Acute Toxicity and Bioaccumulation of Arsenic in Freshwater Clam *Corbicula fluminea*

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ABSTRACT: Arsenic is a potent human carcinogen of skin, lung, and urinary bladder. Freshwater clam *Corbicula fluminea* is a commercially important native species in Taiwan. *C. fluminea* is also a suitable biomonitoring test organism. Little is known, however, about the actual effects of arsenic on *C. fluminea*. The objectives of this study were to provide information on the acute toxicity and bioaccumulation kinetics of arsenic in *C. fluminea*. We carried out a 14-day exposure experiment to obtain bioaccumulation parameters. Uptake was very rapid when *C. fluminea* was first exposed and then slightly decayed during the uptake phase of the experiment and an uptake rate constant of 1.718 $\pm$ 6.70 (mean $\pm$ SE) mL g^{-1} d^{-1} was estimated. The elimination of arsenic from *C. fluminea* obeyed first-order depuration kinetics (r^2 = 0.85, $p < 0.05$) with a calculated half-life of 6.80 days. The derived bioaccumulation factor of 16.84 suggests that arsenic has a high potential for bioaccumulation in *C. fluminea*. This had important implications for dietary exposure of arsenic to humans who eat contaminated clams, because the soft tissue usually constitutes the majority of tissue consumed. The 96-h LC50 value was estimated to be 20.74 (95% CI: 11.74–30.79) mg L^{-1} obtained from a 7-day acute toxicity bioassay. We also kinetically linked an acute toxicity model and a Hill sigmoid model to reconstruct an internal effect concentration based dose-response profile to assess the effect of soft tissue arsenic burden on the *C. fluminea* mortality. This result could be used to support the establishment of an ecological risk assessment to prevent possible ecosystem and human health consequences. Environ Toxicol 23: 702–711, 2008.
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INTRODUCTION

The freshwater clams *Corbicula fluminea* are appreciated for their delicacy and are generally used as an ingredient of soup in Taiwan. Many food products and health drinks containing the freshwater clam extract are now available on the market with a widely varying ornithine (Uchisawa et al., 2004). Recently, it has been reported that ornithine promotes the secretion of the growth hormone and builds muscle (Bucci et al., 1990; Davenport et al., 1990).

Ornithine is thus attractive as an ingredient of dietary supplements. Wu and Shiau (2002) indicated that a freshwater clam or hard clam extract (or referred to as clam essence) contained more ornithine than those in a chicken or beef essence. This evidence makes the freshwater *C. fluminea* commercially important and has a high market value to Taiwan's aquaculture (http://www.fa.gov.tw) with wide farming distribution in the western (Chunghua, Yunlin, Chaiyi, and Tainan) and eastern (Hualien) coastal areas.

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However, the coastal regions of Taiwan where the clam farms are situated, are subjected to contaminated discharges from rivers. Hung et al. (2001) analyzed the correlations among the trace metal concentrations in bivalves, water, and sediments collected in these areas and indicated that significant correlations were found between bivalves and waterborne metals of copper (Cu), cadmium (Cd), plumbum (Pb), and zinc (Zn). Therefore, if waterborne metals are elevated, pollutant-induced changes in the mobility can occur, which has potential risks on the health of clam, resulting in severe economic losses nationwide due to bans on harvesting of contaminated clam and the need for costly monitoring programs.

Lin et al. (2001, 2005), Liao et al. (2003), Huang et al. (2003), and Liu et al. (2005, 2006, 2007) have conducted a long-term investigation during 1998–2007 in blackfoot disease (BFD)-endemic areas of Taiwan. They indicated that arsenic was detected in many farm fish and shellfish ponds. Taken together, they reported that arsenic concentrations in aquaculture waters ranged from 40 to 900 $\mu g\ L^{-1}$, whereas arsenic levels in fish (tilapia *Oreochomis mossanbicus*, milkfish *Chanos chanos*, and large-scale mullet *Liza macrolepis*) and shellfish (hard clam *Meretrix lusoria* and oyster *Crassostrea gigas*) ranged from 1 to 350 and 4 to 23 $\mu g\ g^{-1}$ dry wt, respectively.

Ingested inorganic arsenic that was known to have adverse health effects were thought to contribute to some complex diseases such as skin lesions, diabetes, cardiovascular disease, and cancers of several organs (lung, bladder, kidney) in arseniasis-endemic area in southwestern and northeastern Taiwan (Smith et al., 2002; Hsurh et al., 2003; Chen et al., 2005; Chiou et al., 2005; Navas-Acien et al., 2005; Lamm et al., 2006).

At present, data on the actual effects of arsenic on *C. fluminea* are limited. Little is known about the bioaccumulation kinetics of arsenic in *C. fluminea*. The objectives of this study were to provide information on the acute toxicity and bioaccumulation kinetics of arsenic in *C. fluminea*. We also kinetically linked an acute toxicity model and a Hill sigmoid model to assess the effect of soft tissue arsenic burden on the *C. fluminea* mortality.

Specifically, we carried out two experiments with *C. fluminea*. One experiment involved conduction of a 14-day exposure experiment to obtain bioaccumulation parameters. The second experiment conducted a 7-day acute toxicity bioassay to derive dose-response relationships between mortality effect and equilibrium arsenic levels in soft tissue of *C. fluminea*. Information on uptake and depuration kinetics could contribute to human and ecological risk assessment and fate and transport modeling of arsenic in freshwater bivalves.

