Organ Distribution and Bioaccumulation of Microcystins in Freshwater Fish at Different Trophic Levels from the Eutrophic Lake Chaohu, China

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ABSTRACT: This article reports the organ distribution and bioaccumulation of hepatotoxic microcystins (MCs) in freshwater fishes at different trophic levels from the large, shallow, eutrophic Lake Chaohu in September 2003, when there were heavy surface blooms of toxic cyanobacteria. Among all fish, intestines and blood had the highest average content of MC-RR + MC-LR (22.0 and 14.5 μ g g⁻¹ DW, respectively), followed by liver, bile, and kidney (7.77, 6.32, and 5.81 μ g g⁻¹ DW, respectively), whereas muscle had the least (1.81 μ g g⁻¹ DW). MC content in muscle was highest in carnivorous fish (*Culter ilishaeformis*, 2.22 μ g g⁻¹ DW) and omnivorous fish (Carassius auratus, 1.96 µg g⁻¹ DW) and was lowest in phytoplanktivorous fish (Hypophthalmichthys molitrix, 1.65 μ g g⁻¹ DW) and herbivorous fish (*Parabramis pekinensis* 0.660 μ g g⁻¹ DW). However, the amount of MC in the gut of H. molitrix (137 μ g g⁻¹ DW) was more than 20 times that in the other fish (<6.50 μ g g⁻¹ DW). The MCs showed a tendency to accumulate up the food chain, and piscivorous fish at the top of the food chain were at high risk of exposure to MCs in Lake Chaohu. Our study is the first to report MC concentrations in the bile and blood of wild fish. One hundred grams of fish muscle would contain 2.64–49.7 μ g of MC-LR equivalent, or about 1.3-25 times the recommended tolerable daily intake of MC-LR by humans, indicating that fish are already severely contaminated by MCs and that the local authorities should warn the public of the risk of poisoning by eating the contaminated fish. © 2005 Wiley Periodicals, Inc. Environ Toxicol 20: 293–300, 2005. Keywords: microcystin; fish; bioaccumulation; organ distribution; food chain

INTRODUCTION

Hepatotoxic microcystins (MCs) are natural toxins produced by freshwater cyanobacteria such as *Microcystis* (primarily

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M. aeruginosa), *Anabaena*, *Oscillatoria*, and *Nostoc* (Carmichael, 2001). More than 60 chemical forms have been reported (Sivonen and Jones, 1999). MCs inhibit eukaryotic protein phosphatase types 1 and 2A, resulting in excessive phosphorylation of cytoskeletal filaments, ultimately leading to liver failure, and have been implicated in the deaths of birds, wild animals, livestock, and fish (Carmichael, 1994; Kaebernick and Neilan, 2001). The World Health Organization established 0.04 μ g kg⁻¹ body weight (BW) day⁻¹ as a tolerable daily intake (TDI) of MC-LR, one of the most potent MCs (at least in acute terms), and

provided a guideline value of 1 μ g L⁻¹ as the maximum allowable concentration of MC-LR in drinking water (Falconer et al., 1999; Kuiper-Goodman et al., 1999).

Although it is hard for humans to ingest a lethal acute dose of MCs, chronic toxicity is possible with long-term frequent exposure (Magalhães et al., 2003). Until now, more attention has been paid to human uptake of MCs through drinking water (Chorus and Bartram, 1999) than to bioaccumulation of MCs in aquatic animals in natural waters. Fish, standing at the top of the aquatic food chain, are likely to be most affected by exposure to toxic cyanobacteria, and so their consumption may pose great risk to humans.

Freshwater fish comprise only a minor proportion of fish products in some countries but comprise 40%–50% of total fish products in China. So far, only a few studies have measured MC content in wild fish—in Brazil (Magalhães et al., 2001), Egypt (Mohamed et al., 2003), and Portugal (Vasconcelos, 1999) in only a few species. Such studies are still lacking in China, where people commonly consume freshwater fish regardless of the danger of MCs.

