Mercury and Methylmercury Concentrations in Muscle Tissue of Fish Caught in Major Rivers of the Czech Republic

K. KRUŽÍKOVÁ[1], T. RANDÁK[2], R. KENŠOVÁ[1], H. KROUPOVÁ[2], D. LEONTOVYČOVÁ[3], Z. SVOBODOVÁ[1,2]

[1)] Department of Veterinary Public Health and Toxicology, Faculty of Veterinari Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic
[2)] Research Institute of Fish Culture and Hydrobiology Vodňany, University of South Bohemia, Czech Republic
[3)] Czech Hydrometeorological Institute Prague

Received February 13, 2008
Accepted June 11, 2008

Abstract

Kružíková K., T. Randák, R. Kenšová, H. Kroupová, D. Leontovyčová, Z. Svobodová: Mercury and Methylmercury Concentrations in Muscle Tissue of Fish Caught in Major Rivers of the Czech Republic. Acta Vet Brno 2008, 77: 637-643.

The aim of the study was to evaluate mercury contamination at twelve outlet sites of rivers in the Czech Republic (Labe, Ohře, Vltava, Berounka, Sázava, Otava, Lužnice, Svratka, Dyje, Morava and Odra). As an indicator, we used muscle tissue of the chub (*Leuciscus cephalus*) caught at selected sites in 2007. A total of 96 fish were examined. Total mercury was determined by atomic absorption spectrophotometry using the AMA 254 analyzer and methylmercury was determined by gas chromatography with electron-capture detection. Total mercury (THg) and methylmercury (MeHg) concentrations ranged 0.039–0.384 mg·kg^{-1} fresh weight and 0.033–0.362 mg·kg^{-1} fresh weight, respectively. Mercury bound in methylmercury (Hg_{Me}) made up on average about 82.2% of total mercury. The highest mercury concentrations were found in fish from Obříství, a site on Labe (THg 0.263 ± 0.086 mg·kg^{-1}; MeHg 0.256 ± 0.084 mg·kg^{-1}). Mercury concentrations in fish from rivers that cross the borders of the Czech Republic (Labe, Odra and Morava) were low. The Czech Republic therefore does not contribute significantly to river pollution outside its national borders. Hazard indices of the sites monitored were well below 1, and reached 1.365 only in Obříství on Labe for fisherman's family members (i.e. in the case of annual consumption of 10 kg fish). This indicates possible hazards involved in eating meat of fish caught in that location. Based on PTWI for methylmercury, the maximum amount of fish meat allowed for consumption per week was calculated. The site with the lowest value was Obříství on Labe (0.44 kg). The results of this study present a partial contribution to health risk assessment on the major rivers in Czech Republic.

Leuciscus cephalus, methylmercury/mercury ratio, THg, MeHg, hazard index, PTWI

Mercury is a global pollutant and is distributed in the natural environment including bioorganisms. Among naturally and anthropogenically occurring types of mercury, methylmercury (MeHg) is the most hazardous for human health, especially for the developing foetus (Yasutake et al. 2005). As it advances in the food chain, MeHg accumulates in fish, and fish thus become the main source of human contamination with methylmercury (WHO 1990). Mercury bound in methylmercury (Hg_{Me}) makes up 70–95% of total mercury (THg) in fish tissues (Mason et al. 1995; Houserová et al. 2006b). Mercury (like PCBs and other compounds) has been shown to affect the endocrine systems and reproductive success of several fish species (Friedmann et al. 2002).

The monitoring of mercury contamination of surface water and the evaluation of health hazards related to fish consumption have been given much attention in the Czech Republic (Maršálek et al. 2007), with most attention paid to the longest Czech river, Labe (Dušek et al. 2005; Maršálek et al. 2006; Žlábek et al. 2005).

