ORGANOCHLORINE AND PAHS IN BROWN TROUT (*Salmo trutta fario*) POPULATION FROM TICHÁ ORLICE RIVER DUE TO CHEMICAL PLANT WITH POSSIBLE EFFECTS TO VITELLOGENIN EXPRESSION

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SUMMARY

The goal of this study was to determine the primary cause of reproductive disturbances in salmonids from the Tichá Orlice river. Three sites at the Tichá Orlice river were monitored during 2000-2003.

Samples of fish muscle, bile and bottom sediment were collected on monitored sites for chemical analyses of PCB, HCB, DDT and its metabolites, HCH, OCS, 1-OHPY (bile) and PAH (bottom sediment) concentrations. Vitellogenin (Vtg) concentration in brown trout (*Salmo trutta fario*) blood plasma was assessed using ELISA (Rainbow trout vitellogenin EIA kit). In comparison with the control site, locality at the source of pollution and on another locality downstream were found considerably polluted. This contamination was manifested also by repetitious findings of high Vtg concentrations in blood plasma, resulting in adverse effects on fish reproduction.

KEYWORDS: vitellogenin, males, PCB, HCB, chlorinated pesticides, OCS, 1-OHPY, PAH.

INTRODUCTION

River Tichá Orlice is a sinistral tributary of the Elbe river. The broodstock of brown trout and the grayling from the headwater area of this river and its tributary Kralický brook was used for artificial reproduction. At the beginning of the 1990's, low effectiveness of spawning appeared, the results of reproduction were not satisfactory and high losses of spawners were noticed.

Monitoring of Tichá Orlice river contamination was carried out for the first time in 1989 [1]. The content of organic pollutants (PCB, DDT and its metabolites, HCB, HCH isomers) was measured in muscle of brown trout (*Salmo trutta fario*) at down stream localities. Elevated concentrations of PCB were noticed in Tichá Orlice river compared to findings of pollutant levels common in fish from other rivers of the Czech Republic.

The next monitoring was proceeded during 1993-1998, particularly upstream localities of Tichá Orlice river and on its tributary Kralický brook [2, 3]. Lusková et al. [1] pointed out the „Tesla Králíky" company as the primary source of mercury contamination of brown trout. The company has produced electronics and was located on Kralický brook (production technology included use of mercury). Moreover, the results of long-term monitoring of Tichá Orlice river [3] proved, that Kralický brook was a significant source of organic pollutants (PCB, PAH, OCS, DDT and its metabolites, HCB, HCH).

The above-mentioned results of chemical monitoring of Tichá Orlice river are highly important, but they do not provide evidence for the complex effect of contamination on the physiological condition of fish. A significant biochemical marker, vitellogenin, was chosen to determine negative effects of pollution on the reproductive ability of fish. Vitellogenin is a biochemical marker of surface water contamination of chemicals with estrogenic effects [4]. Vitellogenin is a lipoglycophosphoprotein synthesized in the liver of mature female under the influence of steroid hormone estradiol. Afterwards, the synthesis of vitellogenin starts also in the liver of males, if estrogen-like chemicals occur in aquatic environment. It can lead to degenerative alterations of gonads and disturbances of reproduction [5].

Presence of vitellogenin in the blood plasma of males consequently indicates the occurrence of chemicals that can mimic the effect of estrogens in aquatic environment [6, 7].

Many field studies were performed till present, using determination of vitellogenin in male blood plasma for assessment of xenoestrogenic contamination [8-12].

The aim of this study was to define the pollution of Tichá Orlice river and its disrupting consequenses to fish reproduction.

MATERIALS AND METHODS

Samples of brown trout (*S. trutta fario*) male blood plasma, bile, muscle and samples of sediment were collected at respective sampling sites (see Fig. 1) from 2000 to 2003. Sampling was done in June every year. Sampling sites Lichkov, Kralický brook and Červená Voda are located at 93[rd] km, 100[th] and 103[rd] km of Tichá Orlice river, respectively. Samples of fish tissues were stored deep frozen at -18°C until analysis (not more than 1 month).

Fish have been sampled at the particular locality since introduction into the river (half year old) until sampling age of 2-3 years. The main characteristics of fish species examined within this study are summarized in Table 1.

