行政院國家科學委員會補助專題研究計畫期中報告

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本成果報告包括以下應繳交之附件：

□赴國外出差或研習心得報告一份

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□國際合作研究計畫國外研究報告書一份

執行單位：國立台灣大學生物環境系統工程學系

中　華　民　國　92　年　2　月　10　日

行政院國家科學委員會專題研究計畫期中報告

台灣烏腳病地區養殖魚類砷累積之生態毒物模擬(1/3)

計畫編號：NSC 91－2313－B－002－339
執行期限：91/08/01~92/07/31
執行單位：國立台灣大學生物環境系統工程學系
主 持 人：廖中明

摘要

第一年研究工作為針對台灣西南沿海烏腳病疫區地下水養殖魚類進行採樣調查，並追蹤砷在吳郭魚(*Oreochomis mossambicus*)體內的生物累積。當地養殖池水中砷濃度為 17.8 – 49 μg L^{-1}，急性毒實驗顯示 24 及 96 小時魚體半致死濃度分別為 69,060 μg As L^{-1} 及 28,680 μg As L^{-1}，魚體器官組織中生物濃縮因子以腸最高，其值為 2270，其大小排序如下：腸>胃>肝 鰓>肉。結果顯示魚體內臟累積砷含量(12.65±10.17 μg g^{-1} dry wt)較魚體肌肉組織砷累積含量(3.55±0.42 μg g^{-1} dry wt)顯著。

關鍵詞：砷，吳郭魚，急性毒，生物累積，烏腳病。

Abstract

The general objective of our work in the first year is to determine bioaccumulation of arsenic (As) in tilapia from blackfoot disease (BFD) area in Taiwan. The average As pond water concentrations ranged from 17.8 – 49 μg L^{-1}. Acute toxicity tests over 96 h showed that As concentration to cause toxicity to tilapia ranged from 69,060 μg As L^{-1} in 24 h toxicity test to 28,680 μg As L^{-1} in 96 h toxicity test. The highest bioconcentration factor (BCF) is found in the intestine (maximum value: 2270). The order of BCF was intestine > stomach > liver gill > muscle. Significantly higher concentrations of As are obtained in the viscera of tilapia (12.65±10.17 μg g^{-1} dry wt (mean ± SD)) than that of in muscle tissue (3.55±0.42 μg g^{-1} dry wt).

Keywords: Arsenic; Tilapia; Acute toxicity; Bioaccumulation; Blackfoot disease

Introduction

Arsenic (As) is potentially a toxic trace element and is widespread in the environment as a consequence of both anthropogenic and natural processes. Humans are exposed to As from many sources such as food, water, air and soil. US FDA (1993) while examining the food category indicated that fish and other seafood account for 90% of the total As exposure. Koch et al. (2001) demonstrated that total As in freshwater fish ranged from 0.28 – 3.1 for Whitefish (*Coregonus clupeaformis*), 0.98 – 1.24 for Sucker (*Catostomus commersoni*), 0.46 – 0.85 for Walleye (*Stizostedion vitreum*), and 1.30 – 1.40 μg g^{-1} dry wt for Pike (*Esox lucius*), respectively.

Chen et al. (2001) indicated that long-term exposure to ingested inorganic As in artesian well water has been found to induce blackfoot disease (BFD), a unique peripheral vascular disease that ends with dry gangrene and spontaneous amputation of affected extremities in southwestern coastal area of Taiwan, consisting mainly of four towns, Putai, Yichu, Peimen and Hsuehchia, located at Chiayi and Tainan counties. There exists a dose-response relationship between As concentration in drinking water and risk of BFD. Recently, a number of studies on acquired and genetic susceptibility to As have been carried out in the BFD-endemic

areas of southwestern Taiwan to find out the cause of BFD (Chen et al., 2001). Nowadays, most of the people living in these areas do not drink water from artesian wells because tap water has been made available in this area. However, artesian well water is still used for aquaculture.

Han et al. (1998) reported that the consumption of contaminated fish/shellfish has been as an important route of human exposure to trace elements (As, Cu, Zn, Pb, Cd, Hg) in Taiwan in that oyster (*Crassostrea gigas*) and tilapia, tuna, and shrimp are the most popular. Farming tilapia (*Orechromis mossambicus*) is a promising practice in the BFD area because of its high market value. The fish are fed with artificial bait, which does not contain As. These fish are maintained in the ponds for at least 6 months before harvest. At present, data on the actual effects of As to tilapia are limited. Generally, the accumulation of metals in aquatic organisms has been linked to decreased survival and reduced reproductive ability. If As levels in pond water are higher, severe healths effects may occur on the health of cultured fish, reducing their market prices leading to closure of fish farms. Suhendrayatna et al. (2002) suggested that tilapia could be used as a bioindicator for studying the accumulation and transformation of As in freshwater organisms.

