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Organ-Specific Toxicokinetics and Dose–Response of Arsenic in Tilapia *Oreochromis mossambicus*

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Abstract. We appraised organ-specific toxicokinetics and dose responses of arsenic burdens in tilapia *Oreochromis mossambicus.* We kinetically linked an Area-under-the-curve (AUC)–based acute toxicity model and a pharmacodynamic model to derive dose–response relationships between equilibrium organ-specific arsenic concentrations and mortality effects. The AUC-based acute toxicity model was also used to derive organ-specific internal effect concentration (IEC)–time-response relationships, which can also be applied to predict a time–mortality profile. We conducted a 7-day exposure experiment to obtain toxicokinetic parameters, whereas the AUC-based acute toxicity model was verified with $LC_{50}(t)$ data obtained from a 7-day acute toxicity bioassay. Our results demonstrated that 96-hour LC_{50} and incipient LC_{50} for tilapia exposed to arsenic are 28.68 (95% confidence interval to 24.92 to 32.44) and 25.55 mg L^{-1}, respectively. Dose–response relationships followed the Hill equation, which could be expressed as organ-specific bioconcentration factors and incipient LC_{50}. Organ-specific dose-response relationships showed that muscle, gill, and liver have a relatively steep sigmoid dose-response profile in that IEC_{50} were 26.6, 62.5, and 78.5 μg g^{-1} dry wt (dw), respectively. Organ-specific arsenic internal lethal burdens were the highest in the gill and the lowest in the muscle in waterborne-exposed tilapia. The IEC and target-organ concentrations derived in this study can be used in site-specific risk assessment.

Arsenic is widespread in the environment as a consequence of both anthropogenic and natural processes. It is a ubiquitous but potentially toxic trace element. Inorganic and organic forms of arsenic are present in the environment, and the former seems to be more toxic and slightly more accumulated in some freshwater aquatic species than the latter (Oremland and Stolz 2003). Humans are exposed to arsenic from many sources such as food, water, air, and soil. The United States Food and Drug Administration, (1993) while examining the food category, indicated that fish and other seafood account for 90% of total arsenic exposure. Donohue and Abernathy (1999) reported that total arsenic in marine fish, shellfish, and freshwater fish tissues ranged from 0.19 to 65, 0.2 to 125.9, and 0.007 to 1.46 μg g^{-1} dw, respectively. Koch *et al.* (2001) demonstrated that total arsenic in freshwater fish ranged from 0.28 to 3.1 for whitefish (*Coregonus clupeaformis*), 0.98 to 1.24 for sucker (*Catostomus commersoni*), 0.46 to 0.85 for walleye (*Stizostedion vitreum*), and 1.30 to 1.40 μg g^{-1} dry wt for pike (*Esox lucius*).

Chen *et al.* (2001a) indicated that long-term exposure to ingested inorganic arsenic 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 the southwestern coastal area of Taiwan, which consists mainly of four towns—Putai, Yichu, Peimen, and Hsuehchia—located in the Chiayi and Tainan counties. Recently, a number of studies on acquired and genetic susceptibility to arsenic were carried out in the BFD-endemic areas of southwestern Taiwan to find out the cause of BFD (Chen *et al.* 2001a). Most 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 and shellfish, has been as an important route of human exposure to trace elements (arsenic, copper, zinc, lead, cadmium, mercury) in Taiwan in that oyster (*Crassostrea gigas*), tilapia, tuna, and shrimp are the most popular ones. Farming tilapia *Orechromis mossambicus* is a promising practice in the BFD area because of its high market value. Consumers in Taiwan mainly eat the muscle of tilapia. The fish are fed with artificial bait, which does not contain arsenic. These fish are maintained in the ponds for at least 6 months (from April to October) before harvest. At present, data on the actual effects of arsenic to tilapia are limited. Generally, the accumulation of metals in aquatic organisms has been linked to decrease survival and reproductive ability. If arsenic levels in pond water are higher, severe healths effects may occur on the health of cultured fish by decreasing their market prices and leading to closure of fish farms.

Currently, a lack of reliable data exists on uptake and effects of arsenic in tilapia, and little is known about the actual uptake and elimination processes. The objectives of this article are twofold: (1) to provide knowledge of the organ-specific toxi-

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cokinetics and distributions of arsenic in tilapia and (2) to construct dose-response relationships of arsenic in target organs of tilapia by kinetic coupling of an acute toxicity model and a pharmacodynamic model.

In particular, we carried out two experiments with tilapia *O. mossambicus.* One experiment involved conduction of a 7-day exposure experiment to obtain toxicokinetic parameters of target organs including gill, muscle, liver, intestine, and stomach. The second experiment conducted a 7-day acute toxicity bioassay to derive dose-response relationships between mortality effect and equilibrium arsenic levels in different target organs of tilapia.

Materials and Methods

Exposure Experiments

The present laboratory study was designed to examine the accumulation ability of arsenic in the muscle, gill, stomach, intestine, liver, and whole body of tilapia. The arsenic contamination level was determined by a preliminary test of exposing tilapia to different arsenic concentrations of 0.25, 0.5, 1, 2, 4, and 6 mg L^{-1}. The median lethal tolerance (LT_{50}) of tilapia exposed to concentrations $\leq$1 mg L^{-1} arsenic was $>$21 days. Therefore, we conducted an uptake experiment in arsenic concentration of 1 mg L^{-1} for 7 days based on the suggestion by Suhendrayatna *et al.* (2001). The arsenic concentrations used in this experiment were approximately 20 to 50 times higher than that in the environment conditions to produce high arsenic level in target organs of tilapia.

