Methylmercury, total mercury and total selenium in four common freshwater fish species from Ya-Er Lake, China

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Abstract

Snakehead fish (*Ophiocephalus argus cantor*), silver carp (*Hypophthalmichthys molitrix*), crucian carp (*Carassius carassius*), and common carp (*Cyprinus carpio*) are four common freshwater fish species in China. In this study, the level of methylmercury (MeHg), total mercury (T-Hg), and total selenium (T-Se) in muscle samples of these four fish species from Ya-Er Lake, China, were analyzed using atomic fluorescence spectrometry coupled with high-performance liquid chromatography, and inductively coupled plasma mass spectrometry. The concentrations of MeHg in all the fish species were significantly correlated with those of T-Hg. Higher T-Hg and MeHg concentrations had accumulated in the snakehead fish, which is a strongly predatory fish, than in the other three species. The concentration ratios of MeHg and T-Hg in the muscles of these four fish species were almost equal. Conversely, there was negative correlation between the concentrations of T-Hg and T-Se, which implies that there is a competition between these two elements with respect to bioaccumulation. It is noteworthy that of all the muscle samples tested, the level of T-Hg exceeded the maximum allowable limit in fish [0.4 mg kg⁻¹ (w/w) recommended by the World Health Organization] in 38.46% of those of the silver carp, 18.18% of those of the crucian carp, and 100% of those of snakehead fish. These results show that the consumption of contaminated fish is a potential threat to human health and that necessary preventive measures to safeguard public health should be emphasized.

Introduction

Natural and anthropogenic sources of mercury as well as increasing levels of mercury found in the food chain have resulted in mercury pollution becoming a global problem. Minamata disease, which appeared among inhabitants of Minamata Bay, Japan, was caused by the consumption of fish and shellfish containing high concentrations of MeHg (Tsubaki & Irukayama 1977), and in Iraq 459 people died following their consumption of Hg-contaminated bread (Bakir *et al.* 1973). Mercury pollution has also been reported in

Quebec, Canada (Eyssen & Ruedy 1983), the Amazon in Brazil (Nriagu *et al.* 1992), and many other areas of the world (Michael 2003).

In water bodies, inorganic mercury (I-Hg) can be converted into highly toxic organomercury compounds by microbial action. Methylmercury (MeHg), the most toxic species of the organomercury compounds, usually represents more than 85% of the total mercury (T-Hg) presenting in fish (May *et al.* 1987; Bloom 1992), and most of the mercury presenting in humans originates from the consumption of fish. As a result, many national agencies have issued fish guidelines on safe levels

of exposure to mercury. The U.S. Food and Drug Administration (FDA) has set an action level of $1 \mu\text{g g}^{-1}$ (w/w) for concentration of MeHg in fish. Canada and several U.S. states have even set an advisory consumption level of $0.5 \mu\text{g g}^{-1}$ (w/w) for MeHg in fish (EPA 2001). However, mercury levels above the regulatory limits have been observed many times, particularly in species occupying high trophic levels (Monteiro & Lopes 1990; Nakagawa *et al.* 1997; Storelli & Marcotrigiano 2001; Storelli *et al.* 2002).

Until recently, in China relatively little attention has been devoted to examining the levels of mercury in freshwater fish, despite the economic and culinary important position of these fish in Chinese diets. Ya-Er Lake is located in the eastern part of Wuhan, Hubei province, China. It is a shallow, eutrophic lake along the middle-lower reaches of the Yangtze River (Figure 1) which has been heavily polluted by mercury as a result of the operations of a huge chlor-alkali industrial plant nearby which involves the use of mercury cells (Figure 2). The objectives of the present study were to obtain data on the T-Hg and MeHg

concentrations in the muscles of four commonly consumed freshwater fishes obtained from Ya-Er Lake, to determine the T-Se concentration and the possible correlation of T-Se with T-Hg due to its detoxifying effect, and finally to evaluate the hazard of mercury-contaminated fish consumption to public health.

Materials and methods

Apparatus

The T-Hg concentrations were measured by atomic fluorescence spectrometry (AFS) (model AF-610A; Raleigh Analytical Instruments, Beijing) using a 253.7 nm line source with a specially made hollow cathode lamp (Tian-gong Analytical Instrument Factory, Beijing) operating at 280 V of PMT voltages and 40 mA of the lamps' current.

