ACTA VET. BRNO 2011, 80: 227–233; doi:10.2754/avb201180020227

Assessment of mercury contamination of the Svitava and Svratka rivers and muscle of chub *(Leuciscus cephalus* L.*)* in the urban agglomeration of Brno in the Czech Republic

Kamila Kružíková, Renáta Kenšová, Jana Blahová, Zdeňka Svobodová

Department of Veterinary Public Health and Toxicology, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic

Received May 13, 2010
Accepted October 26, 2010

Abstract

This study focused on the total mercury and methylmercury content in the muscle of chub (*Leuciscus cephalus* L.), the total mercury in the river sediments and the evaluation of health risks associated with fish contamination. Chub were caught at seven localities on the Svratka and Svitava rivers in the agglomeration of Brno in 2008. The results were compared to those obtained from the same sites in 2007. Total mercury was determined by atomic absorption spectrophotometry using an AMA 245 analyzer, and methylmercury was determined by gas chromatography (using an electron-capture detector) after acid digestion and toluene extraction in chub muscle. The highest concentrations of total mercury and methylmercury (0.12 ± 0.14 and 0.07 ± 0.02 mg·kg^{-1} fresh weight, respectively) were found in Svratka before junction (south of Brno), whereas the lowest concentration of mercury and methylmercury in chub (0.06 ± 0.01 and 0.04 ± 0.01 mg·kg^{-1}) was detected in Svitava before junction with the Svratka River. Total mercury in sediments ranged from 0.01 to 1.05 mg·kg^{-1} dry weight, the highest value was detected in the sediment from Rajhradice. The lowest content (0.01 mg·kg^{-1}) was at Knínički. Hazard indices calculated for the selected localities showed no health risk for either a standard consumer or a fishing family. Fish from the Svitava and Svratka rivers show very low mercury concentration and hazard index and their consumption poses no health risk from total mercury and methylmercury contamination.

Czech river, Leuciscus cephalus, methylmercury, sediment, total mercury

Mercury pollution is a ubiquitous problem with atmospheric deposition contaminating watersheds in areas far from anthropogenic or natural atmospheric point sources (Swain et al. 1992). Several forms of mercury are present in the aquatic environment, including elemental, ionic, and organic (Morel et al. 1998). In most freshwater, ionic mercury in the divalent state (Hg^{II}) is the predominant form, whereas in most fish species more than 95% of mercury occurs as methylmercury (MeHg). Thus, the conversion of ionic mercury to MeHg is an important link in the bioaccumulation of mercury in fish and ultimately in its toxicity to humans and wildlife (Eisler 2006). Methylmercury is the most toxic form and bioaccumulates in fish primarily through dietary uptake (WHO 1990). The level of bioaccumulation is a function of age, species, and tropic transfer. Methylmercury exposure can affect growth, reproduction, development and survival in fish (Weiner and Spry 1996). Halbach (1995) reported that bioaccumulation of mercury in fish and its toxicity in humans can be attributed to the high affinity of mercury for sulphur-containing proteins such as metallothioneins and glutathione. The highest concentration of mercury in fish is contained in muscle, and thus muscle is a good indicator of mercury contamination (Čelechovská et al. 2007).

A project of the Ministry of Education Youth and Sport of the Czech Republic comprised a two year study (2007 and 2008) focusing on mercury contamination of the Svitava and Svratka rivers (Czech Republic). In addition to mercury contamination, the project included assessment of persistent organic pollutants using selected

Address for correspondence:
Ing. Kamila Novotná Kružíková, Ph.D.
Department of Veterinary Public Health and Toxicology
University of Veterinary and Pharmaceutical Sciences Brno
Palackého 1–3, Brno, Czech Republic

Phone: +420 541 562 783
Fax: +420 541 562 790
E-mail: novotnak@vfu.cz
http://www.vfu.cz/acta-vet/actavet.htm

biochemical markers in chub (Blahová et al. 2009, 2010), and passive sampling for monitoring endocrine disruptors (Grabic et al. 2010). The present report summarizes mercury contamination in chub in 2008, and compares the results with those from 2007 (Kružíková et al. 2009).

The main aims of the present study were to determine total mercury (THg) and MeHg (methylmercury) concentrations in chub muscle from 7 localities on the Svitava and the Svratka rivers in the Brno agglomeration, to assess THg concentration in sediment at these localities, to evaluate health risks associated with consumption of fish from these sites and to compare data from 2007 and 2008.