MATERIALS AND METHODS

Acclimation

We collected 140 *C. fluminea* from clam farms situated at Hualien of eastern Taiwan with a mean shell length of 27.6 ± 2.4 mm (mean ± SD) and a mean body weight of 6.19 ± 0.86 g wet wt. Before any experiments, tested clams were acclimated in the synthetic water obtained from Hualien clam farms under the laboratory conditions for at least 3 weeks to rescue clams behavior such as burrowing and siphon retraction. The water was air-equilibrated by bubbling, with an artificial photoperiod (day: 10:00–22:00 and night: 22:00–10:00). Seventy clams were hatched per tank (indoor rectangular fiberglass aquaria measuring 45 × 21 × 26 cm^3), containing 15 L of water in a flow-through circulation system. The tested clams were continuously fed by a pump during the acclimation period with the cultured algae *Platymonas* sp.

Acclimated water conditions were as follows: Temperature = 24.26°C ± 1.26°C, pH = 7.96 ± 0.14, DO = 8.3 ± 0.28 $mg\ L^{-1}$, salinity = 0.10, and turbidity = 22.88 ± 0.13. Water ionic compositions were Ca^{2+} = 24.8 $mg\ L^{-1}$, Mg^{2+} = 1.0 $mg\ L^{-1}$, Na^{+} = 4.9 $mg\ L^{-1}$, K^{+} = 2.7 $mg\ L^{-1}$, H^{+} = 7.21, NH_4^{+} = 0.26 $mg\ L^{-1}$, Cl^{-} = 7.6 $mg\ L^{-1}$, NO_2^{-} = 0.047 $mg\ L^{-1}$, and NO_3^{-} = 0.318 $mg\ L^{-1}$. No mortality was observed during acclimation. Ionic components in water samples were measured followed by Standard Methods (APHA, 2005).

Exposure Experiment

The present laboratory study was designed to examine the accumulation ability of arsenic in the soft tissue of *C. fluminea*. We conducted the exposure experiment from June 10 to 23, 2007. The clams were acclimatized for 2 weeks before they were exposed to arsenic. We used sodium arsenite ($NaAsO_2$) (SIGMA, Taiwan; purity ≥90%) as the arsenic chemical. The arsenic contaminated level was determined by a preliminary test of exposing *C. fluminea* to different arsenic concentrations of 0, 2, 5, 10, 25, 50, 100, 200, 500, and 1000 $mg\ L^{-1}$. Here we used median lethal tolerance concentration (LT50) defining the median internal/external no-effect concentration to determine the exposure dosage used in exposure experiment (Kooijman, 1998). We found that LT50 of *C. fluminea* exposed to concentrations at ≤5 $mg\ L^{-1}$ arsenic was longer than 21 days. Therefore, we conducted an uptake experiment in arsenic concentration of 5 $mg\ L^{-1}$ for 7 days and then transferred to clean water and reared for 7 days of depuration. Mortality was less than 5% during the exposure experiment. The arsenic concentrations used in this experiment were nearly 20 to 50 times higher than that of the environmental conditions to produce high arsenic level in soft tissue of *C. fluminea*.

The entire arsenic solution was replaced daily in each tank to avoid the regression of ambient water quality. We checked the water level in each aquarium every 6 h and refilled with distilled water to keep levels constant. The tested clams were not fed during the exposure experiment.

To analyze arsenic uptake by the clams, five clams were sequentially removed from each two tanks at daily basis. Each removed clam 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. The frozen soft tissue of clam were dehydrated in an oven (105°C) for 24 h and grounded into fine powder. Aliquots of dry soft tissue powder weighing 100 mg were placed into a 250-mL beaker. Nitric acid (SIGMA, Taiwan, purity = 65%, 10 mL) was added and then covered with a glass for an overnight digestion.

After the initial digestion, the beaker was heated in a water bath at 95°C for 2–4 h to reduce the total volume to 3 mL. This volume of solution was transferred to a volumetric flask (50 mL). The rinsed solution (5 mL of 0.01 N of HNO_3) for the watch glass was also added to the flask. The flask was then filled with 0.01 N of HNO_3 to dilute to a 25 mL of final solution with double deionized water. After filtration, this 25 mL solution was transferred to test tubes for arsenic analysis.

Acute Toxicity Bioassays

Laboratory static bioassays were conducted to determine the 12-, 24-, 48-, 72-, and 96-h external median lethal concentration (LC50) values for *C. fluminea* exposed to arsenic. The experimental design and calculations for the acute toxicity were based on well-known procedures given by Finney (1978) and Sparks (2000). Ten *C. fluminea* were randomly selected and transferred into each test aquarium ($22 \times 15 \times 18$ cm^3) containing 5 L of water where arsenic was administrated as single dose. The sodium arsenite ($NaAsO_2$) stock solution was prepared with deionized water. The nominal concentrations of arsenic tested were 0 (control), 2, 5, 10, 25, 50, 100, and 200 mg L^{-1}, whereas the measured arsenic concentrations were 1.5 ± 0.1, 3.6 ± 0.1, 7.2 ± 0.4, 16.5 ± 0.5, 47 ± 6, 74 ± 6, and 143.5 ± 3.5 mg L^{-1}, respectively. The rather great differences between measured and nominal concentrations might be explained by the purity of sodium arsenite and nitric acid used to prepare and analyze the tested arsenic nominal concentrations. Gross mortality of clam to each concentration were recorded every 30 min for the first 24 h and every 1 h thereafter to the end of the experiment, and the dead clam being removed every 0.5–1 h. Clam was not fed throughout the test. Control and each test concentrations were conducted in two replicate tanks. The water quality management protocol was the same as deployed in the exposure experiments. No mortality occurred in the control.