The high-pressure liquid chromatography (HPLC) (model LC-10AT; Shimadzu, Japan) and AFS hyphenation system have been described previously (Liang *et al.* 2003). A ZORBAX ODS

Fig. 1. Map showing Ya-Er Lake and the sampling site.

Fig. 2. The old mercury-cell production technique in chlor-alkali industry. The cathode is a layer of Hg (I) that flows along the bottom of the tank. Anodes, at which chlorine gas is found, are situated in the sodium chloride solution just above the Hg (I). Sodium formed at the cathode dissolves in the Hg (I), and the sodium amalgam is decomposed with water to produce NaOH (aq) and Hg (g). The regenerated Hg (I) is recycled.

column (4.6×150 mm, 5 μ m; Agilent Technologies, USA) was utilized to separate MeHg in the samples.

Inductively coupled plasma-mass spectrometry (ICP-MS) (model 7500i; Agilent Technologies) was used for the determination of T-Se. The working parameters were: RF power, 1300 W; sampling depth, 7 mm; carrier gas flow rate, 1.05 l min⁻¹; makeup gas flow rate, 0.1 l min⁻¹; spray chamber temperature, 2 °C; sample uptake rate, 0.4 ml min⁻¹. The orifice of the nickel sampling cone and skimmer cone was 1.0 and 0.4 mm, respectively.

Sampling sites and dissection of fish

Fish samples were obtained in November 2002. Four of the most commonly consumed freshwater fish species in Ya-Er Lake were captured in the same day with netting: silver carp (body weight, 0.84±0.25 kg; *n* = 13), crucian carp (body weight, 0.29±0.08 kg; *n* = 11), common carp (body weight, 0.33±0.16 kg; *n* = 10), and snakehead fish (body weight, 0.77±0.40 kg; *n* = 6). Muscle samples were removed from the fish using stainless steel instruments and stored in plastic bags in accordance with FAO methods (FAO 1983). All of the stainless steel materials were washed with HNO₃ and rinsed with deionized water in order to avoid metal contamination from the background. The samples were frozen in liquid nitrogen and further storage at -80 °C until analysis.

Analytical methods

For T-Hg and T-Se analysis, approximately 0.49–0.51 g of muscle sample was weighed in a Teflon tube and then digested using 1.0 ml HNO₃ by heating the closed tube in an aluminum block and maintaining the temperature at 40 °C overnight. After cooling, the tube was transferred to an oven and heated first at 50 °C for 1 h followed by 4 h at 160 °C. The solutions were then diluted to 24 ml with Milli-Q water for the AFS determinations. MeHg was determined following the method described by Liang *et al.* (2003). Briefly, 3 ml 25% (m/v) KOH (in methanol) was added to 0.5 g well-proportioned wet samples in a 50-ml centrifuge tube and shaken mechanically overnight. Then 3 ml of 6 mol l⁻¹ HCl, 4 ml acidic KBr/CuSO₄ (3:1), and 5 ml CH₂Cl₂ were added into the tube in sequence, followed by 2 h of shaking to extract the organic mercury into the CH₂Cl₂ phase. Following centrifugation at 2000 rpm for approximately 10 min, the CH₂Cl₂ phase was transferred into a 7-ml glass tube and extracted with 1 ml sodium thiosulfate; 45 min of shaking was needed to hasten the extraction process. A few minutes were allowed for phase separation, then the water phase was pipetted off and injected directly into the HPLC-AFS system for separation and determination. Acid-washed glassware, analytical grade reagents, and double-distilled deionized water were used during the analysis. In order to check on the purity of the chemicals used, a number of

chemical blanks were run; there was no evidence of any contamination in these blanks. An analytical quality control of T-Hg and MeHg was achieved using certified dogfish muscle reference material (DORM-2). The values found [T-Hg: 4.56 ± 0.46 mg kg⁻¹ (w/w); MeHg: 4.24 ± 0.14 mg kg⁻¹ (w/w)] agreed with the certified values [T-Hg: 4.64 ± 0.26 mg kg⁻¹ (w/w); MeHg: 4.47 ± 0.32 mg kg⁻¹ (w/w)]. For T-Se, analytical quality control was achieved using mussel standard reference material (GBW 08571). The T-Se contents found [3.57 ± 0.15 mg g⁻¹ (w/w)] agreed with the certified values [3.65 ± 0.09 mg g⁻¹ (w/w)].