The experiments were carried out with 42 fish of a specific size class (mean body length = 12.9 $\pm$ 1.54 cm (mean $\pm$ SD) and mean weight = 32.75 $\pm$ 4.2 g wet weight [ww]). They were supplied by Taiwan Fisheries Research Institute, Lukang, Chunghwa, and considered to be uncontaminated by arsenic. The fish were visibly free of any deformities, lesions, or disease. Fish were allowed to acclimate to laboratory condition for 2 weeks before exposure. During the acclimation and experimental periods, the fish were fed once a day, 7 days/week at a rate of 0.5% of fish biomass. This low feeding rate was chosen to avoid arsenic contamination of feed remaining in the aquaria.

All experiments were carried out in 54-L indoor retangular fiberglass aquaria filled with 50 L and arsenic concentration of 1 mg L^{-1}. The sodium arsenite ($NaAsO_2$) stock solution was prepared with deionized water. Dissolved oxygen in each tank was maintained at close to saturation by aeration (7.21 $\pm$ 0.1 mg L^{-1}). The temperature in each aquarium was maintained at 24.7 $\pm$ 0.24°C using submerged heaters. The pH value was maintained at 7.75 $\pm$ 0.02. The photoperiod was 16 hours light to 8 hours dark with a light intensity of 1400 $\pm$ 100 lux. All experiments were assigned to two tanks. To maintain the ideal experimental condition, we removed feces every 3 hours and collected forage debris 1 hour after feeding. 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 hours and refilled with distilled water to keep levels constant.

Exposure-water characteristics were measured 3 times weekly during the test. The 10-mL water samples in each test media were acidified (pH $<$1) with 5 mL 1 N HNO_3 and then stored at $-$4°C in the dark until they were analyzed. The measured arsenic concentration was 0.94 $\pm$ 0.072 mg L^{-1}. No fish died during the acclimated period. Neither mortality nor weight losses were observed during arsenic exposures. To analyze arsenic uptake by the fish, three fish were sequentially removed from each tank after 0, 1, 2, 4, and 7 days of exposure.

Each removed 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 of the intestine were removed. The dissected tissues samples were cleaned with deionized water and were freeze-dried overnight and then ground to fine powder in a grinder (model no. 536-89; Tai-Hsiang 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.

Acute Toxicity Assays

Laboratory static bioassays were conducted to determine the 24-, 48-, 72-, 96-, 120-, and 144-hour LC_{50} values for tilapia 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). Six fish of a specific size class (mean body length = 12.67 $\pm$ 5.65 cm [mean $\pm$ SD] and mean body weight = 31.72 $\pm$ 6.5 g wet wt) were randomly selected and transferred into each test aquarium.

The nominal concentrations of arsenic tested were 0 (control), 1, 2, 4, 10, 30, 50, and 80 mg L^{-1} (Hwang and Tsai 1993). The measured arsenic concentrations were 0.98 $\pm$ 0.05, 1.97 $\pm$ 0.04, 4.26 $\pm$ 0.09, 10.36 $\pm$ 0.06, 31.04 $\pm$ 0.12, 47.65 $\pm$ 0.06, and 81.53 $\pm$ 0.08 mg L^{-1}. Gross mortality of fish to each concentration was recorded every 1 hour for the first 12 hours and every 2 hours thereafter for 96 hours, and dead fish were removed every 1 to 2 hours. Tilapia were not fed throughout the test. Control and 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 controls.

The LC_{50} values were determined from maximum likelihood estimates of linear functions relating log arsenic concentration to probit transformations of percent mortality (Finney 1978). The LC_{50} values were determined using mean assayed arsenic concentrations and cumulative mortality. All of the observations were used in probit analysis.

Data Analysis

The value of tilapia growth rate was adopted from our unpublished data, which was estimated from the tilapia exposed to uncontaminated water in that the tilapia had ages and sizes similar to that of the fish used in the present exposure and acute bioassays. The resulting value of the average growth rate of tilapia was 0.099 d^{-1}. A constant first-order growth rate (g, d^{-1}) was used for all organs. We assumed that the contaminant had no effect on the growth rate. Growth rates for the organs were taken as same as the whole body. All target-organ concentrations were corrected for growth dilution by determining the percent increase in fish weight at each sampling interval relative to t = 0.

The method to determine uptake and depuration rate constants for each target organ was used by fitting concentration data to the integrated form of the kinetic equation for constant water exposure using iterative nonlinear regression,

$$C_f(t) = C_f(0)e^{-(k_2+g)t} + \frac{k_1}{k_2 + g} C_w(1 - e^{-(k_2+g)t}), \quad (1)$$

where C_f is the time-dependent arsenic concentration in the target organ of tilapia ($\mu g\ g^{-1}$), k_1 is the tilapia uptake rate constant (mL g^{-1} d^{-1}), k_2 is the depuration rate (d^{-1}) constant of arsenic, and t is time

in days. Depuration half-life ($t_{1/2}$) was calculated as $\ln 2/k_2$. The organ-specific bioconcentration factor (BCF) can be calculated as BCF = $k_1/(k_2 + g)$ and represents the net accumulation ability that is the result of the competition between uptake and depuration associated with growth dilution. 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 fish and duration of exposure (Cho *et al.* 2003; Clason *et al.* 2003). To compare absorption efficiencies of arsenic in different target organs of tilapia, the area-under-the-organ-concentration–versus–time-curve (AUC) (μg d g^{-1}) for the arsenic exposure period was estimated. AUC can be derived from the solution of a first-order, one-compartment, uptake-depuration model as

$$\mathrm{AUC} \equiv \int_0^1 C_f(t)dt = \int_0^1 \mathrm{BCF}C_w(1 - e^{-(k_2+g)t})dt,$$

$$= \left(\frac{\mathrm{BCF}}{k_2+g}\right)C_w((k_2 + g)t + e^{-(k_2+g)t} - 1), \quad (2)$$

in that the first-order, one-compartment, uptake-depuration model is described as $dC_f(t)/dt = k_1C_w - (k_2 + g)C_f(t)$ and its solution is subject to the initial condition of $C_f(t = 0) = 0$ is $C_f(t) = \mathrm{BCF}C_w(1 - e^{-(k_2+g)t})$.

