Bull. Environ. Contam. Toxicol. (2005) 75:637–644

DOI: 10.1007/s00128-005-0800-2

Bulletin of Environmental Contamination and Toxicology

Risk Assessment of Arsenic Exposure from Consumption of Cultured Milkfish, *Chanos chanos* (Forsskål), from the Arsenic-Contaminated Area in Southwestern Taiwan

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Received: 22 April 2005/Accepted: 29 July 2005

Arsenic (As) is ubiquitously distributed in nature and transported mainly by water. It is potentially a toxic element, and remains a significant human health concern (Ng et al. 2003). Arsenic is known to increase the risk of producing or inducing cancer. A significant exposure-response between As concentration and the mortality from various cancers has been reported (Chiou et al. 1995). Clinical manifestations of chronic arsenic poisoning, including non-cancer diseases, have been implicated. The main source of As exposure is through ingestion of water, especially groundwater, which contains As (Ng et al. 2003).

Arsenic has been well documented as one of the major risk factors for blackfoot disease (BFD) (Chiou et al. 1995). First observed in the 1930s and peaking in the 1950s, BFD correlated with the consumption of groundwater by local inhabitants mainly living in the four towns, Putai, Yichu, Peimen and Hsuehchia, southwestern Taiwan. Several follow-up studies indicated that an increase in fatal cancers was significantly associated with the use of As-contaminated groundwater. Today most people living in these areas do not drink water from wells after tap water has been made available; however, the groundwater is still used for aquaculture (Lin et al. 2001, 2004). Since As is toxic and can be accumulated in aquatic animals, it may threaten humans consuming contaminated fish. Milkfish farming is a promising practice with high market value in Taiwan. Most of the milkfish aquaculture is located in the coastal region of southwestern Taiwan. Part of that region, which is situated in and around the four towns mentioned above, has groundwater contaminated with As. This area is known as the BFD area (Lin et al. 2004). A high amount (38,000-49,000 ton ha^{-1}) of freshwater is needed for milkfish culture. Several studies have been conducted to demonstrate that to use groundwater for aquaculture may cause an overexposure of As (Lin et al. 2001, 2004; Liao and Ling 2003). Since milkfish is common seafood in Taiwan, ingestion of As contaminated fish could result in As accumulation in inhabitants and lead to adverse health effects (Lin et al. 2004). The purpose of our study was to estimate the risk of the intake of aquacultural milkfish from the ponds, using As-contaminated groundwater, in the southwestern region of Taiwan.

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MATERIALS AND METHODS

Ponds for milkfish culture in the As-contaminated area (also named the BFD area), in the four towns, Putai, Yichu, Peimen and Hsuehchia, were selected for sampling; three ponds for each town, in total twelve ponds, were considered as repeats. The groundwater used for culture in these ponds has a salinity of 0%. Samples of water and adult milkfish (body length 35-40 cm) from three ponds per town were analyzed to determine the As level. With three repeats for each sample, three fish samples and three 500 ml water samples per pond were collected. The milkfish samples were immediately placed on ice and kept at 4°C within a period of 2 h during transfer to the laboratory, and then stored at -20°C. The water samples were fixed by adding 5 ml 1N HNO_3 before As analysis.

Water and milkfish samples were sent to the Super Micro Mass Research and Technology Center, Cheng Shiu Institute of Technology for analysis of total As. The frozen flesh of milkfish was dehydrated in a dryer at 40°C for 96 h, and then ground 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 a 12-h digestion. The beaker with flesh solution, after the digestion, was heated with a water bath at 70-80℃ for 2-4 h until the total volume reduced to 1-2 ml. The solution was transferred to a volumetric flask (50 ml), and then filled with 0.01N of HNO_3 to make a 50 ml of final solution. After filtration, this 50 ml solution was transferred to test tubes for As 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 ranged from 95% to 97%.