In 2007, attention focused on important outlet sections of rivers and their variables at the

Address for correspondence:
Ing. Kamila Kružíková
University of Veterinary and Pharmaceutical Sciences
Palackého 1-3
612 42 Brno
Czech Republic

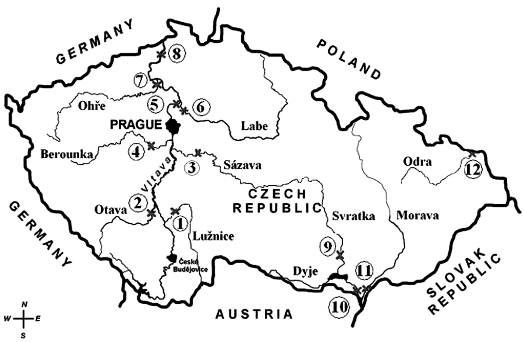
Phone: +420 5 4156 2783
Fax: +420 5 4156 2790
E-mail: kruzikovak@vfu.cz
http://www.vfu.cz/acta-vet/actavet.htm

borders with our countries. That also included sites that characterize the rivers before they leave the Czech Republic and enter neighbouring states. Thus, it was possible to estimate the role of the Czech Republic in the contamination of rivers that transverse several countries.

The aim of our study was to analyse the total mercury and methylmercury content in muscle of chub caught in eleven most important rivers in the Czech Republic, to assess mercury contamination contributions of individual localities and to evaluate health hazards of eating fish from the selected rivers.

Materials and Methods

A total of 96 indicator fish (*Leuciscus cephalus* - males) were collected at the selected localities in 2007. The chub came from the twelve sites on eleven major rivers in the Czech Republic, i.e. Lužnice - Bechyně (river km 11), Otava - Topělec (river km 20), Sázava - Nespeky (river km 27.5), Berounka - Srbsko (river km 29), Vltava - Zelčín (river km 5), Labe - Obříství (river km 122), Ohře - Terezín (river km 3), Labe - Děčín (river km 21), Svratka - Židlochovice (river km 23), Dyje - Pohansko (river km 16), Morava - Lanžhot (river km 9.5), Odra - Bohumín (river km 9) (Fig. 1). River kilometres of the Labe sites (Obříství and Děčín) and Odra site (Bohumín) were measured from the border with Germany and Poland.



Legend:
1. Lužnice - Bechyně
2. Otava - Topělec
3. Sázava - Nespeky
4. Berounka - Srbsko
5. Vltava - Zelčín
6. Labe - Obříství
7. Ohře - Terezín
8. Labe - Děčín
9. Svratka - Židlochovice
10. Dyje - Pohansko
11. Morava - Lanžhot
12. Odra - Bohumín

Fig 1. The map shows localities where fish were caught

The fish were collected after they were stunned with electrofishing equipment. They were immediately weighed and muscle tissue samples were taken for analysis of total mercury and methylmercury. The age of fish was determined from their scales. The sex of fish was determined macroscopically. Samples of muscle tissue were put into polyethylene bags, labelled and stored in a freezer at -18 °C.

Fish muscle mercury (THg) concentrations were determined by cold vapour atomic absorption spectrometry on AMA 254 (Altec Ltd., Czech Republic) analyser.

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

The limit of detection (LOD) was set as a sum of triple the standard deviation of a blank and a blank mean value. Detection limit for THg and MeHg determination methods were 1 $mg \cdot kg^{-1}$ and 21 $mg \cdot kg^{-1}$, respectively. Accuracy of the results of THg and MeHg was validated using standard reference material BCR-CRM 464 (Tuna Fish, IRMM, Belgium).

Total mercury and methylmercury concentrations are given in $mg \cdot kg^{-1}$ fresh weight (FW). For the methylmercury/mercury ratio evaluations, the methylmercury value was expressed as Hg_{Me}.

The hazard index was calculated according to Kannan et al. (1998) using a reference dose (RfD) for THg (0.3 $mg \cdot kg^{-1}$ body weight per day) set forth by US EPA.

