A Dose-Based Modeling Approach for Accumulation and Toxicity of Arsenic in Tilapia *Oreochromis mossambicus*

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ABSTRACT: We proposed an approach to relate metal toxicity to the concentrations of arsenic (As) in specific target organs of tilapia *Oreochromis mossambicus*. The relationships among As exposure, uptake, accumulation, and toxicity of tilapia were investigated using kinetic and dynamic modeling. The biouptake rate of waterborne As through the gills of fish was dependent on exposure concentrations, in that the relationship was well described by incorporating Michaelis–Menten type uptake kinetics. The fitted bioaffinity parameter and limiting uptake flux were 3.07 $\pm$ 2.21 μg/mL^{-1} (mean $\pm$ SD) and 2.17 $\pm$ 0.38 μg/mL^{-1}/d^{-1}, respectively, suggesting that a low As binding affinity of tilapia gills, yet a relatively high binding capacity was obtained. The toxicity of As was analyzed by determining the lethal exposure concentration associated with a mortality of 50% (LC50) at different integration times. Our results demonstrate that 96-h and incipient LC50s for tilapia are 28.68 (95% CI: 15.98–47.38) and 25.55 μg/mL^{-1}, respectively. The organ-specific internal residue associated with 50% mortality was estimated by combining the model-predicted toxicokinetic parameters and the area-under-curve (AUC)-based time-integrated concentration toxicity model. A physiologically based toxicokinetic model was constructed to elucidate the principle mechanisms that account for the observed data and to predict the kinetics of As in tilapia under different water exposure scenarios. We employed the Hill equation model to predict the organ-specific dose–response relationships. We used the liver as a surrogate of target sites to assess the As toxicity to tilapia because of its higher sensitivity to As toxic effects. The predicted mortalities never reach 50% when the tilapia were exposed to waterborne As $<$2 μg/mL^{-1}. The predicted mortality is, however, slightly higher than the observed values before the 10th day in that the profile reached the 70% maximum mortality, which is comparable to the observed data when the tilapia were exposed to 4 μg/mL^{-1}. Our results show that a dose-based toxicokinetic and toxicodynamic modeling approach successfully links metal exposure to bioavailability, bioaccumulation, and toxicity, under variable exposure scenarios. Environ Toxicol 21: 8–21, 2006.

Keywords: arsenic; tilapia; biouptake; toxicokinetics; toxicodynamics

INTRODUCTION

Arsenic (As) is widely distributed in water, soil, and organisms from natural and anthropogenic sources. Long-term ingestion of the groundwater contaminated by inorganic As has been found to induce blackfoot disease (BFD) in the southwestern coastal area of Taiwan (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. Farming tilapia (*Orechromis mossambicus*) is one of the most promising aquatic prod-

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ucts in the BFD area because of its high market value. Liao et al. (2003) conducted a series of field survey to investigate the As content in pond water and its accumulation in farmed tilapia from BFD area. Their study pointed out that the As concentrations in pond water ranged from 8.1 to 251.7 $\mu g/L^{-1}$ in that As content in several farming ponds persistently exceeds the water quality criteria for total As in the freshwater ecosystems (150 $\mu g/L^{-1}$) documented by the Criterion Continuous Concentration (USEPA, 2002). If As levels in pond water become high, severe effects may occur on the health of farmed fish, increasing the expenditure of cultivators, and even pose a potential risk to the public who are consume the farmed tilapia from BFD area (Liao and Ling, 2003).

We traditionally employed the environmental concentration as the surrogate for the chemical dose at the target site to produce a given chemical effect to aquatic animals, e.g., the median lethal concentration (LC50) and the lowest observed effect concentration. However, the recently promulgated concept of the body residue hypothesis states that the use of environmental chemical concentrations to gauge hazard could be misleading because the environmental concentration necessary to cause effect varies with the biouptake route, duration of exposure, type of exposure medium, and species used for testing (McCarty and Mackay, 1993). The chemical dose required to induce effects at the target site should not change significantly with routes of exposure or duration, indicating that the toxicity mechanism does not change and the damage dose does not accumulate over time (McCarty and Mackay, 1993; Sijm et al., 1993; Fisher et al., 1999). Therefore, the target organ/tissue or whole body residue remains an easier and more reliable way of referencing the dose with the continued assumption that the total accumulation reflects the concentration at the target site.

Aquatic animals have been observed in the field to accumulate metals at elevated concentrations in specific organs, such as the liver and intestine, and consequently impose the toxicity of metal to the animals (Labrot et al., 1999; McGeer et al., 2000; Hollis et al., 2001). Understanding this selective accumulation of As into target tissue of tilapia is important in predicting the time variable behavior of As under various exposure conditions. The construction and application of a physiologically based toxicokinetic (PBTK) model of As transfers in tilapia can provide a basis for increasing this understanding. The PBTK models have been developed in several aquatic species in the past years (Nichols et al., 1996; Thomann et al., 1997); however, only a few PBTK models in an aquatic animal have related the toxicological effect to the target organ concentration.

At present, data on biouptake, bioaccumulation, and the caused adverse effect mechanisms of As to tilapia are limited. The objectives of the present study were to characterize the time course of uptake process, toxicokinetics, and toxicodynamics of As in tilapia under water exposure route. We developed an algorithm to assess the risk under variable exposure scenarios, including use of Michaelis–Menten (M–M) type flux to quantitatively model and explicate the transport and biouptake mechanisms of gills of freshwater tilapia, followed by PBTK modeling to describe and predict the As distribution and selective accumulation in target organs and finally predict the sequent acute toxic effects by a toxicodynamic (TD) model.

MATERIALS AND METHODS

Bioaccumulation Tests

The present laboratory study was designed to examine the accumulation ability of As in the gills, carcass (head, muscle, bone, skin, and scale), alimentary canal, and liver of tilapia. The As contamination level was determined by a preliminary test. The median lethal tolerance of tilapia exposed to concentrations $\leq 1\ \mu g/mL^{-1}$ As was longer than 21 d. Therefore, we conducted an uptake experiment in As concentration of $1\ \mu g/mL^{-1}$ for 7 d, based on the suggestion by Suhendrayatna et al. (2001, 2002). The As concentrations are 20–50 times higher than those in field conditions so as to produce high As level in target organs of tilapia and to assess the toxicokinetics of As under sublethal exposure scenarios.