The present study was conducted in the large, shallow, eutrophic Lake Chaohu, where commercial fisheries are important, but cyanobacterial blooms occur in the warm season every year. The purpose of this study was to describe the distribution in organs and the bioaccumulation of two common MCs (MC-LR and MC-RR) in various fish at different trophic levels in order to determine whether MCs accumulate up the food chain and to evaluate the potential risk of human consumption.

MATERIALS AND METHODS

Lake Chaohu is in southeastern China $(31^{\circ}40'N, 117^{\circ}36'E)$, where the climate is subtropical. It is the fifth-largest freshwater lake in China, with a surface area of 760 km² and a mean depth of 3.06 m. Heavy surface blooms of cyanobacteria (mainly Microcystis and Anabaena) have occurred regularly in the warm seasons of each year in recent decades. In September 2003 we collected eight species of fish near Zhongmiao (Fig. 1), where the wind frequently piles up dense cyanobacterial blooms. Table I lists the scientific name, body weight, total length, body length, feeding type, and main foods of the collected fish. The fish were classified into four types according to their food habits: phytoplanktivorous fish [Hypophthalmichthys molitrix (Hm)], herbivorous fish [Parabramis pekinensis (Pp)], omnivorous fish [Cyprinus carpio (Cc) and Carassius auratus (Ca)], and carnivorous fish [Culter ilishaeformis (Ci), Culter erythropterus (Ce), Pseudobagrus fulvidraco (Pf), and Coilia ectenes (Co)]. Because the one C. ectenes collected was a juvenile, it was assumed to feed on planktonic crustaceans.

The fish were dissected in the field into intestines (including gut contents), muscle, liver, blood, kidneys, and



Fig. 1. Map of Lake Chaohu (asterisk indicates the sampling site).

bile. The guts of all cyprinid species (which do not have a stomach) and the stomach of *P. fulvidraco* were full. As the single *C. ectenes* was too small, we just took muscle samples. We also collected gut wall samples of *C. ilishaeformis*. The organ samples were immediately preserved in a portable refrigerator (around 0°C) and then transported to the laboratory. In the laboratory all samples were immediately frozen at -40° C, then freeze-dried for MC analysis.

The method used to extract the MCs from muscle, blood, liver, intestine, and gut contents was as follows. Lyophilized samples were homogenized in a mortar and extracted 3 times with 10 mL of BuOH:MeOH:H₂O (1:4:15) for 24 h with stirring. The dry weight of the extracted tissues ranged between 0.1 and 0.6 g for muscle and between 0.05 and 0.20 g for the other organs. The extracts were centrifuged at $34,920 \times g$ (AG-508R, KUBOTA, Japan) for 60 min. Supernatant diluted 1:1 with water was directly applied to a 5-g reversedphase ODS cartridge (Chromatorex ODS, 100-200 mesh, packed into a polypropylene cartridge; Fuji Silysia Chemical, Japan), which had been preconditioned by washing with 50 mL of 100% MeOH and 50 mL of H₂O. The column was washed with water (50 mL), then with water-MeOH (4:1, 100 mL). Elution from the column with 90% MeOH (100 mL) yielded the fraction of interest. The fraction was evaporated to dryness, the residue was dissolved in 100% MeOH (5 mL), and the solution was applied to a 2-g silica gel cartridge (Sep-Pak, Waters, Milford, MA, USA), which had been preconditioned by washing with 10 mL of 100% MeOH. The column containing the toxins was washed with 100% MeOH (10 mL) and then eluted with 70% MeOH (20 mL). This elution fraction also was evaporated to dryness, then the residue was dissolved in 100% MeOH, and the solution was subjected to high-performance liquid chromatography (HPLC). The HPLC system consisted of a Shimadzu (Kyoto, Japan) LC-9A pump coupled to an SPD-10A UV-visible detector (238 nm), an SPD-M10A photodiode array detector, a

