Bull Environ Contam Toxicol (2009) 83:125–129
DOI 10.1007/s00128-009-9718-4

Risk Assessment on Mixture Toxicity of Arsenic, Zinc and Copper Intake from Consumption of Milkfish, *Chanos chanos* (Forsskål), Cultured Using Contaminated Groundwater in Southwest Taiwan

Ming-Chao Lin

Received: 5 April 2008 / Accepted: 17 March 2009 / Published online: 25 March 2009

Abstract Studies on bioaccumulation of arsenic, zinc, and copper in freshwater-cultured milkfish were carried out to assess the risks on human health. The arsenic, zinc, and copper levels in milkfish showed significant positive correlations to the arsenic, zinc, and copper concentrations in pond water. The hazard index of arsenic, zinc, and copper mixture for intake of milkfish (1.75 ± 0.65) demonstrated that intake of in this way contaminated milkfish will result in non-carcinogenic risk. The target cancer risk of arsenic for intake of the milkfish ($2.74 \times 10^{-4} \pm 1.18 \times 10^{-4}$) indicated that the inhabitants were exposed to arsenic pollution with carcinogenic risk.

Keywords Bioaccumulation · Fish · HI · THQ · TR

Organisms living in an aquatic environment are constantly exposed to chemicals in the ambient water. Among these chemicals, zinc (Zn) and copper (Cu) are essential nutrients for normal physiological processes, while arsenic (As) is a non-essential nutrient for most of the animals (Terra et al. 2008). These chemicals may become toxic to organisms when their concentrations become abnormally high and exceed physiological thresholds (Gioda et al. 2007). It has been demonstrated that As, Zn, and Cu can be found in aquatic-derived foodstuffs, such as fish (Falco et al. 2006; Peshut et al. 2008). Reports concerning these elements in fish are extensive; many of these studies were completed to assess the potential threat to public health from the intake of these contaminated fish (Lin et al. 2005; Jang et al. 2006; Ling and Liao 2007). Consumption of As-polluted fish might cause an overexposure of As and pose a risk to human health via the food chain (Lin et al. 2005; Chou et al. 2006). For Zn and Cu there is a range of intake over which their supply is adequate to the body (12–15 mg kg^{-1} day^{-1} and 1.5–3 mg kg^{-1} day^{-1} for Zn and Cu, respectively) (García-Rico et al. 2007). However, beyond this range, deficiency and toxic effects are observed. A high supplementation of Cu had been related with liver damage. Zinc reduces immune function and levels of high density lipoproteins.

Various in situ studies have been made to assess the levels of As, Zn, and Cu in fish as well as the concentrations in their environments. Most of the researches were concerned with the effects and bioconcentration patterns of individual toxins. In fact, organisms are rarely exposed to single chemicals in isolation in their environment. More typically, exposures occur to multiple chemicals simultaneously. As effluent from many sources enters natural waters, the negative impact on the aquatic ecosystem is due to a mixture of metals, rather than individual component toxins (Falco et al. 2006). Thus far, only a few researches have been conducted to assess the risk on human health associated with the mixture toxicity of these chemicals in fish, especially cultured fish. Milkfish (*Chanos chanos*) is economically important seafood, commonly cultured in the coastal region of southwest Taiwan, which is subjected to water polluted with As, Zn, and Cu. The aim of this study was to investigate the presence of those chemicals in those cultured milkfish and evaluated its possible health risks.

M.-C. Lin (✉)
Graduate Institute of Environmental Management/Department of Natural Biotechnology/General Education Center, Nanhua University, Chiayi, 622, Taiwan, ROC
e-mail: mingchaolin@mail.nhu.edu.tw

Materials and Methods

Samples of milkfish (range 4.0–6.0 cm in length and 0.41–1.41 g in weight) and ambient water were obtained from eight culture ponds in the BFD area. Three fish and three 500 mL water samples per pond were collected. Once collected, the fish samples were placed on ice immediately and kept at 4°C during transfer to the laboratory. The water samples were fixed by adding 5 mL 1 N HNO_3.