To determine the maximum possible consumption of fish meat, the provisional tolerable weekly intake limit (PTWI) of 1.6 µg MeHg per kg body weight per week was used (WHO 1990).

Statistical analysis of the data was performed using the program STATISTICA 8.0 for Windows (StatSoft CR). The data were assessed by non-parametric Kruskal-Wallis test because data normality was not proven. Whenever the Kruskal-Wallis test showed significant differences between profiles ($P < 0.05$), multiple comparisons of all profiles were subsequently performed.

Results and Discussion

Total mercury and methylmercury concentrations in fish

Total mercury and methylmercury was found in all 96 examined samples, but in five samples from Lužnice - Bechyně and one sample from Berounka - Srbsko, methylmercury concentrations were below the detection limit. The main characteristics of the caught fish are given in Table 1.

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

Locality	n	Body weight (g) mean ± SD	Age (years) mean ± SD
1 Lužnice - Bechyně	9	182.2 ± 105.12	3.1 ± 0.60
2 Otava - Topělec	7	93.6 ± 34.12	2.9 ± 0.38
3 Sázava - Nespeky	9	105.6 ± 44.82	2.4 ± 0.53
4 Berounka - Srbsko	9	265.0 ± 72.24	3.4 ± 0.53
5 Vltava - Zelčín	9	282.7 ± 84.41	4.1 ± 0.78
6 Labe - Obříství	10	272.0 ± 170.20	4.0 ± 1.15
7 Ohře - Louny	9	347.8 ± 75.00	4.4 ± 0.53
8 Labe - Děčín	9	343.9 ± 104.01	4.3 ± 0.71
9 Svratka - Židlochovice	7	266.4 ± 50.47	3.4 ± 0.53
10 Dyje - Pohansko	7	257.1 ± 96.95	3.9 ± 1.21
11 Morava - Lanžhot	4	426.0 ± 381.13	4.5 ± 0.58
12 Odra - Bohumín	7	194.3 ± 52.08	3.6 ± 0.53

n: number of fish examined

Medians of total mercury and methylmercury concentrations from individual sites are given in Figs 2 and 3. The lowest concentrations of mercury and methylmercury were 0.097 ± 0.032 mg·kg^{-1} and 0.076 ± 0.035 mg·kg^{-1}, respectively. These concentrations were found in chub from Dyje - Pohansko. On the other hand, the highest concentrations of total mercury and methylmercury (0.263 ± 0.086 mg·kg^{-1} and 0.256 ± 0.084 mg·kg^{-1}, respectively) were found in chub from Labe - Obříství.

THg concentrations at Obříství (Labe) were significantly higher ($P < 0.05$) than those found at the sites on the Berounka, Otava, Lužnice, Dyje and at the Labe site of Děčín. Concentrations from the Berounka site were significantly lower ($P < 0.05$) also compared to the Vltava site.

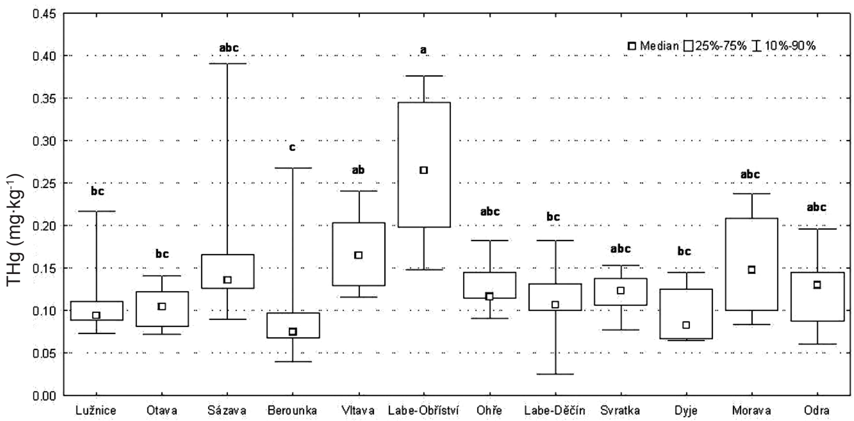


Fig. 2. Content of THg in selected localities
[a, b, c,] Groups with different alphabetic superscripts differ significantly ($P < 0.05$)

