

Arsenic Accumulation and Acute Toxicity in Aquacultural Juvenile Milkfish (*Chanos chanos*) from Blackfoot Disease Area in Taiwan

M.-C. Lin,¹ H.-H. Cheng,¹ H.-Y. Lin,¹ Y.-C. Chen,¹ Y.-P. Chen, G.-P. Chang-Chien,² Y.-H. Chou,³ C.-M. Liao,³ C.-F. Dai,⁴ B.-C. Han,⁵ C.-W. Liu³

¹ Graduate Institute of Environment Management, Nanhua University, Chiayi, 622 Taiwan, Republic of China

² Department of Chemical Engineering, Cheng Hsiu Institute of Technology, Kaoshiung, 833 Taiwan, Republic of China

³ Department of Bioenvironmental Systems Engineering, National Taiwan University, Taipei, 10617 Taiwan, Republic of China

⁴ Institute of Oceanography, National Taiwan University, Taipei, 10617 Taiwan, Republic of China

⁵ Department of Public Health, Taipei Medical University, Taipei, 110 Taiwan, Republic of China

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Milkfish (*Chanos chanos*) is one of the most commercially important aquacultural species in Taiwan. Most milkfish ponds are located in the southwest coasts of Taiwan, where the inhabitants used to suffer from the blackfoot disease (BFD) (Chen et al. 1985; Lin et al. 2001). BFD, a peripheral vascular disorder, was reported to correlate with the consumption of groundwater that contains high concentration of arsenic (Chen et al. 1980, 1985, 1986). Arsenic has been well documented as one of the major risk factors for black-foot disease as well as cancers of the lung, liver, and bladder among residents in BFD area (Chen et al. 1986). A significant exposure-response relationship between As concentration and the mortality from various cancers has been reported (Wu et al. 1989). Arsenic was known to increase the risk of cancer (Chiou 1995). Chen et al. (1985) demonstrated that both the standardized mortality ratios (SMRs) and cumulative mortality rate for cancers of bladder, kidney, skin, lung, liver and colon in the residents from BFD area were significantly greater than that in the general population in Taiwan.

Nowadays, people living in these areas do not drink water from wells; however, the groundwater is still used for aquaculture (Lin et al. 2001). Milkfish culture needs a high amount (38,000–49,000 ton ha⁻¹) of freshwater; and therefore, the fish in the ponds using groundwater may be contaminated with As. Arsenic was reported to be toxic to fish (Donohue and Abernathy 1999). In addition, As can be accumulated in fish tissues, and humans who consume these tissues may be threatened by As (Lin et al. 2001). Since milkfish is common seafood in Taiwan, ingestion of contaminated fish could result in As accumulation in humans and lead to adverse health effects. Thus, it is important to determine the As content in fish from these culture ponds. The tolerance of milkfish to As toxicity as well as the As accumulation from the ambient water in the fish need to be determined.

The process of accumulation of water-borne chemicals by fish and other aquatic animals through nondietary routes is defined as bioconcentration (Franke et al. 1994). The bioconcentration factor (BCF), relating the concentration of a chemical in water to its concentration in the aquatic animal at steady-state equilibrium, is generally used to estimate the propensity of an organism to

accumulate chemicals (Franke et al. 1994). 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 environmental and human risk assessment. In this work, the bioaccumulation of As in milkfish was studied to assess the potential hazards of As in the aquacultural environment. The acute toxicity of As and the BCF value of the fish were determined.

MATERIALS AND METHODS

Samples of juvenile milkfish (range 4.0-6.0 cm in length and 0.41-1.41 g in weight) and ambient water were obtained from 9 culture ponds in BFD area. Three fish and three 500 ml water samples per pond were collected. The milkfish were placed on ice immediately, and kept at 4°C during transfer to the laboratory. The water samples were fixed by adding 5 ml 1N HNO₃.

A total of 80 non-polluted milkfish (body lengths range from 4 – 6 cm) were collected from the Tainan Fisheries Research Institute for laboratory exposure experiments. The fish were transferred into 6 tanks of approximately 80 L volume, containing 60 L of filtered water. The temperature, salinity, pH and dissolved oxygen were maintained at 24.0±0.5°C, 0, 7.0±0.2 and 8.0±0.1 µg ml⁻¹, respectively; which were similar to the conditions of the culture ponds in BFD area (24.0±3.2°C, 0, 7.2±1.1 and 8.0±0.9 µg ml⁻¹, respectively). The milkfish were held for 2 weeks before they were exposed to As.