Chemical and Statistical Analyses

A Perkin-Elmer Model 5100PC atomic absorption spectrometer (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 material (dogfish 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.

We employed the nonlinear option of Statistica software (StatSoft, Tulsa, OK) to perform all curve fittings. Statistica software was also used to calculate the coefficient of determination (r^2) and statistical analyses (analysis of variance and Students *t* test). Statistical significance was determined at $p < 0.05$.

Arsenic Acute-Toxicity Models

We employed an AUC-based, time-integrated concentration (TIC) toxicity model to estimate the internal residues of arsenic in tilapia as a function of a few constants and variables that were verified with acute-toxicity data. The main factors were exposure time and concentration, BCF, depuration rate constant (k_2), and growth rate constant (g).

The AUC-based TIC toxicity model employed in determining the time-dependent median lethal concentrations [$\mathrm{LC}_{50}(t)$], which can be expressed as (Liao and Lin 2001)

$$\mathrm{LC}_{50}(t) = \frac{\mathrm{AUC}_f}{\mathrm{BCF}}\left(\frac{k_2 + g}{(k_2 + g)t + e^{-(k_2+g)t} - 1}\right) + \mathrm{LC}_{50}(\infty), \quad (3)$$

where AUC_f is the area under the whole-body burden of arsenic in tilapia versus time curve (μg d g^{-1}). With sufficient $\mathrm{LC}_{50}(t)$ data, it is possible to calculate best-fit values of two toxicologic parameters [i.e., AUC_f and $\mathrm{LC}_{50}(\infty)$], as appears in Equation 3, by nonlinear regression technique.

Substitution of C_w in a one-compartment, uptake-depuration model by $\mathrm{LC}_{50}(t)$ in Equation 3, and regarding $C_f(t)$ as the internal lethal body burden at the site of action that causes 50% mortality [$C_{\mathrm{L},50}(t)$], leads to the following expression for $C_{\mathrm{L},50}(t)$ (Liao and Lin 2001),

$$C_{\mathrm{L},50}(t) = \mathrm{AUC}_f\left(\frac{(k_2 + g)(1 - e^{-(k_2+g)t})}{(k_2 + g)t + e^{-(k_2+g)t} - 1}\right) +$$

$$\mathrm{BCF}(1 - e^{-(k_2+g)t})\mathrm{LC}_{50}(\infty). \quad (4)$$

Equation 4 shows that the internal lethal body burden in tilapia can be expressed as functions of biokinetic parameters k_2 and BCF, growth rate g, and toxicologic parameters AUC_f and $\mathrm{LC}_{50}(\infty)$. When the exposure time approaches infinity, Equation 4 yields a relation among $\mathrm{LC}_{50}(\infty)$, $C_{\mathrm{L},50}(\infty)$, and BCF as

$$C_{\mathrm{L},50}(\infty) = \mathrm{LC}_{50}(\infty) \times \mathrm{BCF}. \quad (5)$$

Thus, the AUC-based TIC toxicity model in Equation 5 is based on a direct relationship between adverse effects and extent of inhibited molecules in the target tissue, i.e., mortality is assumed to occur at a fixed percentage of inhibited molecules. Furthermore, the AUC-based TIC toxicity model assumes that the concentration of inhibited molecules in the target tissue is constant, i.e., the percentage of lethally inhibited molecules is related to a critical amount of occupied target sites.

Dose-Response Models

In pharmacodynamic modeling, the relationship between dose effect and concentration is commonly expressed by the Hill equation or is referred to as the sigmoid $E_{\max}$ model (Lalonde 1992; Bourne 1995; de Vries 1996),

$$\mathrm{Effect}\ (E) = \frac{E_{\max} \times C^n}{\mathrm{EC}_{50}{}^n + C^n}, \quad (6)$$

where C is the dose concentration in the receptor, $E_{\max}$ is the maximum dose effect, EC_{50} is the concentration that causes an effect equal to 50% of the $E_{\max}$, and n is a slope factor or is referred to as the Hill coefficient, which indicates the number of ligand molecules required to bind to the receptor to produce a functional effect (Weiss 1997). The Hill equation in Equation 6 is derived based on the receptor theory (Lalonde 1992). With sufficient data over a suitable concentration range, it is possible to calculate best-fit values of three parameters appearing in Equation 6 by nonlinear regression.

We combined Equations 4 and 6, incorporated with internal lethal body burden derived from the TIC toxicity model, to construct dose-response profiles. The mathematic model for dose-response relationships between mortality and arsenic levels in different target organs of tilapia can be obtained by refining the Hill equation in Equation 6 as $E \equiv M$, $C \equiv C_f$ and $\mathrm{EC}_{50} \equiv \mathrm{L}_{\mathrm{L},50}(\infty)$ and associating it with Equation 5 as

$$M_i = \frac{M_{\max} \times C_{f,i}{}^n}{C_{\mathrm{L},50\mathrm{i}}{}^n(\infty) + C_{f,i}{}^n} = \frac{M_{\max} \times C_{f,i}{}^n}{(\mathrm{BCF}_i \times \mathrm{LC}_{50}(\infty))^n + C_{f,i}{}^n}, \quad (7)$$

where M_i is mortality for target organ i, $C_{f,i}$ is the internal arsenic concentration in target organ i, BCF_i is the bioconcentration factor for the target organ i, and $M_{\max}$ is the tilapia maximum mortality exposed to waterborne arsenic. We obtained the waterborne arsenic-dependent, organ-specific, time-mortality curves (i.e., $M(t)$) as functions of arsenic concentration in water (C_w) and organ-specific toxicokinetic parameters ($k_2 + g$, BCF_i) and $\mathrm{LC}_{50}(\infty)$ by altering Equation 7 to a time-dependent function and associating it with $C_{f,i}(t)$ in Equation 1 for each target organ and $C_{\mathrm{L},50}(t)$ in Equation 4 as