	No. of	BW (g)	TL (cm)	BL (cm)	Feeding Type and Main Foods	
Name of Fish	Samples					
Cyprinidae						
Hypophthalmichthys molitrix	Hm-1	2900	61.4	58.8	Planktivorous;	
(Hm; Hypophthalmichthyinae)	Hm-2	1500	42.7	34.5	algae and zooplankton	
Parabramis pekinensis	Pp-1	470	33.4	26.3	Herbivorous; macrophytes	
(Pp; Abramidinae)	Pp-2	100	21.8	17.2		
Carassius auratus	Ca-1	88.1	17.0	13.5	Omnivorous; attached algae,	
(Ca; Cypriniae)	Ca-2	Ca-2 64.2 16.9 12.9		12.9	detritus, benthic diatoms, and	
					filamentous algae	
Cyprinus carpio	Cc-1	725	36.8	21.9	Omnivorous; zoobenthos,	
(Cc; Cypriniae)	Cc-2	625	35.3	29.1	organic detritus, even algae	
Culter erythropterus	Ce-1	120	22.0	18.0	Camivorous; juveniles feed on	
(Ce; Abramidinae)	Ce-2	105	24.0	19.0	Cladocera, Copepoda, and aquatic	
					insects, adults feed mainly on small fish	
Culter ilishaeformis	Ci-1	650	_	_	Carnivorous; juvenile fish feed on	
(Ci; Abramidinae)					aquatic insects, Cladocera, and	
					Copepoda, adults feed mainly on fish	
Bagridae						
Pseudobagrus fulvidraco (Pf)	Pf-1	74.6	19.8	16.4	Carnivorous; fish 5-8 cm feed on	
					zooplankton- and- aquatic insect,	
					fish >8 cm feed on molluskcs	
					and small fish.	
Clupeidae						
<i>Coilia ectenes</i> (Co)	Co-1	18	_	_	Carnivorous; juveniles feed on Amphipoda,	
					Cladocera, and Copepoda, adults feed on small fish and shrimp	

TABLE I. Scientific name, body weight, total length, body length, feeding type, and main foods of collected fish

*Information on feeding type and main foods of these fish are from the Ichthyology Laboratory of the Institute of Hydrobiology of Hubei Province (1976).

C-R6A integrator, and an ODS column (Cosmosil 5C18-AR; 4.6 mm × 150 mm, Nakalai, Japan). The sample was separated with a mobile phase consisting of a methanol:0.05 M phosphate buffer (pH 3.0; 58:42) at a flow rate of 1 mL min⁻¹. The MC concentration was quantified against MC-RR and MC-LR standards (Wako Ltd., Japan). Liquid chromatography (electrospray ionization)/mass spectrometry (LC/ESI-MS) analysis of MCs was conducted under the following conditions: curved desolvation line (CDL) temperature, 200°C; nebulizing gas (N₂) flow, 1.5 L/min, injected volume, 5 μ L; detector gain, 1.5 kV.

The *Microcystis* cells from the surface blooms of the lake were lyophilized, and the MCs were extracted and analyzed by the methods described in Zheng et al. (2004).

For *H. molitrix* and *C. carpio*, we analyzed toxins in two individuals of each species. For *Coilia ectenes*, we measured only the toxins in muscle. For the other fish, organs from two fish were pooled, so each value represents the average MC content of each organ from two individuals.

RESULTS

Figure 2 shows MC content in organs of each species. Among all fish, intestines and blood had the highest average content of MC-RR + MC-LR (22.0 and 14.5 μ g g⁻¹ DW, respectively), followed by liver, bile, and kidney (7.77, 6.32, and 5.81 μ g g⁻¹ DW, respectively). Muscle had the least (1.81 μ g g⁻¹ DW). Bile and blood had the highest average proportions of MC-LR among total MCs (48.3% and 45.4%, respectively), followed by kidney, gut contents, and muscle (29.9%, 25.5%, and 18.2%, respectively). Liver had the lowest (12.8%). MC-LR content varied greatly among species: some fish (Pf, Ci) did not have MC-LR in any organ, and some (Hm, Ce) had MC-LR in their gut contents but not in any other organs; *C. carpio* had MC-LR only in the liver. *C. auratus* had substantial MC-LR in their organs.