Chemical and Data Analysis

A Perkin-Elmer Model 5100PC atomic absorption spectrometer (Perkins-Elmer, Shelton, CT) equipped with an HGA-300 graphite furnace atomizer was used to analyze arsenic. Analytical quality control was achieved by digesting and analyzing identical amounts of rehydrated (90% H_2O) standard reference materials (dogfish muscle, DORM-2; NRC-CNRC, Canada). Recovery rate was 95.3% ± 2.6%, and the levels of detection were 0.62 μg As L^{-1} for water samples and 0.05 μg As g^{-1} for soft tissue samples.

The LC50 values were determined using mean assayed arsenic concentrations and cumulative mortality and then estimated by maximum likelihood estimates of linear functions relating log arsenic concentration to probity transformations of percent mortality (Finney, 1978). All of the observations were used in probit analysis (USEPA Probit Analysis Program, Version 1.5).

Uptake and depuration rate constants were determined by fitting the integrated form of the kinetic rate equation to concentration data for constant arsenic exposure, using nonlinear regression (Chou et al., 2006),

$$C_b(t) = C_b(t=0)e^{-k_2 t} + \frac{k_1}{k_2} C_w e^{-k_0 t}(1 - e^{-k_2 t}), \qquad (1)$$

where C_b is the time-dependent arsenic concentration in the soft tissue of clam (μg g^{-1} dry wt), $C_b(t = 0)$ is initial concentration of arsenic in clam soft tissue (μg g^{-1} dry wt), the k_1 is the clam uptake rate constant (mL g^{-1} d^{-1}), k_2 is the depuration rate of arsenic (d^{-1}), k_0 is the dilution rate of water arsenic concentration to clam (d^{-1}), C_w is the dissolved arsenic concentration in the water (mg L^{-1}), t is the time in day. Here we defined the dilution rate as a rate constant describing the reducing rate of arsenic accumulation level when *C. fluminea* exposed to arsenic at a valve closing status.

Typically, the first-order one-compartment model assumes that k_2 is not a function of arsenic concentration in soft tissue. Therefore, k_2 is often determined by depurating contaminated organisms in uncontaminated water and determining k_2 directly in that test organism. Thus after the clams were transferred to clean water tank, the depuration rate constant could be estimated by the linear regression of log-transformed soft tissue arsenic concentrations on depuration time (days) as $\ln C_b(t) = \ln C_b(t = T) - k_2 t$ where T is the time when depuration begins.

Depuration half-life ($t_{1/2}$) was calculated as $\ln 2/k_2$. The bioconcentration factor (BCF) can be calculated as BCF = k_1/k_2, representing the net accumulation ability that is the result of the competition between uptake and depuration processes. An inherent assumption in a first-order, diffusion-based bioaccumulation model is that the rate constants are independent of concentration of chemical in water or organism and duration of exposure (Cho et al., 2003; Clason et al., 2003).

Here we employed a biologically based damage assessment model (DAM) (Lee et al., 2002; Tsai et al., 2006) to describe LC50(t) profile of arsenic in *C. fluminea* as a

function of few constants and variables that were verified with acute toxicity data,

$$\mathrm{LC50}(t) = \frac{\mathrm{DL50}/k_a}{\left(\frac{e^{-k_r t} - e^{-k_2 t}}{k_r - k_2} + \frac{1 - e^{-k_r t}}{k_r}\right)} \mathrm{BCF}^{-1}, \quad (2)$$

where k_a is the damage accumulation rate (g μg^{-1} d^{-1}), k_r is the damage recovery rate constant (d^{-1}), and DL50/k_a is a coefficient that reflects the compound equivalent toxic damage level required for 50% mortality (μg^{-1} d g^{-1}).

DAM was employed in this study because DAM depicts the modes of action including rapid reversible binding to the target site as well as to those that act with irreversible binding. Lee et al. (2002) indicated that the existing well-established acute toxicity models, the critical body residue (CBR) and the critical area under the curve (CAUC) models, are extreme cases of the DAM. The CAUC model (see Appendix) could be applied to depict the acute toxicity and to estimate incipient LC50s and internal effect concentrations (IECs) of waterborne chemicals in organisms. The CBR acute toxicity model could be directly derived from CAUC model (see Appendix).

Based on the acute toxicity test, mortality functions can be estimated from observed mortality in exposure regimes where mortality was an increasing function of arsenic concentration in water. Therefore, we used mortality as an endpoint to investigate the arsenic effect on *C. fluminea*. Here we employed a log-logistic model to fit the mortality data to derive the dose-response relationship by nonlinear regression analysis,

$$M(t, C_w) = \frac{M_{max}}{1 + \left(\frac{\mathrm{LC50}(t)}{C_w}\right)^n}, \quad (3)$$

where $M(t, C_w)$ is the exposure time- and water arsenic concentration-dependent mortality (%), M_{max} is the maximum mortality, and the exponent n is a fitted Hill coefficient or slope of the toxicity curve which is a measure of cooperativity. This model is equivalent to the sigmoid E_{max} model used in pharmacology and first proposed by Hill (1910).

