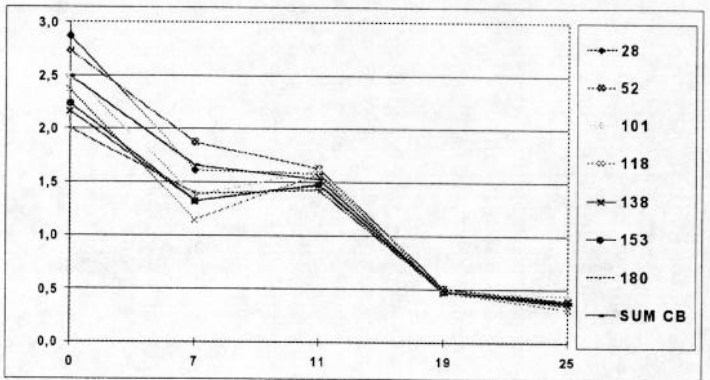

Figure 4b Changes in distribution of PCBs in carps fresh tissue (muscle/liver ratio)

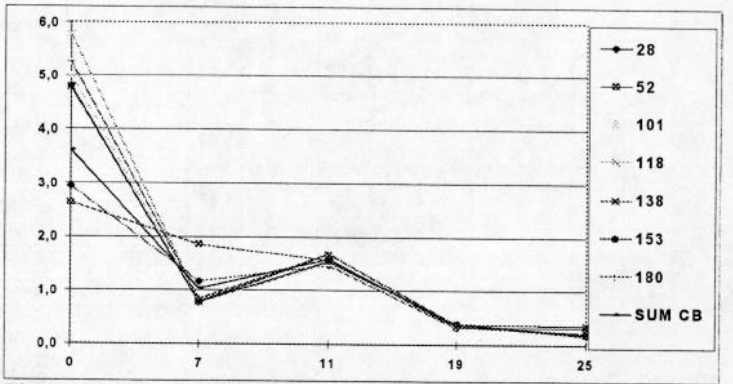

Figure 5 b Changes in distribution of PCBs in carps fresh tissue (muscle/gonads ratio)

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