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Total Mercury and Methylmercury Contamination in Fish from Various Sites along the Elbe River

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Abstract

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The aim of the study was to evaluate total mercury Hg and methylmercury MeHg contamination in muscle tissues of fish collected in 2002 from the Labe (Elbe) river at sites upstream of Pardubice and downstream of Pardubice and Hřensko, and in 2004 from the Labe river upstream and downstream of the Spolana factory in Neratovice, and from the Vltava river downstream of Lenora. Eighty eight fish of the following species were sampled: bream (*Abramis brama* L.), perch (*Perca fluviatilis* L.), chub (*Leuciscus cephalus* L.) and barbel (*Barbus barbus* L.). Total mercury content in chub, perch and bream was in the range of 0.05 - 1.96 mg·kg^{-1} w.w., 0. 09 - 1.46 mg·kg^{-1} w.w. and 0.35 - 0.82 mg·kg^{-1} w.w., respectively. Methylmercury content in chub, perch and bream was in the range of 0.04 - 2.11 mg·kg^{-1} w.w., 0.1 - 1.73 mg·kg^{-1} w.w. and 0.371 - 0.650 mg·kg^{-1} w.w., respectively. Significant correlation ($p < 0.05$) was found between contents of THg and MeHg and both the weight and the age of fish. Significant differences ($p < 0.05$) between THg and MeHg contents were found between individual sites. In 2002, for example, the most contaminated fish were found downstream of Pardubice, followed by fish from upstream of Pardubice and from Hřensko. In 2004, fish from downstream and upstream of the Spolana factory in Neratovice were more contaminated than fish from the Vltava river downstream of Lenora. The methylmercury-to-total mercury ratio in muscle tissue was close to 1.0.

The Vltava river, MeHg/THg ratio, Perca fluviatilis, Leuciscus cephalus, Abramis brama, Barbus barbus

The Elbe river is one of the longest European rivers. It flows through two countries (i.e. the Czech Republic and Germany), it is 1103.5 km long and its drainage area extends over 148.268 km ^{2}, of which 51.336 km^2 are in the Czech Republic and 96.932 km^2 in Germany. In both countries, the Elbe and its tributaries traverse not only through a number of big industrial hubs, but also through important rural areas with intensive agriculture. Intensive research into pollution levels in the Elbe started in 1991 under the Elbe I (1991-1994) project, and continued with the Elbe II (1995 - 1998) and Elbe III (1999 - 2002) projects. In those projects, data from chemical and in recent years also biochemical, monitoring of hazardous substances in various components of the aquatic ecosystems of the river were collected (Nesměrák 1994; Blažková et al. 1998; Blažková 2002; Čelechovská et al. 2005; Široká et al. 2005; Žlábek et al. 2005) .

The aim of the present study was to evaluate the current mercury contamination in a number of sites along the Elbe, and to extend the evaluation to concentrations of organically bound mercury. Mercury in water ecosystems occurs in several forms including elementary (Hg^0), inorganic (Hg^+ or, rather, Hg^0 added to Hg^{2+} and Hg^{2+}) and organic forms of mercury, primarily monomethylmercury (CH_3Hg^+) and dimethylmercury [$(CH_3)_2Hg$]. The type of mercury predominantly occurring (in up to 100%) in tissues of a majority of fish

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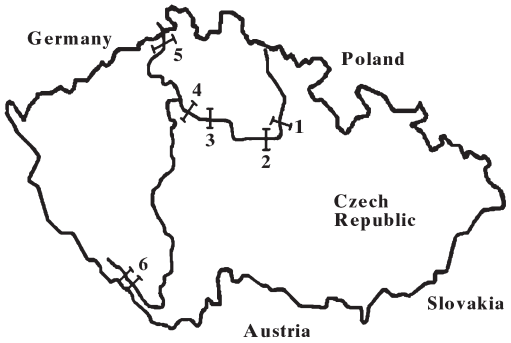

Fig. 1. Localities where fish were caught: 1 - upstream of Pardubice, 2 - downstream of Pardubice, 3 - upstream of Spolana factory, 4 - downstream of Spolana factory, 5 - Hřensko, 6 - Vltava river downstream of Lenora.

species is monomethylmercury (MeHg) (Mason et al. 1995; Kannan et al. 1998; Porcella 1994), whose neurotoxicity (Igata 1986) makes it the most toxic form of mercury (WHO 1990). It is produced by microbial methylation in sediments (Jernelöv 1973; Compeau and Bartha 1985), infiltrates the food chain and is consequently accumulated in fish. MeHg bioaccumulation in fish tissues increases with fish age and thus also their size (Norstrom et al. 1976; Jewett et al. 2003). Fish are the main source of methylmercury contamination of people (FAO/WHO 1990). This makes them the main target in aqueous system contamination monitoring for both environmental and food safety purposes.