We reconstructed an IEC-based dose-response model to describe the relationships between mortality and arsenic level in soft tissue of *C. fluminea* by appropriately transforming Eq. 3 as,

$$M(C_b) = \frac{M_{max}}{1 + \left(\frac{\mathrm{CL50}(\infty)}{C_b}\right)^n}, \quad (4)$$

where CL50(∞) is the equilibrium internal lethal body burden at the site of action that causes 50% mortality and can be derived from DAM as t approaches infinity (Lee et al., 2002),

$$\mathrm{CL50}(t) = \frac{\mathrm{DL50}/k_a}{\left(\frac{e^{-k_r t} - e^{-k_2 t}}{k_r - k_2} + \frac{1 - e^{-k_r t}}{k_r}\right)} \left(1 - e^{-k_2 t}\right). \quad (5)$$

An IEC-based time-mortality profile thus can be derived by substituting Eqs. 1 and 5 into Eq. 4 represented as functions of accumulation parameters (k_2 and BCF) and biological parameters (DL50/k_a and k_r) associated with varied waterborne arsenic concentrations,

$$M(t) = \frac{M_{max}}{1 + \left(\frac{\mathrm{CL50}(t)}{C_b(t)}\right)^n}. \quad (6)$$

We employed the function of nonlinear regression of the TableCurve 2D (Version 5, AISN Software, Mapleton, OR) package to perform all curve fittings. Statistical significance was judged by p values less than 0.05. A Monte Carlo technique was performed to generate 2.5- and 97.5-percentiles as the 95% confidence interval (CI) for all fitted models. We employed Crystal Ball® software (Version 2000.2, Decisionerring, Denver, CO) to implement the Monte Carlo simulation.

RESULTS

LC50 Data and Bioaccumulation Parameters

Table I gives the selected time intervals of 12-, 24-, 48-, 72-, and 96-h LC50 values with 95% CI for *C. fluminea* exposed to arsenic. LC50 values decreased progressively as the duration of exposure increased. A limited number of studies have investigated arsenic toxicity to freshwater *C. fluminea*. Our results, however, indicate that the 96-h LC50 value of arsenic to *C. fluminea* was estimated to be 20.74 (95% CI: 11.74–30.79) mg L^{-1}, which is lower than that of arsenic to farm tilapia *Oreochromis mossambicus* (28.68; 95% CI 24.92–32.44 mg L^{-1}) (Liao et al., 2003) and higher than that of juvenile milkfish *Chanos chanos* (7.29; 95% CI 3.10–10.47 mg L^{-1}) (Chou et al., 2006) in BFD-endemic areas.

In the uptake phase, the accumulation was very rapid when *C. fluminea* was first exposed and then slightly

TABLE I. LC50 values (mean with 95% CI) of *C. fluminea* exposed to arsenic for selected time intervals

Time (h)	LC50 (mg L^{-1})
12	215.15 (156.34–309.92)
24	104.10 (77.45–140.84)
48	35.79 (22.72–52.78)
72	26.79 (16.11–39.74)
96	20.74 (11.74–30.79)

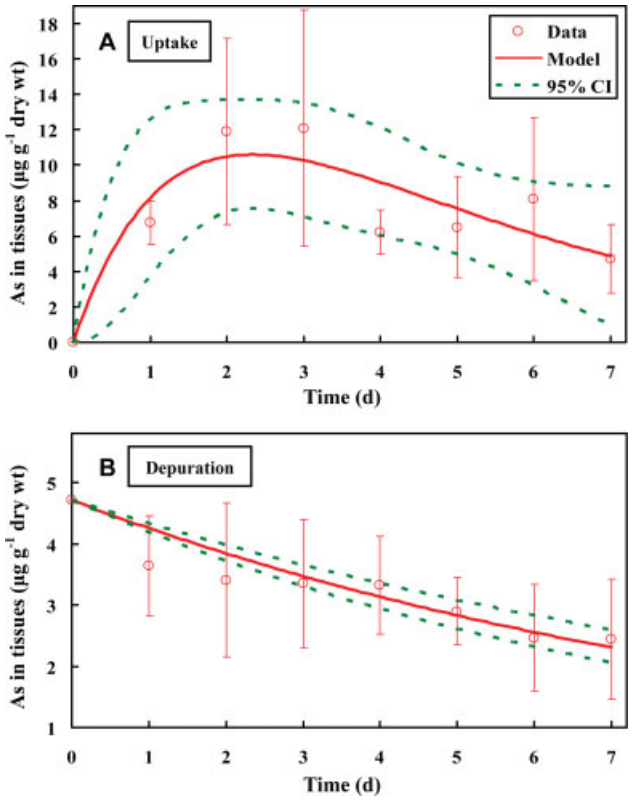

Fig. 1. Exposure experiment. (A) Uptake and (B) depuration kinetics of arsenic by freshwater clam *C. fluminea* exposed to 5 mg L^{-1} for 7 days and another 7 days for elimination. Error bar denotes the standard deviation from mean ($n = 5$). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

decayed, indicating the requirement of extensive time periods for an equilibrium state to be reached [Fig. 1(A)]. A similar trend [Fig. 1(B)] was observed for the depuration process. Thorsen et al. (2007) indicated that uptake in bivalve generally was very rapid when the bivalve was first exposed and then leveled off; suggesting that bivalve sometimes required extensive time periods for an equilibrium state to be reached.