Table 1. Estimates for uptake rate constant k_1, k_2, BCF, $t_{1/2}$, and AUC_u during a 7-day arsenic-exposure and an 8-day elimination periods for *O. mossambicus*

Target Organ	k_1[a] (mL g^{-1} d^{-1})	k_2[a] (d^{-1})	BCF[b] (mL g^{-1})	$t_{1/2}$[c] (d)	AUC_u[d] (μg d g^{-1})
Gill	0.31 ± 0.086 (0.96)[e]	0.028 ± 0.11 (0.96)[e]	2.44	24.76	6.26
Liver	0.61 ± 0.15 (0.96)	0.1 ± 0.11 (0.96)	3.07	6.93	9.44
Muscle	0.24 ± 0.08 (0.93)	0.13 ± 0.029 (0.69)	1.04	5.33	3.43
Intestine	0.67 ± 0.16 (0.97)	0.61 ± 0.098 (0.97)	4.19	1.14	11.06
Stomach	1.53 ± 0.73 (0.93)	0.063 ± 0.2 (0.93)	9.56	11.0	30.08
Whole body	0.49 ± 0.11 (0.96)	0.075 ± 0.09 (0.96)	5.03	9.24	8.15

[a] Mean ± 1 SE.
[b] $BCF = k_1/(k_2 + g)$ where $g = 0.099$ d^{-1} adopted from our unpublished data.
[c] $t_{1/2} = \ln 2/k_2$.
[d] $AUC_u = \int_0^t C_u(t)dt$ where $C_u(t)$ is time-course uptake-phase concentration.
[e] Coefficient of determination (r^2).
AUC_u = Area under curve for uptake period.
BCF = Bioconcentration factor.
k_1 = Uptake rate constant.
k_2 = Elimination rate constant.
$t_{1/2}$ = Half-life for elimination.

$$M(t) = \frac{M_{\max} \times C_{f,i}{}^n(t)}{C_{\mathrm{L,50i}}{}^n(t) + C_{f,i}{}^n(t)} \tag{8}$$

Based on the acute-toxicity test, however, mortality functions were estimated from observed mortality percentages in exposure regimes in which mortality was an increasing function of arsenic concentration in water. Therefore, in fitting the Hill equation to observed mortality in terms of the specific-interval acute-toxicity data, the mortality functions used must be expressed as the functions of waterborne arsenic concentration (C_w) and $LC_{50}(t)$ data as

$$M(t) = \frac{M_{\max} \times C_w{}^n}{\mathrm{LC}_{50}{}^n(t) + C_w{}^n}. \tag{9}$$

With sufficient data of percent mortality over a suitable arsenic concentration in water associated with the specific interval of LC_{50} data, we can estimate best-fit values of the Hill coefficient appearing in Equation 9 by nonlinear regression.

Results

Toxicokinetic Parameters and $LC_{50}(t)$ Data

Table 1 lists the experimentally determined toxicokinetic parameters for arsenic calculated from target organs of tilapia exposure data. The 7-day water exposure experiment of arsenic in target organs of gill, liver, muscle, intestine, and stomach of tilapia had significantly correlated nonlinear regression profiles ($r^2 = 0.69$ to 0.97, $p < 0.05$) resulting from the best fit of the first-order one-compartment, uptake-depuration model (Table 1 and Fig. 1).

The 7-day bioaccumulation experiments demonstrate that uptake rate constants (k_1) are between 0.24 and 1.53 mL g^{-1} d^{-1}. The highest k_1, which occurs in stomach, is two times higher than that of intestine and liver. Muscle is the major biomass, yet it shows the lowest uptake ability. The depuration rate constants (k_2) range from 0.028 to 0.61 d^{-1}. Our study results show that intestine was the organ having the best depuration ability followed by muscle, liver, gill, stomach, and gill. BCF values of target organs were all >1 (range 1.04 to 9.56), and thus they show the potential to accumulate arsenic when tilapia were exposed to a given waterborne arsenic concentration. Stomach and intestine had higher BCF values than the other organs; therefore, they were the dominant accumulative organs in tilapia. The BCF of intestine was 4.19, which is similar to that of the whole body value of 5.03. Muscle had the lowest ability to accumulate arsenic.

The depuration half-lives ($t_{1/2}$) were in the range of 1.14 to 24.76 days. Among these target organs, gill had the maximum $t_{1/2}$ value, indicating that it will take a longer time to eliminate arsenic than the other organs. According to the perspective of toxicology, if the contaminants stay in the target organ longer, there would be higher opportunity to cause adverse effect on that organ. Our analysis shows that the AUC_us range from 3.43 to 30.08 μg d g^{-1}. The AUC_u value of stomach is much higher than that of the other organs during uptake periods, indicating the high accumulating potential of stomach for arsenic, whereas less As is accumulated by muscle. These toxicokinetic parameters can be used to describe the uptake and depuration ability of target organs of tilapia as well as predict the dose-response relationships of these target organs.

The selected time intervals of 24-, 48-, 72-, 96-, 120-, and 144-hour LC_{50} values with 95% confidence intervals (CI) of arsenic to tilapia are listed in Table 2. LC_{50} decreases progressively as the duration of exposure increases. A limited number of studies have investigated arsenic toxicity to tilapia. Our 96-hour LC_{50}s of arsenic to tilapia is 28.68 (95% CI 24.92 to 32.44) mg L^{-1}, which is close to the range of 96-hour LC_{50} of arsenic to seawater tilapia (26.5; 95% CI 23.2 to 33.8 mg L^{-1}), yet it is lower than that of arsenic to freshwater tilapia (71.7; 95% CI 67.8 to 76.4 mg L^{-1}) reported by Hwang and Tsai (1993).