Table 1. The main characteristics of caught fish

species	n	weight (g) mean ± SD (min – max)	age mean ± SD (min – max)
October 2002			
upstream Pardubice			
perch	4	85 ± 44 (50 - 50)	1.3 ± 0.3 (1 - 2)
chub	5	366 ± 366 (130 - 990)	3.4 + 2.2 (2 - 7)
downstream Pardubice			
perch	11	170 ± 114 (110 - 510)	2.3 ± 0.9 (2 - 5)
chub	7	348 ± 102 (250 - 490)	4.0 ± 0.8 (3 - 5)
bream	3	707 ± 287 (390 - 950)	5.7 ± 1.5 (4 - 7)
Hřensko			
chub	8	454 ± 336 (190 - 1240)	4.4 ± 1.8 (2 - 8)
bream	7	979 ± 201 (750 - 1250)	7.6 ± 1.3 (6 - 9)
barbel	5	1910 ± 266 (1700 - 2350)	10
May 2004			
upstream Spolana factory			
chub	10	463 ± 321 (230 - 1100)	5.0 ± 1.6 (3 - 8)
downstream Spolana factory			
chub	16	572 ± 339 (200 - 1300)	4.9 ± 1.8 (3 - 8)
Vltava river			
chub	12	874 ± 176 (190 - 1215)	6.9 ± 1.5 (3 - 9)

Materials and Methods

In October 2002, a total of 15 perch (*Perca fluviatilis* L.), 19 chub (*Leuciscus cephalus* L.), 10 bream (*Abramis brama* L.) and 5 barbel (*Barbus barbus* L.) were caught at selected sites along the Labe. The sites selected along the Labe were: upstream of the city of Pardubice - river km 245, downstream of Pardubice - river km 233 and Hřensko - river km 3. River kilometres were measured from the German border.

In May 2004, a total of 38 chub were caught at selected sites along the Labe and its tributary, the Vltava. They were the following sites along the Labe: downstream of the Spolana factory - river km 116, upstream of the Spolana factory - river km 121, and a section at the Vltava downstream of Lenora: river km 373 - 366. A site at the upper reaches of the Vltava

downstream of the town of Lenora was chosen to determine the natural background mercury concentration in the river system in the Czech Republic.

The fish were collected after they were stunned with an electric generator set. The caught fish were immediately weighed, their age was determined from their scales, and muscle tissue samples were taken for mercury content assessment. The sex of the fish was determined macroscopically and checked by the histological examination of gonads. The main characteristics of fish species examined in this study are summarized in Table 1. Muscle tissue samples were put into polyethylene bags, labelled and stored in a freezer at - 18 °C.

Total mercury (THg) content in fish tissues was determined by the direct method of cold vapours using an AMA 254 (Altec Ltd.) analyzer. Methylmercury (MeHg) was determined in the form of MeHgCl by gas chromatography (Maršálek and Svobodová 2005). Samples were prepared by acidic digestion and extraction to toluene. For the determination, Hewlett Packard 5890 Series II gas chromatograph was used. A capillary column DB 608 (30m) (Caricchia et al. 1997) and an electron capture detector (ECD) were used. Evaluation was made using HP 3365 ChemStation Series II software (Hewlett Packard). All solvents used met residual trace analysis quality parameters. Total mercury (THg) and methylmercury (MeHg) contents are given in $mg \cdot kg^{-1}$ wet weight (w.w.)