Muscle samples of brown trout were analyzed for the content of 7 indicator PCB congeners (IUPAC NOs: 28, 52, 101, 118, 138, 153 and 180), HCB, DDT and its metabolites, HCH and OCS. Homogenized samples were dessicated by anhydrous sodium sulphate and the flowing powder was extracted by 340 ml hexane – dichloromethane solvent mixture (1 : 1, v/v) in a Soxhlet apparatus for 8 h (7 cycles/h). Removing of co-extracted lipids from crude extract was accomplished by gel permeation chromatography (GPC) employing BioBeads SX-3 and chloroform as a mobile phase. After solvent evaporation from the respective GPC fractions and dissolving the residue in isooctane, quantification of target analytes was carried out by high resolution two-dimensional gas chromatography (two capillaries operated in parallel: DB-5 and DB-17, 60m x 0.25 mm (i.d.) x 0.25 mm (both) employing two electron capture detectors (HRGC/2xECD). Splitless injection technique was used (1 µl injeceted).

Quantitation of target compounds was performed by multi-level calibration. Limits of quantification (LOQ - mg/kg lipids) for fish are: PCB 28 – 0.5, PCB 52 – 0.6, PCB 101 – 1.0, PCB 118 – 0.6, PCB 138 – 1.0, PCB 153 – 0.6, PCB 180 – 0.7, HCB-0.2, a-HCH – 0.4, b-HCH – 0.7, g-HCH – 0.4, o,p´-DDE – 0.5, p,p´-DDE – 0.5, o,p´-DDD – 0.8, p,p´-DDD – 1.0, o,p´-DDT – 0.7, p,p´-DDT – 0.9. Limits of quantitation (LOQ mg/kg dry weight) for sediment are: PCB 28 – 1.2, PCB 52 – 1.4, PCB 101 – 1.6, PCB 118 – 2.4, PCB 138 – 1.5, PCB 153 – 1.7, PCB 180 – 1.8, HCB – 0.2, a-HCH – 0.2, b-HCH – 0.3, g-HCH – 0.2, o,p´-DDE – 0.5, p,p´-DDE – 0.3, o,p´-DDD – 0.6, p,p´-DDD – 0.6, o,p´-DDT – 0.6, p,p´-DDT – 1.7.

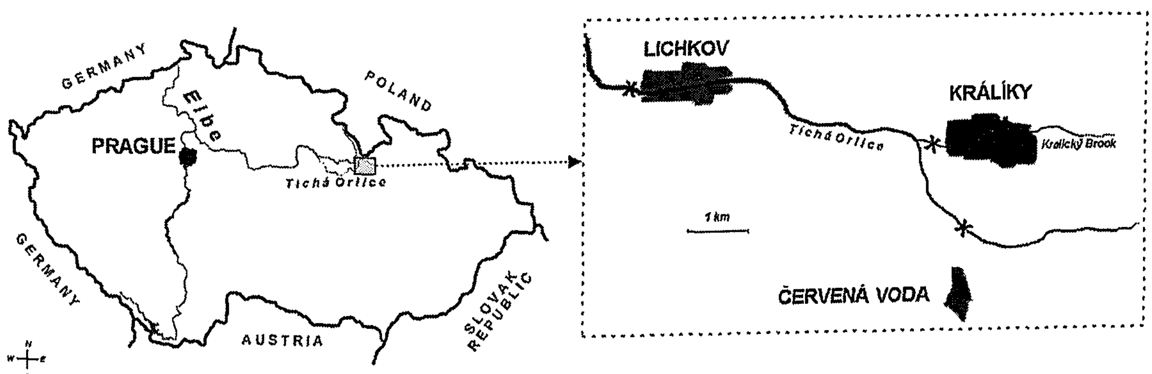

FIGURE 1 - Map of Czech Republic and location of sampling sites within presented study.

TABLE 1 - The main characteristics of sampled brown trout males (*S. trutta fario*) population (n = number of fish examined).

Locality	2000		2001		2002		2003	
	Weight (g)	Age (years)	Weight (g)	Age (years)	Weight (g)	Age (years)	Weight (g)	Age (years)
	mean±SD (n)	*mean*	*mean±SD (n)*	*mean*	*mean±SD (n)*	*mean*	*Mean±SD (n)*	*mean*
Červená Voda	101±21 (6)	2,5	106±29 (8)	2,4	126±39 (5)	3,0	91±12 (7)	2,1
Králíky	126±20 (7)	2,7	147±25 (10)	3,2	208±128 (5)	3,0	118±66 (7)	2,4
Lichkov	139±46 (3)	2,8	142±20 (9)	3,0	149±40 (5)	2,6	119±43 (6)	2,5

The method used for samples analysis is accredited according to ISO/IEC 17025, the results are traceable to respective certified reference materials (CRM) and proficiency testing scheme FAPAS (CSL, York, UK) There was exactly analysed metabolites of DDT: o,p´-DDE; p,p´-DDE; o,p´-DDD; p,p´-DDD, o,p´-DDT; p,p´-DDT.