No MCs were detected in the gut contents of three species: the herbivorous *P. pekinensis* and the carnivorous *C. ilishaeformis* and *P. fulvidraco*, but MC-RR was present in the stomach of *P. fulvidraco* (1.26 μ g g⁻¹ DW). The highest amount of MC in gut contents was in the phytoplanktivorous *H. molitrix* (137 μ g g⁻¹ DW), followed by the two omnivorous fish (6.22–6.46 μ g g⁻¹ DW). No MCs were detected in the gut wall sample of C. *ilishaeformis*.

MC-RR was present in muscle samples of all species, but MC-LR was detected in the muscle of only two species (Ca, Co). *C. auratus* had the highest MC content in muscle (MC-RR, 0.97 μ g g⁻¹ DW; MC-LR, 2.29 μ g g⁻¹ DW). On average, MC content in the muscle was highest in the



Fig. 2. Microcystin (MC) content in organs of each fish species (Hm = Hypophthalmichthys molitrix, Pp = Parabramis pekinensis, Cc = Cyprinus carpio, Ca = Carassius auratus, Ci = Culter ilishaeformis, Ce = Culter erythropterus, Pf = Pseudobagrus fulvidraco, Co = Coilia ectenes).

carnivorous fish (Ci, 2.22 $\mu g g^{-1}$ DW), followed by the omnivorous fish (Ca, 1.96 $\mu g g^{-1}$ DW), and lowest in the phytoplanktivorous fish (Hm, 1.65 $\mu g g^{-1}$ DW) and herbivorous fish (Pp, 0.66 $\mu g g^{-1}$ DW).

Except for *Coilia ectenes*, from which no blood sample was taken, MC-RR was present in blood samples of all fish, but MC-LR was detected only in *C. auratus*.

MC-RR was present in the liver of all fish. The value was highest in the carnivorous *Culter erythropterus* (11.6 μ g g⁻¹ DW) and lowest in the phytoplanktivorous *H. molitrix* (1.16 μ g g⁻¹ DW). MC-LR was present only in *P. pekinensis* and *C. carpio*.

We could not collect bile or kidney samples of *C.* ectenes and *P. fulvidraco*. The highest MC contents in bile were found in *C. auratus* (MC-RR, 6.31 μ g g⁻¹ DW; MC-LR, 16.3 μ g g⁻¹ DW), whereas MCs were absent in the bile of *C. carpio* and *C. ilishaeformis*. The highest MC content in kidney was found in *C. auratus* (MC-RR, 5.18 μ g g⁻¹ DW; MC-LR, 8.98 μ g g⁻¹ DW) and *Culter erythropterus* (MC-RR, 13.7 μ g g⁻¹ DW), whereas MCs were absent in the kidney of *C. ilishaeformis*.

Qualitative identification of MC-RR and MC-LR in the intestine of *H. molitrix* is shown in Figure 3.



Fig. 3. Chromatograms measured in the intestine of *H. molitrix*: (A) high-performance liquid chromatogram monitored at 238 nm; (B) mass chromatograms monitored at *m/z* 498.3, 519.8, 995.6, 1039.0, and 1045.5; (C) LC/ESI-MS mass spectra of microcystin-RR; (D) LC/ ESI-MS mass spectra of microcystin-LR.

DISCUSSION

There is only limited information on microcystin contamination in wild fish. Magalhães et al. (2001) measured the seasonal changes in MC-LR equivalents by ELISA in liver, muscle, and viscera of *Tilapia rendalli* in a Brazilian lagoon populated with phytoplankton dominated by toxic *Microcystis*. Mohamed et al. (2003) measured seasonal TABLE II. Comparisons of maximum MC-LR equivalents (μ g g⁻¹ WW, WW-wet weight) of the muscle of different fish collected in the field and the critical amount of edible muscle necessary to ingest to reach a tolerable daily intake (TDI) of MC (0.04 μ g kg⁻¹ BW, or 2 μ g for an adult weighing 50 kg BW) determined by WHO (Kuiper-Goodman et al., 1999)