A questionnaire interview was conducted from March 2002 to January 2003. We interviewed 141 residents, including the owners of the 12 milkfish ponds, in the four towns mentioned above, and received from them their consumption habits on milkfish. 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 bioconcentration factor (BCF), relating the concentration of As in water to its level in fish (Lin et al. 2004), was used to estimate the propensity of As accumulation in milkfish:

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

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

The risk of As 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 carcinogenic and non-carcinogenic risks of As exposure. It has been well known that inorganic As is more toxic than organic As. Potential human health risks associated with inorganic As uptake from various kinds of seafood have been evaluated by Edmonds and Francesconi (1993), Macintosh et al. (1996) and Han et al. (1998). In their studies, inorganic As in seafood was assumed to be 10% of total As. Huang et al. (2003) has conducted a study measuring the As species in cultured tilapia (*Oreochromis mossambicus*) from the As-contaminated area. It showed that inorganic As in the fish is 7.4% of the total As which is close to 10%. Therefore, we used 10% as the percentage to evaluate the inorganic As in milkfish.

Target cancer risk (TR) and target hazard quotients (THQ) were used to indicate carcinogenic and non-carcinogenic risks. The method to estimate TR and THQ was provided in USEPA Region III Risk-Based Concentration Table (USEPA 2003). The models for estimating TR and THQ are shown as follows:

$$\mathrm{TR} = (C_b \times \mathrm{IRF} \times 10^{-3} \times \mathrm{CPSo} \times \mathrm{EFr} \times \mathrm{EDtot}) \;/\; (\mathrm{BWa} \times \mathrm{ATc}) \qquad (2)$$

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

where TR is the target cancer risk; C_b is the As level in fish ($\mu g\ g^{-1}$); IRF is the fish ingestion ($g\ d^{-1}$); CPSo is the carcinogenic potency slope, oral ($1.5\ \mu g\ g^{-1}\ d^{-1}$); EFr is the exposure frequency ($350\ d\ yr^{-1}$); EDtot is the exposure duration, total (30 yrs); BWa is the body weight, adult (70 kg); ATc is the averaging time, carcinogens (25,550 d); THQ is the target hazard quotient; RfD is the reference dose ($3 \times 10^{-4}\ \mu g\ g^{-1}\ d^{-1}$); ATn is the averaging time, non-carcinogens ($\mathrm{EDtot} \times 365\ d\ yr^{-1}$).

The health protection standard of lifetime risk for TR is 1×10^{-6}, and the standard for THQ is 1 (USEPA 2003). The acceptable consumption of milkfish was calculated, based on the As level in fish and the acceptable values for TR, using Eq. 2. The actual milkfish consumption and the upper limit for TR were inserted to Eq. 2 to calculate the risk-based concentration of As in milkfish (RBC_f). Furthermore, BCF and RBC_f values were used to calculate the risk-based concentration of As in water (RBC_w).

$$RBC_f = 1 \times 10^{-6} \times (\mathrm{BWa} \times \mathrm{ATc}) / (\mathrm{IRF} \times 10^{-3} \times \mathrm{CPSo} \times \mathrm{EFr} \times \mathrm{EDtot}) \qquad (4)$$

$$RBC_w = \mathrm{BCF} / RBC_f \qquad (5)$$

where RBC_f is the risk-based concentration of As in milkfish ($\mu g\ g^{-1}$); RBC_w is the risk-based concentration of As in water (μg ml^{-1})

RESULTS AND DISCUSSION

The average total As concentration in pond water was 65.5 ± 76.4 μg L^{-1} (Table 1). The concentrations in Putai 3, Yichu 3 and Hsuehchia 1, which have higher As concentrations (153.7 ± 5.9 μg L^{-1}, 166.2 ± 4.9 μg L^{-1} and 345.2 ± 12.5 μg L^{-1}, respectively) among all of the sampled ponds, are higher than the standard of 50 μg L^{-1} for As in aquacultural water in Taiwan. Since different ponds contained varied As concentrations (F = 1285.2, n = 36, P < 0.05), the As levels in milkfish were different from each other (F = 47.7, n = 36, P < 0.05). The average total As level in milkfish was 0.7 ± 0.7 μg g^{-1}. The mean BCF for total As accumulation in milkfish was 11.6 ± 4.4. The resulting data of analysis of variance (ANOVA) indicated that the values of BCF of milkfish in different ponds showed no significant difference (F = 1.9, n = 36, P < 0.05).

