A biotic ligand model-based toxicodynamic approach to predict arsenic toxicity to tilapia gills in cultural ponds

Bo-Ching Chen · Wei-Yu Chen · Chung-Min Liao

Abstract Farming of tilapia *Oreochromis mossambicus* is an important aquacultural activity in Taiwan. Due to the elevated arsenic (As) concentration in pond water, it is important to assess the bioavailability and toxicity of As to tilapia for protection of aquatic life and human health. In the present study, we developed a biotic ligand model (BLM)-based toxicodynamic approach to dynamically predict both acute and chronic effective concentrations of As to tilapia in two tilapia farms located at Pudai and Chiangchun counties in southwestern Taiwan. Parameters revealed in the mechanistic model were obtained by fitting this model to the toxicokinetic and toxicodynamic data from our previous laboratory experiments. Based on our extended BLM concepts, the site-specific water effect ratios and ambient water quality criteria can be determined with known water chemistry. The proposed methodology was capable of bridging the gap between laboratory toxicity bioassays and field investigations. With respect to risk assessments, our research may also provide an useful means of generating and adjusting the site-specific ambient water quality criteria.

Keywords Arsenic · *Oreochromis mossambicus* · Biotic ligand model · Water effect ratio · Toxicodynamic

B.-C. Chen
Department of Post-Modern Agriculture, MingDao University, 52345 Changhua, Taiwan, Republic of China

W.-Y. Chen · C.-M. Liao (✉)
Department of Bioenvironmental Systems Engineering, National Taiwan University, 10617 Taipei, Taiwan, Republic of China
e-mail: cmliao@ntu.edu.tw

Introduction

Arsenic (As) is ubiquitous in the environment from both anthropogenic and natural processes (Liao et al. 2003). Humans may be exposed to As through many sources such as food, water, air and soil; dietary intake is the major exposure route (Yost et al. 2004). It has been generally recognized that inorganic As is a potent human carcinogen of skin, lung, bladder and kidney (Vahter 2002; Chen et al. 2005). Previous investigation indicated that there is a strong relationship between As concentrations in artesian well water and black foot disease (BFD) of residents in southwestern coastal areas of Taiwan (Chen et al. 2001). Nowadays, most of the people living in these areas do not drink well water because tap water has been made available in this area. Groundwater, however, is still used for aquaculture.

Farming of tilapia (*Oreochromis mossambicus*) is the most popular aquacultural type in the BFD-endemic areas because of its high market value. In our previous studies, the 24–96 h LC50 (median lethal concentration) of As to tilapia ranged from 69.06 to 28.68 mg l^{-1}, indicating a low toxicity of As to tilapia in the aquacultural systems (Liao et al. 2003; Liao and Ling 2003). The As concentrations in various tissues of tilapia in BFD-endemic areas, however, are relative higher than the background levels (Liao et al. 2003). A probabilistic risk assessment further indicated that the consumption of cultured tilapia from the BFD-endemic areas possibly poses a potential risk to human health (Liao and Ling 2003). At present, however, data on the uptake mechanisms of As to tilapia are limited. Consequently, the development of a mechanistic approach to delineate and predict the uptake and elimination processes of As to tilapia is important not only for generating site-specific ambient water quality criteria, but also for assessing

potential human health risks through consumption of cultured fish from the BFD-endemic areas.

Owing to direct contact with ambient water, gills are proposed to be the first and most important targets of fish exposed to waterborne metals (Playle 1998; Wong and Wong 2000; Tao et al. 2000). Several studies also indicated that the major route of uptake for metals that concentrate in fish is across the gill epithelium (Pelgrom et al. 1997; Bury et al. 1999). They concluded that gill uptake is expected to dominate total biouptake from water. Szebedinszky et al. (2001) demonstrated that initial uptake from water into the gills is followed by subsequent transfer to the blood for distribution throughout the body. Hence, in order to clarify the movement of a metal into an organism, it is fundamental to develop a clear understanding of the mechanism of metal uptake through the organism gills in the aquatic ecosystems.

