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Organochlorine Pollutants in Chub, *Leuciscus cephalus*, from the Svratka River, Czech Republic

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Abstract The aim was to evaluate the extent of pollution of the Svratka River with organochlorinated pollutants using the chub (*Leuciscus cephalus*) as a suitable bioindicator of the river contamination. The sum of 7 PCB congeners was found in range of 7.6–93.8 ng/g (with median of 31.7 ng/g) wet weight (ww), DDTs of 2.9–93.8 (29.9) ng/g ww, HCHs isomers of 0.1–5.3 (1.0) ng/g ww, HCB of 0.3–8.4 (2.2) ng/g ww and OCS of <0.1-0.5 (0.1) ng/g ww. Our results confirm predominance of metabolite DDE and higher-chlorinated PCB congeners and are comparable with similar studies and findings within the Czech Republic.

Keywords *Leuciscus cephalus* · PCBs · DDT metabolites · HCB · OCS

Persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs) or pesticides including dichlorodiphenyltrichloroethane and its metabolites (DDTs), hexachlorobenzene (HCB) or isomers of hexachlorocyclohexane (HCHs) have been known as global contaminants of the environment for decades. Despite the fact they were banned from production or strongly restricted in use many years ago the POPs still continue to be found in various samples from the environment. POPs have been shown to disturb and damage many systems in the animals and humans incl. nervous, hormonal and immune systems (Tyler et al. 1998). There have been several monitoring campaigns carried out in the Czech Republic concerning the issue of POPs in the ambient air (Dvorska et al. 2008) or in aquatic ecosystems incl. rivers or water reservoirs (Maršálek et al. 2004; Svobodová et al. 2003, 2004). In aquatic ecosystems, fish generally are suitable bioindicators of the contamination due to their low metabolic activities and to their position within the food chain. They concentrate pollutants in their tissues directly from water, but also through their diet, thus enabling the assessment of transfer of pollutants through the trophic web (Fisk et al. 2001). Among others, chub is one of the most common cyprinid fish species in the Czech watercourses which is very suitable for both ecotoxicological studies and pollution monitoring (Široká et al. 2005; Christoforidis et al. 2008; Randak et al. 2009). Chub were also used for determining levels of major brominated flame retardants and related chlorinated organic pollutants in two main Czech rivers, Elbe and Vltava (Hajslova et al. 2007).

The River Svratka (Czech Republic) is a tributary of the River Dyje, it is 173.9 km long with a catchment area of 7118.7 km^2. For it runs through Brno, the second largest city in the Czech Republic, increased levels of anthropogenic contaminants incl. organochlorine pollutants in this aquatic ecosystem may be expected. Chub from the Svratka River were used to assess the level of contamination using specific biochemical markers as CYP 450 and EROD (Havelkova et al. 2008) and biliary 1-hydroxypyrene as a biomarker of exposure to polycyclic aromatic hydrocarbons (Blahova et al. 2008).

The main aims of our study were (a) to assess the current load of selected freshwater fish species with POPs in the River Svratka, (b) to compare differences in levels of POPs in fish from distant and not connected localities within the

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same watercourse and (c) to assess potential variations due to different sampling periods.