The frozen flesh of milkfish was dehydrated in a dryer (40°C) for 96 h, and then grounded into powder. Aliquots of dry flesh powder weighing 0.5 g were placed into a 250 mL beaker. Nitric acid (65%, 10 mL) was added for an overnight digestion. The beaker with flesh solution, after the digestion, was heated with a water bath at 70–80°C for 2–4 h until the total volume was reduced to 1–2 mL. The solution was transferred to a volumetric flask (50 mL), and then filled with 0.01 N of HNO_3 to make a 50 mL of final solution. After filtration, this 50 mL solution was transferred to test tubes for Zn and Cu analysis using ICP-MS (Agilent 7500a). Analytical quality control was achieved by digesting and analyzing identical amounts of rehydrated (90% H_2O) standard reference materials (DORM-2, Dogfish Liver-2-organic matrix, NRC-CNRC, Canada). Recovery rates were ranged from 95% to 97%.

When steady-state chemical concentrations of tissue are attained, the equilibrium bioconcentration factor (BCF) of the milkfish can be calculated from the ratio of the chemical concentration in fish to that in water. The BCF can also be calculated from the ratio of the uptake rate constant to the depuration rate constant as,

$$\mathrm{BCF} = \frac{C_b}{C_w} \tag{1}$$

where C_b (μg g^{-1}) is the chemical concentration in biota; C_w (μg ml^{-1}) is the chemical concentration in water.

A questionnaire interview was conducted to analyze the consumption habits on milkfish of the residents in the four towns mentioned above. One hundred and forty residents, including the owners of the eight milkfish ponds, were interviewed. A brief questionnaire was filled in with demographic information and data on nutritional habits. The interview questionnaire included detailed questions about milkfish consumption to determine the amount and frequency of consumption. The personal, dietary, and residential information was also obtained.

The risks of As, Zn, and Cu accumulation from the ambient water to humans via the milkfish was assessed. All information from the 53 residents, who consume the local cultured milkfish, was classified to evaluate the non-carcinogenic risks of As, Zn, and Cu exposure. Target hazard quotient (THQ) was used to indicate the non-carcinogenic risks. The method to estimate THQ was provided in USEPA Region III Risk-Based Concentration Table (USEPA 2006). The model for estimating THQ was shown as follows:

$$\mathrm{THQ} = (C_b \times \mathrm{IRF} \times 10^{-3} \times \mathrm{EFr} \times \mathrm{EDtot}) / (\mathrm{RfD} \times \mathrm{BWa} \times \mathrm{ATn}) \tag{2}$$

where THQ is the target hazard quotient; C_b is the chemical level in fish (μg g^{-1}); IRF is the fish ingestion rate (g day^{-1}); EFr is the exposure frequency (350 day $year^{-1}$); EDtot is the exposure duration, total (30 year); RfD is the reference dose (3×10^{-4} g day μg^{-1}); BWa is the body weight, adult (70 kg); and ATn is the averaging time, non-carcinogens (EDtot × 365 day $year^{-1}$). The health protection standard of lifetime risk for THQ is 1 (USEPA 2006). The hazard index (HI) can be expressed as the sum of the hazard quotients (USEPA 2006):

$$\mathrm{HI} = \mathrm{THQ}_{As} + \mathrm{THQ}_{Zn} + \mathrm{THQ}_{Cu} \tag{3}$$

where HI is the hazard index; THQ_{As} is the target hazard quotient for As intake; THQ_{Zn} is the target hazard quotient for Zn intake; and THQ_{Cu} is the target hazard quotient for Cu intake.