The biotic ligand model (BLM), derived from the gill surface interaction model and the free ion activity model, has been widely used to predict the toxicological effects of metals on aquatic organisms in the last decade (Paquin et al. 2000; Niyogi and Wood 2004; Bielmyer et al. 2007). In the BLM, the metal toxicity is resulted from the free metal ions reacting with the binding sites (i.e., biotic ligand) at the site of action for aquatic organisms. The surface membrane of the gill, considered a negatively charged ligand, is widely recognized as the biotic ligand of fish (De Schamphelaere and Janssen 2002; Paquin et al. 2002; Morgan and Wood 2004). In practice, the BLM has been successfully applied to predict both acute and chronic toxicity of metals on aquatic organisms (De Schamphelaere and Janssen 2002; Schwartz and Vigneault 2007).

Given that both physiological parameters of aquatic organisms and geochemistry parameters of ambient water are considered, this approach is of potential utility to develop and refine the ambient water quality criteria (Paquin et al. 2002; Niyogi and Wood 2004). The integration between BLM and toxicology can further be used to describe metal-gill binding interactions and to predict metal toxicities to aquatic organisms in the field situation.

The purpose of this study is to develop a biotic ligand model-based toxicodynamic approach which can greatly improve our ability to predict the acute and chronic toxicity of As to tilapia in the field situation. Parameters calibrations were achieved by fitting the mechanistic model to the toxicokinetic and toxicodynamic data from our previous laboratory experiments. Model simulations were performed by coupling physiological parameters of tilapia and geochemical parameters of ambient water to predict toxicological effects of As to tilapia of field circumstances. Results of the present study may be helpful while generating site-specific ambient water quality criteria.

Materials and methods

Model development

In light of the BLM concept, the concentration of metal actually bound to the biotic ligand (BL) sites depends on several factors. The crucial factors include the activity of the free metal ion, the complexation capacity of the BL, the concentration of unoccupied BL sites, the affinity (stability) constant for metal ion binding to BL sites at equilibrium, and the concentrations of all cations that compete with the metal for binding sites on the BL (De Schamphelaere and Janssen 2002). In the present study, we link the gill arsenic burden in the BLM scheme and a one-compartment uptake-depuration model at steady-state condition (Liao et al. 2007) to estimate the concentration of unoccupied BL sites at the surface membrane of tilapia gills.

$$[AsBL]_T = [BL^-] \, [a] \, \{As^{5+}\} \approx \frac{k_1}{k_2} \, \{As^{5+}\}$$
$$= BCF \, \{As^{5+}\}, \tag{1}$$

where $[AsBL]_T$ is the steady-state gill arsenic burden ($\mu mol \, g^{-1}$), $[BL^-]$ is the concentration of unoccupied gill BL sites ($\mu mol \, g^{-1}$), $\{As^{5+}\}$ is the activity of the free arsenic ion (nM) based on As^{5+} being the most prevalent in most surface waters (Ferguson and Gavis 1972). k_1 ($l \, g^{-1} d^{-1}$) and k_2 (d^{-1}) are the uptake and depuration rate constants of tilapia to arsenic, respectively, BCF ($l \, g^{-1}$) is the bioconcentration factor, and $[a]$ (nM^{-1}) is an affinity (stability) constant-dependent parameter.

De Schamphelaere and Janssen (2002) have developed a BLM-scheme-based equation to describe the response time (t_R)-dependent effective concentration at X% effect as

$$ECX(t_R)_{AsBL} = \frac{f_{AsBL}^{X\%}(t_R)}{\left(1 - f_{AsBL}^{X\%}(t_R)\right)} \left(\frac{[b]}{[a]}\right), \tag{2}$$

where $ECX(t_R)_{AsBL}$ is the response time-dependent effective concentration at X% effect, $[b] = 1 + K_{CaBL}\{Ca^{2+}\} + K_{MgBL}\{Mg^{2+}\} + K_{NaBL}\{Na^+\} + K_{HBL}\{H^+\}$ in that K_{CaBL}, K_{MgBL}, K_{NaBL}, K_{HBL} are the stability constants for the binding of these cations to the BL (M^{-1}); and {ion} denotes the activity of each ion of water chemistry characteristics (M), and $f_{AsBL}^{X\%}(t_R)$ is the response time (t_R)-dependent fraction of the total number of arsenic binding sites occupied by arsenic at X% effect. Toxicological endpoint considered in the present study was median lethal concentration (LC50) and growth inhibition concentration at 10% effect (IC10) for acute and chronic exposure condition, respectively.