MeHg concentrations at Obříství on Labe were significantly higher ($P < 0.05$) than those found at sites on the Lužnice, Berounka and Dyje. A significant difference ($P < 0.05$) was also found between the Lužnice and the Vltava sites.

Long-term high mercury contamination of the aqueous environment has been observed in Labe. Contamination monitoring there has been going on for many years, and has been documented in a number of studies (Žlábek et al. 2005; Dušek et al. 2005; Maršálek et

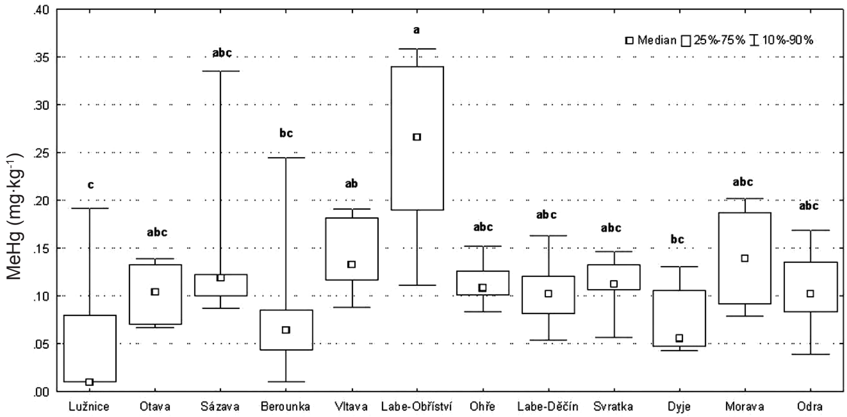


Fig. 3. Content of MeHg in selected localities.
[a, b, c,] Groups with different alphabetic superscripts differ significantly ($P < 0.05$)

al. 2006). Of all the sites monitored in the present study, the highest concentrations of Hg and MeHg (0.263 mg·kg^{-1} and 0.256 mg·kg^{-1}, respectively) were found at Obříství on Labe (river km 122). Dušek et al. (2005) monitored Labe between 1991 and 1996. Their data showed increased mercury concentrations (0.306 mg·kg^{-1} in muscle tissues of omnivorous fish) in the vicinity of the Spolana Neratovice factory (river km 122). Their finding was corroborated by Maršálek et al. (2006), who in 2004 found up to 0.933 and 0.83 mg·kg^{-1} muscle in chub caught upstream (river km 121) and downstream (river km 116) of the Spolana Neratovice factory, respectively. Žlábek et al. (2005) also reported higher Hg concentrations in chub caught in 2003 at Lysá nad Labem on Labe (river km 160) and at Obříství (river km 116) (0.9 and 1.5 mg·kg^{-1}, respectively). Similarly to other authors, they reported different Hg concentrations at different sites along Labe, which has been corroborated by our finding of 0.118 mg·kg^{-1} muscle at Děčín, which is 100 km farther downstream Labe than the already mentioned Obříství site (0.263 mg·kg^{-1}). A comparison with the findings of Žlábek et al. (2005) shows a threefold decrease in the level of contamination of Labe at that site compared with data from 2003.

In their paper on mercury concentrations found in different species of fish in Odra, Svobodová and Hejtmánek (1976) reported mercury concentration of about 0.18 mg·kg^{-1} in chub muscle tissue, which is similar to mercury concentration values found in our study (0.128 mg·kg^{-1}) in 2007.