Materials and Methods

Sampling sites

Mercury contamination was assessed in the Rivers Svitava and Svratka which run through Brno, the second largest city in the Czech Republic (population 366,680). Brno is an important industrial city with highly developed engineering, chemical, textile, and food-processing industries. Domestic waste, sewage, and other effluents from industrial sources are the most likely source of persistent pollutants in the area's aquatic ecosystem.

The Svratka River runs 29 km through the city and is the major source of water for the Kníničky reservoir, a popular recreation area in the northwest part of Brno. The Svitava River flows through the city for about 13 km and merges into the Svratka River downstream of Brno and is the main tributary to the Svratka River. Seven sites were chosen to evaluate the influence of Brno on mercury contamination of fish and sediments. Two sites were on the Svitava River: (1) Bílovice nad Svitavou, 18 river km; and (2) Svitava before junction, 0.6 river km. Five sites were on the Svratka River: (3) Kníničky, 56.2 river km; (4) Svratka before junction, 40.9 river km; (5) Modřice, 38.7 river km; (6) Rajhradice, 35.0 river km; and (7) Židlochovice, 30.0 river km. Sites 1 and 3 situated upstream of Brno characterize conditions upper of the city. Sites 2 and 4 are situated downstream of Brno and above the confluence of the two rivers. Site 5 is located downstream of a sewage treatment plant and characterizes the Svratka River in areas of waste water effluent. Sites 6 and 7 situated below Brno represent the cumulative effects of all other sites. Weirs are situated between sites 1 and 2, 3 and 4, 5 and 6 and 6 and 7. Fig. 1 shows the locations of the sites.

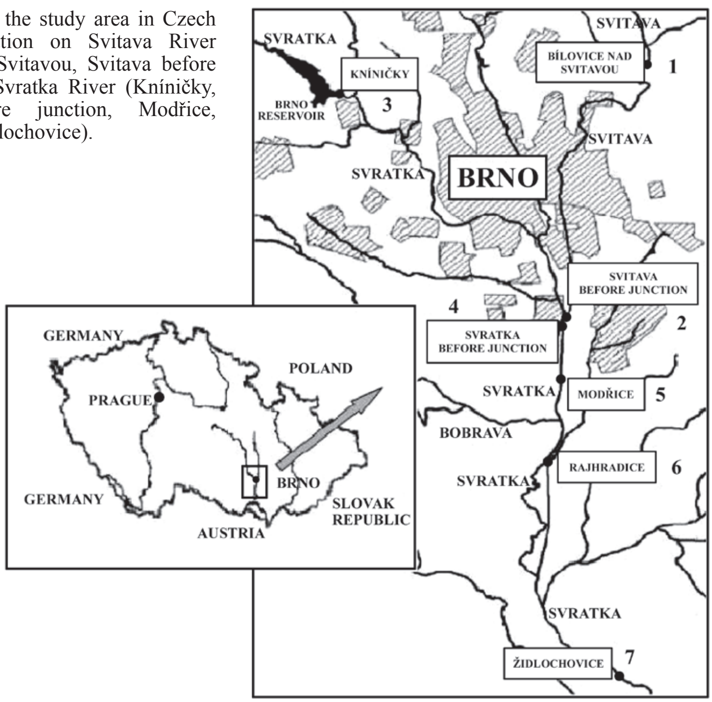

Fig. 1. Map of the study area in Czech Republic: location on Svitava River (Bílovice nad Svitavou, Svitava before junction) and Svratka River (Kníničky, Svratka before junction, Modřice, Rajhradice, Židlochovice).

Sampling of fish and sediments

In 2008, 119 chub (*Leuciscus cephalus* L.) were captured by electrofishing, immediately weighed, measured for total length, and scales collected for age determination. Sex was determined macroscopically. For THg and MeHg analysis, muscle samples were taken from the cranial area dorsal to the lateral line, placed in polyethylene bags, labelled, and stored at -18 °C. Table 1 shows the main biometric characteristics of fish collected in 2008.

Sediment was sampled from the same locations in February, March, and September. At each location (both years), composite bottom sediment was collected into dark glass bottles, lyophilized in the laboratory, and stored at -18 °C. Sampling was validated in accordance with ISO 5667 12 norm.

Table 1. Biometric values of sampled chub (*Leuciscus cephalus* L.)