The process of accumulation of waterborne metals by fish and other aquatic animals through nondietary routes is defined as bioconcentration (Hemond and Fechner-Levy, 2000). The bioconcentration factor, relating to the concentration of metals in water to its concentration in the aquatic animal at equilibrium, is generally used to estimate the propensity to accumulate metals in the organisms (Hemond and Fechner-Levy, 2000). Fish are targets for BCF assessments because of their importance as a human food source and the availability of standardized testing protocols. Measured or predicted BCFs are a requisite component for both aquacultural ecosystems and human health risks assessment (Liao et al., 2003).

The present study aims to assess toxicity thresholds and to investigate the As accumulation in tilapia from BFD area in southwestern Taiwan. In specific terms, the objectives are: (a) to establish the acute toxicity of As to tilapia, (b) to investigate the relationship between As accumulation in fish tissues and As in pond water, and (c) the allometric relationship between As concentrations in fish tissues and fish body size.

Materials and Methods

Sample Collection and Preparation

The details about sampling are shown in Table I. Tilapias (*Orechromis mossam -bicus*) were collected from fishponds in Hsuehchia and in Yichu, situated in the BFD area in the southwestern Taiwan region on June-November 2002. After the fish were stocked in the ponds, we used nylon nets to collect the samples of stocked tilapia from the fishponds. Fish samples were kept at 0°C cooler and were transported to the laboratory as quickly as possible. Thus, four sets of fish samples from Yichu and

Table I. Sampling date and size ranges of fish samples collected from fish ponds situated in BFD area in southwestern Taiwan

Sampling date	Fish pond	No. of sample	Length (cm)	Weight (g wet wt)
June 2002	Y-1, Y-2	6	15.68[a] (13.55 – 17.01)[b]	82.41 (51.43 – 109.50)
July 2002	Y-1, Y-2	6	23.50 (22.31 – 23.78)	302.15 (255.89 – 310.38)
August 2002	Y-1, Y-2, Y-3, H-1, H-2	15	25.59 (24.09 – 26.18)	396.62 (326.80 – 427.31)
November 2002	Y-1, Y-2, Y-3, H-1, H-2	15	18.37 (16.89 – 19.48)	137.33 (104.28 – 165.72)

[a]average value.

[b]Minimum and maximum values.

Hsuehchia have been collected. Each time three 500 ml water samples per pond were collected. One-liter polyethylene bottles cleaned with 10% nitric acid and then rinsed with deionized water were used as containers for the collection of water samples. The collected water was filtered through a 125 μm nylon mesh to remove large suspended particles and macro-invertebrates immediately after collection, and then acidified by adding 5 ml 1N HNO_3 before As analysis.

Tissue Distribution Assays

After measuring length and weight each fish was individually wrapped in a plastic bag and stored frozen. Dissections were performed on a clean bench on defrozen material using a titanium knife and Teflon forceps. An adequate portion of the gill, intestine, liver, stomach, and muscle of each individual was collected. The contents in the intestine were removed. The dissected tissues samples were cleaned with deionized water and were freeze-dried overnight, and then grounded to fine powder in a grinder (Tai-Hsiang S36-89, Taiwan). A 500 mg portion of the powder was digested in 10 mL concentrated HNO_3 (65% wt) overnight at room temperature. The resulting solution was evaporated and the residue redissolved in 0.1 N HCl. Arsenic uptake results were described in terms of bioconcentration factor (BCF). BCF was calculated from the concentration of the As accumulated in the fish (C_f, μg g^{-1}) divided by the As concentration in pond water (C_w, μg g^{-1}) as: BCF = C_f / C_w.