Target cancer risk (TR) was used to indicate carcinogenic risks. The method to estimate TR was also provided in USEPA Region III Risk-Based Concentration Table (USEPA 2006). The model for estimating TR was shown as follows:

$$\mathrm{TR} = (C_b \times \mathrm{IRF} \times 10^{-3} \times \mathrm{CPSo} \times \mathrm{EFr} \times \mathrm{EDtot}) / (\mathrm{BWa} \times \mathrm{ATc}) \tag{4}$$

where TR is the target cancer risk; CPSo is the carcinogenic potency slope, oral (kg day mg^{-1}); ATc is the averaging time, carcinogens (25,550 days). The health protection standard of lifetime risk for TR is 1×10^{-6} (USEPA 2006). Since Zn and Cu do not cause any carcinogenic effects, only the TR value for intake of As was calculated to indicate the carcinogenic risk. The health risks (TR and THQ) were evaluated, based on the values of RfD (3×10^{-4} mg kg^{-1} day^{-1}, 0.3 mg kg^{-1} day^{-1} and 0.04 mg kg^{-1} day^{-1} for As, Zn, and Cu, respectively) and CPSo (1.5 kg day mg^{-1} for As) provided by USEPA (USEPA 2006).

Results and Discussion

The As, Zn, and Cu levels in milkfish showed significant positive correlations ($R > 0.9$) to the As, Zn, and Cu concentrations in pond water: $C_b = -0.26 + 0.02\ C_w$, $C_b = 25.43 + 1.02\ C_w$, and $C_b = 1.22 + 0.01\ C_w$, respectively (Fig. 1), where C_b is the chemical concentration in milkfish (μg g^{-1}); C_w is the chemical concentration in water

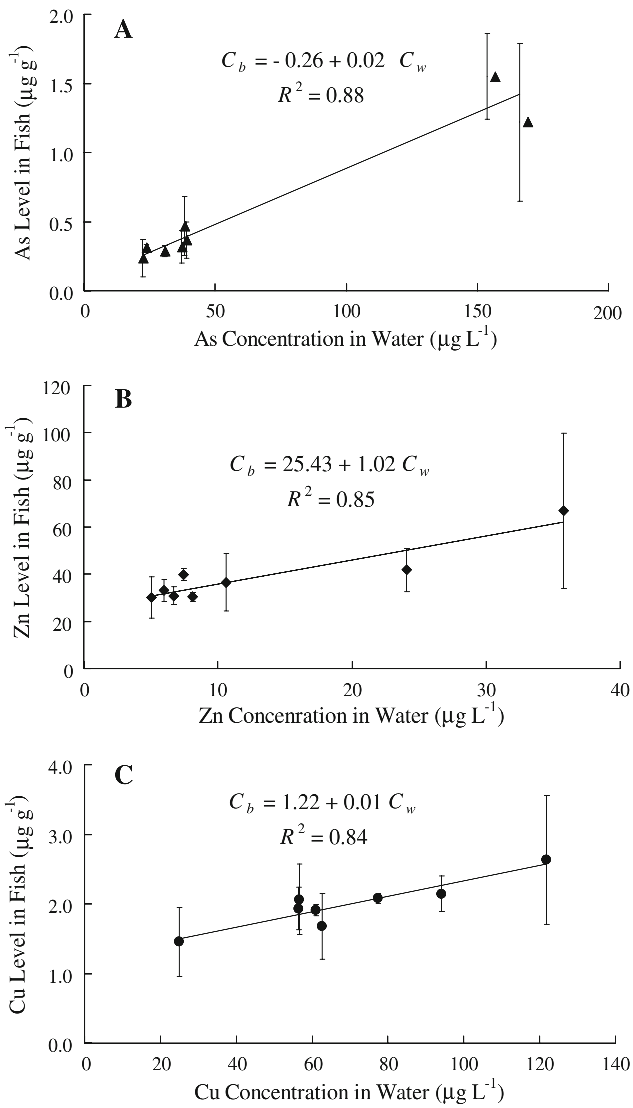

Fig. 1 Plot of the chemical level in cultured milkfish and the chemical concentration in pond water (**a** arsenic; **b** zinc; and **c** copper)