The experiments were carried out with 42 fish of a specific size class (mean body length = 10.67 ± 1.22 cm (mean ± SD) and mean weight = 35.46 ± 4.8 g/wet/wt). They were supplied by Taiwan Fisheries Research Institute, Cigu Township, Tainan, which are hatched in laboratory and considered to be uncontaminated by As. Tilapia were visibly free of any deformities, lesions, or diseases. Fish were kept in cool ice during transport from Tainan to the Ecotoxicological Modeling Center, Department of Bioenvironmental Systems Engineering, National Taiwan University, Taipei, Taiwan. Fish were allowed to acclimate to laboratory conditions for 2 weeks before exposure. All experiments were carried out in 54 L indoor rectangular fiberglass aquaria, full of 50 L As concentration of $1\ \mu g/mL^{-1}$. Dissolved oxygen in each tank was maintained at close to saturation by aeration (7.43 ± 0.4 $\mu g/mL^{-1}$). The temperature in each aquarium was maintained at 26.7 ± 0.34°C, using submerged heaters. The pH value was maintained at 7.73 ± 0.02. The photoperiod was 16 h light:8 h dark with an intensity of 1400 ± 100 lux. All experiments were assigned to two tanks. The As solution was replaced daily to avoid the regression of ambient water quality. The measured As concentration was 0.94 ± 0.072 $\mu g/mL^{-1}$. To analyze the As uptake by the fish, five fish were sequentially removed from each tank after 0, 1, 2, 4, and 7 d of exposure. An adequate portion of the gills, carcass, alimentary canal, and liver of each individual was collected. The

dissected tissue 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.

A Perkin-Elmer Model 5100PC atomic absorption spectrometer (Perkins-Elmer, Shelton, CT, USA) 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 per liter for water samples and 0.05 μg As per gram for tissue samples.

Data Analysis

The method to determine uptake and depuration rate constants for each target organ/tissue was by fitting concentration data to the integrated form of the kinetic equation for constant water exposure, using iterative nonlinear regression (Newman, 1998),

$$C_i(t) = C_i(0)e^{-k_{2i}t} + \frac{k_{1i}}{k_{2i}} C_w(1 - e^{-k_{2i}t}) \qquad (1)$$

where $C_i(t)$ is the time-dependent As concentration in the target organ i of tilapia ($\mu g/g^{-1}$), k_{1i} is the organ-specific uptake rate constant ($mL/g^{-1}/d^{-1}$), k_{2i} is the depuration rate constant (d^{-1}), t is the time in d, and C_w is the bulk water concentration of As ($\mu g/mL^{-1}$). The organ-specific bioconcentration factor (BCF_i) can be calculated as $BCF_i = k_{1i}/k_{2i}$, representing the net accumulation ability that is the result of the competition between uptake and depuration processes. An inherent assumption in first-order, diffusion-based bioaccumulation model is that the rate constants are independent of the concentration of chemical in water or fish and the duration of exposure (Cho et al., 2003; Clason et al., 2003).

We employed the nonlinear option of the Statistica® software (StatSoft, Tulsa, OK, USA) to perform all curve fittings. The Statistica was also used to calculate the coefficient of determination (r^2) and statistical analyses (analysis of variance and Student's t-test). Statistical significance was justified if p-values were less than 0.05.

Acute Toxicity Tests

Laboratory static bioassays were conducted to determine the 24 h, 48 h, 72 h, 96 h, 120 h, and 144 h LC50 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). Six fish of a specific size class (mean body length = 12.67 ± 5.65 cm (mean ± SD) and mean body weight = 31.72 ± 6.5 g/wet/wt) were randomly selected and transferred into each test aquarium.

The nominal concentrations of As tested were 0 (control), 1, 2, 4, 10, 30, 50, and 80 $\mu g/mL^{-1}$ (Hwang and Tsai, 1993). The measured As concentrations were 0.98 ± 0.05, 1.97 ± 0.04, 4.26 ± 0.09, 10.36 ± 0.06, 31.04 ± 0.12, 47.65 ± 0.06, and 81.53 ± 0.08 $\mu g/mL^{-1}$, respectively. Gross mortality of fish to each concentration was recorded every 1 h for the first 12 h and every 2 h thereafter for 144 h, and dead fish being removed every 1–2 h. Tilapia were not fed throughout the test. Control and test concentrations were conducted in two replicate tanks. The water quality management protocol is the same as deployed in the bioaccumulation tests. No mortality occurred in the controls.

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

Acute Toxicity Model

The area-under-curve (AUC)-based time-integrated concentration (TIC) toxicity model is based on a direct relationship between adverse effects and extent of inhibited molecules in the target tissue (Legierse et al., 1999; Lee et al., 2002). Liao and Ling (2003) suggested that the TIC model is applicable to describe the metal toxicity to fish since the toxicity is indeed dependent on the TIC of metals in fish. The TIC toxicity model employed in determining the time-dependent LC50 can be expressed as (Legierse et al., 1999; Lee et al., 2002) as follows:

$$\mathrm{LC50}(t) = \frac{\mathrm{AUC}_i}{\mathrm{BCF}_i}\left(\frac{k_{2i}}{k_{2i}t + e^{-k_{2i}t} - 1}\right) + \mathrm{LC50}_i(\infty) \qquad (2)$$

where AUC_i is the area under the internal burden of As in target organ i of tilapia versus time curve ($\mu g/d/g^{-1}$). With sufficient LC50(t) data, it is possible to calculate best-fit values of two toxicological parameters [AUC_i and $LC50_i(\infty)$] that appeared in Eq. 2 by an nonlinear regression technique.

Substitution of C_w in the one-compartment bioaccumulation model

$$C_i(t) = \mathrm{BCF}_i C_w(1 - e^{-k_{2i}t})$$

by LC50(t) in Eq. 2 and by regarding $C_i(t)$ as the internal lethal body residue at the target organ i that causes 50% mortality, $\mathrm{CL50}_i(t)$, leads to the following expression for $\mathrm{CL50}_i(t)$ as (Legierse et al., 1999; Lee et al., 2002)

$$\mathrm{CL50}_i(t) = \mathrm{AUC}_i\left(\frac{k_{2i}(1 - e^{-k_{2i}t})}{k_{2i}t + e^{-k_{2i}t} - 1}\right) + \mathrm{BCF}_i(1 - e^{-k_2 t})\mathrm{LC50}_i(\infty). \quad (3)$$

Equation 3 shows that the internal lethal body residue in tilapia can be expressed as functions of toxicokinetic parameters, k_{2i} and BCF_i, and toxicological parameters AUC_i and $\mathrm{LC50}_i(\infty)$. When the exposure time approaches infinity, Eq. 3 gives a relation among LC50(∞), CL50, and BCF as

$$\mathrm{CL50}_i(\infty) = \mathrm{LC50}_i(\infty) \times \mathrm{BCF}_i. \quad (4)$$

These estimated organ-specific $\mathrm{LC50}_i(\infty)$, AUC_i, and $\mathrm{CL50}_i(t)$ values provide the essential toxicokinetic parameters for predictions of the relationship between target organ residues and induced mortalities.