Table 2. Content of total mercury, methylmercury and total mercury-to-methylmercury ratio

Species	n	MeHg ($mg \cdot kg^{-1}$) mean ± SD (min – max)	THg ($mg \cdot kg^{-1}$) mean ± SD (min – max)	MeHg/THg mean ± SD (min – max)
upstream Pardubice				
perch	4	0.118 ± 0.024	0.109 ± 0.020	1.01 ± 0.02
		(0.098 - 0.152)	(0.093 - 0.137)	(0.98 - 1.03)
chub	5	0.098 ± 0.017	0.169 ± 0.123	1.00 ± 0.02
		(0.089 - 0.122)	(0.118 - 0.388)	(0.99 - 1.03)
downstream Pardubice				
perch	11	0.496 ± 0.419	0.437 ± 0.347	1.04 ± 0.08
		(0.230 - 1.730)	(0.231 - 1.455)	(0.88 - 1.16)
chub	7	0.381 ± 0.145	0.359 ± 0.145	1.00 ± 0.04
		(0.182 - 0.581)	(0.159 - 0.567)	(0.95 - 1.06)
bream	3	0.478 ± 0.150	0.516 ± 0.260	0.91 ± 0.15
		(0.371 - 0.650)	(0.348 - 0.816)	(0.74 - 1.00)
Hřensko				
chub	8	0.101 ± 0.075	0.120 ± 0.063	0.74 ± 0.19
		(0.044 - 0.272)	(0.050 - 0.266)	(0.46 - 0.97)
bream	7	0.343 ± 0.112	0.361 ± 120	0.95 ± 0.05
		(0.183 - 0.472)	(0.188 - 0.531)	(0.89 - 1.00)
barbel	5	0.654 ± 230	0.608 ± 0.203	0.95 ± 0.05
		(0.360 - 0.789)	(0.398 - 0.881)	(1.07 - 0.16)
upstream Spolana factory				
chub	10	0.93 ± 0.61	0.86 ± 0.56	1.00 ± 0.09
		(0.36 - 2.11)	(0.34 - 1.96)	(0.85 - 1.16)
downstream Spolana factory				
chub	16	0.85 ± 0.41	0.86 ± 0.38	0.90 ± 0.12
		(0.43 - 1.86)	(0.48 - 1.78)	(0.64 - 1.07)
Vltava river				
chub	12	0.75 ± 0.25	0.74 ± 0.26	0.96 ± 0.07
		(0.24 - 1.14)	(0.22 - 1.09)	(0.83 - 1.07)

BCR 463 and BCR 464 (IRMM Belgium) reference materials were used to validate the methods and to determine the uncertainties. Detection limits for total mercury and methylmercury determination methods were 0.001 $mg \cdot kg^{-1}$ and 0.01 $mg \cdot kg^{-1}$, respectively. Extended uncertainty for total mercury and MeHg determination methods were 10% and 12%, respectively. The methods were successfully tested in the inter-laboratory comparison test IMEP-20 Trace Elements in Tuna Fish.

The analysis of variance (ANOVA) was used for the testing of species and tissue differences and the Spearman correlation was used for testing the relation of THg and MeHg contents to the weight and age of the fish. Statistical calculations were carried out using QC Expert software (Trilobyte Ltd.).

Table 3. Correlation between mercury content and the weight or age of fish ($p < 0.05$), 1 - upstream of Pardubice, 2 - downstream of Pardubice, 3 - Hřensko, 4 - upstream of Spolana factory, 5 - downstream of Spolana factory and 6 - Vltava river downstream of Lenora

locality	weight correlation				age correlation			
	R (THg)	significant	R (MeHg)	significant	R (THg)	significant	R (MeHg)	significant
chub *(Leuciscus cephalus* L.*)*								
1	0.90	yes	0.90	yes	0.60	no	0.60	no
2	0.32	no	0.32	no	0.36	no	0.36	no
5	0.74	yes	0.76	yes	0.62	no	0.62	no
3	0.57	no	0.65	yes	0.65	yes	0.70	yes
4	0.90	yes	0.87	yes	0.89	yes	0.90	yes
6	0.68	yes	0.73	yes	0.66	yes	0.81	yes
perch *(Perca fluviatilis* L.*)*								
1	0.00	no	0.00	no	0.00	no	0.00	no
2	0.71	yes	0.69	yes	0.44	no	0.41	no
bream *(Abramis brama* L.*)*								
2	1.00	yes	1.00	yes	1.00	yes	1.00	yes
5	-0.32	no	-0.36	no	-0.39	no	-0.54	no

Results

THg and MeHg were found in all the samples examined (Table 2). Because no significant sex-related differences in THg and MeHg contents were found, fish of both sexes were evaluated together in the rest of the study.