Fitting Toxicity Model to $LC_{50}(t)$ Data

The optimal fits of the AUC-based TIC toxicity model to the $LC_{50}(t)$ data listed in Table 2 are presented in Figure 2. The

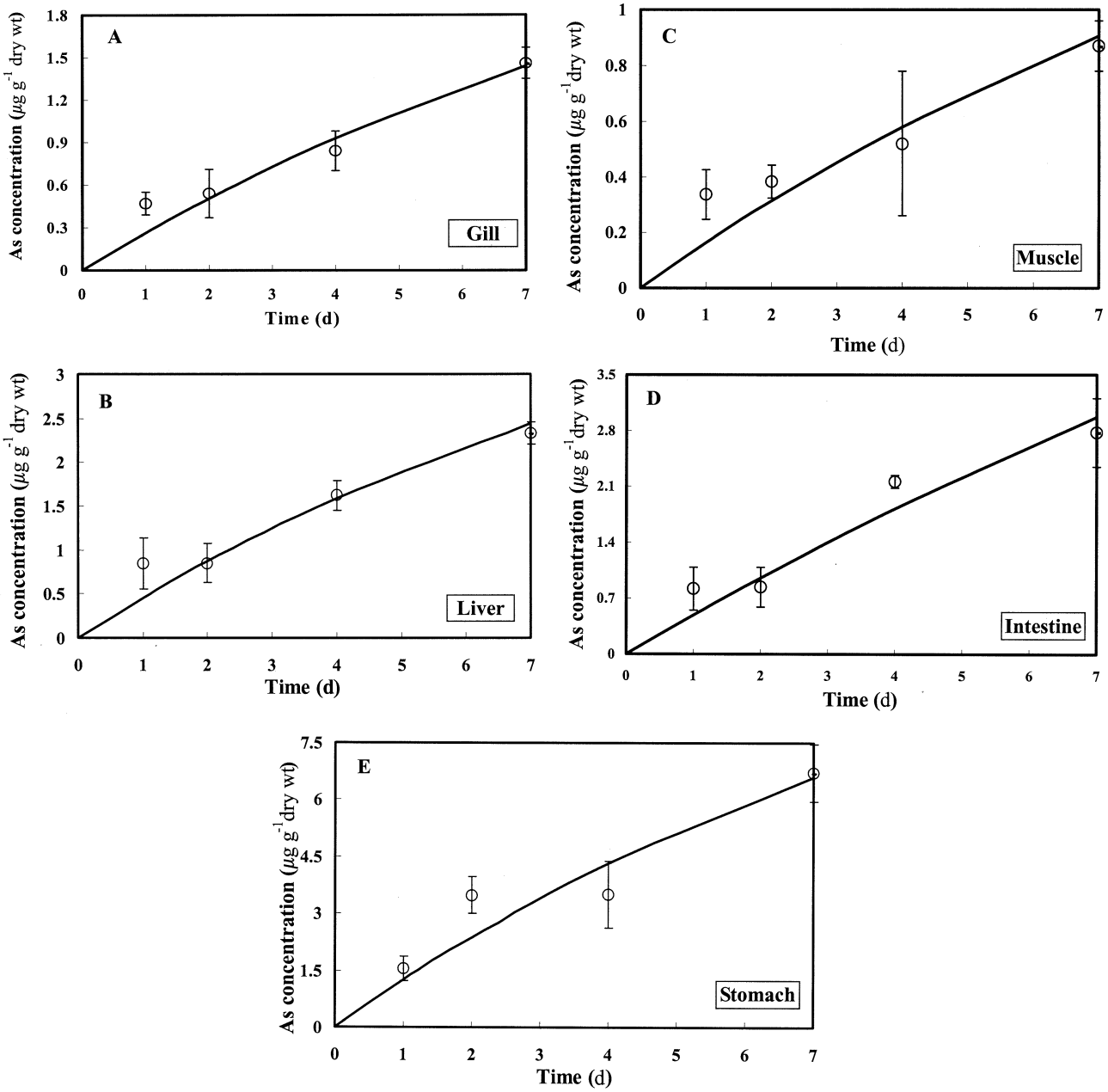

Fig. 1. Exposure experiment in (**A**) gill, (**B**) liver, (**C**) muscle, (**D**) intestine, and (**E**) stomach of tilapia *O. mossambicus* exposed to 1 mg L^{-1} arsenic for 7-day uptake. Symbols are averages with standard errors ($n = 6$). The solid lines are best-fit regression curves from one-compartment kinetic models of each target organ

input parameters used are whole-body $k_2 = 0.077\ d^{-1}$ and $g = 0.099\ d^{-1}$. The estimated values for $LC_{50}(\infty)$ and AUC_f/BCF are 25.55 mg L^{-1} and 46.36 (mg d L^{-1})/(L kg^{-1}), respectively. The coefficients of determination (r^2) indicate the quality of fit for the TIC toxicity model ($r^2 = 0.80$, $p < 0.05$).

Dose-Response Relationships

A dose-response relationship between equilibrium arsenic concentration in each target organ of tilapia and mortality was derived using Equation 7 and the estimate of Hill coefficient (n) obtained from optimal fitting to Equation 9 by nonlinear regression. The optimal fits of Equation 9 to the observed percent mortality of tilapia versus waterborne arsenic concentration of the 96-hour acute toxicity test, which is presented in Figure 3A, results in the estimated Hill coefficient, $n = 4.07$ ($r^2 = 0.93$, $p < 0.05$).