Biouptake Model

The clarifying of the mechanism of metal uptake through the gills is fundamental in aquatic toxicology because the initial uptake of chemical from water into the gill is followed by subsequent transfer to the blood for distribution throughout the body (Suhendrayatna et al., 2002). To describe the relationship between the As concentrations in the exposure solution and metal uptake, we consider the actual biouptake by gills that follow a M–M type of steady-state flux. This model gives a mechanistic description of the biouptake process characterized by a transport system, which can be represented by the uptake flux J_u (Redeker and Blust, 2004)

$$J_u = J_{u,\max}\left(\frac{C_w}{K_M + C_w}\right) \quad (5)$$

where $J_{u,\max}$ is the limiting uptake flux (μg/mL^{-1}/d^{-1}) and K_M is the bioaffinity constant of As (μg/mL^{-1}). Suhendrayatna et al. (2001, 2002) conducted a series of experiments to examine organ accumulation of As by *O. mossambicus* exposed to different level of sodium arsenite for 7 d. The optimal fit of Eq. 5 to the uptake flux of tilapia versus waterborne arsenite concentration of the 7 d exposure experiments, resulting in the estimated limiting uptake flux $J_{u,\max}$ and bioaffinity constant of the metal K_M.

PBTK Model

The PBTK modeling provides estimates of the time course of chemical concentration in the organ of interest. The PBTK models depict the complex chemical transportation and accumulation by a physiologically realistic compartmental structure. Nestorov (2003) pointed out that in contrast to the conventional toxicokinetic model, for example, the one compartmental bioaccumulation model, the structure of a PBTK model is developed from the anatomical and physiological structure of the target species instead of the available chemical-related toxicokinetic data.

The PBTK approach considers the body as series of compartments with physiologically based terms. The derivation of the required differential equations is based on the principle of mass balance and basic physiology. The following assumptions were made to develop the PBTK model: (1) each tissue compartment was well mixed and homogeneous, (2) tissue compartments were interconnected only through the circulatory system, (3) the system operates by first-order kinetics, and (4) a steady-state can be reached. To represent the principal features of the accumulation and transfer of As in tilapia, a five-compartment model was constructed as shown in Figure 1. The five compartments are blood (No. 1), carcass (primarily white muscle, skin and skeleton) (No. 2), gills (No. 3), alimentary canal (stomach, pyloric caeca, intestine, and other viscera) (No. 4), and liver (No. 5), respectively. We applied mass–balance differential equations to describe As uptake by the gills from water, delivery by blood to the tissues, distribution into the tissues, metabolism in tissues, and depuration from the fish into the water. An equilibrium assumption was applied to relate the dissolved As concentration in a specific compartment to the total As in that compartment. Thus, for the compartment i, the partition constant π_i (mL/g^{-1}) $= C_i/C_{\mathrm{b}i}$, where C_i is the total As concentration in compartment i (μg/g^{-1}) and $C_{\mathrm{b}i}$ is the As concentration in the blood leaving compartment i (μg/mL^{-1}), which is assumed in equilibrium with As concentration in the compartment. This expression is then substituted into each of the diffusive exchange terms. Thus, for the blood compartment (No. 1) (Fig. 1), the mass balance is given by

$$V_1\frac{dC_1}{dt} = Q_{12}\left(\frac{C_2}{\pi_2} - f_d C_1\right) + Q_{13}\left(\frac{C_3}{\pi_3} - f_d C_1\right) + Q_{14}\left(\frac{C_4}{\pi_4} - f_d C_1\right) + Q_{15}\left(\frac{C_5}{\pi_5} - f_d C_1\right) \quad (6)$$

where Q_{ij} is the diffusive exchange (mL/d^{-1}) of dissolved As, f_d is the dissolved fraction of total As concentration in

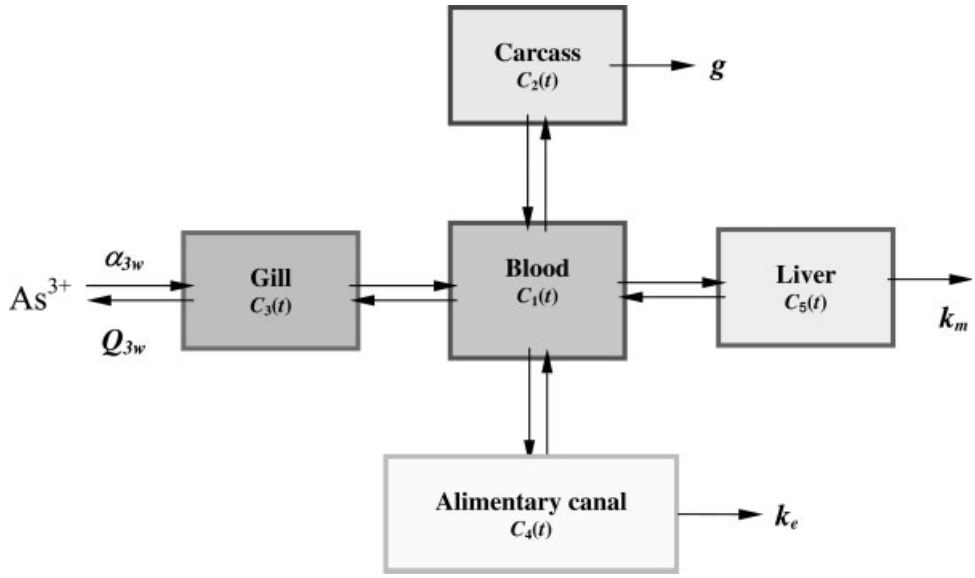

Fig. 1. Schematic of five-compartment PBTK model of tilapia, where g is the growth rate, k_e is the egestion rate, and k_m is the liver metabolite rate.

blood, and V_1 is the blood volume (mL). The loss rate from the liver and the alimentary canal was assumed to be a first order to the tissue and whole body wet weight. The resulting model equations are summarized in Table I. We performed all model exercises in Matlab (Version 5.2, the Mathworks, Natick, Massachusetts, USA). All steady-state analytical solutions and eigen values were derived or checked using the appropriate Matlab Symbolic Math Toolbox functions. We could not determine time-dependent solutions analytically, and thus integrated the differential equations numerically in Matlab.

