Mercury and Methylmercury Contamination of Fish from the Skalka Reservoir: A Case Study

P. MARŠÁLEK[1,2], Z. SVOBODOVÁ[1,3], T. RANDÁK[3], J.ŠVEHLA[4]

[1]University of Veterinary and Pharmaceutical Science, Brno
[2]State Veterinary Institute, Brno
[3]University of South Bohemia in České Budějovice, Research Institute of Fish Culture and Hydrobiology, Vodňany
[4]University of South Bohemia, Faculty of Agriculture, České Budějovice

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Abstract

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The aim of this case study was to investigate the total mercury and methylmercury contamination of fish from the Skalka reservoir, Czech Republic. The reservoir had been polluted with mercury-containing sewage water for several decades. In total, 30 fish was sampled in August 2003. The fish caught included 5 roach (*Rutilus rutilus*), 8 bream (*Abramis brama*), 2 silver bream (*Blicca bjoerkna*), 7 big head carp (*Aristichthys nobilis*), 4 asp (*Aspius aspius*), 3 eel (*Anguilla anguilla*) and 1 wels catfish (*Silurus glanis*). Muscle tissue, the liver and the gonads were used for mercury determination. Total mercury in muscle tissue of the asp (as the representative of predatory species) and the bream (as the representative of non-predatory species) averaged 3.11 ± 0.20 mg·kg^{-1} and 0.96 ± 0.22 mg·kg^{-1}, respectively. All total mercury in muscle tissue of the asp and the bream was in methylmercury form. The content of total mercury and methylmercury was significantly ($p < 0.05$) higher in muscle tissue and the liver than in the gonads. The methylmercury-to-total mercury ratio in muscle tissue, the liver and the gonads averaged 1.012, 0.545 and 0.907, respectively. The results showed that mercury contamination of the Skalka reservoir continues to be very high, that consumption of predatory fish in particular poses a major health risk and that methylmercury was the predominant contaminant in fish tissues.

Total mercury, methylmercury, MeHg/THg ratio, bream, big head carp, roach, asp, muscle, liver, gonads, freshwater fish

For several decades, the rivers of Reslava and Ohře and the Skalka reservoir near Cheb, west Bohemia, were polluted with mercury-contaminating sewage water from a factory that manufactured mercury-based technical chemicals and preparations in Marktredwitz (Germany). Its sewage water was discharged to the stream Kösseine and went to the rivers Reslava and Ohře. The monitoring of mercury contamination levels in the Ohře, its tributaries and the Skalka reservoir was started in 1974 due to initiative of the State Water-Management Inspectorate (Hejtmánek et al. 1975; Svobodová et al. 1976). Depending on fish species, the range of average muscle tissue total mercury (THg) content was 0.14 – 0.49 mg·kg^{-1} wet weight (w.w.), 0.20 – 0.91 mg·kg^{-1} w.w. and 0.10 – 0.52 mg·kg^{-1} w. w. in the Skalka reservoir, in the Reslava and in the Ohře, respectively. Although mercury contamination of sewage water fell below the statutory limit 0.05 mg·l^{-1} in the second half of the 1970s (Vondrák et al. 1984), the 1980s saw a marked increase in fish mercury contamination. Levels of mercury contamination in fish caught in 1990, 1995 and 1996 in the rivers Reslava and Ohře and in the Skalka reservoir mostly greatly exceeded the statutory limit 0.5 mg·kg^{-1} w.w. and some levels were very high (e.g. 4.43 mg·kg^{-1} w.w. in muscle tissue of eel and 3.40 mg·kg^{-1} w.w. in perch). Mercury contamination ascertained in the perch liver reached 39.7 mg·kg^{-1} w.w. (Černá and Hrabětová 1996; Svobodová et al.

Address for correspondence:
Mgr. Petr Maršálek
University of Veterinary and Pharmaceutical Sciences
Department of Veterinary Public Health and Toxicology
Palackého 1-3, 612 42 Brno, Czech Republic

Phone: +420 737 611 508, +420 541 562 788
Fax: + 420 541 562 780
E-mail: petrmarsalek@seznam.cz
http://www.vfu.cz/acta-vet/actavet.htm

1997). The most likely cause of the increase was the burst sewage water pipeline in the factory in 1984. The Marktredwitz factory was then closed down. The entire basin was contaminated again in 1994 during Kösseine stream rehabilitation works when the contaminated sediments were exposed and washed away. The situation was aggravated by the changes in water level height, particularly in 1976 and 1982, when the reservoir was completely drained and filled with water again, followed by a twofold increase in total mercury levels in fish.