Sample site	n	Total length (cm)	Weight (g)	Age (years)
1 Bílovice nad Svitavou*	15	25.2 ± 3.3	182 ± 97.5	4 ± 1.3
2 Svitava before junction*	17	23.9 ± 4.5	241 ± 107.3	3 ± 0.9
3 Kníničky**	14	25.1 ± 7.5	242 ± 101.2	3 ± 1.1
4 Svratka before junction**	17	29.4 ± 4.9	311 ± 179.0	4 ± 0.9
5 Modřice**	24	26.1 ± 4.8	365 ± 239.0	3 ± 1.1
6 Rajhradice**	17	29.2 ± 4.7	310 ± 179.5	3 ± 0.8
7 Židlochovice**	15	26.9 ± 3.3	231 ± 89.2	2 ± 0.7

*the Svitava River; **the Svratka River

Determination of THg and MeHg

The THg content in muscle and in sediments was determined by cold vapour atomic absorption spectrometry using AMA 254 analyzer (Altec Ltd., Czech Republic)..

The MeHg content was determined in the form of methylmercury chloride by gas chromatography (Caricchia et al. 1997). Samples were prepared by acid digestion and extraction with toluene (Maršálek and Svobodová 2006). A Shimadzu capillary gas chromatograph with an electron captured detector GC 2010A (Shimadzu Kyoto, Japan) was used for analysis. A capillary column DB 608 (30 m × 0.53 mm × 0.83 µm; J&W Scientific Chromservis, Czech Republic) was used. Data evaluation was made with GC Solution software (Shimadzu Kyoto, Japan).

Limits of detection for THg and MeHg were 1 $\mu g \cdot kg^{-1}$ and 21 $\mu g \cdot kg^{-1}$, respectively. The limit of detection was set as a sum triple the standard deviation of a blank and a blank mean value. The accuracy for THg and MeHg values was validated using standard reference material BCR-CRM 464 (Tuna Fish, IRMM, Belgium).

The total mercury and MeHg concentrations in fish muscle are given in mg kg^{-1} fresh weight (FW), and THg in sediment is given as dry weight (DW).

Health hazard assessment

The hazard index was calculated according to Kannan et al. (1998) using a reference dose for THg (0.3 $\mu g \cdot kg^{-1}$body weight day^{-1}) set by the United Stated Environmental protection Agency (US EPA). To determine the maximum safe consumption of fish, a provisional tolerable weekly intake limit (PTWI) of 1.6 µg MeHg kg^{-1} body weight $week^{-1}$ was used (WHO 1990). The amount of fish safe to be eaten per week was calculated.

Statistical analysis

The statistical analysis of the data was performed using STATISTICA 8.0 for Windows (StatSoft CR). The data were analyzed with the parametric ANOVA Tukey's HSD test.

Results

Total mercury and methylmercury in fish

The content of THg and MeHg was measured in chub, an omnivorous fish suitable for the monitoring of aquatic ecosystems. The sampled fish were of similar age. The main biometric data for sampled chub are shown in Table 1. No significant differences in the age of the fish were found. Since no significant sex-related differences in THg and MeHg content were found, data for both sexes were combined.

Mercury and methylmercury content in the muscle of indicator fish in 2008 is given in Fig. 2. No significant differences in the mercury content (THg and MeHg) were found in fish from the various sites. The lowest value of THg and MeHg was found at site 2 (0.06 ± 0.016 and 0.037 ± 0.011 $mg \cdot kg^{-1}$, respectively) and at site 5 (0.06 ± 0.083 and 0.04 ± 0.039

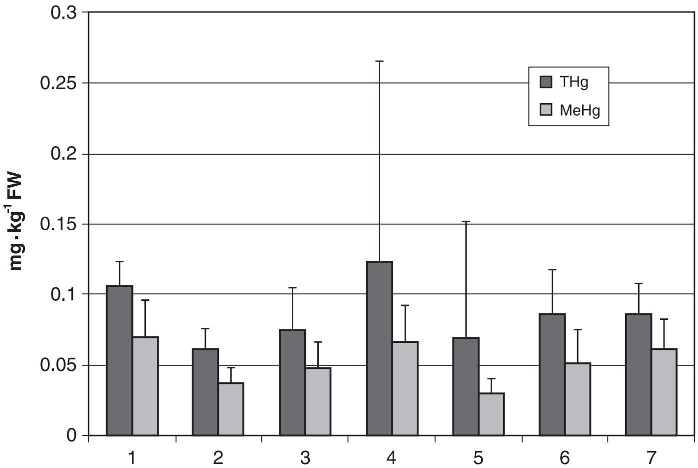

Fig. 2. Total mercury and methylmercury content in the muscle of chub *(Leuciscus cephalus* L.*)* in 2008

mg·kg^{-1}, respectively). The highest THg and MeHg concentrations were detected on the Svratka River (site 4) (0.12 ± 0.14 and 0.06 ± 0.016 mg·kg^{-1}, respectively).