Acute Toxicity Assays

Laboratory static bioassays were conducted to determine the 24-h, 48-h, 72-h, and 96-h LC_{50} values for tilapia exposed to As. The experimental design and calculations for the acute toxicity were based on well-known procedures given by Finney (1978) and Sparks (2000). The tests were carried out in 50 L rectangular fiberglass aquaria of well-aerated and reconstituted dilution water (pH 7.8 – 8.0). The tested fish were collected from fish ponds Y-1 and Y-2. Six fish of a specific size class (mean body length = 17.67±1.65 cm (mean ± SD) and mean body weight = 148.72±6.5 g) were randomly selected and transferred into each test aquarium. Dissolved oxygen in each tank was maintained at close to saturation by aeration. The temperature in each aquarium was maintained at 24.7±0.2°C using submerged heaters. The photoperiod was 16 h light:8 h dark with an intensity of 1400±100 lux. The sodium arsenite ($NaAsO_2$) stock solution was prepared with deionized water. The fish were visibly free of any deformities, lesions or disease and acclimated in tap water for one week prior to experiment. The nominal concentrations of As tested were 0 (control), 1, 2, 4, 10, 30, 50, and 80 mg L^{-1} (Hwang and Tsai, 1993). Gross mortality of fish to each concentration was recorded every 1 h for the first 12 h and every 2 h thereafter for 96 h, and dead fish being removed every 3 – 8 h. Tilapias were not fed throughout the test. Control and each test concentration were tested in duplicate. No mortality occurred in the controls.

The LC_{50} values were determined from maximum likelihood estimates of linear functions relating log As concentration to probit transformations of percent mortality (Finney, 1978). The LC_{50} values were determined using mean assayed As concentrations and cumulative mortality. Statistical comparisons between LC_{50}s were based on the standard error of the difference. When it became apparent no statistically significant differences in LC_{50}s between bioassay replicates ($p > 0.05$), the replicates were pooled and a single LC_{50} was calculated for As. Chi-square tests were performed to test the homogeneity of mortality between replicates.

Chemical and Statistical Analyses

A Perkin-Elmer Model 5100PC atomic absorption spectrometer equipped with an HGA-300 graphite furnace atomizer was used to analyze As. Analytical quality control was achieved by digesting and analyzing identical amounts of rehydrated (90% H_2O) standard reference material (Dog

fish muscle, DORM-2, NRC-CNRC, Canada). Recovery rate was 94.6±3.6% and the levels of detection were 0.62 μg As L^{-1} for water samples and 0.05 μg As g^{-1} for tissue samples.

The curve fitting was performed using the nonlinear regression option of the Statistica® software (StatSoft, Tulsa, OK, USA). We also employed Statistica® to determine the coefficient of determination (r^2) and to perform statistical analyses including analysis of variance and Student's *t* test. Statistical significance was determined if *p* values were less than 0.05.

Results and discussion

Acute Toxicity Study

LC_{50}s for 24-h, 48-h, 72-h, and 96-h are listed in Table II. LC_{50} lowers progressively as the duration of exposure increases. A limited number of studies have investigated As toxicity to tilapia. Our 96-h LC_{50}, 28.68 (95% CI: 24.92 – 32.44) mg L^{-1}, is closed to the range of 96-h LC_{50} As to seawater tilapia (26.5; 95% CI: 23.2 – 33.8 mg L^{-1}), yet lower than that of to freshwater tilapia (71.7; 95% CI: 67.8 – 76.4 mg L^{-1}) reported by Hwang and Tsai (1993). Thus, our results indicate that tilapias reared in water from artesian wells (fish ponds Y-1 and Y-2) (mean length = 17.67 ± 1.65 cm) are more sensitive than freshwater tilapias used by Hwang and Tsai (1993) (mean length = 9.51 ± 0.81 cm),which were first collected from seawater ponds in the Tanan Branch of Taiwan Fisheries Research Institute and then reared in freshwater for over 1 month.

Table II. LC_{50} of arsenic to tilapia for selected time intervals in that values of 95% confidence interval (CI) are given in parentheses

Time (h)	LC_{50} (mg L^{-1})
24	69.06 (65.81 – 72.31)
48	51.52 (48.11 – 54.93)
72	38.44 (34.85 – 42.03)
96	28.68 (24.92 – 32.44)

Several studies have reported acute toxicity of As to other fish species. Our result of the 96-h LC_{50} As for tilapia is closed to the range of 96-h LC_{50} As to rainbow trout *Oncorhynchus mykiss* (23 – 26.6 mg L^{-1}) (Spehar et al., 1980), to bluegill *Lepomis macrochirus* (29 – 35 mg L^{-1}) and to stonefly (*Pteronarcys californica*) (38 mg L^{-1}) (Johnson and Finley, 1980).