Materials and Methods

During August and October 2007, specimens of the European chub (*Leuciscus cephalus*) were sampled using an electrical device. Basic characteristics of the specimens caught are in Table 1. In total four sets of fish were taken from two different sites at the Svratka River, mutually parted by a weird, so that the fish were not supposed to migrate between them (see the map in Fig. 1). The first sampling site was located 0.5 km upstream to the Brno Waste water treatment plant Modřice (WWTP Modřice), the second site was near the village of Rajhradice (4 km downstream to the WWTP Modřice). Immediately after catching, fish were weighed and tissues (muscle, skin and entrails – i.e. the whole content of the abdominal cavity) were sampled. Labelled samples were placed in microtone bags and stored in a freezing box at −18°C until analysis. Homogenised samples were desiccated by anhydrous sodium sulphate and extracted by petroleum ether solvent (6 h.) in a Soxtec apparatus. Extracts were evaporated by means of a rotary vacuum evaporator and an aliquot part of fat portion (about 0.2 g) was dissolved in *n*-hexane (3 mL) and cleaned up on a Florisil column (90 mL of *n*-hexane:diethyl ether mixture, 94:6, v/v). After solvent evaporation and dissolving the residue in 1 ml of *n*-hexane, quantification of target analytes was carried out by high-resolution gas chromatography (two capillary columns HT-8 and DB-17 ms operated in parallel) employing two electron capture detectors (HRGC/2 × μ-ECD). Individual tissue samples were analysed for the content of DDT and its metabolites, HCH isomers, HCB, OCS, and 7 indicator PCB congeners (No. 28, 52, 101, 118, 138, 153, and 180). Limits of quantifications (LOQ) for all analytes were 0.13 ng/g tissue sample (for 5% lipid content).

The results were tested using ANOVA in MS Excel 2003 in order to assess the differences of POPs levels among investigated fish in terms of the sampling site, sampling period or individual tissues. Hygienic limits concerning fish

Fig. 1 Map of the Czech Republic with sampling localities

quoted in the Decree of the Ministry of Health of the Czech Republic No. 381/2007 Coll. and 306/2004 were used.

Results and Discussion

Shown in Fig. 2 are the concentrations of organochlorine pollutants determined in fish tissues from two sampling localities and two sampling periods.

Mean levels of PCBs in muscle of chubs from Modřice were 41.9 and 30.8 ng/g ww for the spring and autumn sampling, respectively, and at Rajhradice, the means were determined as 38.8 and 38.6 ng/g ww, resp. There were no statically significant differences in levels of PCBs between both sampling localities ($p < 0.05$) and between the sampling periods ($p < 0.05$) within each locality. Observed differences and varying ranges of values in individual sets of samples might be caused by different weights and metabolism of fish rather than by 'outer' factors including the assumed contribution of the wastewater treatment plant in Modřice (WWTP).

An interesting value for a complex evaluation is the weighed mean, taking the different body weights of fish from each set into consideration (Table 2). Then, the weighed means for the Modřice locality were calculated as 42.5 and 30.7 ng/g wet weight (in spring and autumn, resp.) and for Rajhradice as 40.6 and 38.9 ng/g ww,

Table 1 The main characteristic of sampled chub (*Leuciscus cephalus*)

Date	Locality	n	Body length (mm) (mean ± SD)	Body weight (g) (mean ± SD)	Age (years) (min–max)
Apr 2007	Rajhradice	9	266 ± 25	252 ± 62	4.0 (3–5)
Apr 2007	Modřice (WWTP)	10	247 ± 10	204 ± 33	3.5 (3–4)
Oct 2007	Rajhradice	11	241 ± 19	135 ± 36	2.6 (2–4)
Oct 2007	Modřice (WWTP)	10	262 ± 43	157 ± 74	3.1 (2–4)