Total mercury in sediment

Sediments for THg determination were sampled 3 times during 2008 (Table 3). The characteristics of the sediments were not consistent from site to site. As in 2007, sediments from site 1 and 3 were arenaceous while other sediments were sloughy.

In 2008, the highest concentration of THg was found on site 6 and reached 1.05 ± 0.13 mg·kg^{-1} DW. In 2007 the highest concentration of THg in the sediment was found at site 4 (1.21 ± 0.24 mg·kg^{-1}). Sites with the lowest amounts were similar in both years (site 3 and 1).

Discussion

Total mercury and methylmercury in fish

Kružíková et al. (2009) reported mercury content in fish sampled in 2007 at the same sites. In 2007 significant differences among sites were found, in contrast to results in 2008. A reduction of the mercury content was observed at all sites compared to 2007 with the exception of site 4. A large decrease in THg and MeHg was found at site 6 (2.25 times) from 0.18 to 0.08 mg·kg^{-1} THg. This was not found in the mercury content of the sediment from this site. Although the mercury concentration in 2007 did not exceed the hygienic

Table 2. Average total mercury content (mg·kg^{-1}) and % methylmercury to total mercury in chub (*Leuciscus cephalus* L.) in 2007 and 2008

	Total Hg		Methyl Hg to total Hg (%)	
Sample site	2007***	2008	2007***	2008
1 Bílovice nad Svitavou*	0.13 ± 0.06	0.11 ± 0.019	55.8 ± 25.1	70.5 ± 25.9
2 Svitava before junction*	0.11 ± 0.03	0.06 ± 0.016	68.2 ± 21.6	61.6 ± 19.1
3 Kníničky**	0.11 ± 0.04	0.07 ± 0.030	75.7 ± 26.3	66.4 ± 18.3
4 Svratka before junction **	0.10 ± 0.03	0.12 ± 0.143	85.6 ± 18.7	55.1 ± 30.2
5 Modřice**	0.08 ± 0.02	0.06 ± 0.083	52.8 ± 40.2	61.9 ± 24.3
6 Rajhradice**	0.18 ± 0.08	0.08 ± 0.032	87.8 ± 14.4	59.5 ± 19.5
7 Židlochovice**	0.13 ± 0.02	0.08 ± 0.022	74.2 ± 18.8	70.6 ± 16.5

* Svitava River; ** Svratka River, *** Results form Kružíková et al. (2009)

Table 3. Total mercury levels (mg·kg^{-1} dry weight) in sediment in 2008

Sample site	2007*** Mean± SD	2008 February	March	September	Mean ± SD
1 Bílovice nad Svitavou*	0.11 ± 0.03	0.031	0.061	0.046	0.05 ± 0.01
2 Svitava before the junction*	0.59 ± 0.23	0.190	0.205	0.905	0.43 ± 0.41
3 Kníničky**	0.06 ± 0.01	0.017	0.013	0.010	0.01 ± 0.01
4 Svratka before the junction**	1.21 ± 0.21	0.491	0.781	0.239	0.50 ± 0.27
5 Modřice**	0.71 ± 0.18	0.426	0.636	0.637	0.57 ± 0.12
6 Rajhradice**	1.15 ± 0.18	0.993	0.950	1.194	1.05 ± 0.13
7 Židlochovice**	0.51 ± 0.19	0.859	0.766	0.318	0.65 ± 0.29

* The Svitava River; ** the Svratka River, ***results from Kružíková et al. (2009)

limit, it is a felicitous finding that mercury contamination was reduced. The THg content was below the hygiene limits set by Commission Regulation (EC) No. 1881/2006 in either 2007 or 2008. The limit for total mercury in fish products and muscle meat of non-predator fish is 0.5 mg kg^{-1} and 1.0 mg kg^{-1} for selected fish species.

Many studies have examined the levels of mercury contamination in Czech rivers such as the Elbe (Dušek et al. 2005; Žlábek et al. 2005; Maršálek et al. 2006; Maršálek and Svobodová 2006), the Ohře, the Vltava, the Morava, and the Sázava (Kružíková et al. 2008a). In general, it has been demonstrated that mercury contamination of Czech rivers is low and does not exceed the limit of 0.5 mg·kg^{-1} FW, which is in agreement with the present study.

