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Total and Inorganic Arsenic in Natural and Aquacultural Freshwater Fish in Thailand: A Comparative Study

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Abstract Total and inorganic arsenic were determined in 108 samples of four freshwater fish species collected from natural water sources and aquaculture systems in the central region of Thailand between March and May 2010. Concentrations of total and inorganic arsenic (dry wt) and percentages of inorganic arsenic in four aquacultural fish species were not significantly different from those found in natural fish. Inorganic arsenic levels found in the four fish species from both sources in this study were much lower than the Thai regulatory standard of 2 µg/g, and hence are considered safe for human consumption.

Keywords Total arsenic · Inorganic arsenic · Freshwater fish · Natural water · Aquaculture

Arsenic is a toxic element which is found as a contaminant in the environment and food. It has long been known that food is the major non-occupational source of arsenic exposure in humans (WHO 2001). Arsenic in food is present in two major forms: organic and inorganic. Inorganic arsenic species [As(III) and As(V)], but not organic arsenic, are classified as Group 1 human carcinogens by the International Agency for Research on Cancer (IARC 1998).

Marine animals have been reported to contain the highest levels of total arsenic in comparison with other foods (Lawrence et al. 1988; Yost et al. 1998). Lorenzana et al. (2009) reviewed worldwide reports regarding inorganic arsenic, and indicated that inorganic arsenic relative to total arsenic in marine fish does not exceed 7.3 %. When compared to marine fish, freshwater fish contain lower arsenic concentrations but have higher percentages of inorganic arsenic (Lawrence et al. 1988; Tao and Bolger 1998). At present, few countries have established regulatory limits for inorganic arsenic in aquatic animals. China has established national standards for inorganic arsenic in aquatic fish and other aquatic products of animal origin at 0.1 and 0.5 µg/g (wet wt), respectively (USDA 2010). Thailand has also set a regulatory limit for inorganic arsenic in freshwater and marine animals at 2 µg/g (wet wt) (MOPH 2003). Although Thailand has already established a regulatory standard, there have been a limited number of internationally published reports on the concentrations of inorganic arsenic in freshwater animals in Thailand.

In Thailand, aquacultural freshwater fish constitute approximately 70 % of the total annual freshwater fish catch. From 2006 to 2009, yearly aquacultural fish production amounted to 527,400, 525,100, 522,500, and 521,900 tons, respectively, while the total fish catches were 741,400, 750,700, 751,100, and 728,700 tons, respectively; the percentages of aquacultural fish were 71.1 %, 69.9 %, 69.6 %, and 71.6 %, respectively (DOF 2011). Several concerns have been raised regarding the levels of contaminants in aquacultural fish compared to those in natural fish. Arsenic was the first contaminant to be investigated for these concerns, since inorganic arsenic is ubiquitous in the environment and is a known human carcinogen.

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Four fish species with the highest annual catches from natural water sources and aquaculture ponds in the central region of Thailand were analyzed in this study. Thailand has four administrative regions: central, north, northeast and south. The central region was selected for this study since this region has the highest annual freshwater fish production in the country. Between 2005 and 2009, the annual catches in the central region ranged from 48 % to 52 % of Thailand's total freshwater fish production (DOF 2011). The four fish species consisted of tilapia, silver barb, striped catfish, and striped snakehead.

According to fisheries statistics compiled by the Thailand Department of Fisheries (DOF 2011), tilapia (*Oreochromis niloticus*) is the most common commercial freshwater fish in Thailand, with an annual yield of 258,000 tons. Aquacultural tilapia accounts for 85 % of the total annual production. Approximately 10 % of the tilapia is exported as fresh-frozen fillets and smoked fish products. The annual aquacultural production of silver barb (*Puntius gonionotus*) is 47,200 tons, which is approximately 51 % of the total annual catch of 93,200 tons. The striped catfish (*Pangasius hypophthalmus*) is one of the largest freshwater fish species in Thailand; the fish can reach 150 cm in length, and a weight of up to 10 kg. Aquacultural striped catfish production (30,200 tons/year) represents approximately 86 % of the total catch (35,300 tons/year). The annual production of aquacultural striped snakehead (*Channa striata*), 7,800 tons, accounts for 23 % of the total annual catch (33,300 tons).

This study evaluated arsenic contamination in these four freshwater fish species from natural water sources and aquaculture production systems in the central region of Thailand, and also investigated whether the contamination levels are within the Thai regulatory standard.

Materials and Methods

Standard Reference Material (SRM) 2977 (mussel tissue) was purchased from the National Institute of Standards and Technology (NIST; Gaithersburg, MD). SRM 1566a (oyster tissue) was a gift from Mr. Janewit Wongsanoon of the Environmental Research and Training Centre (Thailand). Certified Reference Material (CRM) No. 6 (mussel tissue) of the National Institute for Environmental Studies (NIES; Tsukuba, Japan) was a gift from Dr. Chanchai Jaengsawang of the Thailand Department of Medical Sciences.