Figure 3B depicts a clear dose-response relationship between internal arsenic concentration in each target organ of tilapia and mortality. Figure 3B shows that muscle, gill, and liver have relative steep sigmoid dose-response profiles with mortalities

Table 2. LC_{50} of arsenic to tilapia for selected time intervals[a]

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)
120	21.41 (17.59–25.23)
144	15.98 (12.07–19.89)

[a] 95% CIs given in parentheses.
CI = Confidence interval.

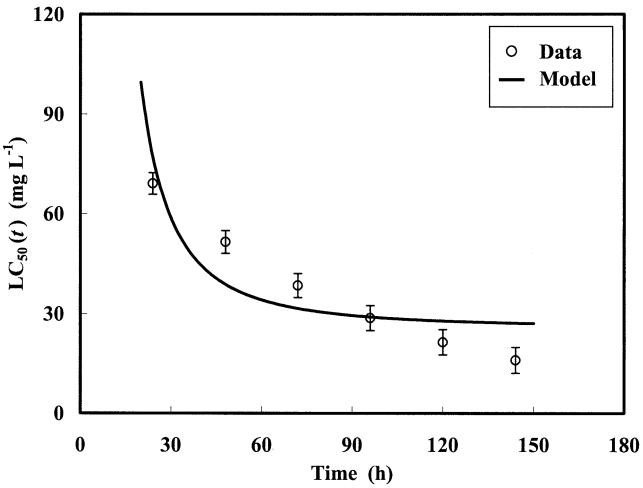

Fig. 2. Optimal fit of the AUC-based TIC acute toxicity model to the $LC_{50}(t)$ data (mean ± 95% CI) listed in Table 2. CI = confidence interval

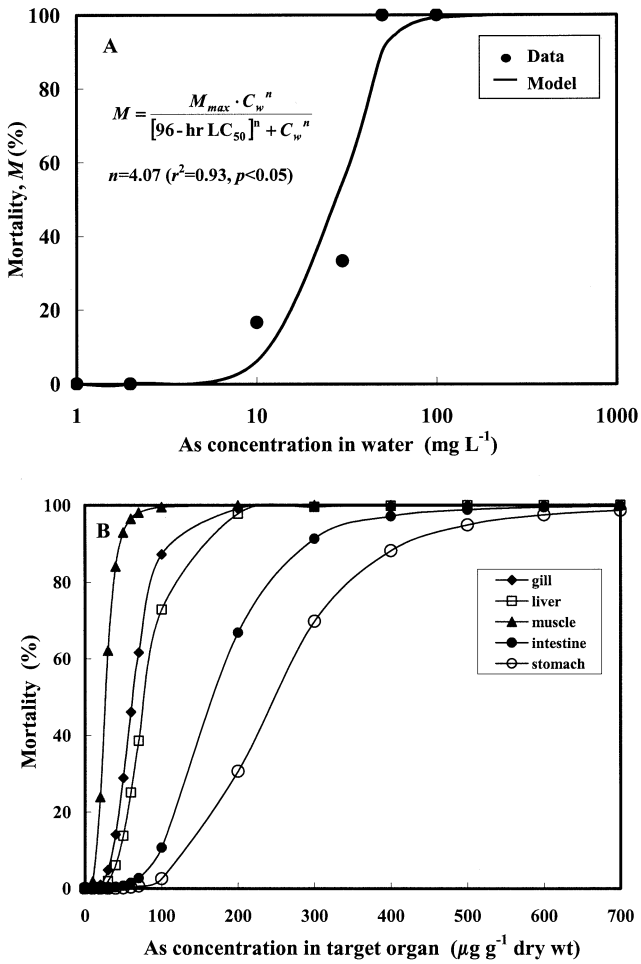

Fig. 3. (**A**) Optimal fit of Hill equation to observed percent mortality of tilapia versus waterborne arsenic concentrations in the 96-hour acute toxicity bioassay. (**B**) Derived organ-specific, dose–response relationships between equilibrium internal effect concentration of arsenic and mortality effects for tilapia *O. mossambicus*

approaching 100%, whereas intestine and stomach have "lazier" sigmoid dose-response profiles. The IEC_{50} and IEC_{10} for muscle, gill, and liver are 26.6, 15.5; 62.35, 36.35; and 78.45, 47.74 μg g^{-1} dw, respectively, whereas for intestine and stomach they are 168.4, 98.3 and 244.4, 142.62 μg g^{-1} dw, respectively. The United States Environmental Protection Agency (2000) recommended that IEC_{10} could be used as a surrogate threshold of regulatory end point in ecological risk assessment. We suggested that gill can be used as a surrogate to assess arsenic toxicity to tilapia because of its higher sensitivity to toxic effects and the sampling convenience.

Predictions of Gill Mortality and Internal Lethal Body Burden

For this article, we selected gill as our example to gain insight into time-mortality predictions because gill is the primary interface between the environment and tilapia's internal milieu. We substituted $C_f(t)$ of gill obtained from Equation 1 and $C_{L,50}(t)$ in Equation 4 into Equation 8 to obtain time-mortality profiles as functions of toxicokinetic parameters of gill [$k_2 + g$, BCF and $LC_{50}(\infty)$] and varied waterborne arsenic concentrations ranging from 1 to 50 mg L^{-1} (Fig. 4A). Figure 4A shows that the predicted mortalities never reached 50% when tilapia were exposed to waterborne arsenic <2 mg L^{-1}, which is in high agreement with the data from the acute-toxicity bioassays. When the tilapia were exposed to arsenic at 4 mg L^{-1}, the predicted mortality was slightly higher than the observed values before 10 days and reached 70% of maximum mortality. Similar results also occurred in 10 and 50 mg L^{-1} arsenic. Our pharmacodynamic model described the dose-response relationships well, although some values were overestimated during initial time spans in some higher arsenic concentration exposure scenarios. It reveals that the assumptions of input toxicokinetic parameters are independent of waterborne arsenic concentrations and should be addressed in the future studies, especially as applied to the exposure conditions that are dramatically different from the bioassay conditions.