Figure 1 also shows high values of the standard deviation. This may be due to the small amounts of clam tissues and limited sample sizes ($n = 5$) we analyzed, and consequently, the variability in final outcomes obtained from the chemical analyses was significant. Table II summarizes the experimentally determined bioaccumulation parameters in Eq. 1 describing the arsenic bioaccumulation process in soft tissue of *C. fluminea* exposed to arsenic. A simple, well-established, one-compartment uptake-depuration model in Eq. 1 was thus fitted by the nonlinear technique to the 14 days exposure data with a relative high coefficient of determination (r^2) of 0.81 (Table II). Estimates of depuration rate constant k_2 was also determined from the depura-

TABLE II. Bioaccumulation parameters (mean ± SE) for arsenic to soft tissue of freshwater clam *C. fluminea* estimated from 14-day exposure experiment

	Uptake Phase	Depuration Phase
k_1 (mL g^{-1} d^{-1})[a]	1.718 ± 6.70	
k_2 (d^{-1})[a]	0.392 ± 1.76	0.102 ± 0.007
k_0 (d^{-1})[a]	0.263 ± 0.33	
BCF (mL g^{-1})[b]	4.38	16.84
$t_{1/2}$ (d)[c]	1.78	6.80
r^{2} [d]	0.81	0.85

[a] Uptake rate constant (k_1), depuration rate constant (k_2), and dilution rate (k_0) estimated from Eq. 1, whereas at depuration phase k_2 estimated using the model ln concentration = $a + b$ (time) fitted to depuration concentration data.

[b] Equilibrium bioaccumulation factor calculated from the equation: BCF = k_1/k_2 in that BCF of depuration phase (16.84) is calculated as the ratio of uptake rate constant (k_1) from uptake phase to depuration rate constant (k_2) obtained from depuration phase.

[c] Depuration half-life calculated from $\ln 2/k_2$.

[d] Coefficient of determination.

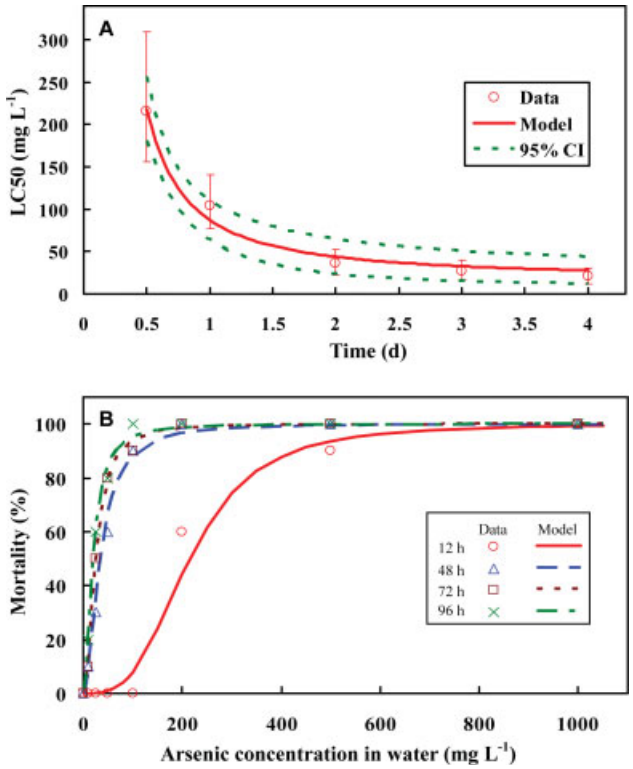

Fig. 2. Acute toxicity modeling. (A) Optimal fit of damage assessment model (DAM) to LC50(t) data. (B) Fitted dose-response curves by Hill sigmoid model demonstrating the relationships between exposure time-specific water arsenic concentration and mortality of *C. fluminea*. Error bar denotes the standard deviation from mean ($n = 10$). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

tion phase with a r^2 of 0.85. All of these regressions were significant ($p < 0.05$).

Our results show that the uptake rate constant (k_1) and depuration constant (k_2) were estimated to be 1.718 ± 6.70 mL g^{-1} d^{-1} and 0.392 ± 1.76 d^{-1} in uptake phase, whereas in depuration phase k_2 was estimated to be 0.102 ± 0.007 d^{-1} (Table II). The dilution rate of water arsenic concentration to clam (k_0) was also estimated to be 0.263 ± 0.33 d^{-1}. The BCF of *C. fluminea* soft tissue in uptake phase (4.38) was less than that of the depuration phase (16.84) (Table II). BCF values were all greater than 1, indicating the potential to accumulate arsenic when *C. fluminea* exposed to a given waterborne arsenic concentration.

The depuration half-life in uptake phase (1.78 d) was less than that in depuration phase (6.80 d), indicating that it will take a longer time to eliminate arsenic in depuration process. These bioaccumulation parameters not only can describe the uptake-depuration ability of *C. fluminea*, but also can be further used to predict the dose-response relationships between mortality and steady-state arsenic levels in water or in *C. fluminea*.