Acute toxicity assays were conducted to determine the median lethal time (LT₅₀ value) and lethal time (LT₁₀₀ value), as well as the median lethal concentration (LC₅₀ value) for milkfish. The tolerance of As toxicity was examined by exposing fish to As concentrations ranging from 0 to 1000 µg ml⁻¹. The concentrations were prepared from arsenite (Na₃AsO₃). Eight healthy milkfish were exposed to As for each concentration. The mortality was recorded every 1 h for the first 12 h and every 3 h thereafter up to 4 d. Death was defined as cessation of opercular movement; and dead fish were removed from the tanks immediately and kept at -20°C before they were analyzed. During the experiments, water samples were taken daily from each tank, acidified by adding 5 ml 1N HNO₃, and stored for analysis of As concentration. After sampling, the tank water was renewed immediately to maintain the As concentration.

Samples were sent to the Super Micro Mass Research and Technology Center, Cheng Shiu Institute of Technology for analysis of total As. The frozen muscle of milkfish were dehydrated in a dryer (40°C) for 96 h and grounded into powder. Aliquots of dry muscle powder weighing 0.5 g were placed into a 250 ml beaker. Nitric acid (65%, 10 ml) was added and then covered with a glass for an overnight digestion.

After the initial digestion, the beaker was heated with a water bath at 70-80°C for 2-4 h to reduce the total volume to 1-2 ml. This volume of solution was transferred to a volumetric flask (50 ml). The rinsed solution (5 ml of 0.01N of HNO₃) for the watch glass was also added to the flask. The flask was then filled with 0.01N of HNO₃ to make a 50 ml of final solution. After filtration, this 50 ml solution was transferred to test tubes for As analysis. Arsenic analysis was carried out by using an Agilent 7500a ICP-MS. Analytical quality control was achieved by digesting and analyzing identical amounts of rehydrated (90% H₂O) standard reference materials (DORM-2, Dogfish Liver-2-organic matrix, NRC-CNRC, Canada). Recovery rates ranged from 95% to 97%.

The bioconcentration factor derived using dry weight (BCFD), relating the concentration of As in water to its concentration in the fish was used to estimate the propensity of accumulating ability of milkfish: $BCFD = C_b / C_w$, where C_b ($\mu\text{g g}^{-1}$ dry wt) is the As concentration in biota, i.e., milkfish; C_w ($\mu\text{g ml}^{-1}$) is the As concentration in water.

RESULTS AND DISCUSSION

Field samples showed that the level of As in the pond water was $0.027 \pm 0.001 \mu\text{g ml}^{-1}$ (Mean $\pm$ SD) and that in milkfish was $15.20 \pm 5.10 \mu\text{g g}^{-1}$ dry wt. The value of BCFD of milkfish was 556.16 ± 187.98 dry wt.

Table 1. The median lethal time (LT₅₀ value), lethal time (LT₁₀₀ value) and the median lethal concentration (LC₅₀ value) for milkfish acutely exposed to waterborne As

Nominal As concentration ($\mu\text{g ml}^{-1}$)	Mean (SE) assayed As concentration ($\mu\text{g ml}^{-1}$)	LT ₅₀ value (h)	LT ₁₀₀ value (h)	LC ₅₀ value ($\mu\text{g ml}^{-1}$)
0	ND ^a	> 96	> 96	15.20
5	4.8 (1.1)	> 96	> 96	40.80
10	10.2 (2.3)	61.33	> 96	89.40
15	14.6 (3.7)	20.50	> 96	86.02
20	19.5 (2.4)	23.25	34.58	117.72
25	25.5 (3.0)	4.50	20.12	97.38
50	51.9 (2.2)	2.33	14.04	94.42
100	104 (10.1)	1.00	1.26	173.63
250	237 (17.2)	0.52	0.90	284.86
500	487 (16.5)	0.20	0.25	550.57
1000	1014 (22.2)	0.10	0.12	971.14