Bioaccumulation and Tissue Distribution

Arsenic concentrations in pond water and in various tissues of tilapia from fish farms in BFD area are illustrated in Fig. 1. Fig. 2 gives the BCF values for various tissues. The mean As pond water concentrations in Hseuehchia (H-1, H-2) and Yichu (Y-1, Y-2, Y-3) were 17.8±1.86 and 49±7.49 μg L^{-1} (Table III), respectively, and were below both the current drinking water standard of 50 μg L^{-1} and the US EPA established water quality criteria for protection of aquatic biota (100 μg L^{-1}) (Hellawell, 1988). However, due to increasing concerns of potential for As-related problems in aquatic-receiving systems, current drinking water standards are under scrutiny.

Our study reported that As concentration are toxic to tilapia at 24 – 96 h LC_{50} tests and the values were from 69,060 μg L^{-1} in 24 hr LC_{50} test to 28,680 μg L^{-1} in 96 hr LC_{50} test (Table II), indicating a low risk of toxicity to tilapia in these aquacultural ecosystems. Average As concentrations in fish tissues were 29.3, 10.9, 5.37, 5.04, and 3.55 μg g^{-1} in intestine, stomach, liver, gill, and muscle, respectively (Table III). The overall mean BCFs of As in intestine (1394) was found to be highest than that of the stomach, liver, gill, and muscle of 421, 180, 163, and 143, respectively. Takatsu and Uchiumi (1998) and Mason et al. (2000) also showed that As concentrations are lower in muscle as compared to other tissues in *Tribolodon bakonensis*.

No statistically significant differences in As concentrations in fish and pond water was found ($p > 0.05$), yet appeared to be linearly related ($r^2 = 0.5$). Schendrayatha et al. (2002) indicated that the direct