HNO_3 and HCl were obtained from Merck Chemicals (Darmstadt, Germany). Dimethylarsinic acid (DMA), hydrazine sulfate, hydrobromic acid, and other chemicals were purchased from Sigma–Aldrich (St. Louis, MO). Deionized water (18 MΩ cm) was used to prepare standard solutions, reagents, and samples throughout the study. To decontaminate possible arsenic residues, all glassware was treated with 10 % (v/v) HNO_3 for 20–24 h and washed three times with deionized water.

To minimize possible individual variations in arsenic concentrations, fish samples of the same size from each species were used in this study. Natural fish samples were purchased from fishermen who had caught the fish from the Chao Phraya River or Tha Chin River. Aquacultural fish samples were purchased from four aquaculture production facilities. Fish samples were put in polyethylene bags, placed in ice boxes, and transported to the laboratory. All samples were collected in the central region of Thailand between March and May 2010.

Sizes and weights of individual fish were measured before the muscle (approximately 10 g excluding the skin) was cut from each side of the fish. The muscle was washed once with distilled water, homogenized, weighed, freeze-dried, weighed, ground, and kept at 4°C until analysis. The moisture content of individual fish was calculated using the muscle weights before and after lyophilization. Total and inorganic arsenic were analyzed on an individual fish basis.

Sample preparation for determination of total arsenic was performed by the acid digestion procedure described by Muñoz et al. (1999). Total As in the final solutions was determined by hydride generation–atomic absorption spectrometry (HG-AAS). Duplicate analyses were performed for individual samples.

The extraction procedure for inorganic arsenic was performed using the method described by Muñoz et al. (1999). Inorganic arsenic in lyophilized samples was dissolved in concentrated HCl, reduced with hydrobromic acid and hydrazine sulfate to As(III), and extracted with chloroform. The As(III) was extracted back with dilute HCl, digested with concentrated HNO_3, and analyzed by HG-AAS. Duplicate analyses were performed for individual samples.

Determination of total and inorganic arsenic concentrations in the final solutions was performed with a Perkin-Elmer AAnalyst 300 atomic absorption spectrometer (Norwalk, CT) interfaced with an AS-90 autosampler and a FIAS-400 flow injection system. The operating conditions for atomic absorption spectrophotometry were as previously described by Ruangwises et al. (2012).

The limits of quantification (LOQs) for total and inorganic arsenic were performed using the Q2B procedure of the US Food and Drug Administration (USFDA 1996). For determination of the LOQ for total arsenic, samples were fortified with an arsenic mixture [As(III):DMA, 1:1] equivalent to total arsenic at concentrations of 0.5, 1.0, 2.5 and 5.0 µg/g; blank samples were not fortified with arsenic. Concentrations of total arsenic in arsenic-fortified samples and blank samples were quantified for total arsenic. Six linear regression lines were obtained by least-square linear regression analysis of the residual peak heights of standard arsenic versus the four fortified total arsenic concentrations. For determination of the

LOQ for inorganic arsenic, samples were fortified with an inorganic arsenic mixture [As(III):As(V), 1:1 w/w] at concentrations of 0.25, 0.5, 0.75 and 1.0 µg/g; blank samples were not fortified with inorganic arsenic. Concentrations of inorganic arsenic in arsenic-fortified samples and blank samples were quantified for inorganic arsenic. Six linear regression lines were obtained by least-square linear regression analysis of the residual peak heights of standard arsenic versus the four fortified inorganic arsenic concentrations.

To assess the accuracy of determination of total arsenic, SRM 2977 (mussel tissue) and CRM No. 6 (mussel tissue) were analyzed for total arsenic. The concentration of inorganic arsenic in SRM 2977 (mussel tissue) was determined and compared with the values previously reported in the literature.

The differences of each parameter between natural and aquacultural fish were analyzed using Student's t test. A statistical significance level of $p < 0.05$ was accepted for all comparisons. SPSS Statistics version 17.0 for Windows was used for statistical analysis.