^a ND: not detectable

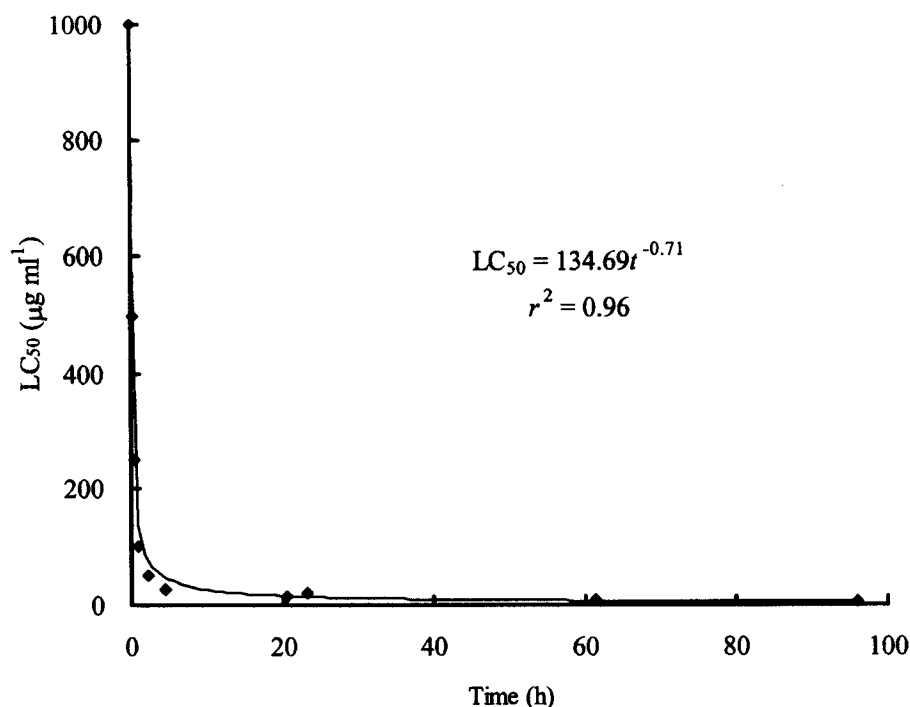


Figure 1. Plot of the relation between the median lethal concentration (LC_{50} value) for milkfish and the exposure time.

The actual arsenic concentrations assayed, the median lethal time (LT_{50} value) and lethal time (LT_{100} value) are listed in Table 1. The median lethal exposure concentration (LC_{50} value) for milkfish showed a significant negative relation to exposure time: $LC_{50}(t) = 134.69t^{-0.7175}$ (Fig. 1), where $LC_{50}(t)$ is the median lethal concentration ($\mu\text{g ml}^{-1}$) and t is the exposure time (h). Milkfish can tolerate As concentration in water higher than $10 \mu\text{g ml}^{-1}$ for more than 96 h.

Lethal internal residue of As in fish body that causes 50% mortality ($C_{L,50}$ value) is negatively related to median lethal time (LT_{50} value) as $C_{L,50} = 247.47LT_{50}^{-0.36}$ (Fig. 2), $C_{L,50}$ is the lethal internal residue of As in fish body that causes 50% mortality ($\mu\text{g g}^{-1}$ dry wt) and LT_{50} is the median lethal time. The lethal internal residue of As in fish body that causes 50% mortality ($C_{L,50}$ value) is a linear function of As concentration in water as $C_{L,50} = 63.97 + 0.92C_w$ (Fig. 3), where C_w is As concentration in water ($\mu\text{g ml}^{-1}$).

Due to dense human population and industrial expansion, the aquatic environments of Taiwan are suffering an ever-increasing impact from human activities (Lin and Liao 1999). In spite of natural As in the underground water, pollutants of As were discharged into the underground water from polluted rivers,