We employed Equation 4 to predict the internal lethal body burden for each target organ at the site of action that causes 50% mortality [$C_{L,50}(t)$ values] for tilapia when exposed to 1 mg L^{-1} waterborne arsenic concentration. Figure 4B indicates that $C_{L,50}(t)$ values for gill, stomach, intestine, liver, and muscle would reach equilibrium after 30 days of simulation, and

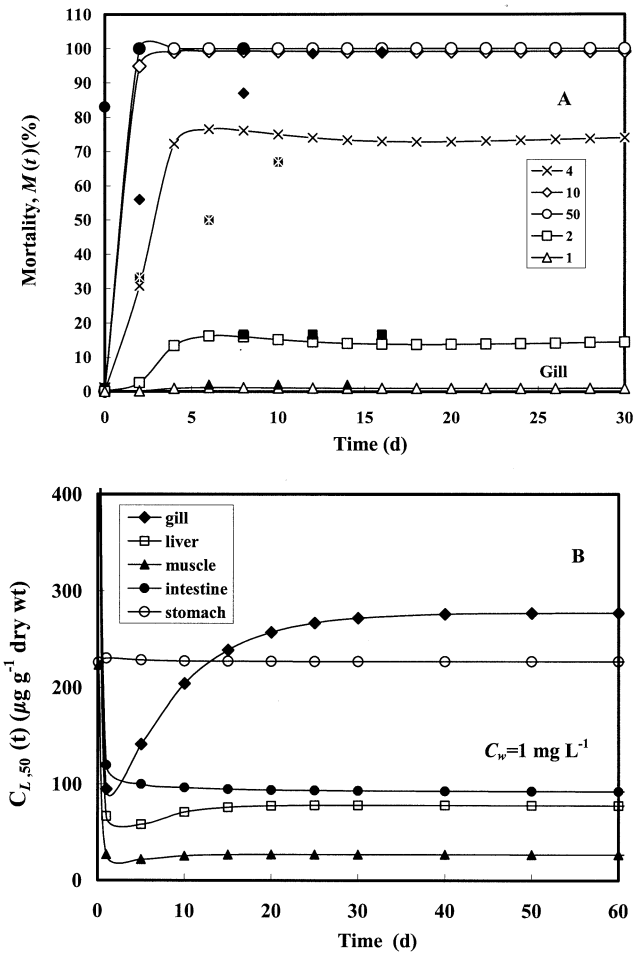

Fig. 4. (**A**) Prediction of time–mortality profiles of tilapia exposed to waterborne arsenic, ranging from 1 to 50 mg L^{-1}, by using gill as biomarker. Solid symbols are the measured data from the acute toxicity bioassay, and open symbols with the same shape are the predicted values from Equation 8. (**B**) Predicted internal effect concentration–time response relationships in target organs of tilapia when exposed to 1 mg L^{-1} waterborne arsenic, which causes 50% mortality

the equilibrium values would be 275.75, 227.78, 93.1, 78.07, and 26.88 μg g^{-1}, respectively. The $C_{L,50}(t)$ values decreased initially and then increased to steady-state condition with the extension of duration. The predicted results revealed that the target organs of tilapia are capable of regulating arsenic toxicity by way of internal regulation mechanisms. Gill has greater tolerance to arsenic toxicity than the other target organs. However, our acute-toxicity bioassays could not explicate the relationships between the internal medium lethal concentrations ($C_{L,50}$) and time. Other literature also reveals that the hypothesis of constant body residue for mortality has been challenged (Legierse *et al.* 1999; Yu *et al.* 1999; Lee *et al.* 2002). Yu *et al.* (1999) reported that the $C_{L,50}$ of series of halobenzenes in fish and juvenile crabs decreased with increasing natural logarithm of exposure time in a linear manner. Legierse *et al.* (1999) showed that the lethal body residues of organophosphorus compounds in pond snails and guppies decreased with increasing exposure time, and the decreasing trend could not be modeled using the critical body residue (CBR) model. In our study, the AUC-based TIC model predicted the trends of $C_{L,50}(t)$ values, which agreed well with the organ-specific toxicokinetic parameters. It will be critical to determine the relationship between exposure time and body residue to produce time-dependent mortality accurately.

Discussion

Waterborne Arsenic Toxicity to Tilapia

Because few previous studies have evaluated arsenic toxicity to tilapia, we did not have an *a priori* estimate of internal lethal body burdens. Mechanisms of arsenic toxicity in tilapia have not been investigated extensively. Several studies have reported acute toxicity of arsenic to other fish species. Our result of the 96-hour LC_{50} arsenic (95% CI 24.92 to 32.44 mg L^{-1}) for tilapia is close to the range of the 96-hour LC_{50} arsenic to rainbow trout *Oncorhynchus mykiss* (23 to 26.6 mg L^{-1}) (Spehar *et al.* 1980) and to bluegill *Lepomis macrochirus* (29 to 35 mg L^{-1}) (Johnson and Finley 1980).