Model Parameterization and Validation

The PBTK model is composed of terms involving physiological and physicochemical parameters. Physiological parame-

TABLE I. PBTK model equations applied to five-compartment of tilapia shown in Figure 1

No.	Compartment	Equation
1	Blood	$V_1\frac{dC_1}{dt} = Q_{12}\left(\frac{C_2}{\pi_2} - f_dC_1\right) + Q_{13}\left(\frac{C_3}{\pi_3} - f_dC_1\right) + Q_{14}\left(\frac{C_4}{\pi_4} - f_dC_1\right) + Q_{15}\left(\frac{C_5}{\pi_5} - f_dC_1\right)$
2	Carcass	$w_2\frac{dC_2}{dt} = Q_{21}\left(f_dC_1 - \frac{C_2}{\pi_2}\right) - gw_2C_2$
3	Gill	$w_3\frac{dC_3}{dt} = Q_{31}\left(f_dC_1 - \frac{C_3}{\pi_3}\right) + Q_{3w}\left(\alpha_{3w}C_w - \frac{C_3}{\pi_3}\right)$
4	Alimentary canal	$w_4\frac{dC_4}{dt} = Q_{41}\left(f_dC_1 - \frac{C_4}{\pi_4}\right) - k_ew_4C_4$
5	Liver	$w_5\frac{dC_5}{dt} = Q_{51}\left(f_dC_1 - \frac{C_5}{\pi_5}\right) - k_mw_5C_5$

See text for detailed symbol descriptions.

ters needed for a PBTK model for tilapia include tissue weights, growth rate, blood volume, and the exchange rates between tissue compartments. Tissue weights and blood volume are adapted from published bioaccumulation data (Liao et al., 2003). It is not possible to estimate all of the parameters for the PBTK model independent of the experimental data, because individual experiments for tilapia are not available. It is possible, however, to estimate from the literature, the order of parameters for the exchange rate between compartments and the physicochemical parameters, including organ-specific partition coefficient, gills sorption factors, and the fraction of As in the available plasma form. Therefore, a preliminary database from Thomann et al. (1997) regarding cadmium bioaccumulation in rainbow trout was adopted to estimate a range of model parameters with the 7 d organ-specific bioaccumulation data in lab and 300 d field data reported in previous article (Liao et al., 2003). Thomann et al. (1997) pointed out that the final set of parameters used to calibrate the data is hardly unique and variations may give equally reasonable calibration results.

The validity of the PBTK model of As was supported by the reasonable agreement between the model predictions and data for the concentration–time profiles of As in a variety of tissues. To compare modeled and observed results, the best fit was evaluated using root-mean-squared-error (RMSE), computed from

$$\mathrm{RMSE} = \sqrt{\sum_{n=1}^{N} (C_{m,n} - C_{s,n})^2 / N},$$

where N denotes the number of measurements, $C_{m,n}$ is the measurement data, and $C_{s,n}$ is the simulation result corresponding to data point n.

TD Model

The relationships between mortality and As doses in different target organs were fitted using an empirical three-parameter Hill equation model (Lalonde, 1992; Bourne, 1995) based on acute toxicity data associated with the relationship described in Eq. 4,

$$M_i = \frac{M_{\max} \times C_i^n}{\mathrm{CL50}_i^n(\infty) + C_i^n} = \frac{M_{\max} \times C_i^n}{(\mathrm{BCF}_i \times \mathrm{LC50}_i(\infty))^n + C_i^n} \quad (7)$$

where M_i is mortality for target organ i (%), C_i is the As concentration in target organ i (μg/g^{-1}), BCF_i is the bioconcentration factor for target organ i (mL/g^{-1}), $M_{\max}$ is the tilapia maximum mortality exposed to waterborne As, and n is a slope factor, or is referred to as the Hill coefficient, which means the number of chemical molecules that are required to bind to the receptor to produce functional effects or be thought of as an interaction coefficient, reflecting the extent of cooperativity among chemical and receptor (Weiss, 1997).

We can obtain the organ-specific time–mortality curves [$M_i(t)$] as functions of As concentration in target organ (C_i) and organ-specific toxicokinetic parameters (k_{2i}, BCF_i), and $\mathrm{LC50}_i$ (∞) by altering Eq. 7 to a time-dependent function and associated with C_i (t) from PBTK model for each target organ and $\mathrm{CL50}_i(t)$ in Eq. 3 as

$$M_i(t) = \frac{M_{\max} \times C_i^n(t)}{\mathrm{CL50}_i^n(t) + C_i^n(t)}. \quad (8)$$

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 the As concentration in water rather than in target tissue. Therefore, the mortality functions have to be expressed as the functions of C_w and LC50(t) data

$$M(t) = \frac{M_{\max} \times C_w^n}{\mathrm{LC50}^n(t) + C_w^n}. \quad (9)$$

We can estimate the best-fit value of Hill coefficient appeared in Eq. 9 by nonlinear regression, with sufficient data of percent mortality over a suitable As concentration in water associated with the specific interval of LC50 data.

RESULTS

Biokinetic Parameters and As Toxicity

Table II summarizes the toxicokinetic parameters for As calculated from target organs of tilapia exposure data. The 7 d water exposure experiment of As in the gills, liver, alimentary canal, and carcass of tilapia had significant correlated nonlinear regression profiles ($r^2 = 0.93$–0.96, $p < 0.05$), resulting from the best fit of the first-order one-compartment bioaccumulation model (Fig. 2). The organ-specific uptake rate constants (k_{1i}) range between 0.12 and 0.84 mL/g^{-1}/d^{-1}. The highest k_{1i} occurs in the alimentary canal, following by the liver, gills, and carcass, respectively. Carcass is the major biomass of tilapia yet shows relative lower uptake ability than other target organs. The depuration rate constants (k_{2i}) range from 0.001 to 0.20 d^{-1}. Our study revealed that the liver and alimentary canal are the organs having the best depuration ability, followed by gills and carcass. All of the organ-specific BCF_i values are above one (1.1–4.2), indicating that these target organs have potential to accumulate As when the tilapia are exposed to waterborne As. Many laboratory and field studies have revealed that many trace metals (Zn, Cu, Cr, Ni, Hg, Cd, and Pb) were accumulated in the intestine/stomach/liver than in the carcass of tilapia (Kureishy and D'silva, 1993; Liang et al.,

TABLE II. Estimates for uptake rate constant (k_{1i}), elimination rate constant (k_{2i}), and bioconcentration factor (BCF_i) during a 7-d As-exposure period for target organs of tilapia *O. mossambicus*

Target Organ	k_{1i}[a] ($mL/g^{-1}/d^{-1}$)	k_{2i}[a] (d^{-1})	BCF_i[b] (mL/g^{-1})
Gill	0.31 ± 0.07 (0.97)[c]	0.13 ± 0.09 (0.97)[c]	2.38
Liver	0.61 ± 0.13 (0.96)	0.20 ± 0.09 (0.96)	3.05
Alimentary canal	0.84 ± 0.18 (0.96)	0.20 ± 0.09 (0.96)	4.2
Carcass	0.12 ± 0.02 (0.93)	0.001 ± 0.06 (0.93)	1.1

[a] Mean ± 1SE.
[b] $BCF_i = k_{1i}/k_{2i}$.
[c] Coefficient of determination (r^2).