Results and discussion

T-Hg, MeHg, and T-Se in fish

T-Hg + MeHg + I-Hg = (T-Hg – MeHg) + T-Se concentrations in the muscle tissues of the four species listed in Table 1. The statistical analysis showed significant differences ($p < 0.05$) between the T-Hg and MeHg contents among the four species with the exception of crucian carp and silver carp. The observed variability of T-Hg and MeHg levels among fish of the same species was in accordance with fish size, sex, longevity, and growth rate, while among the different fish species, the T-Hg and MeHg concentrations were also affected by feeding habits, trophic level, and habitat of the fish. Elevated muscle Hg concentration in the snakehead fish was indicative of a food-chain effect of Hg in the lake. The snakehead fish, a bottom dweller, is a strongly predatory fish preying on fingerling and shrimp, so it could bioaccumulate high levels of T-Hg and MeHg, whereas common carp and crucian carp feed on a wide

variety of plankton with the result that they could assimilate much less T-Hg and MeHg.

There were significant correlations between T-Hg and MeHg concentrations in the muscles of each fish species (Fig. 3). Of the T-Hg concentrations in the muscles, the mean MeHg fraction was standardly less than 50%, although the fraction was highly variable. This variability and mean value are in contrast to the consistent fraction of 95% reported in the muscle of 14 species of fish using ultra-clean techniques (Bloom 1992) and the fraction of 93% reported in the muscles of four other fish species using more standard techniques (May *et al.* 1987). However, the values are in agreement with proportions of MeHg to T-Hg (33–98%) found by Ipolyi *et al.* (2004) in mussel samples.

Levels of T-Se ranged from 388.53 to 1502.25 µg kg⁻¹ (w/w), with the greatest mean concentration found in common carp [1170.59 ± 173.56 µg kg⁻¹ (w/w)] and the lowest mean concentration found in silver carp [559.49 ± 82.68 µg kg⁻¹ (w/w)]. The muscle T-Se content was significantly different in the four fish species ($p < 0.05$), except between the snakehead fish and silver carp. It is worth noting that in the fish muscle the concentration of T-Hg decreased exponentially with an increase of T-Se, with the negative correlation being significant in common carp (Fig. 4). The first report of the protective effect of selenite against mercury toxicity appeared in 1967 (Parizek & Ostadalova 1967). Since then, numerous studies have shown that selenium supplementation counteracts the toxicity of exposure to mercury. The ability of selenium compounds to decrease the toxic action of mercury has been established in all species of mammals, birds, and fish investigated to date (Beijer & Jernelov 1978;

Table 1. T-Hg, MeHg, I-Hg, T-Se concentrations [µg kg⁻¹ (w/w)] MeHg/T-Hg ratios and T-Se/I-Hg molar ratios in the fish muscles.

Species	<i>n</i>	T-Hg	MeHg	I-Hg	MeHg/T-Hg	T-Se	T-Se/I-Hg
Silver carp	13	205.48–928.32	57.44–360.17	37.65–622.96	0.27–0.72	388.58–708.74	1.47–44.29
(<i>Hypophthalmichthys molitrix</i>)		428.98 ± 241.14	195.15 ± 100.50	233.83 ± 164.64	0.48 ± 0.16	559.49 ± 82.68	10.45 ± 11.25
Common carp	10	23.57–210.43	5.44–126.06	5.87–84.37	0.18–0.85	897.92–1502.25	25.04–507.88
(<i>Cyprinus carpio</i>)		78.88 ± 63.67	39.29 ± 39.21	39.59 ± 27.44	0.44 ± 0.21	1170.59 ± 173.56	132.25 ± 141.96
Crucian carp	11	130.74–1360.40	52.33–643.80	78.41–716.60	0.29–0.55	614.58–868.41	2.02–25.46
(<i>Carassius carassius</i>)		422.95 ± 383.98	184.84 ± 182.47	238.11 ± 204.48	0.43 ± 0.08	759.01 ± 85.17	12.12 ± 7.28
Snakehead fish	6	428.81–1199.39	164.38–498.86	243.65–700.53	0.38–0.54	505.00–642.36	1.86–5.49
(<i>Ophiocephalus argus cantor</i>)		827.11 ± 333.96	371.12 ± 134.19	455.98 ± 206.23	0.46 ± 0.06	565.94 ± 52.52	3.59 ± 1.79

Fig. 3. T-Hg and MeHg concentrations [$\mu\text{g kg}^{-1}$ (w/w)] in the muscle samples of the four fish species.