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 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 (WHO 1990). This makes them the main target in aqueous system contamination monitoring for both environmental and food safety purposes.

The aims of the present study were:

- to assess the contemporary mercury contamination levels in the Skalka reservoir and to compare them with historical figures
- to compare methylmercury-to-total mercury ratios in different tissues of the fish species studied
- to assess the health risks posed by fish consumption from the Skalka reservoir.

Materials and Methods

Location

The Skalka reservoir (Fig. 1) is located in a valley in western Bohemia on the river Ohře near the border with Germany. The reservoir was built in 1964, and its surface area is 3.78 km^2. The reservoir is managed by the Povodí Ohře company. The main purpose of the reservoir is to provide for at least minimum flow rates in the river Ohře. The reservoir should also provide a degree of protection of areas downstream of its dam against floods, generate hydroelectricity, and serve as a place for angling, recreation and water sports.

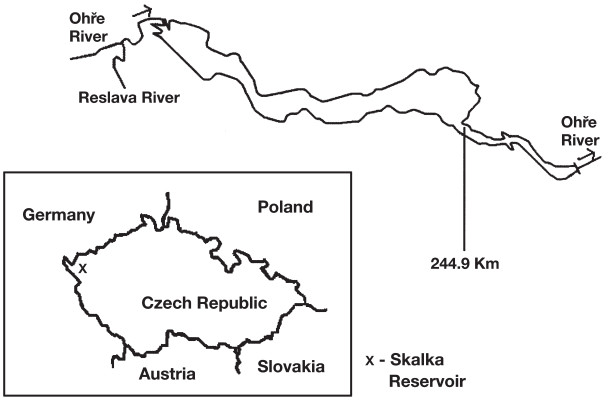

Fig. 1. Skalka reservoir near Cheb and the site where fish were caught

Material

The fish from the Skalka reservoir was caught in August 2003. The fish were caught at 244.9 river km using toils, scoop nets and hook and bait. A total of 30 fish was caught (Table 1). The fish caught included 5 roach (*Rutilus rutilus*), 8 bream (*Abramis brama*), 2 silver bream (*Blicca bjoerkna*), 7 big head carp (*Aristichthys nobilis*), 4 asp

(*Aspius aspius*), 3 eel (*Anguilla anguilla*) and 1 wels catfish (*Silurus glanis*). The fish were weighed and their scales collected for age determination. Samples of muscle tissues, the liver and the gonads were then collected, frozen in plastic bags at -20 °C and analyzed during the next 1 or 2 months.

Table 1. Characteristics of the fish

Species	n	Weight (g) mean ± SD range	Age (years) mean ± SD range
Big head carp	7	10 042 ± 5 290 (3 300 – 18 000)	11.1 ± 5.3 (4 – 19)
Bream	8	764 ± 331 (85 – 1 165)	5.4 ± 1.4 (3 – 7)
Roach	5	221 ± 130 (75 – 370)	3.8 ± 1.3 (2 – 5)
Silver bream	2	255, 300	5
Asp	4	2 001 ± 219 (1 680 – 2 175)	5.5 ± 0.5 (5 – 6)
Eel	3	308 ± 105 (190 – 390)	N/A
Wels	1	2 500	4

Analytical methods

Total mercury content in fish tissues was determined by the direct method of cold vapours using an AMA 254 (Altec Ltd.) analyzer. MeHg was determined in the form of a MeHgCl by gas chromatography. Samples were prepared by acidic digestion and extraction to toluene (AOAC 1992). For the determination, Hewlet Packard 5890 Series II gas chromatograph was used. A capillary column DB 608 (30 m) (Caricchia et al. 1997) and an electron capture detector (ECD) were used. Evaluation was made using HP 3365 ChemStation Series II software (Hewlet 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.)

Result quality assurance

BCR 463 and BCR 464 (IRMM Belgie) 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 testing of species and tissues differences and the Spearman correlation was used for testing of 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.).

Results and Discussion

The results of fish tissues analysis for total mercury (THg) and methylmercury (MeHg) contents are given in Table 2. Fish of both sexes were evaluated together in the rest of the study.