Chen *et al.* (2001b) indicated that tilapia could potentially be able to regulate the concentrations of metals in their organs over time by combining the processes of absorption, excretion, detoxification, and storage, and this can be checked by analyzing the organs of individuals exposed to different metals for different durations. In addition, the rate of metal uptake was organ specific and time dependent in fish. Our results demonstrated that most of the arsenic was accumulated in the intestine, stomach, liver, and gill of tilapia rather than in muscle. Suner *et al.* (1999) also stated that fish liver contains more arsenic than muscle and that this is true for total arsenic as well as inorganic arsenic. Many laboratory and field studies have revealed that many trace metals (zinc, copper, chromium, nickel, mercury, cadmium, and lead) were accumulated in intestine, stomach, and liver rather than in muscle of tilapia (Kurcishy and D'silva 1993; Liang *et al.* 1999), thus demonstrating that intestine, stomach, and liver play a vital role in storing arsenic in tilapia. Suhendrayatna *et al.* (2002) further suggested that tilapia *O. mossambicus* could be used as a bioindicator for studying the accumulation and transformation of arsenic in freshwater organisms. The mechanisms of arsenic toxicity to tilapia are still limited in the literature. We linked the refined Hill equation used in pharmacology with the AUC-based toxicity model to assess the time–variance lethality potential of the tissue compartments of tilapia and identify involved toxicity mechanisms based on this modeling approach. The organ-specific dose-response relationships determined in this study indicate that gill has a more steep sigmoid profile than does liver, thus indicating that gill is a more sensitive organ than liver when tilapia are exposed to waterborne arsenic. The gill is an important site of accumulation for many transition metals (Sorensen 1991) and organic pollutants (Landrum *et al.* 1996). Furthermore, gills are the primary sites of toxicity because metal-induced mortality in freshwater fish occurrs by way of distribution of branchial ion regulation (Lauren and McDonald 1987a, b).

Internal Effect Concentration–Time Response Relationships

The fit of the AUC-based TIC toxicity model may be strongly determined by the input parameters. Therefore, uncertainties in the k_2 and g values, which are input parameters in the TIC toxicity model, affect the validation of the model. Generally, the experimental $LC_{50}(t)$ data for tilapia exposed to waterborne arsenic support the validity of the TIC toxicity model ($r^2 = 0.80$, $p < 0.05$) despite that fact that k_2 was estimated from a 7-day exposure test in 1 mg L^{-1} arsenic solution and that g value was adapted from our unpublished data, which were estimated using uncontaminated water. Our analysis suggests that the use of internal whole-body burden for each individual mode of action as an interpretive and regulatory tool in the environmental risk assessment of waterborne metals might be limited to mode of actions.

Hermens (1989) and McCarty and Mackay (1993) also suggested that the concept of whole-body burden might not hold for chemicals exhibiting an irreversible adverse effect. A specific model of action, however, could also be complicated and misleading in estimating ecosystem concentrations and in comparing these concentrations with LC_{50} data. McCarty and Mackay (1993) also pointed out that the constant whole-body burden with respect to time and species for chemicals indicates the same mode of action; thus, the whole-body burden toxicity concept differs from the concept of the TIC toxicity model employed.

Time–mortality profiles can be expressed as functions of BCF, $k_2 + g$, $LC_{50}(\infty)$, and AUC_f. Our work used the AUC-based TIC toxicity concept to derive the internal effect concentration–time response relationships for each target organ under a specific waterborne arsenic exposure, which can be applied to time–mortality data, and estimated toxicologic parameters from the results from the traditional bioassay. Our results strongly suggest the applicability of the TIC toxicity concept because it demonstrates that toxicity is indeed dependent on the AUC of each target organ of tilapia. Therefore, a one-compartment, first-order toxicokinetic model coupled with an AUC-based toxicity model can describe and predict the time course of arsenic toxicity to tilapia.

Dose-Response Relationships

We employed a receptor-theory–based, pharmacodynamic model–based Hill equation to reconstruct dose–response relationships between organ-specific equilibrium arsenic concentrations in tilapia and their mortality effects. Therefore, the complete dose–response profiles and duration of effect can be predicted for aquatic biota exposed to any waterborne metal. To obtain accurate and precise parameter estimates for E_{max}, EC_{50}, and n, the observation of effects must include comprehensive ranges of concentrations (i.e., if $C < EC_{50}$), then E_{max} or EC_{50} will not be a proper estimate (Derendorf and Hochhaas 1995), and the fitted n value will not be estimated accurately. The 96-hour acute-toxicity bioassay data obviously provided a suitable range for parameter estimations and organ-specific dose–response relationship predictions (Fig. 3A).

Based on the Hill equation and one-compartment models, Wagner (1968) calculated AUC as a measure of the total pharmacologic response over 24 hours and then evaluated how AUC was affected by changing the dosing interval. Therefore, by kinetic coupling an appropriate toxicokinetic model (i.e., the time course of accumulation of the waterborne arsenic) with an AUC-based pharmacodynamic model (i.e., the time course of the adverse biologic response by each target site of tilapia to the accumulated arsenic), the complete dose–response profiles and duration of effect can be predicted for aquatic biota exposed to any waterborne metal.

The organ-specific, dose–response relationships indicate that gill has a more steep sigmoid profile than that of liver, thus indicating that gill is a more sensitive organ than liver in tilapia exposed to waterborne arsenic. The organ-related difference in the mortality dose–response observed in this study is noteworthy. The gill is an important site of accumulation for many transition metals (Sorensen 1991) and organic pollutants (Landrum *et al.* 1996). Furthermore, the gills are the primary sites of toxicity because metal-induced mortality in freshwater fish occurs through the distribution of branchial ion regulation (Lauren and McDonald 1987a, b). Under these conditions, gill appears to be a more sensitive and robust biomarker of short-term arsenic exposure than liver, intestine, and stomach (Daglish and Nowak 2002). Parsimoniously, tilapia gill may serve as a surrogate sensitive biomarker of short-term exposure to waterborne arsenic.

In conclusion, we may employ the derived internal effect and target organ concentrations in the site-specific risk assessment. We further suggest that replacing exposure-based external effect concentrations by internal effect concentrations is an effective means to measure inherent toxicity.

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