Results and Discussion

The Q2B analytical procedure described by the USFDA (1996) was performed for calculating the limit of quantification (LOQ), using the equation $LOQ = 10\,\sigma/S$, where σ is the standard deviation of y-intercepts and S is the slope of linear regression analysis. The LOQs for total and inorganic arsenic in freshwater fish were 0.025 and 0.012 µg/g (dry wt), respectively. Based on the average moisture content of 75 % found in 108 samples in this study, the LOQs for total and inorganic arsenic were 0.00625 and 0.003 µg/g (wet wt), respectively. Concentrations of total arsenic found in SRM 2977 (mussel tissue) and CRM No. 6 (mussel tissue) were 8.69 ± 0.37 µg/g (n = 6; reference value 8.83 ± 0.91 µg/g) and 8.73 ± 0.32 µg/g (n = 5; reference value 9.2 ± 0.5 µg/g), respectively. The concentration of inorganic arsenic found in SRM 1566a (oyster tissue) was 0.586 ± 0.049 µg/g (n = 6), which was in agreement with the previously published values of 0.647 ± 0.027 µg/g (Muñoz et al. 2000).

The overall recoveries for total and inorganic arsenic were 96.8 % and 96.6%, respectively. The precision of the method, expressed as %RSD (% relative standard deviation), was calculated using the equation %RSD = 100 SD/AV, where SD is the standard deviation and AV is the average arsenic concentration recovered from the arsenic-fortified samples. The %RSD ranged from 1.2 to 4.8 for total arsenic, and 1.4 to 4.2 for inorganic arsenic.

Table 1 summarizes the total lengths and weights of fish samples, concentrations of total and inorganic arsenic, and percentages of inorganic arsenic with respect to total arsenic of the four freshwater fish species. Total arsenic concentrations found in tilapia from natural water sources and aquaculture systems (based on wet wt) were 0.203 ± 0.039 and 0.236 ± 0.041 µg/g, respectively, whereas inorganic arsenic concentrations were 0.025 ± 0.004 and 0.029 ± 0.005 µg/g, respectively. Average percentages of inorganic arsenic relative to total arsenic were 12.5 and 12.7, respectively. Concentrations of total and inorganic arsenic in natural and aquacultural silver barb, respectively, were 0.217 ± 0.040 and 0.028 ± 0.006 µg/g, and 0.196 ± 0.032 and 0.025 ± 0.006 µg/g.

Levels of total arsenic (0.195 ± 0.039 µg/g), inorganic arsenic (0.025 ± 0.005 µg/g), and percentage of inorganic arsenic (12.9 %) in aquacultural striped catfish were comparable to those (0.201 ± 0.029, 0.023 ± 0.004 µg/g, and 11.9 %) in natural fish. Total and inorganic arsenic concentrations in natural and aquacultural striped snakehead, respectively, were 0.354 ± 0.087 and 0.080 ± 0.017 µg/g, and 0.367 ± 0.130 and 0.073 ± 0.012 µg/g. Percentages of inorganic arsenic in striped snakehead from natural and aquacultural sources were 22.9 % and 21.5 %, respectively.

In this study, statistical analysis showed that concentrations of total and inorganic arsenic, based on wet wt (except for tilapia) and dry wt, and percentages of inorganic arsenic in the four aquacultural fish species were not significantly different from those found in natural fish. This could be explained by the fact that all four aquacultural fish species in this study were raised in pond systems using water from natural sources. The farmers changed water in the ponds regularly to minimize the accumulation of food residues, excreta, and some pathogens, which could cause health problems in the fish. This practical procedure results in no accumulation of contaminants, including arsenic, in the water and sediment of the ponds. The only statistical differences found in this study were the concentrations of total and inorganic arsenic in tilapia based on wet wt, since the moisture contents in natural tilapia (75.7 ± 1.93 %) were significantly higher than those in aquacultural fish (73.5 % ± 1.59 %).

There are a limited number of internationally published reports on the levels of inorganic arsenic in freshwater fish from Thailand. Jankong et al. (2007) determined concentrations of total and inorganic arsenic in striped snakehead collected from the Suphan River in the central region of Thailand. The reported levels of total and inorganic arsenic (n = 3) were 1.9 ± 1.46 and 0.77 µg/g [sum of 0.04 ± 0.01 for As(III) and 0.73 ± 0.08 for As(V)] (dry wt), respectively, which were slightly greater than the values found in the natural (1.35 ± 0.331 and 0.303 ± 0.066 µg/g) and aquacultural (1.42 ± 0.537 and 0.280 ± 0.048 µg/g) striped snakehead in this study. The calculated percentage of inorganic arsenic in the published report (40.5 %) was also higher than those (21.5 % and 22.9 %) found in this study.