Table 1. Arsenic concentration in pond water (μg L^{-1}), As level in milkfish (μg g^{-1}), and the BCF value for As accumulation in milkfish from the As-contaminated area.

Pond	As in Pond Water (Mean ± SE)	As in Milkfish (Mean ± SE)	BCF (Mean ± SE)
Putai 1	20.3 ± 1.8	0.3 ± 0.1	14.1 ± 7.7
Putai 2	22.5 ± 2.5	0.2 ± 0.0	10.5 ± 1.0
Putai 3	153.7 ± 5.9*	1.6 ± 0.3	10.1 ± 1.7
Yichu 1	39.0 ± 1.3	0.4 ± 0.1	9.5 ± 3.7
Yichu 2	95.2 ± 5.1*	0.4 ± 0.1	4.3 ± 1.3
Yichu 3	166.2 ± 4.9*	1.2 ± 0.6	7.3 ± 3.2
Hsuehchia 1	345.2 ± 12.5*	3.4 ± 0.3	9.7 ± 0.6
Hsuehchia 2	23.6 ± 1.8	0.3 ± 0.0	13.3 ± 2.1
Hsuehchia 3	37.3 ± 0.5	0.3 ± 0.1	8.6 ± 3.2
Peimen 1	38.3 ± 2.8	0.5 ± 0.2	12.4 ± 6.5
Peimen 2	30.8 ± 0.5	0.3 ± 0.0	9.3 ± 1.3
Peimen 3	27.9 ± 0.5	0.2 ± 0.0	7.7 ± 0.7
Average	65.5 ± 76.4*	0.7 ± 0.7	11.6 ± 4.4

*: > 50 μg L^{-1}, higher than the standard for As in aquacultural water in Taiwan

The total As level of adult milkfish (0.7 ± 0.7 μg g^{-1}), determined in this study, is lower than that in juveniles (15.2 ± 5.1 μg g^{-1}) reported by Lin et al. (2004) (t = 8.5, n = 63, P < 0.05). It was suggested that such a decrease could be related to a "growth dilution" factor. Similar phenomena were also discovered in large-scale mullet *Liza macrolepis* (Lin et al. 2001) and tilapia *Oreochromis mossambicus*

(Liao et al. 2003). The lower value of BCF of adult milkfish (11.6 ± 4.4), in comparison to juvenile milkfish (556.2 ± 188.0), indicates that the latter have a higher accumulation effect of As ($t = 8.7$, $n = 63$, $P < 0.05$). Intake of juvenile milkfish may cause a higher risk to health. The high tolerance for As by both adult and juvenile fish suggests that the milkfish can accumulate high amount of As before they show symptoms.

Table 2. The actual and the acceptable consumption (g d^{-1}) of the milkfish in the As-contaminated area.

Pond	Actual Consumption (Mean ± SE)	Acceptable Consumption (Mean ± SE)
Putai 1	294.6 ± 76.7*	5.3 ± 3.6
Putai 2	150.1 ± 72.4*	4.9 ± 0.8
Putai 3	151.1 ± 65.1*	0.8 ± 0.1
Yichu 1	314.8 ± 51.6*	3.3 ± 1.1
Yichu 2	282.5 ±150.8*	3.0 ± 1.0
Yichu 3	178.3 ± 90.1*	1.1 ± 0.4
Hsuehchia 1	173.0 ± 121.9*	0.3 ± 0.0
Hsuehchia 2	138.3 ± 77.6*	3.7 ± 0.3
Hsuehchia 3	58.9 ± 65.4*	4.0 ± 1.8
Peimen 1	186.5 ± 62.9*	2.9 ± 1.7
Peimen 2	107.1 ± 52.3*	4.0 ± 0.5
Peimen 3	95.0 ± 33.0*	5.3 ± 0.4
Average	177.5 ± 81.2*	3.2 ± 1.7