n = number of specimens examined

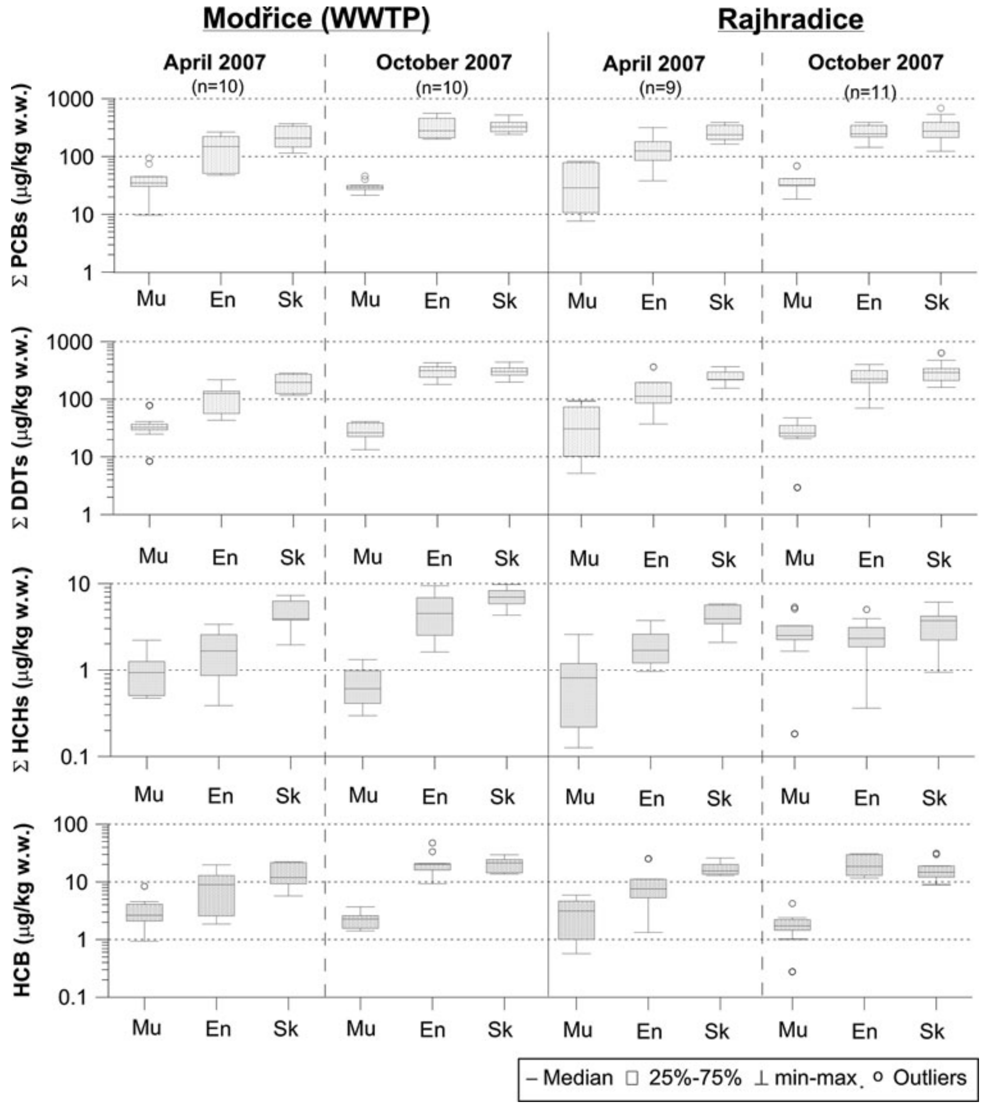

Fig. 2 The log-transformed concentrations (µg/kg wet weight) of organochlorine pollutants in the tissues of chub from the Svratka River (Mu = muscle; En = entrails; Sk = skin)

Table 2 Weighed means of organochlorine pollutants in muscle of the chub (ng/g of tissue, ww)

Compound/locality	PCBs[a]		DDTs[b]		HCHs[c]		HCB	
	Apr[d]	Oct[d]	Apr[d]	Oct[d]	Apr[d]	Oct[d]	Apr[d]	Oct[d]
	Muscle samples							
Modřice	42.5	30.7	34.9	29.4	1.0	0.7	3.2	2.3
Rajhradice	40.6	38.9	40.0	28.8	0.9	2.6	2.9	2.0

[a] Sum of 7 indicator congeners (28, 52, 101, 118, 138, 153, 180)

[b] Sum of DDT and its metabolites (*o,p′*- and *p,p′*-DDT; *o,p′*- and *p,p′*-DDE; *o,p′*- and *p,p′*-DDD)