(μg L^{-1}). The resulting data showed that the As, Zn, and Cu concentrations in pond water were 63.92 ± 34.29 μg L^{-1}, 10.89 ± 7.25 μg L^{-1}, and 77.09 ± 35.07 μg L^{-1}, respectively (Table 1). The As and Cu concentrations in pond water were higher than the safe standards for aquacultural water in Taiwan (As < 50 μg L^{-1} and Cu < 30 μg L^{-1}), while the Zn concentration in pond water was lower than the standard (Zn < 500 μg L^{-1}). The As, Zn, and Cu levels in fish were 0.97 ± 0.78 μg g^{-1}, 37.98 ± 6.49 μg g^{-1}, and 2.09 ± 0.40 μg g^{-1}, respectively (Table 2). According to USEPA (2006), the maximum contamination levels of As, Zn, and Cu in fish were 2.1 × 10^{-3} μg g^{-1}, 410 μg g^{-1}, and 54 μg g^{-1}, respectively. Among the three pollutants in milkfish, only As level exceeded the guideline value, while the levels of Zn and Cu were below those recommended safe values.

Table 1 Arsenic (As), zinc (Zn), and copper (Cu) concentrations in water of milkfish culture ponds (μg L^{-1}) in the BFD area

Location	Concentration in water (Mean ± SE)		
	As	Zn	Cu
Putai	88.10 ± 65.63[a]	21.65 ± 11.06	85.70 ± 8.34[a]
Yichu	102.57 ± 63.62[a]	5.97 ± 1.91	60.01 ± 2.44[a]
Hsuehchia	30.44 ± 6.82	7.45 ± 0.23	40.78 ± 15.92[a]
Peimen	34.58 ± 3.76	8.49 ± 0.23	121.88 ± 7.18[a]
Average	63.92 ± 34.29[a]	10.89 ± 7.25	77.09 ± 35.07[a]

[a] As > 50 μg L^{-1}; Zn > 500 μg L^{-1}; Cu > 30 μg L^{-1}, higher than the standards for aquacultural water in Taiwan

Table 2 Arsenic (As), zinc (Zn), and copper (Cu) levels in milkfish (μg g^{-1}) from culture ponds in the BFD area

Location	Level in fish (Mean ± SE)		
	As	Zn	Cu
Putai	1.891 ± 1.659	46.330 ± 13.752	2.111 ± 0.030
Yichu	1.286 ± 0.932	33.102 ± 4.613	1.839 ± 0.109
Hsuehchia	0.318 ± 0.003	39.916 ± 2.632	1.759 ± 0.305
Peimen	0.402 ± 0.111	32.570 ± 2.651	2.634 ± 0.448
Average	0.974 ± 0.782	37.979 ± 6.494	2.086 ± 0.396

The values of BCF of As, Zn, and Cu accumulated in fish were 9.65 ± 2.34, 4052.47 ± 1135.44, and 34.35 ± 11.71, respectively (Table 3). It indicated that milkfish can accumulate As, Zn, and Cu from the ambient water. The high BCF values showed that those cultured milkfish were contaminated by the ambient water and had a high tolerance against the pollutants. However, the accumulation of these chemicals could still contribute to chronic toxicity in milkfish. When the chemicals reach sufficiently high concentrations in body cells they can alter the physiological functioning of the fish (Beaumont et al. 2000). Simultaneous subthreshold exposures to multiple toxins under this condition could result in an adverse health effect (Gioda et al. 2007).

The THQs of As, Zn, and Cu for intake of the milkfish were 1.28 ± 0.61, 0.34 ± 0.13, and 0.13 ± 0.00,

Table 3 Bioconcentration factor (BCF) of arsenic (As), zinc (Zn), and copper (Cu) in milkfish from culture ponds in the BFD area

Location	BCF (Mean ± SE)		
	As	Zn	Cu
Putai	10.28 ± 8.34	3191.77 ± 2404.89	24.84 ± 2.92
Yichu	7.01 ± 3.40	5541.02 ± 1469.51	30.78 ± 3.81
Hsuehchia	10.94 ± 3.55	5357.59 ± 2125.76	47.43 ± 15.61
Peimen	10.35 ± 4.17	3919.48 ± 593.07	21.62 ± 7.44
Average	9.65 ± 2.34	4052.47 ± 1135.44	34.35 ± 11.71