*: > acceptable consumption

The nutritional habits of the 53 residents from the As-contaminated area showed that the actual consumption on milkfish was 177.5 ± 81.2 g d^{-1} (Table 2). The residents had varied habits of consuming milkfish ($F = 2.9$, $n = 36$, $P < 0.05$). The target cancer risk (TR) of consuming milkfish was $1.1\times10^{-4} \pm 1.4\times10^{-4}$ (Table 3), higher than the acceptable risk 1×10^{-6} ($t = 2.6$, $n = 12$, $P < 0.05$). It shows that the inhabitants from the As-contaminated area are exposed to As pollution with a carcinogenic risk. Han et al. (1998) indicated that the TR for inorganic As intake of consuming fish from supermarkets in various regions in Taiwan is 6.6×10^{-5}, which is lower than that we determined in the As-contaminated area.

The Target Hazard Quotient (THQ) for intake of the milkfish was 0.3 ± 0.3 (Table 3), lower than the safe value 1 ($t = 8.0$, $n = 12$, $P < 0.05$), which does not demonstrate a non-carcinogenic risk for humans. However, the THQ values for Hsuehchia 1 show an over exposure of As for non-carcinogenic risk; the residents in the two towns must be advised to reduce the intake of milkfish. Han et al. (1998)

indicates that the THQ for As-polluted fish from the supermarkets, mentioned above was 0.3, which is close to the value we determined in the As-contaminated area.

The acceptable consumption of the milkfish was 3.2 ± 1.7 g d^{-1} (Table 2), which was lower than the actual milkfish consumption in the four towns 177.5 ± 81.2 g d^{-1} ($t = 7.4$, $n = 24$, $P < 0.05$). The risk-based concentration (RBC_f) for total As in milkfish was 0.011 ± 0.010 μg g^{-1}, lower than the amount we obtained from the fish samples (0.7 ± 0.7 μg g^{-1}) ($t = 2.8$, $n = 24$, $P < 0.05$). The risk-based concentration (RBC_w) for total As concentration in pond water was 0.3 ± 0.3 μg L^{-1} (Table 4), lower than the pond water *in situ* (65.5 ± 76.4 μg L^{-1}) ($t = 2.9$, $n = 24$, $P < 0.05$). It demonstrates that consumption of cultured milkfish from the As-contaminated area may pose a cancer risk to human health.

Table 3. Estimated target cancer risk (TR) and target hazard quotients (THQ) for inorganic As caused by consuming milkfish from ponds in the As-contaminated area.

Pond	TR (Mean ± SE)	THQ (Mean ± SE)
Putai 1	$7.2\times10^{-5} \pm 3.6\times10^{-5}$*	0.2 ± 0.1
Putai 2	$3.1\times10^{-5} \pm 4.4\times10^{-6}$*	0.1 ± 0.0
Putai 3	$2.1\times10^{-4} \pm 4.1\times10^{-5}$*	0.5 ± 0.1
Yichu 1	$3.7\times10^{-5} \pm 1.3\times10^{-5}$*	0.2 ± 0.1
Yichu 2	$5.4\times10^{-5} \pm 1.9\times10^{-5}$*	0.2 ± 0.1
Yichu 3	$1.9\times10^{-4} \pm 8.9\times10^{-5}$*	0.4 ± 0.2
Hsuehchia 1	$5.1\times10^{-4} \pm 4.1\times10^{-5}$*	1.1 ± 0.1**
Hsuehchia 2	$3.8\times10^{-5} \pm 3.1\times10^{-6}$*	0.1 ± 0.0
Hsuehchia 3	$1.7\times10^{-5} \pm 6.1\times10^{-6}$*	0.0 ± 0.0
Peimen 1	$7.7\times10^{-5} \pm 3.5\times10^{-5}$*	0.2 ± 0.1
Peimen 2	$2.7\times10^{-5} \pm 3.9\times10^{-6}$*	0.1 ± 0.0
Peimen 3	$1.8\times10^{-5} \pm 1.5\times10^{-6}$*	0.0 ± 0.0
Average	$1.1\times10^{-4} \pm 1.4\times10^{-4}$*	0.26 ± 0.31