1999), demonstrating that intestine/stomach/liver plays a vital role in storing As in tilapia.

The selected time intervals of 24 h, 48 h, 72 h, 96 h, 120 h, and 144 h LC50 values with 95% CI of As to tilapia are given in Figure 3. LC50 lowers progressively as the duration of exposure increases. A limited number of studies have investigated As toxicity to tilapia. Our 96 h LC50s of As to tilapia is 28.68 (95% CI: 15.98–47.38 $\mu g/mL^{-1}$), which is close to the range of 96 h LC50 of As to seawater tilapia (26.5; 95% CI: 23.2–33.8 $\mu g/mL^{-1}$), yet lower than that of to freshwater tilapia (71.7; 95% CI: 67.8–76.4 $\mu g/mL^{-1}$) reported by Hwang and Tsai (1993).

Biouptake Parameters

We applied the M–M equation to involve the movement of As across the apical membrane into the tilapia gills and intracellular trafficking of As to the basolateral membrane. The shape of the curve obtained from M–M kinetics is hyperbolic, suggesting an initial carrier-mediated uptake process at the gills surface, followed by the rate-limiting extrusive step across the basolateral membrane (Bury et al., 1999). Figure 4 shows the optimal fits of Eq. 5 to the uptake flux of tilapia versus waterborne arsenite concentration of the 7 d exposure experiments by Suhendrayatna (2001, 2002). The limiting uptake flux ($J_{u,max}$) and bioaffinity constant (K_M) are estimated to be 2.17 ± 0.38 $\mu g/mL^{-1}/d^{-1}$ and 3.07 ± 2.21 $\mu g/mL^{-1}$, respectively, with a r^2 of 0.96.

The limiting uptake flux and bioaffinity constant differ widely among metals and among fish species. However, information on biouptake parameters of As uptake for tilapia gills is limited. We compared the present results with metal biouptake data of some species of teleost fish from other studies (Table III). The ratio of $J_{u,max}$ to K_M, or referred to as specific affinity (Harms and Bosma, 1997), is also given in Table III to depict how much the gill membrane reduces the metal concentrations at its surface. The M–M model allows a mechanistic description of the transport process in fish gills to be characterized by two biouptake parameters, K_M and $J_{u,max}$. It can be seen in Table III that the gills exhibit relatively significant differences in both of the binding affinity and the binding capacity for various metals, a result in line with that proposed by Reid and McDonald (1991). The K_M value, the inverse of binding affinity of the transport metal, of As binding to tilapia gills was found to be the highest among various metals and different species of teleost fish, except for that of Ag binding to the gill of rainbow trout.

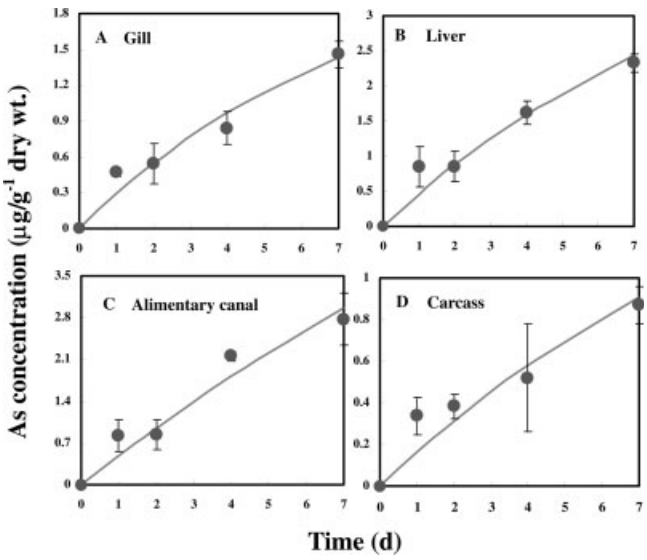

Fig. 2. Experimental-derived As profiles in the (A) gills, (B) liver, (C) alimentary canal, and (D) carcass of tilapia *O. mossambicus* exposed to 1 $\mu g/mL^{-1}$ As for 7 day bioaccumulation. Symbols are averages with standard errors ($n = 3$). The lines are best-fit regression curves from first-order bioaccumulation models of each target organ.

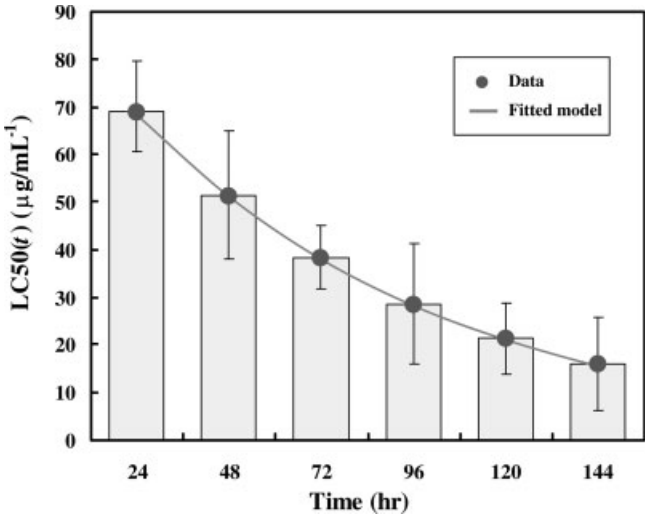

Fig. 3. LC50(t) values (mean ± 95% CI) of As to tilapia for selected time intervals.

The specific affinities in Table III reveal that the class A metals of an oxygen-seeking metal group (i.e., Ca) exhibit a strong tendency to bind to the gills of teleost, whereas the class B metals of a sulfur- or nitrogen-seeking metal group (i.e., Cd, Cu, and Ag) have a relative low gill-metal binding rate. The specific affinities of borderline metals (i.e., Zn and As) are closer to that of class B metals. These results imply that the dominant metal receptors at the gill surface of teleost consist of oxygen-rich centers in that Zn and As may share the similar binding site with the class B metals.

Organ-Specific As Toxicokinetics

Figure 5 displays the results of the model prediction comparing with the measured data of the temporal profiles obtained from the 7 d laboratory bioaccumulation bioassay and 300 d real tilapia farms in BFD area, respectively. In general, the PBTK model accurately described As kinetics in the target organs of tilapia. Table IV lists the final set of the input parameters used in the PBTK model implementation. Table V lists the RMSE values for the model performances, indicating that each RMSE value is more than 1 SD of the gills, liver, alimentary canal, and carcass during bioaccumulation experiment. The predicted values are within the error limits of the field observations and the RMSE values are more than 1 SD in the liver, as shown in Table V.