Table 4 The values (Mean ± SE) of target hazard quotient (THQ) and hazard index (HI) for intake of arsenic (As), zinc (Zn), and copper (Cu), as well as the target cancer risk (TR) for intake of arsenic (As), from the contaminated milkfish

Location	THQ			HI	TR
	As	Zn	Cu		As
Putai	1.22 ± 0.86[a]	0.34 ± 0.08	0.13 ± 0.00	1.69 ± 0.95[a]	$2.35 \times 10^{-4} \pm 1.65 \times 10^{-4}$[b]
Yichu	1.65 ± 0.98[a]	0.25 ± 0.07	0.13 ± 0.02	2.03 ± 1.06[a]	$3.18 \times 10^{-4} \pm 1.88 \times 10^{-4}$[b]
Hsuehchia	1.81 ± 1.76[a]	0.55 ± 0.27	0.13 ± 0.12	2.49 ± 2.14[a]	$3.49 \times 10^{-4} \pm 3.40 \times 10^{-4}$[b]
Peimen	0.44 ± 0.16	0.38 ± 0.15	0.13 ± 0.01	0.95 ± 0.32	$8.47 \times 10^{-5} \pm 3.03 \times 10^{-5}$[b]
Average	1.28 ± 0.61[a]	0.34 ± 0.13	0.13 ± 0.00	1.75 ± 0.65[a]	$2.74 \times 10^{-4} \pm 1.18 \times 10^{-4}$[b]

[a] >1, higher than the safe standard for non-cancer risk

[b] $>1 \times 10^{-6}$, higher than the safe standard for cancer risk

respectively (Table 4). The value of THQ of As for intake of the milkfish were higher than the safe standard 1 (USEPA 2006), while the values for Zn and Cu were both lower than the standard. The HI of As, Zn, and Cu mixture for intake of milkfish 1.75 ± 0.65, higher than the standard 1, demonstrated that ingestion of in this way contaminated milkfish will result in non-carcinogenic risk in inhabitant. The risk was mainly contributed by As (THQ = 1.28 ± 0.61), which may cause chronic non-carcinogenic effects, such as jaundice, cirrhosis, blueness of the extremities, Raynaud's syndrome, blackfoot disease (a type of gangrene), anemia, and hyperkeratosis of the skin. The HI value of Zn and Cu mixture 0.50 ± 0.18, lower than the standard 1, demonstrated that ingestion of the milkfish does not result in overexposure of these two chemicals. Thus far no adverse effect on health of the people from southwestern Taiwan due to exposure to Zn and/or Cu contaminated milkfish is evidenced. The average value of TR was $2.74 \times 10^{-4} \pm 1.18 \times 10^{-4}$ (Table 4), higher than the acceptable risk 1×10^{-6} (USEPA 2006). It shows that the inhabitants consuming milkfish from the As-contaminated area were exposed to As pollution with a carcinogenic risk.

Health problems caused by As in the coastal area of southwest Taiwan have been the subject of much research since their initial discovery in the early 1960s and have formed the basis of many epidemiological risk assessments over the last 40 years (Ling and Liao 2007). Use of groundwater containing high concentrations of As was significantly related to internal organ and skin cancers as well as blackfoot disease (BFD) and other vascular diseases. Routine monitoring has revealed that groundwater in various coastal regions of southwest Taiwan contain hazardous levels of As. Previous studies have shown that the crude mortality rate of cancer was higher in those regions than the average in Taiwan. Taiwan is the classic area for the identification of BFD and other peripheral vascular disorders; many other As-related diseases have also been described from the area. A large-scale correlative study on the association between As complexes in well water and age-adjusted mortality from diabetes, hypertension, and cancers of the nasal cavity, lung, liver, bladder, kidney, and prostate yields consistent findings. Several epidemiological studies confirm that there is an increased risk of cancer in case of exposure to As (Jang et al. 2006; Ling and Liao 2007).