Blood sampling (from *v. caudalis*), centrifugation and deep freezing of blood plasma were performed *in situ* on monitored sites. Vitellogenin concentration in fish blood plasma was assessed using ELISA - Rainbow trout vitellogenin EIA kit (Biosense Laboratories, Norway).

Bile samples were transferred from gall bladders to test tubes, frozen in situ and stored at –18 °C until analysed. Individual samples of bile were analysed by reverse-phase HPLC with fluorescence detection after a release of 1-hydroxypyrene (1-OHPY) from conjugates by enzymatic hydrolysis [13]. Sediments originating from the same localities as fish bile were frozen and stored in plastic bottles at -18 °C until analysed. Determination of PAHs (a standard mixture of 16 priority PAHs) in the purified sample of sediment was accomplished by reverse-phase HPLC with the column temperature at 35 °C and flow-rate at 1.2 mL/min [13].

The sex was determinated macroscopically and confirmed by histologic evaluation of hematoxylin–eosin stained sections. Results were tested using ANOVA Mann-Whitney test.

RESULTS

The values of vitellogenin concentration in blood plasma of the brown trout males from the monitored localities of Tichá Orlice river and its tributary Kralický brook are given in Fig. 2. During the whole period of 2000-2003, the highest values were detected at the Kralický brook downstream of Králíky town. The lowest values were detected at locality Červená Voda (103rd km of Tichá Orlice River). Results of statistical comparison of vitellogenin concentrations in blood plasma of males per single sampling site and per year are shown in Table 2. Statistically high significant differences were found all over the tracked period between Červená Voda and Králíky localities (P<0.01); statistically high significant (P<0.01) or statistically significant (P<0.05) differences were found in the Králíky locality in comparison with values found in blood plasma of males from the Lichkov locality.

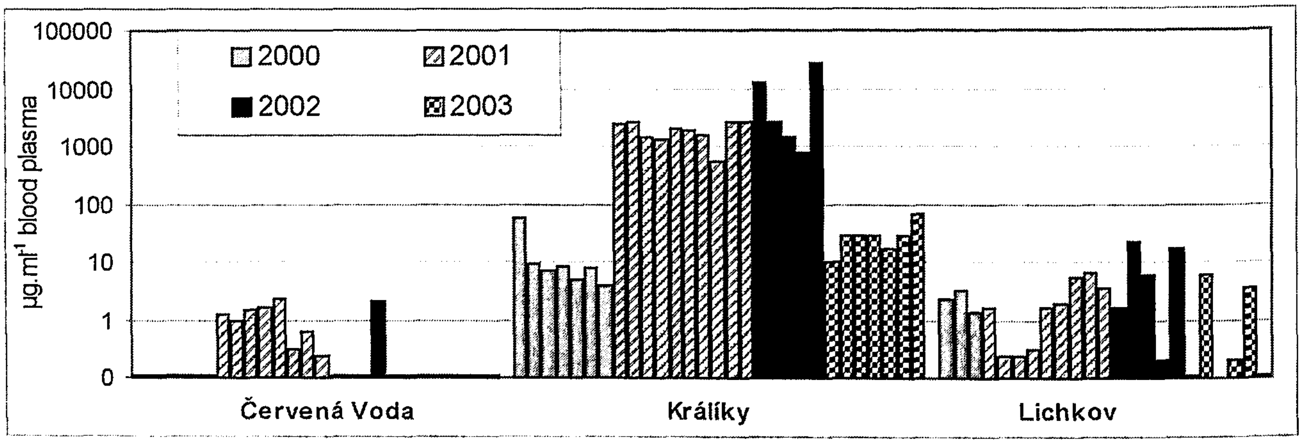

FIGURE 2 - Comparison of vitellogenin concentrations in blood plasma of brown trout males (*S. trutta fario*) from monitored sites of Tichá Orlice river.

TABLE 2 - Comparison of vitellogenin concentration in blood plasma of brown trout males (*S. trutta fario*) among monitored localities (Mann – Whitney test).

Compared localities	Comparison of Localities (p)			
Year	**2000**	**2001**	**2002**	**2003**
Červená Voda versus Kralický brook	0.001	0.001	0.001	0.001
Červená Voda versus Lichkov	0.02	0.71	0.03	0.13
Kralický brook versus Lichkov	0.02	0.03	0.007	0.001

Values of content of industrial pollutants in monitored localities are presented in Fig. 3 (PAH in bottom sediments), in Fig. 4 (1-OHPY concentrations in bile of brown trout males), in Fig. 5. (PCBs in muscle of brown trout males) and in Fig. 6 (HCB in muscle of brown trout males). The highest content of all monitored industrial pollutants in sediment (PAH) and in fish muscle (PCB, OCS) were found in the Kralický brook.