Culvin-Aralar & Furness 1991). However, field studies on the Se-Hg interaction in freshwater fish are relatively scarce, and the data collected from these are often controversial (Chen *et al.* 2001). Various studies have shown that there is either no correlation (Cappon & Smith 1981) or a negative

one (Paulsson & Lundbergh 1991; Chen *et al.* 2001) between the concentrations of selenium and mercury in fish. Sheline & Schmidt-Nielsen (1977) investigated the Hg-Se interaction in the fish *Fundulus heteroclitus* and found little effect of selenium on the overall body retention of mercury.

Fig. 4. T-Hg and T-Se concentrations [$\mu\text{g kg}^{-1}$ (w/w)] in the muscle samples of four fish species.

Table 2. PTWI regulated by FAO/WHO and estimated Chinese weekly intake of T-Hg and MeHg (μg).

Species	T-Hg estimated weekly intake	FAO/WHO PTWI of T-Hg	MeHg estimated weekly intake	FAO/WHO PTWI of MeHg
Silver carp	600.57	< 300	273.21	< 100
Common carp	110.43		55.01	
Crucian carp	592.13		258.78	
Snakehead fish	1157.95		519.57	

Other studies, however, have shown that the addition of selenium is an effective way to reduce mercury concentrations in pike (*Esox lucius*) and perch (*Perca flavescens*) when the appropriate doses and addition periods were used (Turner & Swick 1983). Southworth *et al.* (2000) observed a long-term increase in mercury concentrations in the largemouth bass following the reduction of waterborne selenium. Chen *et al.* (2001) further found a significantly negative correlation between the mercury concentrations in perch and walleye muscles and selenium concentrations. The mechanism of interaction between these two elements in the bioassimilation processes has not yet been clarified although it appears to be related to the element competition between mercury and selenium in fish muscles. One hypothesis is as follows: when an organism is exposed to high concentrations of mercury, selenium could be released from selenium-binding protein of muscle to liver, the primary detoxification organ, to antagonize the toxicity of mercury, thus causing a decrease in muscle selenium levels. However, the interaction of mercury and selenium can be either antagonistic or synergistic, primarily depending on exposure concentrations and the form of mercury involved. The geochemistry of Ya-Er Lake should also be taken into account when speculating on the bioassimilation of mercury and selenium.

An assessment of mercury exposure in man

Freshwater fish is one of the more important protein sources for humans, and China is not an exception. In general, food intake provides the major exposure route of organisms to mercury (D'tri 1990), and the food exposure route provides 90% of the MeHg bioaccumulated in the fish body (Hall *et al.* 1997). In this study, attention must be given to the fact that of all the muscles sampled 38.46% of those of the silver carp, 18.18% of those

of the crucian carp, and 100% of those of the snakehead fish contained T-Hg levels exceeding the maximum allowable limit recommended by WHO for most fish species [0.4 mg kg^{-1} (w/w); WHO 1989], while none of the muscle samples from the common carp contained T-Hg levels exceeding the maximum allowable concentration.

The Joint Food and Agriculture Organization/World Health Organization (FAO/WHO) Expert Committee on Food Additives has established regulatory guidelines regarding dietary mercury intake. It recommends a provisional tolerable weekly intake (PTWI) of $300 \mu\text{g}$ of T-Hg per person, of which no more than $100 \mu\text{g}$ should be present as MeHg (WHO 2003). In the Ya-Er Lake area, fish is the primary protein intake source in the diet of residents, and the average consumption of fresh fish is about 200 g day^{-1} . The weekly intake of T-Hg and MeHg are consequently between 110.43 and $1157.95 \mu\text{g}$ and between 55.01 and $519.57 \mu\text{g}$, respectively (Table 2). As can be seen from the table, the estimated weekly intake of T-Hg and MeHg from three fish species exceeds the established PTWI except for common carp. The presence of such high levels of toxic MeHg in fish muscle, in combination with the fact that the consumption of contaminated fish is the main source of mercury intake in people not occupationally exposed (Galal-Gorchev 1993; Cuadrado *et al.* 1995), amply underlines the need for preventive measures to safeguard public health.

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