The simulation results of 300 d field data reveal that the As concentrations in the gills reached steady-state (1.46 μg/g^{-1}) in 24 d. The predicted values in carcass approached steady-state condition in 120 d, and they are slightly higher than the measured values, resulting from applying the higher partition coefficient or ignoring some eliminating mechanisms, i.e., elimination by skin, in the model. The estimated blood residue approach steady-state in 120 d (Fig. 5I), and the residues of As in the blood are higher relative to the liver, gills, and carcass yet similar to alimentary canal, revealing that the blood of tilapia has higher potential to induce As from external medium and connecting tissues.

Predictions of Dose-Based Mortality

A dose–response relationship between equilibrium As concentration in each target organ of tilapia and mortality was predicted by using Eq. 7, and the estimate of Hill coefficient (n) was obtained by optimal fitting of the Eq. 9 to the measure data by nonlinear regression. The optimal fits of Eq. 9 to the observed percent mortality of tilapia versus waterborne As concentration of the 96 h acute toxicity test result in the estimated Hill coefficient, $n = 4.07$ ($r^2 = 0.93$, $p < 0.05$) (Fig. 6A). Our simulations show that the carcass and liver have relative steep sigmoid dose–response profiles with mortalities approaching 100%, whereas the gills and alimentary canal have lazy sigmoid dose–response profiles (Fig. 6B). Therefore, we used the liver as a surrogate of the target site to assess the As lethal toxicity to tilapia because of its higher sensibility to mortality and higher BCF value.

We substituted $C_i(t)$ of the liver obtained from PBTK model and $CL50_i(t)$ in Eq. 3 into Eq. 8 to obtain the time-mortality profiles as functions of toxicokinetic parameters of liver [k_{2i}, BCF_i, and $LC50_i(\infty)$] and varied waterborne As concentrations ranging from 1 to 50 μg/mL^{-1}. The predicted mortalities by using liver as surrogate target

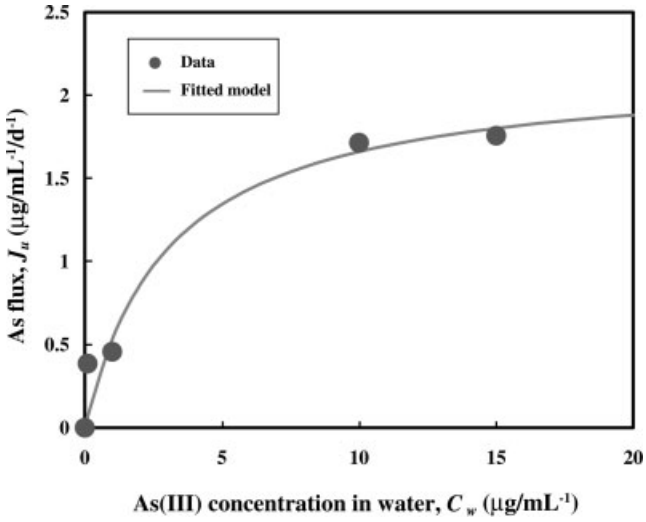

Fig. 4. Optimal fits of published concentration-flux data by Michaelis–Menten equation.

TABLE III. Biouptake parameters of various metals in some species of teleost fish

Species/Metals	Bioaffinity Constant, K_M (μg/mL^{-1})	Limiting Uptake Flux, $J_{u,max}$ (μg/mL^{-1}/d^{-1})	$J_{u,max}/K_M$ (d^{-1})	References
*Tilapia (*O. mossambicus*)*				
Ca	0.001	2.11	2110	Chang et al. (1997)
Ca	0.056	–	–	Flik et al. (1993)
Cd	0.015	0.22	14.47	Wong and Wong (2000)
As	3.07	2.17	0.71	This study
Rainbow trout (*O. mykiss*)				
Zn	0.278	0.38	1.36	Hogstrand et al. (1998)
Zn	1.17	0.52	0.44	Galvez et al. (1998)
Ca	1.35	41.76	30.98	Hogstrand et al. (1998)
Cu	0.76	0.08	0.11	Campbell et al. (1999)
Ag	6.76	–	–	Bury et al. (1999)
Common carp (*C. carpio*)				
Zn	0.22	3.57	16.23	Van Ginneken et al. (1999)
Cd	0.038	1.01	26.58	Van Ginneken et al. (1999)

site never reach 50% when the tilapia are exposed to waterborne As <2 μg/mL^{-1}, which agree with the data of our acute toxicity bioassay (Fig. 7). The predicted mortality was slightly higher than the observed values before 10 d and reached the 70% maximum mortality, which is comparable to the observed data when the tilapia were exposed to 4 and 10 μg/mL^{-1}of As.

DISCUSSION

We investigated the relationships among As exposure, accumulation, and toxicity by employing kinetic and dynamic modeling. The biouptake rates are depended on waterborne As concentrations, and the involved mechanisms could be well described by incorporating of M–M type uptake and integrating with the uptake rate constants, which were derived from compartmental models. The binding affinity of a metal for biological ligands is a function of ligands chemistry and types of bond formation, yet the characteristics of actual binding sites of fish gills are not entirely known (Reid and McDonald, 1991). However, with a low binding affinity, we could expect a less permissive As entry to the branchial membrane of tilapia. The specific affinity ($J_{u,max}/K_M$) represents a measure of affinity and has a unit of d^{-1}, thus to some extent it stands for the rate of gill-metal binding.

We employed the PBTK model to elucidate the major mechanisms that account for the observed data and utilized the model to describe the kinetics of As in tilapia under different exposure concentrations. The advantages of the PBTK model contrast with the traditional kinetics models in two ways. First, the PBTK model involves mechanical information regarding the toxic effect, including the presence of physiological and biochemical parameters, helping us to acquire the inherent bioaccumulation knowledge. Second, the PBTK model allows more reliable residue extrapolations for metals over a wide range of different exposure scenarios. Clason et al. (2003) and McGeer et al. (2003) pointed out that the first-order BCF-based bioaccumulation model for metals is only applicable for residue predictions in lower range of exposure conditions, where the uptake process is not limiting the rate of uptake.

The PBTK model, however, is also associated with certain restrictions and disadvantages that are needed to be identified before extensively applications. Firstly, the schematic representation of a PBTK model is normally fairly complicated, requiring a large number of physiological inputs that may not be easily accessible or even available. The kind of physiological measurements needed, if not available in the literature, are often extremely difficult and time consuming to measure (Nestorov, 2003). In addition, measurement of these physiological parameters are acquired with great effort, and they may be quite variable because of the differences in the experimental set-up and animal preparation (Yang et al., 2000). Thus, the PBTK model can only be applied following the collection of physiological information for this fish, which is vital for accurate estimation of toxicant loading using this model.