Fish name	Sampling Site	Analytic Method	Maximum MC-LR Equivalent	Critical Amount for TDI	Source
Oreochromis niloticus	Fish pond in Egypt	ELISA	0.102	20	Mohamed et al., 2003
Tilapia rendalli	Lagoon in Brazil	ELISA	0.337	6	Magalhães et al., 2001
Fish (no name given)	Sepetiba Bay, Brazil	ELISA	0.04	50	Magalhães et al., 2003
Cyprinus sp.	Guadiana River (southern Portugual)	ELISA	0.28	7	Vasconcelos, 1999
Barbus sp.	Guadiana River (southern Portugual)	ELISA	0.12	17	Vasconcelos, 1999
Lisa sp.	Guadiana River (southern Portugual)	ELISA	0.11	18	Vasconcelos, 1999
Hypophthalmichthys molitrix	L. Chaohu	HPLC	0.066	30	Present study
Parabramis pekinensis	L. Chaohu	HPLC	0.026	76	Present study
Carassius auratus	L. Chaohu	HPLC	0.497	4	Present study
Cyprinus carpio	L. Chaohu	HPLC	0.026	76	Present study
Culter erythropterus	L. Chaohu	HPLC	0.079	25	Present study
Culter ilishaeformis	L. Chaohu	HPLC	0.109	18	Present study
Pseudobagrus fulvidraco	L. Chaohu	HPLC	0.078	26	Present study
Coilia ectenes	L. Chaohu	HPLC	0.182	11	Present study

*A coefficient of 5 was used to convert muscle dry weight to muscle wet weight for fish from Lake Chaohu (Chen, 1990), and as the i.p. LD_{50} in mice for MC-RR is about 5 times higher than that for MC-LR (Gupta et al., 2003), a coefficient of 0.2 was used to convert MC-RR into the MC-LR equivalent for fish from Lake Chaohu.

changes in MC-LR equivalents by ELISA in gut, liver, kidney, and muscle of another tilapia, *Oreochromis niloticus*, in an Egyptian fish pond containing heavy blooms of toxic *Microcystis aeruginosa*. Magalhães et al. (2003) used ELISA to measure the seasonal changes in MC-LR equivalents in the muscle of fish (not named) in Sepetiba Bay, Brazil. Vasconcelos (1999) reported the MC-LR concentrations in the edible parts of three Portugal freshwater fish carp (*Cyprinus* sp.), barbel (*Barbus* sp.), and grey mullet (*Lisa* sp.)—but no details of analytical methods, sampling dates, or sites were given. The present study is the first to compare MC content (by HPLC) in various organs of four trophic levels of fish.

In the present study, MC content in the liver and muscle was highest in carnivorous fish, followed by omnivorous fish, and was lowest in phytoplanktivorous and herbivorous fish, indicating that MCs showed a general tendency to accumulate up the food chain in Lake Chaohu. However, in the gut MC content generally showed a reversed pattern: content was highest in the phytoplanktivorous fish, followed by the omnivorous and carnivorous fish. There are several possible explanations for this: (1) carnivorous fish might accumulate more MCs; (2) phytoplanktivorous fish might degrade MCs more actively; (3) carnivorous fish might take up MCs through routes other than the gastrointestinal tract (e.g., via gills); and (4) a combination of these mechanisms could be occurring. Tencalla et al. (1994)

reported that concentrations of 8-16 mg of freeze-dried algae L^{-1} were nontoxic to trout when present in aquarium water, but trout died within 96 h when gavaged with 1440 mg of freeze-dried algae per kg body weight (BW) (equivalent to 6.6 mg of MC kg^{-1} BW). Therefore, the authors concluded that the main uptake route of MC in trout is the gastrointestinal tract. However, in our study, the herbivorous P. pekinensis had no MCs in their guts (the guts were filled with macrophytes), but they accumulated substantial amounts of MCs in their liver, muscle, bile, and blood. Similarly, the highest MC-RR content in muscle was found in the carnivorous C. ilishaeformis, despite the absence of MCs in their guts. Therefore, it is likely that routes other than the gastrointestinal tract are important for the uptake of MCs by fish such as P. pekinensis and C. ilishaeformis in the lake we studied. On the other hand, our results highlight that piscivorous fish at the top of the aquatic food chain are at high risk of MC exposure, having high accumulation of MCs in their organs, although mechanisms explaining the patterns of MC accumulation in fish at different trophic levels need to be studied experimentally in our future work.