The proportion of methylmercury to total mercury ranged from 55.1 to 70.6% which is slightly lower than previous reports. Houserová et al. (2006) found MeHg to be over 95% of total mercury in fish tissue. The low MeHg/THg proportion indicates that conditions for methylation in the sediments were unfavourable. The rate of methylation is not only a function of total mercury concentrations but also of activity of methylating bacteria such as *Methanobacterium* (Gilmour and Henry 1991; Hamasaki et al. 1995). Compared to the results from 2007, a decrease in the MeHg proportion was observed (Table 2) on sites 1 and 5.

Total mercury in sediment

As in 2007, observations showed that mercury sediment levels did not vary widely within a single year. The exception was the concentration in September samples from site 2 (0.905 mg·kg^{-1}), site 4 (0.239 mg·kg^{-1}), and site 7 (0.318 mg·kg^{-1}). While a marked increase was found on site 2, a decrease was found on sites 4 and 7. The increase could be caused by a single atypical event. Total mercury content increased along the river with the highest

Table 4. Hazard indices for a standard consumer and a member of a fishing family and maximum weekly tolerable mercury intake (kg)

Sample site	Hazard index[1] standard consumer	Hazard index[1] fishing family	Maximum weekly tolerable intake[2]
1 Bílovice nad Svitavou*	0.018	0.137	1.6
2 Svitava before junction*	0.010	0.078	3.0
3 Kníničky**	0.013	0.097	2.3
4 Svratka before junction**	0.021	0.160	1.7
5 Modřice**	0.012	0.090	2.7
6 Rajhradice**	0.014	0.112	2.1
7 Židlochovice**	0.014	0.112	1.8

* The Svitava River; ** the Svratka River, [1]calculation of total mercury according to Kannan et al. (1998); [2]calculation of methyl mercury according to WHO (1990)

values downstream at site 6 in both 2007 and 2008, demonstrating an effect of Brno on mercury contamination in the sediment. The average THg content in sediment from 2008 is lower than in 2007 at the same sites (Table 3). Higher levels were found at sites 2 and 4 in comparison to upstream sites (1 and 3) showing the negative effect of Brno with increased pollution by waste water from households, chemical factories, and industry. Similarly to results in 2007 (Kružíková et al. 2009) the highest value was 1.15 ± 0.18 mg·kg^{-1}.

Although the highest values for THg in the sediment were found on site 6, the highest concentration in fish muscle was detected on site 4. One of the lowest values of THg in sediment was found at this site, indicating that the content in sediment does not correspond with the concentration of THg and MeHg in fish muscle.

All results from 2007 and 2008 were expressed as DW but Svobodová et al. (1988) have reported values of total mercury in sediment relative to organic matter. Characteristics of the analyzed sediment could impact mercury content in the muscle of fish. It is necessary to relate mercury content in the dry sediment to organic matter. Dissolved organic matter is known to promote (Weber 1993) or inhibit (Barkay et al. 1997) the formation of toxic and bioaccumulative methylmercury species.

Health hazard assessment

The hazard index (HI) for a standard consumer and a member of a fishing family were calculated for all sites according to the method set by Kannan et al. (1998) (Table 4). The calculated HI is compared to a value of 1. An HI below 1 indicates no hazard, an index ≥ 1 represents a hazard for the consumer. The average consumption of fish is used for this calculation. Recommended fish consumption is about 17 kg per capita per year. The average consumption of fish per individual worldwide is 16 kg per year, and only 11 kg per year in Europe. In the Czech Republic, the average consumption of fish is much lower than these values being 5.5 kg (for freshwater fish 1.3 kg, 4.2 kg for marine fish) (Ministry of Agriculture 2009). The average consumption of 10 kg per year was used for a fishing family (Table 4).

Hazard indices calculated for tested sites were several times lower than the hazard limit. A low HI indicated no significant health risk associated with the consumption of fish from the sites monitored in this study. The HI for both a standard consumer and a member of a fishing family was found in fish from site 4 but was still much lower than site 1. Similar results were obtained from fish sampled in 2007 (Kružíková et al. 2009).

The maximum weekly tolerable intake (Table 4) indicates the amount of fish meat that can be consumed per week without constituting a health risk. In view of the MeHg contamination, the highest quantity of fish can be consumed from sites 2 and 5, and the lowest from sites 1 and 4. Kružíková et al. (2009) identified the amount of fish that can be safely eaten from site 5 as 2.57 kg per week, a similar result to our study. Fish from the Svitava and Svratka rivers in the Czech Republic show very low HI, and their consumption poses no health risk from THg and MeHg contamination.

Acknowledgements

This research was supported by the project of Ministry of Education, Youth and Sports of the Czech Republic MSM 6215712402 and 2B06093.

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