Total mercury (THg)

The significantly highest THg content in muscle tissue ($p < 0.05$) was found in asp followed by eel and big head carp, bream and roach. There were no significant differences in muscle tissue THg contents between big head carp, bream and roach. THg content in the liver was significantly higher ($p < 0.05$) in asp and eel than in bream and roach. Big head carp liver THg levels showed no significant differences compared with other species. This was due to the high variability of big head carp liver THg levels (Table 2) and particularly by their three high scattered values (11.6, 20.3 and 24.9 $mg \cdot kg^{-1}$). THg content in asp gonads was significantly higher ($p < 0.05$) than in the gonads of big head carp, bream and roach. Differences in contamination were evaluated only in the species where 3 or more samples

Table 2. Total mercury (THg) and methylmercury (MeHg) concentrations in samples of muscle tissue, the liver and the gonads of different fish species (in mg/kg w.w.) and methylmercury-to-total mercury ratios

Species	Tissue	n	MeHg (mg·kg^{-1}) mean ± SD range	THg (mg·kg^{-1}) mean ± SD range	MeHg/THg mean ± SD range
Big head carp	muscle	7	0.90 ± 0.28 (0.56 – 1.26)	0.83 ± 0.21 (0.53 – 1.04)	1.01 ± 0.18 (0.64 – 1.15)
	liver	7	0.70 ± 0.30 (0.29 –1.07)	8.81 ± 10.2 (0.42 – 24.89)	0.33 ± 0.29 (0.02 – 0.64)
	gonads	7	0.19 ± 0.07 (0.11 – 0.31)	0.25 ± 0.13 (0.1 – 0.42)	0.80 ± 0.25 (0.38 – 1.02)
Bream	muscle	8	1.04 ± 0.24 (0.59 – 1.33)	0.96 ± 0.22 (0.55 – 1.23)	1.01 ± 0.06 (0.94 – 1.15)
	liver	8	0.75 ± 0.26 (0.29 – 1.03)	1.50 ± 0.70 (0.63 – 2.98)	0.48 ± 0.10 (0.30 – 0.61)
	gonads	8	0.55 ± 0.26 (0.24 – 1.05)	0.52 ± 0.23 (0.23 – 0.96)	0.98 ± 0.06 (0.83 – 1.03)
Roach	muscle	5	0.87 ± 0.29 (0.65 – 1.37)	0.81 ± 0.28 (0.58 – 1.27)	1.01 ± 0.03 (0.97 – 1.04)
	liver	5	0.73 ± 0.43 (0.44 – 1.37)	0.88 ± 0.52 (0.39 – 1.69)	0.88 ± 0.15 (0.75 – 1.04)
	gonads	5	0.30 ± 0.19 (0.18 – 0.63)	0.28 ± 0.18 (0.16 – 0.59)	1.01 ± 0.03 (0.97 – 1.05)
Silver bream	muscle	2	0.91 – 0.99	0.81 – 0.91	1.01 – 1.05
	liver	2	0.21 – 0.99	0.31 – 1.62	0.57 – 0.63
	gonads	2	0.35	0.31 – 0.80	1.05
Asp	muscle	4	3.41 ± 0.45 (3.00 – 4.05)	3.11 ± 0.20 (2.93 – 3.40)	1.02 ± 0.09 (0.91 – 1.11)
	liver	4	4.36 ± 1.71 (2.84 – 6.81)	6.24 ± 1.67 (4.19 – 8.20)	0.67 ± 0.20 (0.47 – 0.95)
	gonads	4	1.80 ± 0.44 (1.25 – 2.31)	1.71 ± 0.38 (1.17 – 2.08)	0.98 ± 0.05 (0.91 – 1.03)
Eel	muscle	3	1.85 ± 0.23 (1.66 – 2.11)	1.80 ± 0.40 (1.52 – 2.26)	0.96 ± 0.08 (0.87 – 1.02)
	liver	3	2.89 ± 2.01 (1.72 ± 5.21)	4.46 ± 3.27 (2.50 – 8.23)	0.61 ± 0.03 (0.59 – 0.65)
Wels	muscle	1	1.11	0.96	1.07
	liver	1	1.01	1.03	0.91

were available. Higher THg contents in asp and eel reflect their position in the food chain and show that higher total mercury levels will be found in predatory fish than in non-predatory fish species, which has also been corroborated by findings of other authors (Jewett et al. 2003; Brabo et al. 2000; Peňáz et al. 1979). The higher level of THg in asp compared with eel can probably be ascribed to their different diets and also their different ability to migrate (Baruš et al. 1985ab)