In this study, a receptor theory-based TD model representing by a modified Hill equation model is used to construct dose–response relationships between organ-specific equilibrium As concentrations in tilapia and their mortality. Therefore, the complete dose–response profiles and the

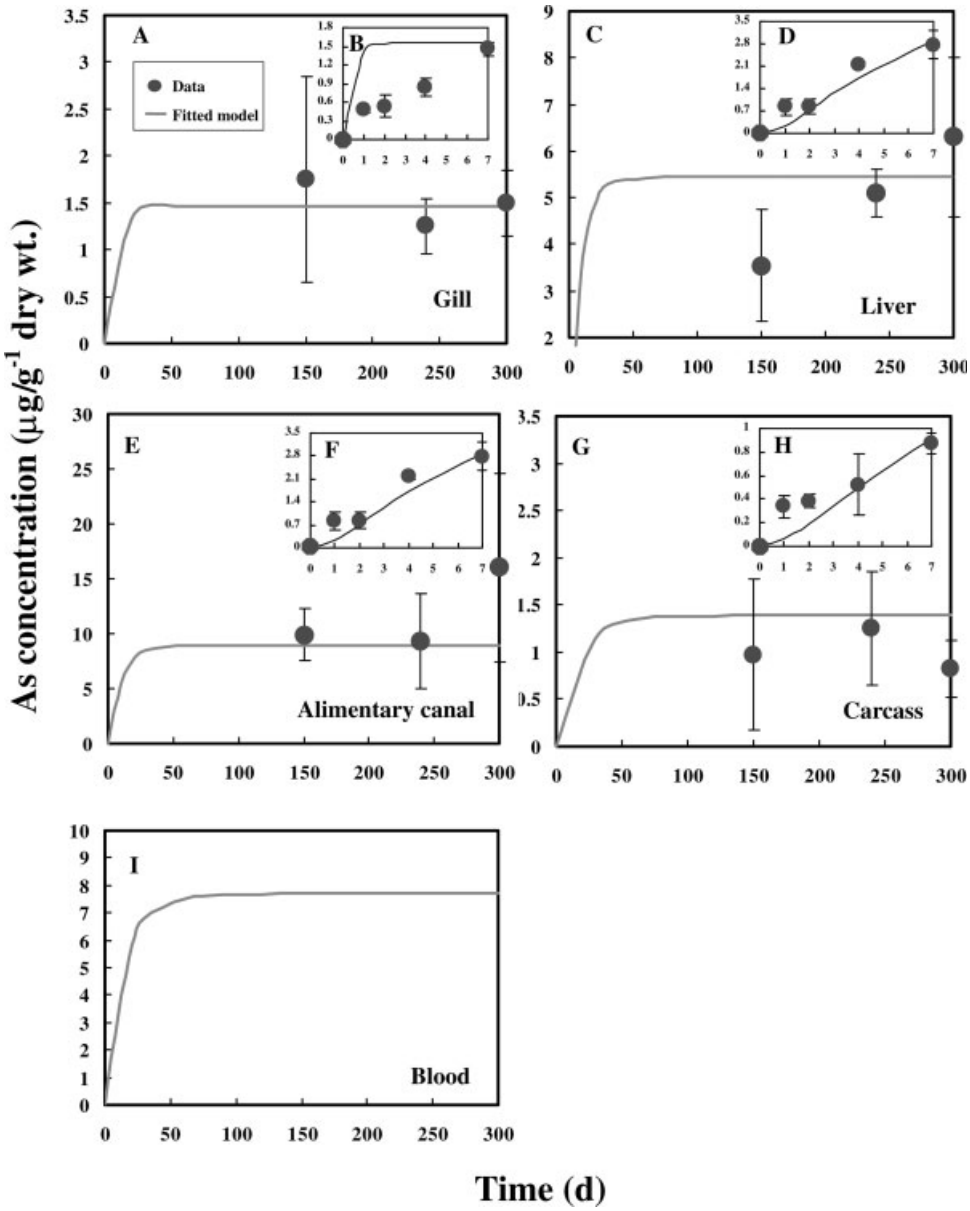

Fig. 5. Comparisons of model to measured As concentrations (mean ± SD) obtained from 300 day field data (A, C, E, and G) and 7 day laboratory exposure experiment (B, D, F, and H) for the gills, liver, alimentary canal, and carcass. The simulation of blood compartment is also shown (I).

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 have to include comprehensive ranges of concentrations, i.e., if $C_w < EC_{50}$, E_{max}, or EC_{50} will not be properly estimated (Venitz, 1995) and the obtained n value will not be correctly estimated. In our study, the acute toxicity bioassay data obviously [Fig. 6A] provided a suitable source for parameter estimations and following organ-specific dose–response relationships predictions. Our TD model describes the dose–response relationships well, although some values are overestimated during initial time span in some higher exposure scenarios. Input parameters to PBTK model are usually a point estimate or a mean value. We assumed that parameters are held constant despite evidence that they changed somewhat as tilapia grew, or in different exposure conditions, because these changes have no significant influence on model outputs of

TABLE IV. Physiologically-based parameters used for PBTK model simulation[a]

Symbol	Description	Estimated Value
Physiological parameters		
Q_{3w}	Gill-water exchange rate (mL/d^{-1})	10
$Q_{12} = Q_{21}$	Blood-carcass exchange rate (mL/d^{-1})	1800
$Q_{13} = Q_{31}$	Blood-gill exchange rate (mL/d^{-1})	260
$Q_{14} = Q_{41}$	Blood-alimentary canal exchange rate (mL/d^{-1})	3500
$Q_{15} = Q_{51}$	Blood-liver exchange rate (mL/d^{-1})	5040
V_1	Blood volume (mL)	2[b]
w_2	Weight of carcass (g)	27.7[b]
w_3	Weight of gills (g)	1.2[b]
w_4	Weight of alimentary canal (g)	0.63[b]
w_5	Weight of liver (g)	0.29[b]
w_t	Whole fish weight (g)	31.8[b]
g	Growth rate (d^{-1})	0.099[c]
k_e	Egestion rate (d^{-1})	0.02
k_m	Liver metabolite rate (d^{-1})	0.0845
Physicochemical parameters		
α_{3w}	Gill sorption factor (--)	8
f_d	Fraction As dissolved in blood	0.2
π_2	Partition coefficient of carcass (mL/g^{-1})	2400
π_3	Partition coefficient of gill (mL/g^{-1})	50
π_4	Partition coefficient of alimentary canal (mL/g^{-1})	1,000,000
π_5	Partition coefficient of liver (mL/g^{-1})	3600
C_w	Water concentration (μg/mL^{-1})	94

[a] Calibrated from Thomann et al. (1997) and field observations adopted from Liao et al. (2003).
[b] This study.
[c] Unpublished data.

interest (Nichols et al., 1998; Nestorov, 2003). Our study reveals that the assumptions of toxicokinetic parameters are constant and have to be addressed in future studies, especially when applying to the exposure conditions that are dramatically different from the bioassays conditions.