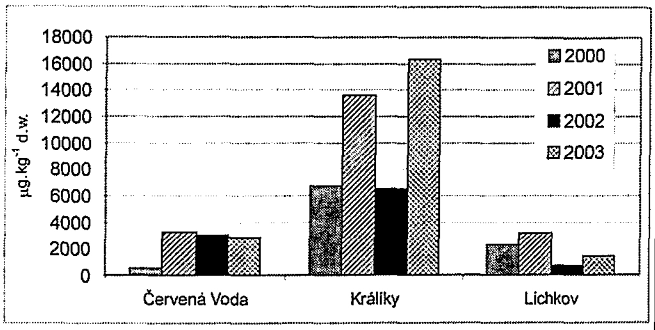

FIGURE 3 - Comparison of the contents of PAH in dry matter of bottom sediments from monitored sites of Tichá Orlice river.

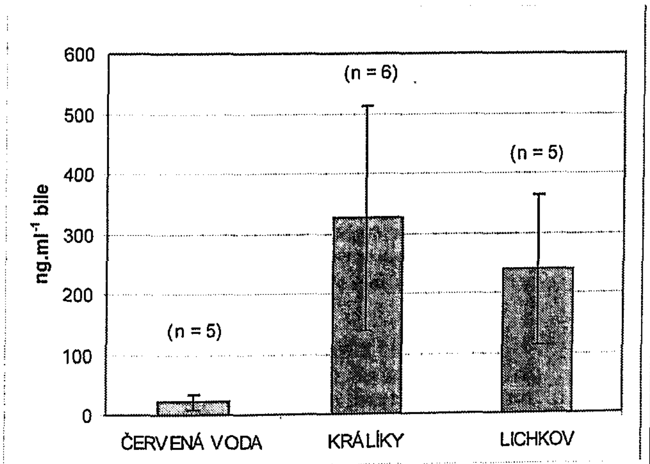

FIGURE 4 - Comparison of 1-OHPY concentrations in bile of brown trout males (*S. trutta fario*) from monitored localities in 2000 (n = number of samples)

Similarly, concentrations of 1-OHPY (biochemical marker of PAH contamination) in bile were the highest in brown trout males from the Kralický brook. The most contaminated site was Kralický brook followed by the Lichkov site and the lowest values were noticed at Červená Voda. Concentration of 1-OHPY in bile of fish from monitored sites was measured only in 2000 (Fig.4). A previous study [14] has confirmed positive correlation between the content of polycyclic aromatic hydrocarbons (PAH) in sediment and 1-OHPY in bile of fish during 1998, 1999 and 2000. The biomarker 1-OHPY was not measured in 2001-2003. Pollution level was assessed on the basis of total PAH concentration in bottom sediment.

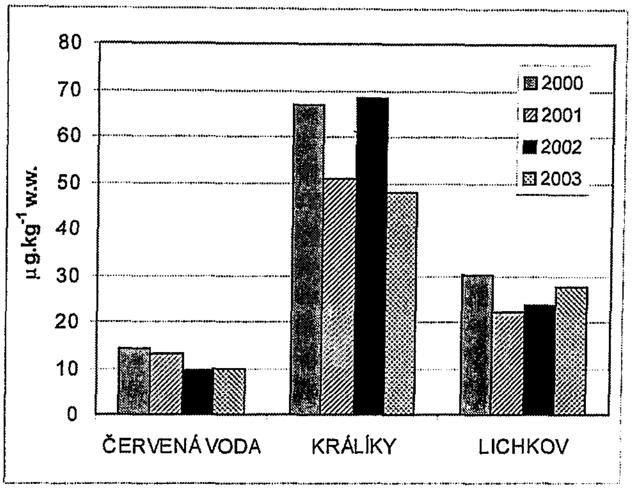

FIGURE 5 - Comparison of the contents of sum of PCBs in brown trout (*S. trutta fario*) muscle from monitored sites of Tichá Orlice river.