The As problems of Taiwan are largely historical, as alternative treated surface water supplies have been provided for the affected communities. Nowadays, people living in these areas do not drink water from wells. However, the groundwater is still used for aquaculture due to the shortage of surface water mainly from limited rivers and creeks. Milkfish culture needs a high amount (38,000–49,000 ton ha^{-1}) of freshwater, groundwater is a major water resource for milkfish aquaculture in southwest Taiwan (Lin et al. 2005). The inhabitants consuming the As-contaminated fish from the ponds using groundwater in the coastal area of southwest Taiwan might be exposed chronically to As, Zn, and Cu pollution with carcinogenic and non-carcinogenic risks. Since using groundwater for aquaculture is such a common situation in the As-contaminated area (Chou et al. 2006), a greater understanding of the As, Zn, and Cu accumulation in human bodies by consuming the contaminated fish and the subsequent health effects is needed. A large amount of artesian well waters were still pumped for the milkfish aquaculture. It is important to establish regulatory limits regarding the presence of As, Zn, and Cu in the pond water for milkfish aquaculture in southwest Taiwan, in order to safeguard the health of residents.

Acknowledgments This work was financially supported by the National Science Council of Republic of China (NSC 94-2313-B-343-001). The earlier draft of this manuscript benefited from the comments of Mr. R. Regout.

References

Beaumont MW, Butler PJ, Taylor EW (2000) Exposure of brown trout, *Salmo trutta*, to a sub-lethal concentration of copper in soft acidic water: effects upon muscle metabolism and membrane potential. Aquatic Toxicol 51:259–272. doi:10.1016/S0166-445X(00)00109-0

Chou BYH, Liao CM, Lin MC, Cheng HH (2006) Toxicokinetics/toxicodynamics of arsenic for farmed juvenile milkfish *Chanos chanos* and human consumption risk in BFD-endemic area of Taiwan. Environ Int 32:545–553. doi:10.1016/j.envint.2006.01.004

Falco G, Llobet JM, Bocio A, Domingo JL (2006) Daily intake of arsenic, cadmium, mercury, and lead by consumption of edible marine species. J Agric Food Chem 54:6106–6112. doi:10.1021/jf0610110

García-Rico L, Leyva-Perez J, Jara-Marini ME (2007) Content and daily intake of copper, zinc, lead, cadmium, and mercury from dietary supplements in Mexico. Food Chem Toxicol 45:1599–1605. doi:10.1016/j.fct.2007.02.027

Gioda CR, Lissner LA, Pretto A, da Rocha JBT, Schetinger MRC, Neto JR, Morsch VM, Loro VL (2007) Exposure to sublethal concentrations of Zn(II) and Cu(II) changes biochemical parameters in *Leporinus obtusidens*. Chemosphere 69:170–175. doi:10.1016/j.chemosphere.2007.04.008

Jang CS, Liu CW, Lin KH, Huang FM, Wang SW (2006) Spatial analysis of potential carcinogenic risks associated with ingesting arsenic in aquacultural tilapia (*Oreochromis mossambicus*) in blackfoot disease hyperendemic areas. Environ Sci Technol 40:1707–1713. doi:10.1021/es051875m

Lin MC, Lin HY, Cheng HH, Chen YC, Liao CM, Shao KT (2005) Risk assessment of arsenic exposure from consumption of cultured milkfish, *Chanos chanos* (Forsskål), from the arsenic-contaminated area in southwestern Taiwan. Bull Environ Contam Toxicol 75:637–644. doi:10.1007/s00128-005-0800-2

Ling MP, Liao CM (2007) Risk characterization and exposure assessment in arseniasis-endemic areas of Taiwan. Environ Int 33:98–107. doi:10.1016/j.envint.2006.08.006

Peshut PJ, Morrison RJ, Brooks BA (2008) Arsenic speciation in marine fish and shellfish from American Samoa. Chemosphere 71:484–492. doi:10.1016/j.chemosphere.2007.10.014

Terra BF, Araújo FG, Calza CF, Lopes RT, Teixeira TP (2008) Heavy metal in tissues of three fish species from different trophic levels in a tropical Brazilian river. Water Air Soil Pollut 187:275–284. doi:10.1007/s11270-007-9515-9

USEPA (2006). USEPA region III risk-based concentration table: technical background information