accumulation of As by tilapia was proportional to the concentration of

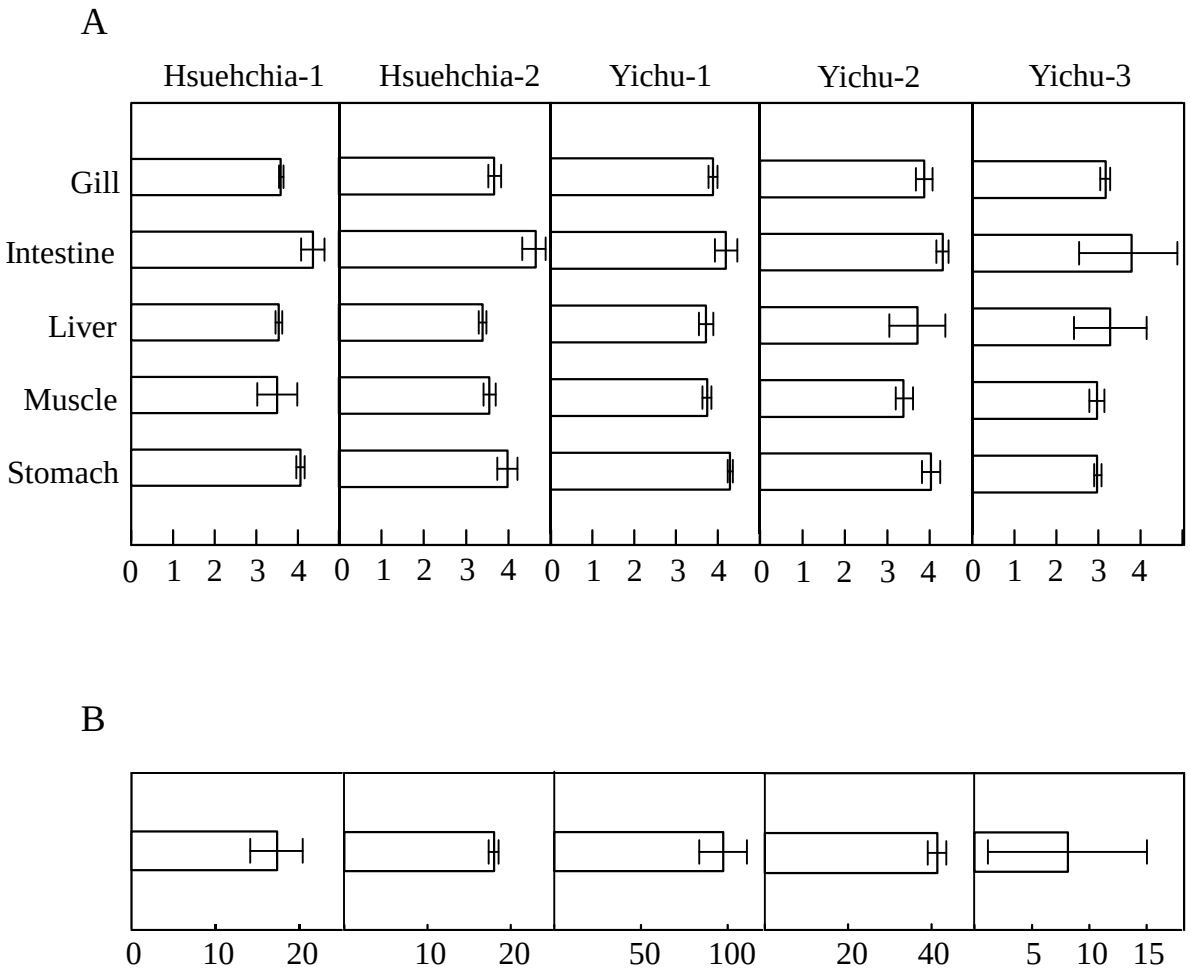

Fig. 1. (A) Arsenic concentrations in various tissues of tilapia and (B) in pond water collected from fish farms Y-1, Y-2, Y-3, H-1, and H-2 situated in BFD area. Error bars show one standard deviation from the mean.

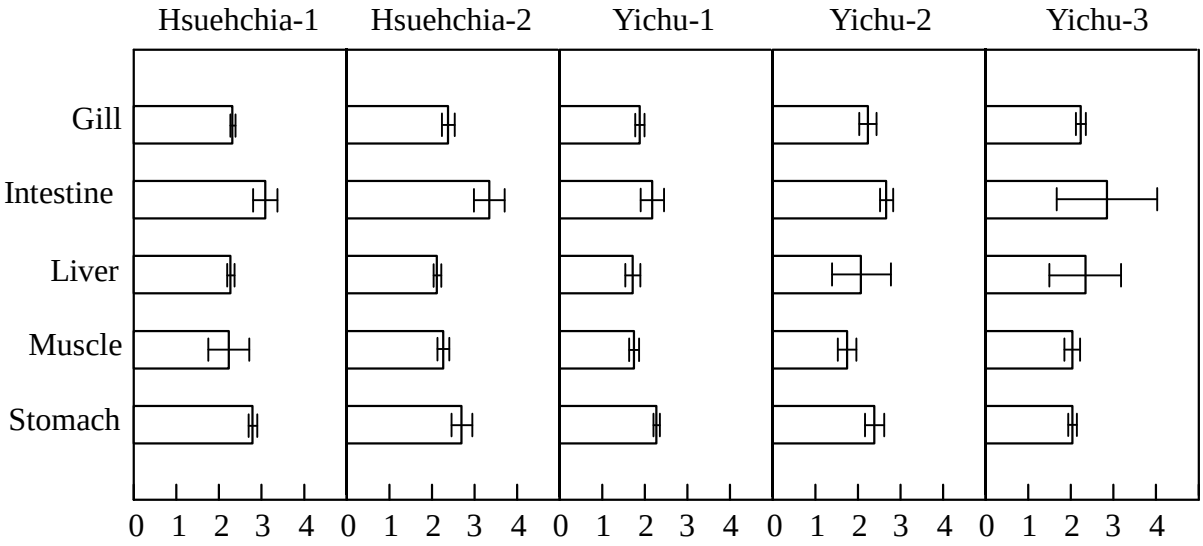

Fig. 2. Bioconcentration factors (BCFs) for arsenic for various tissues of tilapia collected from fish farms Y-1, Y-2, Y-3, H-1, and H-2 situated in BFD area. Error bars show one standard deviation from the mean.

Table III. Average arsenic concentrations (mean ± SD) in pond water (î g L^{-1}) and various tissues of tilapia (î g g^{-1} dry wt) in Hsuehchia and Yichu fish farms situated in BFD area in southwestern Taiwan

Study site	Arsenic concentrations					
	Pond water	Gill	Intestine	Liver	Muscle	Stomach
Hsuehchia	17.8±1.86	4.28±1.21	33.27±14.44	3.00±0.78	3.96±1.56	11.11±3.26
Yichu	49.0±7.49	5.79±3.76	25.34±8.30	7.74±2.73	3.13±2.26	10.67±5.92

arsenicals in water. Hence, generally As concentration in fish tissues increased with As concentration in pond water. Results of two-way ANOVA show that BCFs differed significantly in various tissues ($F = 3.19$, $df = 123$, $p < 0.05$). No significant variations ($F = 1.20$, $df = 123$, $p > 0.05$) between As concentrations and fish individuals were found.