[c] Sum of HCH isomers (α, β, γ)

[d] Apr = April; Oct = October

respectively. The former sampling area showed a decline in the value of weighed mean, however, the change was generally not of great significance and could be caused e.g. by abundance of non-contaminated food between two sampling periods. The decrease in weighed mean values is more marked at the locality of Modřice. The highest concentrations of PCBs ranging from 115.1 to 681.7 ng/g ww were found in skin of all samples, followed by entrails with a range from 37.9 to 559.7 ng/g ww. The lipid content determining the levels of PCBs in the tissues was on average 13.2% in skin, 12.3% in entrails and 2.2% in muscle. Contrary to the relatively invariable lipid content in muscle and skin in all samples (on average 1.62%–2.96% in muscle and 9.93%–15.9% in skin), significantly higher lipid content in entrails ($p < 0.01$) was observed in specimens from the autumn sampling (12.42%–17.78%) than in those from the spring season (9.35%–9.58%), which was true for both sampling sites. Since the levels of PCBs in lipids from different tissues did not vary significantly ($p < 0.01$), the concentrations of PCBs in entrails of the ‘autumn’ specimens were similar to those in skin. This was also true for the other contaminants monitored. Higher lipid contents in entrails fish caught in the autumn might be explained by feeding activity over summer, whereas during hibernation in winter the fat reserves are metabolised to energy.

Predominating PCB congeners in all tissues were the higher chlorinated CB 138, 153, and 180. These three PCBs accounted for 58% and 64% of total ΣPCBs in the samples from spring and autumn, respectively. Relatively high abundance also showed PCB 28 with 20% and 16% in the spring and autumn fish, resp. Even within the same set of fish exist differences in patterns of PCBs and variances in their relative abundances.

Concentrations of DDTs similar to those of PCBs were determined in the muscle tissue of chub specimens as well. Mean levels of ΣDDTs in chub from Modřice were 34.8 (ranging from 8.4 to 78.1) and 28.6 (with range of 13.3–41.1) ng/g ww for the spring and autumn sampling, respectively, and at Rajhradice, they were 38.1 (5.2–93.8) and 27.8 (2.9–47.5) ng/g ww, resp. There were indeed strong correlations ($r = 0.781$–0.985) between DDTs and PCBs in all set of samples except the locality Mořice (autumn sampling), where the correlation was somewhat weaker ($r = 0.468$). On the whole, the values did not significantly differ between the sampling localities ($p < 0.01$) or between two sampling periods ($p < 0.01$), just like PCBs. The weighed means for the Modřice locality were 37.9 and 29.4 ng/g ww (in spring and autumn, resp.) and in Rajhradice they were 40.0 and 28.8 ng/g ww, resp. The trend in decreasing values was similar to that described for PCBs. And again, due to higher lipid content in entrails of the specimens from the autumn sampling, the concentrations of PCBs in entrails were similar to those in skin.

Among DDTs with chlorines located in the p,p'- positions, the metabolite DDE highly predominated in all tissues, followed by p,p'-DDD and p,p'-DDT the contributions of which were quite comparable. The p,p'-DDE accounted for on average 65.5%–69.5% in the four sets of fish, the p,p'-DDD and p,p'-DDT isomers accounted for 14.6%–17.8% and 9.4%–11.2%, respectively. The ratio of DDT/DDE ranged from 0.04 to 0.25 (mean of 0.15), which suggests this load is relatively old and there are no longer significant sources of DDT. Just for comparison, Suchan (2004) monitored the levels of organochlorines in selected fish species from the Labe, Vltava and Tichá Orlice rivers in the years of 2001–2003 and reported the average ratio of 0.1 for DDT/DDE. Thus, the fish in our study do not show any divergence to a general trend in the Czech Republic. The o,p'-isomers of DDTs made up a minor contribution to the total sum of DDTs.