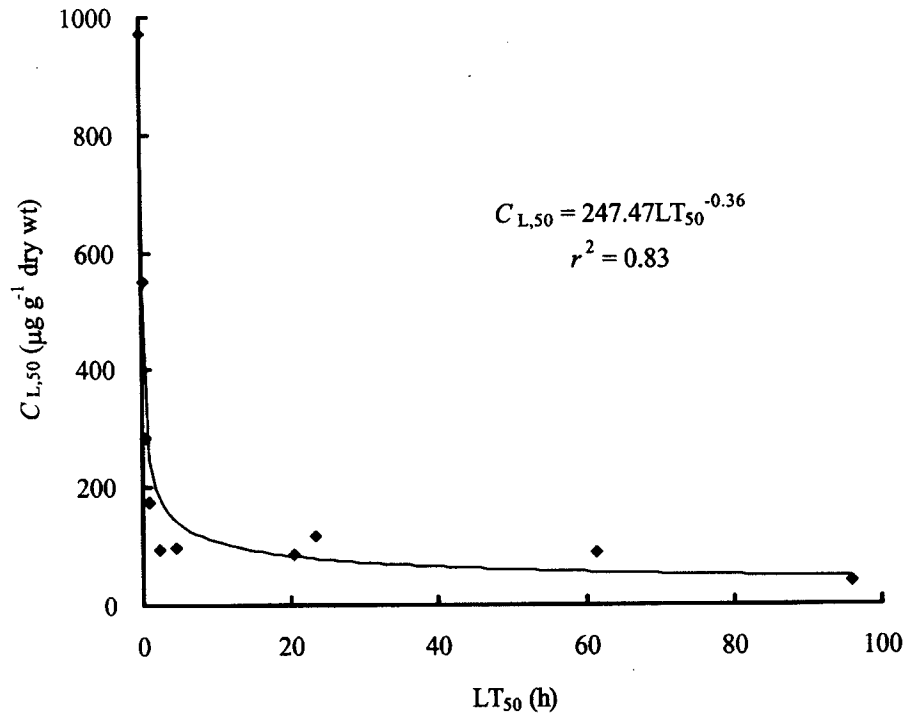


Figure 2. Plot of the lethal internal residue of As in milkfish body that causes 50% mortality ($C_{L,50}$ value dry wt) and the median lethal time (LT_{50} value).

sewage outfalls or local industries because of those human activities. The resulting data indicated that the pond waters in BFD area are contaminated by As. The value of BCFD showed that the milkfish taken from the ponds accumulated a high level of water-borne As. The high tolerance of As for milkfish showed that the fish can accumulate high concentrations of As before they are harmed.

Thus far no adverse effect on health of the people in BFD area due to exposure to As contaminated seafood is evidenced. A wider study involving As analyses of milkfish from non-BFD areas, as well as other aquacultural products should be investigated to assess the extent of As contamination in seafood. The daily intake of As compounds from fish and other aquacultural products need to be studied to determine the relative risk of As toxicity in the population.

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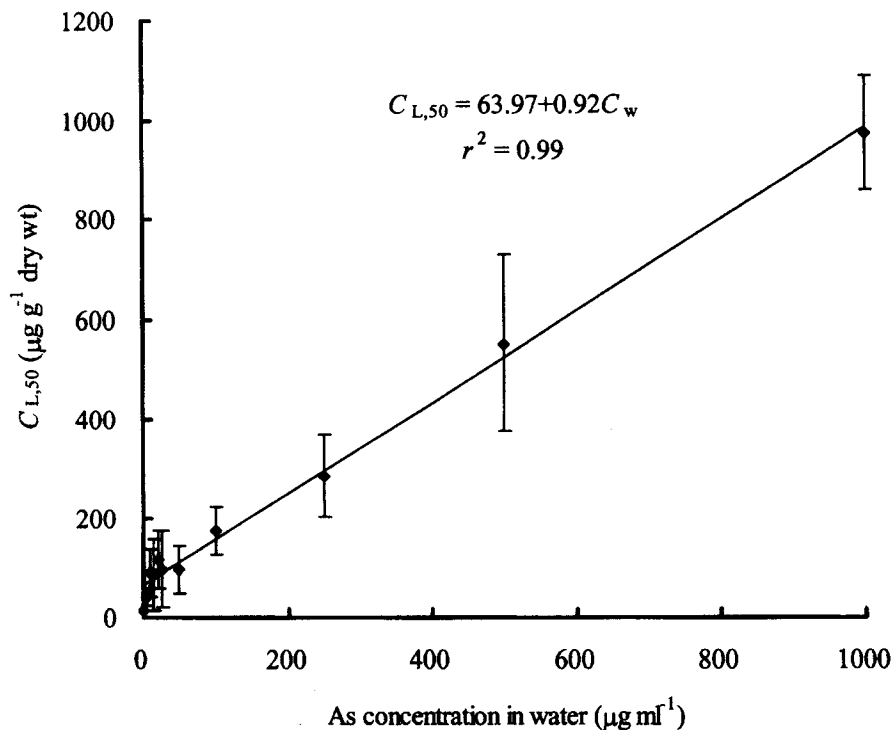


Figure 3. Plot of the lethal internal residue of As in milkfish body that causes 50% mortality ($C_{L,50}$ value dry wt) and the As concentration in water ($\mu\text{g ml}^{-1}$).

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