THg distribution was evaluated for each species separately. Only species with at least three fish available were evaluated. Asp, big head carp and bream had significantly the highest ($p < 0.05$) THg contents in the liver followed by muscle tissue and the gonads. Roach had a significantly higher THg content ($p < 0.05$) in muscle tissue and the liver than in the gonads. Eel showed no significant difference in THg contents between muscle tissue and the

liver. Results in all species studied showed that the lowest THg levels were in the gonads. While THg levels in the liver and muscle tissue were different in the individual species, it was true in most of them that liver THg levels were higher. The distribution in tissues of different fish species from the Skalka reservoir corresponded to that found in fish from the Želivka reservoir, i.e. significantly higher levels in the liver than in muscle tissue were found in asp and bream. Roach had significantly higher levels in muscle tissue than in the liver. The lowest THg level in all species from the Želivka reservoir was found in the gonads (Svobodová et al. 1988)

Methylmercury and methylmercury-to-total mercury ratio

After the conversion of MeHg to elementary mercury content, it was clear that almost 100% of mercury in muscle tissue and the gonads was MeHg (Table 2). The MeHg-to-THg ratio was significantly higher ($p < 0.01$) in muscle tissue (1.01 ± 0.19; median = 1.01) and the gonads (0.94 ± 0.15; median = 1.00) than in the liver (0.57 ± 0.25; median = 0.59). This fact indicates a higher proportion of inorganic mercury in the liver. Jewet et al. (2003) also reported a higher proportion of MeHg in THg in muscle tissue than in the liver, which is probably due to MeHg demethylation taking place there. MeHg values exceeding 100% are due to error in determination of both THg and MeHg. The correlation between THg and MeHg levels was assessed by the linear regression method. Values from each tissue were tested separately. In all three tissues, significant ($p < 0.01$) correlation between THg and MeHg contents was found. The correlation coefficients for muscles, livers and gonads were r = 0.983 (n = 30), r = 0.932 (n = 27) and r = 0.993 (n = 27), respectively. Three THg values had to be discarded from big head carp liver correlation assessments as too widely scattered. A significant correlation between MeHg and THg contents confirms the key role of MeHg in Hg accumulation in fish tissues.

To evaluate the differences in MeHg contamination between different species, results in asp, big head carp, bream, roach and eel were used. A comparison between MeHg levels in muscle tissue of the individual fish species ($p < 0.05$) produced the following order: asp > eel > big head carp, roach and bream. Liver MeHg content was significantly higher ($p < 0.05$) in asp and eel than in big head carp, bream and roach. MeHg content in asp gonads was significantly higher ($p < 0.05$) than in the gonads of big head carp, bream and roach. The results indicate higher MeHg levels in predatory species compared with non-predatory ones, which corresponds to differences in THg levels between the species.

As far as the distribution of MeHg in fish tissues is concerned, big head carp and bream had significantly ($p < 0.05$) the highest MeHg contents in muscle tissue, lower in the liver and the lowest in the gonads. Asp and roach had significantly higher MeHg content ($p < 0.05$) in muscle tissue and the liver than in the gonads. Eel showed no significant difference in MeHg contents between muscle tissue and the liver. Results of MeHg distribution showed that the lowest MeHg levels were in the gonads in all fish species studied. As with THg, a comparison between the liver and muscle tissue shows inter-species differences. Contrary to THg, however, MeHg levels in muscle tissue were higher than, or equal to, MeHg levels in the liver. This fact is probably due to the demethylation of MeHg in the liver and it serves to confirm the results of the MeHg/THg ratio assessment between the individual tissues.

The relation of THg and MeHg contents to the weight and age of the fish was tested by the Spearman correlation method. Significant correlations ($p < 0.1$) between mercury content and fish weight were found for MeHg in muscle tissue and the liver of big head carp (r = 0.75 a r = 0.96; n = 7) and for THg in muscle tissue of big head carp (r = 0.65; n = 7). Significant correlations ($p < 0.1$) between mercury content and fish age were found for

MeHg in muscle tissue, the liver and gonads of big head carp (r = 0.75; 0.96 and 0.67; n = 7) and for THg in muscle tissue of big head carp (r = 0.67; n = 7). No significant correlations were found in tissues of other fish. This may be due to the small number of specimens tested, and also by different life history of individual fish. Significant correlations between THg levels in fish tissues, and particularly in musculature, have been reported by a number of authors (Norstrom et al. 1976; Jewett et al. 2003; Peňáz et al. 1979). In most of the cases, however, they tested a larger number of fish.