Contrary to PCBs and DDTs, the concentrations of HCHs were very low with predominating β- and γ- isomers. The average values of ΣHCHs in muscle tissue of chub were all in units or rather tenths of ng/g ww, except for the autumn samples from Rajhradice, where significantly higher values ($p < 0.01$) ranging from 0.2 to 5.3 (mean of 2.6) ng/g ww were measured. The weighed means for the Modřice locality were 1.02 and 0.7 ng/g ww (in spring and autumn, resp.) and 0.87 and 2.6 ng/g ww (in spring and autumn, resp.) in Rajhradice. Only the γ-HCH (known as an insecticide *lindan* and used formerly in agriculture) showed a rise at this locality which could suggest some fresh source of this contaminant. But on the whole, this increase seems not to be of greater importance. The γ-HCH was the most abundant isomer through the whole set of samples accounting for on average 56.3% of the total HCHs except for the autumnal set of samples from Rajhradice, where it accounted for the above discussed 80.5%. The trend in proportions of α- and β-HCH isomers was not as clear as that of γ-HCH. Due to low levels of HCHs, just above the LOQ in many cases, no correlations to the other compounds measured were calculated.

HCB and OCS were the last two organochlorine pollutants determined in the fish. Similarly to the other pollutants, neither HCB nor OCS showed any differences of statistical importance ($p < 0.01$) between the two sampling localities and sampling periods. HCB was detected in the samples in much higher concentrations than OCS, ranging mostly just above LOQ. However, the HCB levels were in units of ng/g ww, compared to PCBs and DDTs.

Considering the hygienic limits quoted in the Decree of the Ministry of Health of the Czech Republic No. 381/2007 and 306/2004 Coll. (2,000 ng/g ww for PCBs, 500 ng/g ww for DDTs, 50 ng/g ww for HCB and 20 ng/g ww for γ-HCH), the levels in chub from this study were by far lower than the limits. We assume that even older fish of greater weights would not near these values.

When compared to other similar findings (not only) from the Czech Republic, the contamination of chub from the Svratka River with organochlorine pollutants seems not to differ from the common background levels. Chub from the River Labe, caught during 2001–2003, showed averages levels of PCBs from 16.7 to 106 ng/g ww for similar weight categories (115–410 g of bw), the values of DDTs ranged from 15.5 to 39.8 ng/g ww and of HCB ranged from 0.5 to 6.8 ng/g ww. However, the maximum levels measured in 2003 were significantly affected by disastrous floods during 2002. The levels of PCBs in chub from the River Vltava were similar ranging from 23.9 (in 2002) to 100 (in 2003) ng/g ww (Suchan 2004). Recent analysis of chub from the Vltava and Labe Rivers showed somewhat higher levels of PCBs in fish muscle (69.6 ± 67.2 ng/g ww; mean ± SD) compared to this study (Hajslova et al. 2007). Chub from the River Morava in 1996 and 1998 had the average levels of PCBs of 23.1 and 18.2 ng/g ww and DDTs had the mean values of about 11 and 19.2 ng/g ww for the same weight category (Gregor 2000). Low levels of PCBs and DDTs (4.97 and 0.4 ng/g ww, resp.) were determined in chub from the River Nestos, Greece (Christoforidis et al. 2008) and in similar omnivores like carp and roach (9.5 and 6.8 ng/g ww for PCBs, 21.6 and 11.4 ng/g ww) from the Danube Delta (Covaci et al. 2006).

In conclusion, the assumed differences between two sampling localities on the Svratka River, caused by e.g. local wastewater treatment plant, did not prove true and there were only tiny differences among the sets of samples. This was probably due to a relatively short distance between the sampling localities, despite the fact they were separated by a weir not allowing the fish to migrate. Moreover, short sampling period did not enable us to assess temporal trends in levels of POPs. The values did not exceed any hygienic limits for freshwater fish and represented usual background levels in the Czech Republic.

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