Mean mercury concentrations in muscle tissue of chub caught in rivers that run out of the Czech Republic were in the range of 0.09–0.154 mg·kg^{-1}. The lowest mercury concentration was found in the river Morava that runs to Austria.

Hg_{Me}/THg ratio

Fig. 4 shows the differences in percentages of Hg_{Me} to total mercury at individual sites. To calculate Hg_{Me}/THg ratios, MeHg was expressed as Hg weight and related to THg (in %). The highest percentage of Hg_{Me} to total mercury was found at Obříství on Labe (90.4%), and the lowest percentage of Hg_{Me} /THg was found in muscle tissue of fish caught in Vltava and Odra (76 and 77% Hg_{Me} to THg, respectively). No significant differences were found between the monitored sites.

Houserová et al. (2006a) and Maršálek et al. (2005) also reported high percentages of Hg_{Me} to THg ranging from 74 do 100% in their chub studies. In their analysis of muscle tissue of carp (*Cyprinus carpio*), Maršálek et al. (2007) reported high percentages of Hg_{Me} to THg (87.9–98.5%), i.e. similar percentages of methylmercury to total mercury as those found

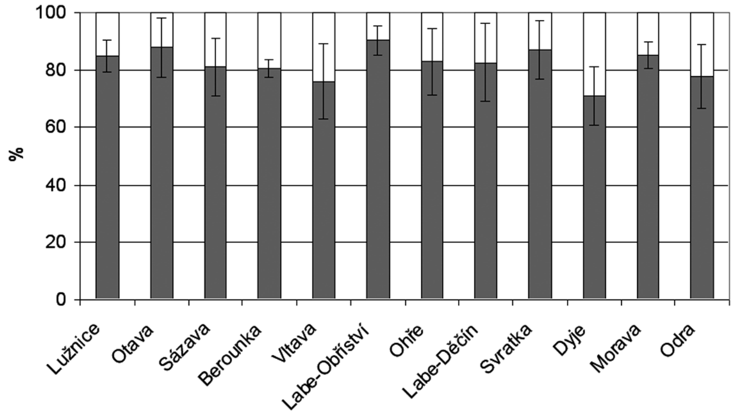


Fig. 4. Mean percentages of Hg_{Me} to total mercury in muscle tissue of fish from individual localities. Dark columns show percentage of Hg_{Me}, THg is 100%.

in our analysis of chub, which ranged from 53.6 to 99.6%. Kannan et al. (1998) also mentioned differences in Hg_{Me}/THg ratios. Such differences suggest different conditions for mercury methylation in river sediment, e.g. redox conditions, types of sediment, microflora, and season of the year (Maršálek 2006).

Health hazard assessment

Potential health hazard caused by mercury in fish was calculated according to the method of Kannan et al. (1998), who described the calculation of a hazard index associated with fish consumption. Hazard index below 1 indicates no hazard for consumers. In hazard index calculations, average consumption of freshwater fish in the Czech Republic was used, i.e. 1 kg per capita (Ministry of Agriculture 2007) and 10 kg per member of fisherman's household. Hazard indices calculated for Hg are given in Table 2. The indices given are low, in fact several times lower than the hazard index of 1, only at Obříství on Labe the hazard index for fisherman's family is 1.365, which indicates a possible hazard associated with fish consumption.

Table 2. Hazard indices for a standard consumer and a member of a fisherman's family, and maximum tolerable weekly intakes of chub meat from monitored localities

	Hazard index for standard consumer*	Hazard index for fisherman's family*	Maximum weekly tolerable intake** (kg)
Lužnice-Bechyně	0.014	0.571	1.00
Otava-Topělec	0.014	0.545	1.13
Sázava-Nespeky	0.022	0.882	0.76
Berounka-Srbsko	0.013	0.503	1.27
Vltava-Zelčín	0.023	0.903	0.80
Labe-Obříství	0.034	1.365	0.44
Ohře-Terezín	0.016	0.664	0.99
Labe-Děčín	0.015	0.612	1.06
Svratka-Židlochovice	0.016	0.628	0.98
Dyje-Pohansko	0.013	0.503	1.47
Morava-Lanžhot	0.020	0.799	0.81
Odra-Bohumín	0.017	0.659	1.04