Fitting DAM to LC50 Data and Dose-Response Profile

The optimal fit of DAM to the LC50(t) data (Table I) is illustrated in Figure 2(A). The input parameters used were soft tissue k_2 = 0.39 d^{-1} and BCF = 4.38 mL g^{-1}, resulting in the estimated parameters DL50/k_a and k_r of 25.94 mg d^{-1} g^{-1} and 3.62 d^{-1}, respectively. A good quality of fit for the DAM was found ($r^2 = 0.97$, $p < 0.05$).

An external effect concentration (EEC)-based dose-response relationship between mortality and water arsenic concentration was derived using Hill model (Eq. 3) by nonlinear regression [Fig. 2(B)]. Generally, Figure 2(B) indicates that all fitting performances were robust with an average r^2 of 0.99 with the fitted Hill coefficients (n) ranging from 1.89 to 3.19. Particularly, the optimal fits of Eq. 3 to the observed percent mortality of *C. fluminea versus* waterborne arsenic concentration of the 96-h acute toxicity test resulted in the estimated Hill coefficient n = 1.89 (r^2 = 0.99, $p < 0.05$).

IEC-Based Dose-Response Profile

A clear IEC-based dose-response relationship between internal arsenic concentration and mortality was reconstructed [Fig. 3(A)] by Eq. 4 with a 10 000 iteration of Monte Carlo simulation providing a knowledgeable uncertainty of 95% CI. The CL50(t) value for soft tissue of *C. fluminea* would reach equilibrium after 30 days of simulation with the equilibrium values of 50 μg g^{-1} dry wt [Fig. 3(B)]. The IEC50 and IEC10 that caused 50 and 10% responses, respectively, for the soft tissue of *C. fluminea* were estimated to be 93.65 (95% CI 32.97–154.33) and 22.28 (95% CI 10.31–48.25) μg g^{-1} dry wt. The United States Environmental Protection Agency (USEPA, 2000) recommended that IEC10 could be used as a surrogate threshold of regulatory endpoint in ecological risk assessment.

The predicted mortalities never reached 80% when *C. fluminea* exposed to waterborne arsenic less than 50 mg L^{-1}, which was agreed well with the data from acute toxicity bioassays [Fig. 3(C)]. When *C. fluminea* were exposed to arsenic greater than 200 mg L^{-1}, the predicted mortality was slightly less than the observed values and reached 100% maximum mortality after day 4 [Fig. 3(C)].

DISCUSSION

Comparison of Existing Acute Toxicity Models

DAM assumes that death occurs when the cumulative damage reaches a critical level, assuming that damage is proportional to the accumulated residue and damage recovery is proportional to the cumulative damage when damage is reversible (Lee et al., 2002). The time-dependent LC50 data were determined by both a damage recovery rate and an elimination rate, suggesting that the critical cumulative damage was the determinant of the time-concentration response relationship. The CL50(t) value decreased initially and then slowly increased to a steady-state condition with the extension of duration, revealing that *C. fluminea* was capable of regulating arsenic toxicity by way of internal regulation mechanisms. Our results show that the predicted concentration-specific time-mortality profiles were notably agreed satisfactory with the observations; although some predictions were underestimated during initial time span exposure scenarios.

The proposed DAM is similar to that of DEBtox model (Bedaux and Kooijman, 1994; Widianarko and van Straalen, 1996). DEBtox model relates survivorship to toxicokinetics by assuming that the probability of dying, i.e., the hazard rate, is related to the concentration of the toxicant in the organism. DEBtox models have also been extensively applied in the fields of ecological risk assessment (Pery et al., 2001; Bonnoment et al., 2002). Based on toxicological principles, the mechanisms through which the dose at the target site elicits the ultimate adverse response were described by pharmacodynamic (PD) scheme and referred to as the action of the effect dose at the target site. Verhaar et al. (1999) and Legierse et al. (1999) have developed a PD-based model, the CAUC model, to describe time course of LC50 data for chemicals which act through the irreversible interaction between chemical and receptors.

Here we compared the predictability of three existing acute toxicity models based on our LC50 data of *C. fluminea* exposed to arsenic. Our results indicate that DAM was the best model (r^2 = 0.98, RMSE = 9.47 mg L^{-1}) than those of CAUC (r^2 = 0.97, RMSE = 17.76 mg L^{-1}) and CBR (r^2 = 0.86, RMSE = 44.70 mg L^{-1}) models to