Results of the evaluation of the correlation between THg and MeHg content and the weight and age of fish are presented in Table 3. They show positive correlation between the content of the two forms of mercury and both the weight and the age of fish in a large number of cases. This fact had to be taken into account in further evaluations of differences in mercury contamination levels between different fish species and sites. The correlation was done by data normalization to the fish weight or age. In evaluating inter-species differences, age normalization was used to eliminate differences in weight gains in the individual species. In evaluating differences among individual sites, weight normalization was used instead, only intra-species differences were evaluated.

Upstream of Pardubice, significantly ($p < 0.05$) higher content of both THg and MeHg in perch compared with chub was found. Downstream of Pardubice, THg and MeHg contents were significantly ($p < 0.05$) higher in perch than in bream or chub. In Hřensko, THg and MeHg contents were significantly ($p < 0.05$) higher in bream and barbel than in chub.

In evaluating differences among the sites, each species was evaluated individually. In chub, THg content was significantly higher ($p < 0.05$) downstream of Pardubice than upstream of Pardubice and in Hřensko. No significant difference was found between upstream of Pardubice and Hřensko. The significantly highest MeHg content in chub ($p < 0.05$) was found downstream of Pardubice, with upstream of Pardubice and Hřensko ranking second and third. In perch, significantly higher ($p < 0.05$) content of both THg and MeHg was found downstream of Pardubice than upstream of Pardubice. In bream, a significantly higher ($p < 0.05$) content of both THg and MeHg was found downstream of Pardubice than in Hřensko.

In 2004, the content of both THg and MeHg was significantly higher ($p < 0.05$) upstream and downstream of the Spolana factory than at the control site on the Vltava river. No significant differences in THg or MeHg content between upstream of the Spolana factory and downstream of the Spolana factory were found. The results failed to support the assumption of higher mercury concentrations downstream of the Spolana chemical factory. The results, however, may have been influenced by fish migrations.

In THg, MeHg makes up almost 100% (Table 2) in most of the cases. Nevertheless, significant differences ($p < 0.05$) between the sites were found. In samples from the year 2002, MeHg levels in chub from Hřensko were significantly lower ($p < 0.05$) than those found in chub from upstream and downstream of Pardubice, and they were also lower compared with bream and perch from all three sites. In samples from 2004, significantly ($p < 0.05$) higher MeHg levels were found in chub from upstream of the Spolana factory and the Vltava than from downstream of the Spolana factory. The MeHg/THg ratio was calculated after the conversion of the MeHg content to elementary mercury because of different molecular weights of MeHg and elementary mercury. Errors in determining the two types of mercury are responsible for cases where MeHg in THg exceeds 100%.

Discussion

The positive correlation between the mercury content and the weight or age of fish has been corroborated by a large number of studies (e.g. Jackson 1990; Jewett et al. 2003; Peňáz et al. 1979). Other studies, however, have reported negative correlation between mercury content and fish size (Dixon and Jones 1994; Leah et al. 1991; Svobodová et al. 1999). When we compare correlations between the two types of mercury and the weight and the age of fish, fish weight seems to be a more suitable variable for correlation assessments. This is particularly true when several fish of the same age but different weight due to different weight gains are available, as was the case of downstream of Pardubice, where there were 9 2-year-old and one 5-year-old perch. Here we found a significant ($p < 0.05$) correlation between the two forms of mercury and weight, while the correlation between THg and MeHg levels and age was not significant (Table 3).

The reasons for different percentages of MeHg in THg in one and the same species from different sites remain unclear, and are probably multifactorial. Kannan et al. (1998) also reported different percentages of MeHg in THg in the same species from different sites. It is probably mainly due to different natural features in individual locations, particularly the conditions for microbiological methylation in sediments. MeHg percentages in Labe sediments range between 1 - 2% (Robertson et al. 1987) and 10% (Wilken and Hintelman 1991). Fish age or sex may also play an important role (Kannan et al. 1998). Different percentages of MeHg in THg in different species are, to a certain extent, also determined by the food consumed by each species (Walker et al. 1976).