* calculation of THg according to Kannan (1998)
** calculation of MeHg according to WHO

The World Health Organisation (WHO) has been observing the MeHg issue and has set a maximum recommended dose of MeHg, the so-called Provisional Tolerable Weekly Intake (PTWI), at 1.6 μg MeHg·kg^{-1} body weight/week. This value can then be used to calculate the amount of fish meat that a consumer from a specific site can eat. From the MeHg contamination point of view, the best rivers of the ones monitored in the present study were Dyje - Pohansko and Berounka - Srbsko, where up to 1.47 kg and 1.27 kg, respectively, of fish captured can be consumed per week. On the other hand, the maximum amounts of fish that can be consumed per week from Labe - Obříství and Vltava - Zelčín are only 0.44 kg and 0.80 kg, respectively.

The limit of 0.5 mg Hg·kg^{-1} wet weight provided by the Commission Regulation (EC) No. 1881/2006 setting the maximum level for certain contaminants in foodstuffs, has not been exceeded in any samples of chub muscle.

Obsah rtuti a methylrtuti ve svalovině ryb z významných českých řek

Cílem předkládané práce bylo zhodnotit dvanáct závěrových profilů řek na území České republiky (Labe, Ohře, Vltava, Berounka, Sázava, Otava, Lužnice, Svratka, Dyje, Morava, Odra) z hlediska jejich zatížení rtutí. Jako indikátoru byla použita svalovina jelce tloušť (*Leuciscus cephalus*) odloveného v roce 2007 na vybraných lokalitách. Celkem bylo analyzováno 96 kusů ryb. Celková rtuť byla měřena metodou atomové absorpční spektrofotometrie na analyzátoru AMA 254 a methylrtuť metodou plynové chromatografie s detekcí elektronového záchytu. Obsah celkové rtuti (THg) ve svalovině se pohyboval v rozmezí 0,039–0,384 mg·kg^{-1} čerstvé tkáně a methylrtuti (MeHg) v rozmezí 0,033–0,362 mg·kg^{-1} čerstvé tkáně. Rtuť vázaná v methyrtuti (Hg_{Me}) zaujímá v průměru cca 82,2% z celkové rtuti. Největší zatížení rtutí bylo zjištěno v rybách z řeky Labe v lokalitě Obříství (THg 0,263 ± 0,086 mg·kg^{-1}; MeHg 0,256 ± 0,084 mg·kg^{-1}). Ryby z řek vytékajících za hranice ČR (Labe, Odra, Morava) mají nízké hodnoty obsahu rtuti. ČR se tak nepodílí významnou měrou na jejich dalším znečištění za hranicemi ČR. Index rizika ze sledovaných lokalit je několikanásobně nižší než 1, pouze v lokalitě Labe - Obříství dosahuje hodnoty 1,365 pro členy rybářské rodiny (tj. při konzumaci 10 kg ryb ročně). To ukazuje na možné riziko konzumace rybího masa z dané lokality. Na základě hodnot PTWI methylrtuti bylo stanoveno nejvyšší množství rybího masa, které lze zkonzumovat za týden. Nejnižší hodnota byla v lokalitě Labe - Obříství (0,44 kg). Výsledky práce jsou dílčím příspěvkem pro hodnocení zdravotního rizika na nejvýznamnějších tocích České republiky.

Acknowledgement

This study was supported by the project MSM 6215712402 (Ministry of Education, Youth and Sports of the Czech Republic), SP/2e7/229/07 and The Biomonitoring Programme of the Czech Hydrometeorological Institute and Ministry of the Environment of the Czech Republic.