It has been reported that percentages of inorganic arsenic in freshwater fish are greater than those in marine fish. Percentages of inorganic arsenic in marine fish are generally less than 7.3 % (Lorenzana et al. 2009), while this value has been

Table 1 Total length, weight, total and inorganic arsenic concentrations, and percentages of inorganic arsenic with respect to total arsenic of four freshwater fish species from natural water sources and aquaculture in Thailand

Species	n	Total length (cm)	Weight (g)	Total arsenic (μg/g)		Inorganic arsenic (μg/g)		% Inorganic Arsenic[a]
				Wet weight	Dry weight	Wet weight	Dry weight	
Tilapia (*Oreochromis niloticus*)								
Natural	14	28.3 ± 1.66[b]	388 ± 10.8	0.203 ± 0.039	0.837 ± 0.154	0.025 ± 0.004	0.103 ± 0.012	12.5 ± 1.66
		(26.1–32.1)	(371–406)	(0.140–0.286)	(0.623–1.22)	(0.021–0.034)	(0.088–0.130)	(9.76–15.1)
Aquacultural	14	29.4 ± 1.72	392 ± 14.4	0.236 ± 0.041*	0.892 ± 0.149	0.029 ± 0.005*	0.111 ± 0.016	12.7 ± 2.61
		(26.7–33.2)	(365–421)	(0.148–0.290)	(0.613–1.12)	(0.022–0.039)	(0.081–0.146)	(9.24–19.3)
Silver barb (*Puntius gonionotus*)								
Natural	14	29.1 ± 1.33	485 ± 10.7	0.217 ± 0.040	0.861 ± 0.207	0.028 ± 0.006	0.112 ± 0.027	13.2 ± 2.74
		(27.5–31.6)	(468–501)	(0.156–0.315)	(0.622–1.38)	(0.019–0.037)	(0.078–0.154)	(9.35–18.8)
Aquacultural	12	29.5 ± 2.14	491 ± 17.6	0.196 ± 0.032	0.825 ± 0.150	0.025 ± 0.006	0.105 ± 0.023	12.9 ± 2.95
		(26.9–34.7)	(471–524)	(0.143–0.252)	(0.629–1.07)	(0.018–0.036)	(0.074–0.140)	(9.25–17.0)
Striped catfish (*Pangasius hypophthalmus*)								
Natural	15	49.8 ± 2.38	1207 ± 63.3	0.201 ± 0.029	0.796 ± 0.114	0.023 ± 0.004	0.093 ± 0.015	11.9 ± 3.07
		(46.7–55.3)	(1089–1286)	(0.135–0.234)	(0.573–0.965)	(0.018–0.032)	(0.072–0.126)	(8.56–18.2)
Aquacultural	15	47.9 ± 2.90	1161 ± 66.9	0.195 ± 0.039	0.806 ± 0.167	0.025 ± 0.005	0.102 ± 0.019	12.9 ± 2.19
		(44.6–53.5)	(1053–1264)	(0.141–0.260)	(0.556–1.16)	(0.017–0.036)	(0.064–0.152)	(9.95–17.5)
Striped snakehead (*Channa striata*)								
Natural	14	38.7 ± 1.76	857 ± 26.7	0.354 ± 0.087	1.35 ± 0.331	0.080 ± 0.017	0.303 ± 0.066	22.9 ± 3.70
		(36.7–42.2)	(817–904)	(0.214–0.516)	(0.924–1.89)	(0.041–0.110)	(0.188–0.414)	(18.3–29.8)
Aquacultural	10	39.6 ± 1.26	872 ± 20.6	0.367 ± 0.130	1.42 ± 0.537	0.073 ± 0.012	0.280 ± 0.048	21.5 ± 5.99
		(37.1–41.5)	(835–902)	(0.204–0.599)	(0.891–2.35)	(0.056–0.093)	(0.213–0.367)	(11.9–31.6)

[a] % inorganic arsenic = (concentration of inorganic arsenic * 100)/concentration of total arsenic

[b] Values are mean ± SD; numbers in parentheses are ranges

* Significantly different from natural tilapia ($p < 0.05$)

reported to reach 40.5 % in freshwater fish (Jankong et al. 2007). Lawrence et al. (1988) determined arsenic concentrations in four freshwater fish species collected from Alberta and five species from Ontario, Canada; the calculated percentages of inorganic arsenic in the fish samples from the two provinces ranged from 15 % to 29 % and 27 % to 29 %, respectively. Shah et al. (2010) reported percentages of inorganic arsenic in the muscle of 10 freshwater fish species collected from Manchar Lake, Pakistan, ranging between 17.3 % and 29.8 %.

The present study shows that concentrations of total and inorganic arsenic and percentages of inorganic arsenic in the four freshwater fish species—tilapia, silver barb, striped catfish, and striped snakehead—collected from aquaculture systems were comparable to those from natural water sources. Concentrations of inorganic arsenic in 108 samples of the four freshwater fish species from both sources were much lower than the Thai regulatory standard of 2 μg/g (wet wt), and hence are deemed safe for human consumption. Further investigations should be performed on heavy metal levels in freshwater fish from both sources to ensure that they are safe for consumption.

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