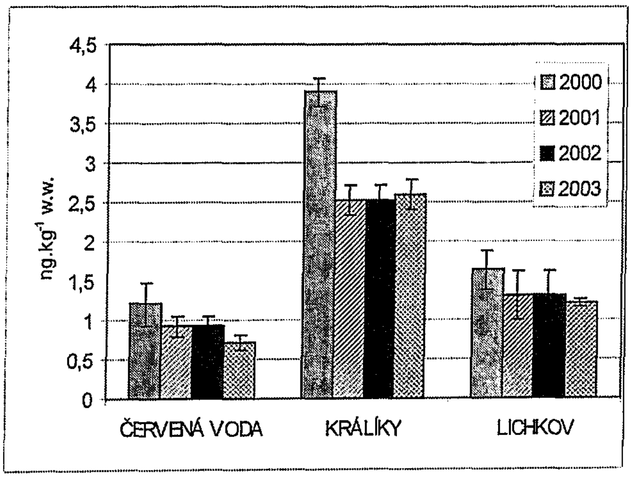

FIGURE 6 - Comparison of the contents of HCB in brown trout (*S. trutta fario*) muscle from monitored sites of Tichá Orlice river.

Values of the content of HCH (range of values - Červená Voda: 0.3-1.0; Kralický brook: 0.2-1.0; Lichkov: 0.1-0.8) and DDT (range of values - Červená Voda: 33.3-42.9; Kralický brook: 44.2-53.0; Lichkov: 27.4-34.3) in brown trout muscle (µg.kg^{-1} w.w) were comparable on all monitored sites of the Tichá Orlice river. Values of the content of OCS were measured in muscle of fish in 2001, 2002 and 2003. The highest content of OCS was detected on the Kralický brook site (0.1 µg.kg^{-1} w.w.), and lower values were detected at the Lichkov locality (0.05 µg.kg^{-1} w.w). The value of the content of OCS at the unpolluted Červená Voda site was 0.03 µg.kg^{-1} w.w.

DISCUSSION AND CONCLUSION

The Tichá Orlice river provided to the research team an almost ideal model situation for studying the surface water loading with pollutants. At all sites under study it was possible to collect the same fish specie – the brown trout (*S. trutta fario*). On its upper stretch (Červená Voda), the fish population was entirely natural. The two downstream sites (Králíky and Lichkov) were managed by the local angling club and are hence purposefully stocked. There was found the practically ideal unpolluted control site (Červená Voda), which showed the minimum loading in almost all parameters as evident from Fig. 1-6. The only exception was the arsenic (As) concentration in brown trout (*S. trutta fario*) muscle [15]. The site Lichkov – several km downstream the pollution source showed approximately half-values in comparison with the site influenced by the direct pollution source (Králíky).

The mercury (Hg) content in fish muscle on sites Králíky and Lichkov was significantly higher in comparison with unloaded site Červená Voda ($P< 0.01$) [15]. This finding is in direct connection with the local long-term industrial pollution.

Kralický brook (the tributary of the Tichá Orlice river), where the source of the long-term loading in the Králíky town and its vicinity is located, showed the highest values of loading in all parameters under study (Fig. 1-6). The values of vitellogenin concentration in the blood plasma of brown trout males (Fig. 2) on this site are equivalent or similar to the values, which are reported by Christiansen et al. [16] on the 9th day following the intraperitoneal treatment by 17β-estradiol (0.5 $mg.kg^{-1}$), nonylphenol (50 $mg.kg^{-1}$) and bisphenole A (50 $mg.kg^{-1}$) in rainbow trout (*Oncorhynchus mykiss*). Also Norman [17] reports the same values of vitellogenin concentration in blood plasma of rainbow trout (*O. mykiss*) males following the 12-day exposition to 17α-ethinylestradiol applied into water (10 $ng.l^{-1}$). The highest vitellogenin values in blood plasma of males from the site Králíky corresponded to the values found in females from all sampling sites of the Tichá Orlice river (vitellogenin in the blood plasma of 1-2 female individuals was also determined on each site as a natural positive control). Recorded high vitellogenin values in the blood plasma on the polluted Králíky site prove significant differences in comparison with the control site Červená Voda and also the site downstream of the source of pollution in Lichkov (Tab. 2 and Fig. 2). It can be concluded from these data that the reproductive ability of males from the site Králíky is significantly influenced. It may confirm the assumption that reported problems with fish reproduction in the Králíky region are due to the surface water loading by endocrine disruptors. Keith [4] records among endocrine disruptors also substances, which were studied on behalf of the monitoring of the Tichá Orlice river: PCB, HCH, PAU, HCB, OCS, DDT, Hg. Increased values of concentrations of these substances in fish bodies and river bottom sediments on monitored sites may be the reason of pathological changes in the physiology of local brown trout (*S. trutta fario*) population, which cause the reproductive disturbances.

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