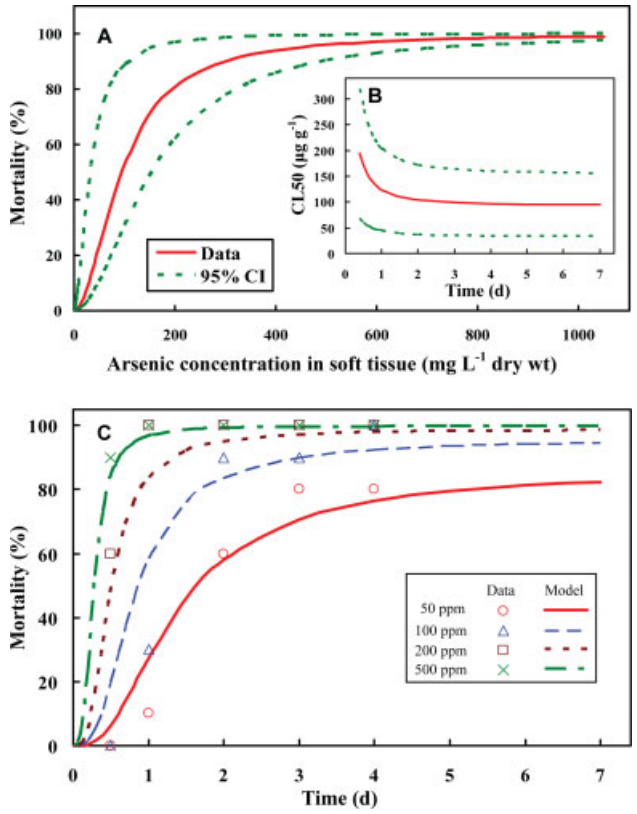

Fig. 3. Internal effect concentration based dose-response profile. (A) Reconstructed dose-response curve demonstrating the relationship between arsenic burden in soft tissue and mortality of *C. fluminea*. (B) DAM predicted internal lethal body burden of arsenic that causes 50% mortality. (C) Prediction of time-mortality profiles of *C. fluminea* exposed to waterborne arsenic ranging from 50 to 500 mg L^{-1}. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

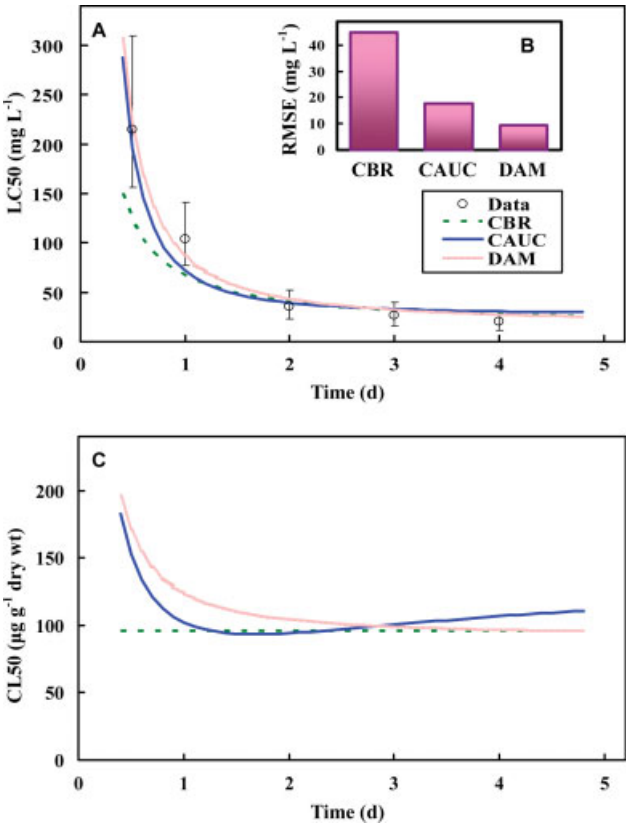

Fig. 4. Acute toxicity model predictability comparisons. (A) A comparison of the predictability of LC50(*t*) curve by three acute toxicity models (DAM, CAUC, and CBR). (B) Model verification justified by root mean squared error (RMSE) varied by three toxicity models. (C) Predicted internal lethal body burden of arsenic in soft tissue of *C. fluminea* that causes 50% mortality by three acute toxicity models. Error bar denotes the standard deviation from mean (n = 10). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

describe the LC50(t)-time relationship in a more accurate way [Fig. 4(A,B)] based on present estimated bioaccumulation parameters (Table III). The DAM-predicted CL50(∞) was estimated to be 21.41 mg L^{-1} (Table III). On the other hand, the predicted equilibrium CL50 values ranged from 93.90 to 95.74 μg g^{-1} dry wt [Fig. 4(C), Table III].

Verhaar et al. (1999) pointed out that the bioaccumulation parameter k_2 should be interpreted on the basis of the first-order bioaccumulation model. The fit of a model might be strongly determined by the input parameters. Therefore, the uncertainties in the k_2 value, which is an input parameter in the three toxicity models (Table III), affect the validation of the models. The experimental LC50 data support the validity of the three models, despite the uncertainties in the input parameter k_2. Our study suggests that using the DAM-based acute toxicity model with biological parameters estimated from the LC50(t) data can predict the time-mortality profile accurately and comparable with traditional PD-based CAUC model. Therefore, to describe and predict toxicity dynamics, two different types of experiments are needed to be conducted using the same treatment levels at the same time, including bioassays for (i) gathering the

TABLE III. Input parameters and parameter estimates for three acute toxicity models (CBR, CAUC, and DAM) comparisons

Parameter	CBR	CAUC	DAM
Input			
k_2 (d^{-1})	0.39	0.39	0.39
BCF (mL g^{-1})	4.38	4.38	4.38
Estimate			
DL50/k_a (μg d g^{-1})	–	–	25.94
k_r (d^{-1})	–	–	3.62
LC50(∞) (μg mL^{-1})	21.83	26.83	21.41
CL50(∞) (μg g^{-1} dry wt)	95.72	95.74	93.90
r^2	0.86	0.97	0.98

time-to-death data at a given exposure level and (ii) exposure experiments to estimate bioaccumulation parameters (McLeod et al., 2007).