Our study is the first to report MC concentrations in the bile and blood of wild fish. In a laboratory toxicity experiment, rainbow trout (*Onchorhynchus mykiss*) were orally dosed with 1220 mg of *Microcystis aeruginosa* kg⁻¹ BW, corresponding to 5.60 mg of MC-LR kg⁻¹ BW. After 3 and

48 h hepatotoxin concentrations in bile (measured by protein phosphatase inhibition assay) were 3.50 and 0.48 μ g MC-LR equivalent mL^{-1} , respectively (Sahin et al., 1996). Assuming a water content of 10% in bile, these were approximately 35.0 and 4.75 μ g MC-LR equivalent per gram. Hepatotoxins became detectable in bile samples as early as 1 h after the oral administration of cyanobacteria and remained detectable for up to 3 days after exposure. In our study MCs in bile varied between 0 and 22.6 μ g g⁻¹ and averaged 6.32 μ g g⁻¹, which is close to the level found in the experiment by Sahin et al. (1996). Because Tencalla et al. (1994) showed that trout died within 96 h when gavaged with freeze-dried *Microcystis* at a dose of 6.6 mg of MC kg⁻¹ BW, the presence of a concentration of 22.6 μ g of MC g⁻¹ in the bile suggests that MC concentrations in the fish of Lake Chaohu are close to a sublethal or lethal level.

In the present study, the MC content in the cyanobacterial blooms in Lake Chaohu was 240 μ g g⁻¹ DW, and the maximum MC content in the fish was 137 μ g MCs g⁻¹ DW in the gut of *H. molitrix* (104 μ g of MC-RR + 33.3 μ g of MC-LR). Similarly, in our previous experiment, MC content was 207 μ g g⁻¹ DW in seston and 78.8 μ g g⁻¹ DW in the gut of *H. molitrix* (Xie et al., 2004). However, in an Egyptian fish pond, MC content reached as high as 1120 μ g g⁻¹ DW in *Microcystis* blooms, but only 8.21 μ g g⁻¹ DW (maximum) in the gut of a phytoplanktivorous tilapia (a 136-fold difference). Tilapia have a stomach pH as low as 1.25 during digestion (Payne, 1978), and it is likely that active degradation of MCs in the stomach occurred, resulting in a low MC content in the guts.

The MC content in the cyanobacterial blooms in Lake Chaohu was 240 μ g g⁻¹ DW, and the average MC content in muscle of the eight fish species was 1.82 μ g of MCs (1.44 μ g of MC-RR + 0.38 μ g pf MC-LR) g⁻¹ DW. In our previous 80-day toxicity experiment, the average MC content was 207 μ g g⁻¹ DW in seston and 1.04 μ g g⁻¹ DW in the muscle of *H. molitrix* (Xie et al., 2004). These results indicate that the MC level in the muscle reached 0.5%–0.75% of that in the suspended seston containing the toxic cyanobacteria. We do not know whether such a relationship is generally true because no data are available on regions outside China.

The World Health Organization has determined a TDI of 0.04 μ g kg⁻¹ BW per day for MC-LR (Chorus and Bartram, 1999). The average portion of fish eaten by a person is about 100–200 g, and therefore a 100-g portion would contain 2.64–49.7 μ g of MC-LR equivalent (Table II) or about 1.3–25 times the recommended TDI for MC-LR. On the other hand, because MCs are heat stable, they are not broken down by cooking (Harada et al., 1996). The present results indicate that there is already severe contamination of Lake Chaohu fish by hepatotoxic MCs during cyanobacterial blooms. Therefore, we recommend that MC content in fishery products of this lake be strictly monitored season-

ally to avoid human intoxication and that the local authorities warn the public to be on the alert for poisoning by contaminated fish.

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