Representing the chemical dose by bioaccumulation data could be a better means of assessing the hazard of real world exposures, if the toxicological significance can be correlated to the bioaccumulation data. It has been reported that body residues for a toxic response of narcotic compound are not discriminatory (Pawlisz and Peter, 1993), if this is universal, body residues may not be useful in referencing the chemical dose to toxicity. Our study indicates that using body residues to assess As toxicity to tilapia are both sensitive and discriminatory.

Another problem using body residues may be associated with the presence of metabolites. Metabolites are usually included in total chemical amounts in laboratory tests, thereby overestimating the critical body residue unless the metabolites contribute equally to the effect. Suhendrayana et al. (2001) indicated that inorganic As will be metabolized into methylated As compounds, which have lower hazard potency than that of inorganic As after been taken from water or diet routs. They reported that the content of methylated As compounds to the total amount in the tissues of tilapia ranges from 25 to 93%; however, our study treated

TABLE V. Root-mean-squared-error (RMSE) (μg/g^{-1}) between measured concentration and simulated concentration in various organs of tilapia

Measured Data	Gill	Liver	Alimentary Canal	Carcass
Bioaccumulation experiment	0.63 (0.15)[a]	0.43 (0.26)	1.22 (0.42)	0.58 (0.15)
Field investigations	0.21 (0.58)	0.89 (0.87)	0.28 (0.72)	0.20 (0.93)

[a] 1 SD value of bioaccumulation experiment and field investigations.

the total As content as the reference dose to cause effects, which may lead us to underestimate the As toxicity. Thus, the extent of metabolism must be determined, and the toxicity of metabolites must be assessed.

In conclusion, using organ-specific concentration as the reference dose has distinct advantages over using environmental concentrations, despite some difficulties and limitations. In referencing toxicity to target tissues residues, bioaccumulation data can be explained more meaningfully. Bioaccumulation data themselves have little meaning beyond confirmation of exposure and bioavailability. Making a connection between accumulated dose and toxicological effects will permit better interpretation of the hazard associated with complex exposure such as occurs with multiple exposure routes, or from matrices such as sediment where bioavailability and exposure routes are not readily predicted. Interpretation of field data will also be improved.

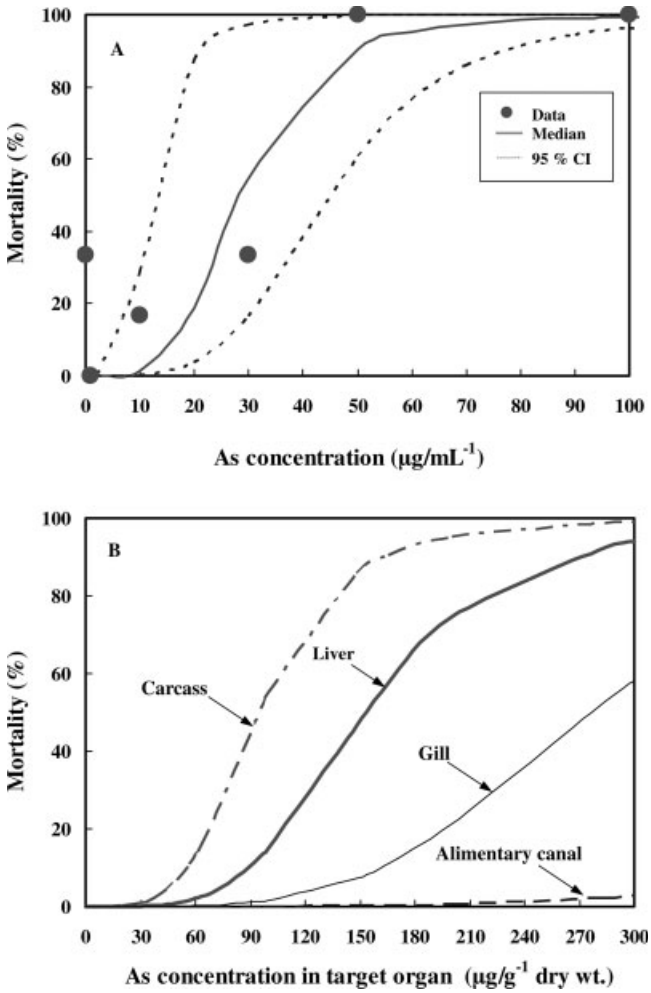

Fig. 6. (A) Optimal fit of Hill equation model to the observed percent mortality of tilapia versus waterborne As concentration in the acute toxicity bioassay with 95% CI, where the 96 h LC50 is 28.68 (95% CI: 15.98–47.38) and (B) derived organ-specific dose–response relationships between equilibrium internal effect concentration of As and mortality effects for tilapia *O. mossambicus*.

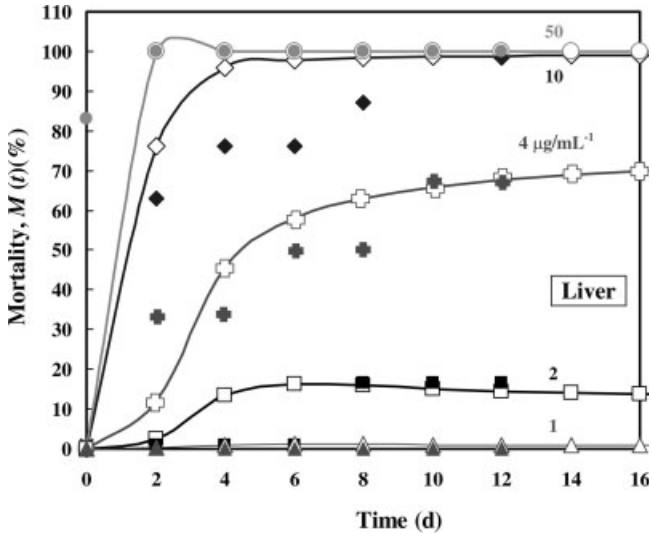

Fig. 7. Prediction of time-mortality of tilapia exposed to waterborne As, ranged from 1 to 50 μg/mL^{-1}, by using the liver as a biomarker. Solid symbols are the measured data from the acute toxicity bioassay, and the corresponding open symbols are the predicted values from Eq. 8.

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