*: > the acceptable value of 1×10^{-6} for carcinogenic risk

**: > the acceptable value of 1 for non-carcinogenic risk

It has been well known that fish can covert the toxic inorganic arsenicals in their bodies into non-toxic methylated forms, such as arsenobetaine. Since no distinction between inorganic and organic As in fish was made in our study, we have followed Edmonds and Francesconi (1993), Macintosh et al. (1996) and Han et al. (1998) using 10% of the total As to calculate the level of inorganic As in milkfish. The target cancer risk (TR) of consuming milkfish ($1.1\times10^{-4} \pm 1.4\times10^{-4}$) is times higher than the acceptable risk (1×10^{-6}); it would still be high enough ($1.1 \times$

$10^{-6} \pm 1.4 \times 10^{-6}$) to increase cancer risk, even if we would have assumed that the level of inorganic As is 0.1% of the total As in milkfish. This study indicates that the government should investigate the safety of milkfish consumption in the BFD area. Other alternative water supplies for aquaculture in this area need to be arranged, based on better watershed management strategies.

Table 4. Estimated RBC_f and RBC_w for total As in the As-contaminated area

Pond	RBC_f (μg g^{-1})	RBC_w (μg L^{-1})
Putai 1	$4.1\times10^{-3} \pm 1.2\times10^{-3}$	0.4 ± 0.2
Putai 2	$8.9\times10^{-3} \pm 4.2\times10^{-3}$	0.9 ± 0.5
Putai 3	$8.8\times10^{-3} \pm 4.6\times10^{-3}$	0.9 ± 0.6
Yichu 1	$3.7\times10^{-3} \pm 6.1\times10^{-4}$	0.4 ± 0.2
Yichu 2	$5.4\times10^{-3} \pm 4.2\times10^{-3}$	1.3 ± 0.9
Yichu 3	$7.5\times10^{-3} \pm 3.3\times10^{-3}$	1.3 ± 0.9
Hsuehchia 1	$1.2\times10^{-2} \pm 1.3\times10^{-3}$	1.3 ± 1.4
Hsuehchia 2	$1.0\times10^{-2} \pm 6.1\times10^{-3}$	0.8 ± 0.4
Hsuehchia 3	$4.3\times10^{-2} \pm 3.6\times10^{-2}$	5.3 ± 3.9
Peimen 1	$6.6\times10^{-3} \pm 2.6\times10^{-3}$	0.6 ± 0.2
Peimen 2	$1.2\times10^{-2} \pm 4.8\times10^{-3}$	1.3 ± 0.5
Peimen 3	$1.3\times10^{-2} \pm 5.5\times10^{-3}$	1.7 ± 0.5
Average (Mean ± SE)	$1.1\times10^{-2} \pm 1.0\times10^{-2}$	1.4 ± 1.3

RBC_f: the risk-based concentration of As in milkfish
RBC_w: the risk-based concentration of As in water

There is no acceptable standard legislation in Taiwan for As levels in fish. The assumed inorganic As level of milkfish in our study (0.07 ± 0.07 μg g^{-1}) is considerably lower than the maximum (2 μg g^{-1}) for inorganic As in various fish species and fish products authorized in New Zealand (Munoz et al. 2000), yet it still increases a risk of cancers. It is recommended that legislation should be established limiting the levels of inorganic As in different fish species.

Thus far no adverse effect on health of the people in the As-contaminated area due to exposure to local cultured milkfish has been reported, although the As level in these milkfish is high and identified as a potential cancer risk in people. More studies concerning As in fish and the risk in human health need to be undertaken. Clinical research concerning cancers and As-contaminated milkfish consumption is also necessary. A wider study involving As analyses of milkfish from non-As-contaminated areas should be initiated to assess the extent of As contamination. In addition, uncertainty analysis should be incorporated into the health risk assessment. Additional investigation into the relationship between As species in milkfish and pond water in the As-contaminated area should be explored in further studies.

Acknowledgments. We thank Dr. G.-P. Chang-Chien for deploying experimental equipment and providing technical assistance, and Miss Yu-Min Yeh for her help with statistical analysis. The earlier draft of this manuscript benefited from the comments of Mr. Rudolf Regout. This study was supported by the National Science Council of Republic of China under Grant NSC 92-2313-B-343-001.

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