Implications to Biomonitoring and Environmental Risk Assessment

Our reconstructed dose-response profile in Figure 3(A) describing the effect of soft tissue arsenic burden on the *C. fluminea* mortality was the pivotal result for regulatory policy in this article. Figure 3(A) therefore can be used to improve the deviation of environmental quality criteria and to support the establishment of an ecological risk assessment in the management of toxic metal in aquaculture systems.

In light of the freshwater bivalve monitoring programs, the use of *C. fluminea* as a surrogate species in metal toxicity testing has supported that *C. fluminea* is a viable indicator of impairment in aquatic ecosystems (Dohery and Cherry, 1988; Tran et al., 2003, 2004, 2007; Fournier et al., 2004; Jou and Liao, 2006). Cherry and Soucek (2007) have also suggested that uses of *C. fluminea* as an *in situ* monitoring test organism may provide a useful biomonitor to better understand contaminant bioavailability. Furthermore, Sebesvari et al. (2005) and Santos et al. (2007) suggested that *C. fluminea* was suitable for the monitoring of arsenic. Therefore, our proposed bioaccumulation models may offer scientists and engineers with a useful tool to better understand arsenic uptake dynamics and thus better inform risk assessment and remediation strategies at contaminated clam farms.

Since natural waters may contain trace metals other than arsenic at appreciable concentrations, it is possible that these metals act to increase arsenic bioavailability by competitive interactions with dissolved humic substances. Further investigation of these interactions is possible by using the present information of acute toxicity and uptake and depuration kinetics of arsenic in *C. fluminea* associated with recent developed biotic ligand mode (Paquin et al., 2002; Niyogi and Wood, 2004) to assess arsenic bioavailability.

When valve closure behavior in response to waterborne metals, clam will reduce nutrient uptake activity by closing their shells to escape toxicant damage and exclude themselves from the outside contaminated environment for maintaining their biotic faculty and increasing their survivability (Wildridge et al., 1998; Kadar et al., 2001). Therefore, we may incorporate the valve daily activities in the clam as a biological endpoint into the clam uptake mechanisms to gain insight into the mechanisms by which the arsenic bioavailability to *C. fluminea* can be reduced. From the perspective of the aquatic ecosystems, rather than developing a single-value waterborne metal concentration for establishing the water quality criteria, it is better to derive a mechanistic model that explicitly incorporates the factors controlling bioavailability and bioaccumulation to enhance predictability for protecting aquatic organisms. In the future, how to link valve behavioral movement to predict arsenic bioavailability and bioaccumulation in freshwater bivalves is worth the study.

In conclusion, our current results indicate that arsenic was rapidly taken up and eliminated from *C. fluminea* soft tissue in the first exposure period with equilibrium BCF of nearly 5 in uptake phase and 17 in depuration phase. The findings suggest that arsenic did bioaccumulate in *C. fluminea*. This may have important implications for dietary exposure of arsenic to humans who eat contaminated clams, because the soft tissue usually constitutes the majority of tissue consumed. To date, arsenic kinetics in different tissues of teleost fish have been well investigated, yet to the best of our knowledge, no study has been conducted to determine the acute toxicity and the uptake and depuration of arsenic in freshwater clam *C. fluminea*. Therefore, our results may be critical and pivotal for providing a direct and quantitative method for evaluation of arsenic that accumulates to hazardous levels in freshwater bivalves. Our results may also provide regulatory authorities for regulatory assessment to prevent possible environmental and human health risks which we seek to reduce or eliminate.

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APPENDIX: CAUC AND CBR MODELS

On the concept of CAUC model, adverse effect is associated with a critical amount of irreversible covalenty occupied target site, and the concentration of inhibited molecules in the target tissue is constant (Verhaar et al., 1999). Legierse et al. (1999) suggested that the critical irreversible target occupation could be expressed with the CAUC, which describes the time-integrated concentration of the molecular inhibition. By employing CAUC model, LC50(t) can be determined as (Legierse et al., 1999),

$$\mathrm{LC50}(t) = \frac{\mathrm{AUC}}{\mathrm{BCF}}\left(\frac{k_e}{k_e t + e^{-k_e t} - 1}\right) + \mathrm{LC50}(\infty), \quad \text{(A1)}$$

where AUC is the area under the concentration of toxicant in organism *versus* time curve (μg h g^{-1}). Substitution of C_w in first-order bioaccumulation model (Eq. 1) by LC50(t) in Eq. A1 and by regarding $C_b(t)$ as the CL50(t), leads to the following expression for CL50(t) as (Legierse et al., 1999),

$$\mathrm{CL50}(t) = \mathrm{AUC}\left(\frac{k_e\left(1 - e^{-k_e t}\right)}{k_e t + e^{-k_e t} - 1}\right) + \mathrm{BCF}\left(1 - e^{-k_e t}\right)\mathrm{LC50}(\infty). \quad \text{(A2)}$$

By the assumption of reversible binding in the critical burden residue (CBR) model, LC50 (t) can also be predicted from knowledge of the exposure time (t), depuration rate constant (k_2), BCF, and CL50 by first-order bioaccumulation model as,

$$\mathrm{LC50}(t) = \frac{\mathrm{CL50}}{\mathrm{BCF}\left(1 - e^{-k_e t}\right)}. \quad \text{(A3)}$$

When the exposure time approaches infinity, Eq. A3 gives a relation among $\mathrm{LC}_{50}(\infty)$, CL50, and BCF,

$$CL50 = LC50(\infty)BCF. \quad \text{(A4)}$$