The results showed clearly higher THg and MeHg concentrations in fish from downstream of Pardubice than in fish from upstream of Pardubice. This identifies the city of Pardubice, an important centre of (primarily chemical) Czech industry, as the probable source of mercury contamination. Compared with upstream of Pardubice and downstream of Pardubice, Hřensko is the least contaminated site. A comparison between fish from individual sites caught there in 2002 and 2004 shows that the most contaminated sites were upstream of the Spolana factory and downstream of the Spolana factory, and the least contaminated one was Hřensko. Dušek et al. (2005) evaluated a total of 1,251 fish of different species caught between 1991 and 1996 at 13 different sites along the Czech section of the Labe. The most seriously polluted sites in their study were Čelákovice and Neratovice (river km 144 and 122, respectively), Děčín and Hřensko (river km 15 and 5, respectively). Žlábek et al. (2005) reported a marked decrease in contamination in the fish caught in Děčín and Hřensko in 1999 - 2003. Mercury contamination levels in the fish caught between river kilometres 160 and 120 (Obříství and Lysá nad Labem) remained the same as in 1991 - 1996, and the sites are referred to as the most contaminated of the sites monitored along the Czech section of the Labe. Results of our study confirm a marked decrease in mercury contamination at Hřensko compared with the results in 1991 - 1996. The highest concentrations of mercury in 2004 were found upstream and downstream of the Spolana

factory, which is in agreement with the findings of previous studies. Contrary to the expectations, elevated levels of contamination with both types of mercury at the control site in the upper reaches of the Vltava were found. There are several possible sources of mercury contamination there, e.g. waste water from the former glass factory in Lenora, or waste water from the former military base. In evaluations, river bottom characteristics and physical and chemical variables of water in the location should also be taken into account. For instance, Žlábek et al. (2005) mentioned that mercury content at different sites along the Labe may change significantly even several times a year.

For the assessment of public health risk posed by the consumption of fish, the maximum weekly recommended amount of MeHg of 1.6 μg MeHg/kg live weight set down by the FAO/WHO was used. Mean MeHg content values in chub muscle tissues were used because chub samples were available from all the sites. The maximum weekly recommended consumption of fish per person from individual sites would then be 0.61 kg of fish from upstream of Pardubice, 0.29 kg of fish from downstream of Pardubice, 1.10 kg of fish from Hřensko, 0.12 kg of fish from upstream of the Spolana factory, 0.13 kg of fish from downstream of the Spolana factory and 0.15 kg of fish from the Vltava river downstream of Lenora. It is clear from the results that eating fish from the Labe river upstream and downstream of the Spolana factory and Lenora poses a particularly high risk to public health, which is also corroborated by the fact that THg in all fish samples from those sites with only 4 exceptions exceeded 0.5 mg/kg, which is the public health limit in many countries.

Kontaminace rtutí a methylrtutí ryb z různých lokalit řeky Labe

Cílem předkládané práce bylo zhodnotit stav zatížení rtutí (THg) a methylrtutí (MeHg) svaloviny ryb z řeky Labe v lokalitách nad a pod Pardubicemi a Hřensko odlovených v roce 2002 a v lokalitách Neratovice nad a pod továrnou Spolana a v lokalitě Lenora na řece Vltavě (přítok Labe) odlovených v roce 2004. Celkem bylo odloveno a analyzováno 88 kusů následujících druhů ryb: cejn velký (*Abramis brama* L.), okoun říční (*Perca fluviatilis* L.), jelec tloušť (*Leuciscus cephalus* L.) a parma obecná (*Barbus barbus* L.). Obsah celkové rtuti v čerstvé tkáni svaloviny se pohyboval v rozmezí 0,05–1,96 $mg \cdot kg^{-1}$ u jelce tlouště, 0,09–1,46 $mg \cdot kg^{-1}$ u okouna říčního a 0,35–0,82 $mg \cdot kg^{-1}$ u cejna velkého. Obsah methylrtuti byl ve svalovině jelce tlouště v rozmezí 0,04–2,11 $mg \cdot kg^{-1}$, okouna říčního 0,1–1,73 $mg \cdot kg^{-1}$ a cejna velkého 0,371–0,650 $mg \cdot kg^{-1}$ čerstvé tkáně. Byla zjištěna signifikantní korelace ($p < 0,05$) mezi obsahem THg a MeHg a věkem, resp. hmotností ryb. Byly zjištěny signifikantní rozdíly ($p < 0,05$) v obsahu obou forem rtuti mezi jednotlivými lokalitami. V v roce 2002 byla nejvyšší úroveň kontaminace zjištěna v lokalitě pod Pardubicemi, následovaná lokalitou nad Pardubicemi a Hřensko. V roce 2004 potom v lokalitách pod a nad Spolanou v Neratovicích byl nalezen vyšší obsah rtuti ve srovnání s lokalitou na řece Vltavě pod Lenorou. Poměr methylrtuti a celkové rtuti se blížil 1,0.

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