The BCF represents the capacity for a species to accumulate a compound to a greater extent than the background level. In our study, BCFs ranged from 2 – 3.5 log units for tilapia (Fig. 2), indicating that process of bioconcentration occurred with As. The BCFs in our study were comparable to the one reported by Mason et al. (2000) in small brook trout exposure to As having bioaccumulation factor ranged from 3 – 3.5 log units in that levels of As decreased with increasing trophic level.

Chen et al. (2001) indicated that tilapia could potentially be able to regulate the concentrations of metals in their tissues with time by combining the processes of absorption, excretion, detoxification and storage, and this can be checked by analyzing the tissues of individuals exposed to different metals for different periods of time. In addition, the rate of metal uptake was organism-specific and time-dependent in fish. Our results demonstrate that most of the As was accumulated in the intestine, stomach and liver (referred to as viscera) of fish than in the muscle. The order of tendency to accumulate As in tilapia tissues was intestine > stomach > liver gill > muscle.

Many laboratory and field studies have revealed that many trace metals (Zn, Cu, Cr, Ni, Hg, Cd, Pb) were accumulated in viscera than in muscle of tilapia (Kureishy and D'silva, 1993; Liang et al., 1999). The results of the present study also reveal that significantly higher concentrations of As are in the viscera than those in muscle, demonstrating that viscera play a vital role in storing As in tilapia. Our results also indicate that As in viscera decreased with the increase in fish body weight. Cossa et al. (1992) believe that higher metal concentrations in juvenile specimens are related to the higher metabolic rates and insufficiently developed mechanisms for the neutralization of toxic trace metals. Furthermore, if the growth of organisms is faster than the metal accumulation, the observed trace metal concentration will decrease with age and weight, even though the overall metal content may be increasing. Mackay (1991), Sijm et al. (1992), Landrum et al. (1994), and Wong et al. (1999) pointed out that the processes that cause the decrease in the metal concentrations in the larger fish may due to a growth dilution effect and also suggested that metabolism seemed to be the key factor determining metal accumulation in fish, such as (a) the metabolic rate is linked with uptake/depuration rates; (b) small fish might have a more rapid short-term uptake of metals, etc.

A limited number of studies have investigated As contents in the muscle of tilapia, especially harvested from BFD area.

The present study was compared with the studies conducted by Han et al. (1998) in Taiwan, demonstrating that mean As contents in muscle of tilapia were higher in the present study (3.13 – 3.96 μg g^{-1} dry wt) than in the tilapia collected from supermarkets or grocery stores of Taipei city (0.13 – 1.45 μg g^{-1} dry wt) by them. Our results demonstrate that As concentrations in pond water do not pose acute risks for As toxicity to cultured tilapia in selected fish farms from BFD area of Taiwan. The highest BCF was found in the intestine. Significantly higher As concentrations were found in the viscera of tilapia than those in the muscle, demonstrating that viscera plays an important role in storing As in tilapia. Arsenic concentrations in the muscle of tilapia were positively correlated with As concentrations in the viscera.

These accumulation data coupled with additional acute toxicity data would provide a database to realistically pursue effective fish farm management practices and for reducing impairment from As or other metals allowing aquaculture production to continue with minimum restraints. Of particular concern is that significant As accumulation was detected in cultured tilapia harvested from BFD area. Therefore, we cannot rule out the possibility of As contamination in tilapia because of its high BCFs in various tissues. Consequently, the consumption of cultured tilapia from BFD area may possibly pose a potential risk to human health. From the present study, we suggest that for reducing the health risk associated with consuming tilapia is to trim and remove viscera of tilapia which can greatly reduce the amount of As. More investigations, however, should be conducted on As contents in tilapia muscle from different sources such as fish farms that not located at BFD area and natural water bodies. On the other hand, we recommend the government should set the safety limits on the As contents in the muscle of fish. Efforts to update the knowledge base on human health effects by consuming tilapia and acceptable exposure levels are needed.

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