References

CARICCHIA AM, MINERVINI G, SOLDATI P, CHIAVARINI S, UBALDI C, MORABITO R 1997: GC-ECD determination of methylmercury in sediment samples using a SPB-608 capillary column after alkaline digestion. Microchem J **55**: 44-55

COMMISSION REGULATION (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants of foodstuffs

DUŠEK L, SVOBODOVÁ Z, JANOUŠKOVÁ D, VYKUSOVÁ B, JARKOVSKÝ J, ŠMÍD R, PAVLIŠ P 2005: Bioaccumulation of mercury in muscle tissue of fish in the Elbe river (Czech Republic): multispecies monitoring study 1991-1996. Ecotox Environ Safe **61**: 256-267

FRIEDMANN AS, COSTAIN EK, MACLATCHY DL, STANSLEY W, WASHUTA EJ 2002: Effect of mercury on general and reproductive health of largemouth bass (*Micropterus salmoides*) from three lakes in New Jersey. Ecotox Environ Safe **52**: 117-122

HOUSEROVÁ P, KUBÁŇ V, SPURNÝ P, HABARTA P 2006a: Determination of total mercury and mercury species in fish and aquatic ecosystems of Moravian rivers. Vet Med-Czech **51**: 101-110

HOUSEROVÁ P, JANÁK K, KUBÁŇ P, PAVLÍČKOVÁ J, KUBÁŇ V, 2006b: Chemical forms of mercury in aquatic ecosystems: properties, levels, cycle and determination (In Czech). Chem Listy **100**: 862-876

KANNAN K, SMITH RG, LEE RF, WINDOM HL, HEITMULLER PT, MACAULEY JM, SUMMERS JK 1998: Distribution of total mercury and methyl mercury in water, sediment and fish from South Florida estuaries. Arch Environ Contam Toxicol **34**: 109-118

MARŠÁLEK P, 2006: Methylmercury in water ecosystems (In Czech). Bulletin VÚRH Vodňany **42**: 117-124

MARŠÁLEK P, SVOBODOVÁ Z 2006: Rapid determination of methylmercury in fish tissues. Czech J Food Sci **24**: 138-142

MARŠÁLEK P, SVOBODOVÁ Z, RANDÁK T, ŠVEHLA J 2005: Total mercury and methylmercury contamination of fish from the Skalka reservoir: a case study. Acta Vet Brno **74**: 427-434

MARŠÁLEK P, SVOBODOVÁ Z, RANDÁK T 2006: Total mercury and methylmercury contamination in fish from various sites along the Elbe river. Acta Vet Brno **75**: 579-585
MARŠÁLEK P, SVOBODOVÁ Z, RANDÁK T 2007: The content of total mercury and methylmercury in common carp from selected Czech ponds. Aquac Int **15**: 299-304
MASON RP, REINFELDER JR, MOREL FMN 1995: Bioaccumulation of mercury and methylmercury. Water Air Soil Pollut **80**: 915-921
MINISTERSTVO ZEMĚDĚLSTVÍ 2007: Situační a výhledová zpráva. Ryby. (Situation and Outlook Report. Fish). October 2007: 1-41
SVOBODOVÁ Z, HEJTMÁNEK M 1976: Total mercury content in the musculature of fishes from the river Ohře and its tributaries. Acta Vet Brno **45**: 45-49
WHO 1990: Methylmercury. In: Environmental health criteria 101. World Health Organisation, Geneva, pp. 1-145
YASUTAKE A, NAGANO M, NAKANO A 2005: Simple method for methylmercury estimation in biological samples using atomic absorption spectroscopy. J Health Sci **51**: 220-223
ŽLÁBEK V, SVOBODOVÁ Z, RANDÁK T, VALENTOVÁ O 2005: Mercury content in the muscle of fish from the Elbe river and its tributaries. Czech J Anim Sci **50**: 528-534