Comparison with historical dates

The results obtained were compared with the results of THg monitoring in selected fish species from the Skalka reservoir carried out in 1995 and 1996 (Černá and Hrabětová 1996; Svobodová et al. 1997). THg levels in muscle tissue of asp caught in 1995 (1.63-3.04 $mg \cdot kg^{-1}$ w.w., mean value 2.37 ± 0.57 $mg \cdot kg^{-1}$ w.w., n = 6) were significantly lower ($p < 0.05$) than THg levels in asp caught in 2003 (Tab. 2). There were no significant differences in THg levels in muscle tissue between bream caught in 1996 (0.32 - 1.38 $mg \cdot kg^{-1}$ w.w., mean value 1.08 ± 0.54 $mg \cdot kg^{-1}$ w.w., n = 5) and the bream caught in 2003 (Table 2). Neither were there any significant differences in THg levels in muscle tissue between roach caught in 1996 (0.38 – 0.70 $mg \cdot kg^{-1}$ w.w., mean value 0.51 ± 0.13 $mg \cdot kg^{-1}$ w.w., n = 5) and the roach caught in 2003. It follows from the comparison that there were no significant changes in the total mercury content in the Skalka reservoir in the period following the 1995 - 1996 monitoring in spite of the fact that the source of contamination had been removed. At a number of other sites, mercury levels in fish muscle tissue decreased following the removal of the source of contamination (Strong 1981; Busch 1983). Transport of mercury in water ecosystems depends, of course, mainly on local conditions, both natural (Lange et al. 1993) and, in the case of artificial lakes, on technical conditions. The water level in the Skalka reservoir may drop as a result of both scheduled and unscheduled water discharges from the reservoir, which exposed approximately a half of the reservoir area (Vondrák et al. 1984). Consequently sediments shift, contaminated sediments get exposed and dry, and the deep-deposited mercury is then released back into the water ecosystem.

Assessment of health risk

In the assessment of heath risks posed by eating fish from the Skalka reservoir, the authors used the maximum THg and MeHg doses allowed by FAO/WHO, i.e. 0.3 mg THg/person/week and 0.2 mg MeHg/person/week. Health risks were evaluated for asp as a representative of predatory species and for bream as a representative of non-predatory species. Using THg values, the maximum weekly recommended consumption of the fish would then be 96 g of asp or 305 g of bream. Using MeHg levels, the figures would be 59 g of asp or 185 g of bream. In view of the MeHg-to-THg ratio, it is much more appropriate to choose MeHg levels for food safety assessments. It follows from the above values that eating the fish poses a major health risk and a regular consumption of particularly of predatory fish from Skalka cannot be recommended under any circumstances. THg content in muscle tissues in all the fish also exceed 0.5 $mg \cdot kg^{-1}$ w.w., which in many countries is the statutory limit for THg levels in fish for human consumption. The assessment of heath risks is valid on condition that fish from Skalka reservoir is the only source of mercury in food.

Kontaminace ryb celkovou rtutí a methylrtutí v nádrži Skalka: případová studie

Cílem této práce bylo zjistit a zhodnotit stav zatížení tkání ryb z údolní nádrže Skalka v České republice celkovou rtutí a methylrtutí. Tato nádrž byla několik desítek let znečišťována odpadními vodami obsahujícími rtuť. V srpnu 2003 bylo odloveno celkem 30

kusů ryb, z toho: 5 kusů plotice obecné (*Rutilus rutilus*), 8 kusů cejna velkého (*Abramis brama*), 2 kusy cejnka malého (*Blicca bjoerkna*), 7 kusů tolstolobika pestrého (*Aristichthys nobilis*), 4 kusy bolena dravého (*Aspius aspius*), 3 kusy úhoře říčního (*Anguilla anguilla*) a 1 kus sumce velkého (*Silurus glanis*). K analýzám byla použita svalovina, játra a gonády. Průměrný obsah celkové rtuti byl ve svalovině bolena dravého (jako zástupce dravých druhů) 3,11 ± 0,20 mg·kg^{-1} a u cejna velkého (jako zástupce nedravých druhů) 0,96 ± 0,22 mg·kg^{-1}. Všechna celková rtuť byla ve svalovině bolena dravého a cejna velkého ve formě methylrtuti. Obsah celkové rtuti a methylrtuti byl signifikantně ($p < 0,05$) vyšší ve svalovině a játrech ve srovnání s gonádami. Průměrný poměr methylrtuti a celkové rtuti byl ve svalovině 1,012, v játrech 0,545 a v gonádách 0,907.Výsledky ukazují na stále vysokou kontaminaci nádrže Skalka rtutí, na vysoké hygienické riziko konzumace zejména dravých ryb a na převažující podíl methylrtuti v tkáních ryb.

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