Incorporating $[a] = BCF \times [BL^-]^{-1}$ obtained from Eq. 1 and assumed $f_{AsBL}^{X\%}(t_R) \approx c + d \exp(-t_R/e)$(Liao et al. 2007) into Eq. 2, we obtain

$$\mathrm{ECX}(t_R)_{\mathrm{AsBL}} = \frac{c + d \exp(-t_R/e)}{(1 - (c + d \exp(-t_R/e)))} \times A_0, \qquad (3)$$

where $A_0 = [b]\,[\mathrm{BL}^-]\,\mathrm{BCF}^{-1}$. The coefficients c, d, e, and A_0 can be estimated by fitting Eq. 3 to $\mathrm{ECX}(t_R)$ data obtained from the bioassays. Subsequently, the concentration of unoccupied gill BL sites ($[\mathrm{BL}^-]$) and BLM parameter $[a]$ can also be calculated by known values of $[b]$ and BCF obtained from laboratory uptake-depuration experiment.

A BLM-based Hill dose-response curve can thus be reconstructed without any prior knowledge of stability constant-dependent $[a]$ value describing the binding behavior of free arsenic ion,

$$R(t_R, \{\mathrm{As}^{5+}\}) = \frac{R_{\max}(t_R)}{1 + \left(\frac{\mathrm{ECX}_{\mathrm{AsBL}}(t_R)}{\{\mathrm{As}^{5+}\}}\right)^{n(t_R)}}, \qquad (4)$$

where $R(t_R, \{\mathrm{As}^{5+}\})$ is the time-dependent response (% response) based on As^{5+}-activity $\{\mathrm{As}^{5+}\}$ (M) at any given response time t_R, $R_{\max}$ is the response time-specific maximum response (%), and $n(t_R)$ is a response time-dependent Hill coefficient which is a measure of cooperativity. A value of $n > 1$ indicates positive cooperativity.

Biokinetic and geochemical parameters

Parameterization of the BLM-based toxicodynamic model involved selecting datasets and deriving input distributions. In the present study, the stability (or affinity) constants (log K) of biotic ligand-cation were adopted from a critical review which summarized several available versions of the acute and chronic BLM developed for fathead minnow *P. promelas* and *D. magna* (Niyogi and Wood 2004). To account for the uncertainty/variability of log K in different versions, we optimal fitted the lognormal distributions for log Ks (Table 1).

The bioaccumulation parameters of k_1, k_2, and BCF for *O. mossambicus* exposed to arsenic were obtained from Liao et al. (2004) (Table 1). The experiments were carried out with 42 fish, aged 8–9 months, of a specific size class (body length 12.94 ± 1.54 cm (mean $\pm$ SD) and body weight 32.75 ± 4.2 g wet weight). Toxicological parameters (i.e., LC50 and IC10) were determined by fitting the Hill equation to the laboratory experimental data obtained from previously acute and chronic bioassays (Liao et al. 2004; Tsai and Liao 2006). Based on the fitted dose-response curves, the 48, 96, 120, and 144 h- LC50 values could be estimated to be 51.52, 28.68, 21.41, and 15.98 mg l^{-1}, respectively. The response time-specific IC10 values were 0.37, 0.35, and 0.36 nM for response time of 2, 3, and 4 weeks, respectively.

Table 1 Distributions and point values of affinity constants of biotic ligand-cation and bioaccumulation parameters used in the proposed model

	Affinity constants (M^{-1})	
	Acute toxicity	Chronic toxicity
log K_{MgBL}	3.58[a]	LN (2.88, 1.11)[c]
log K_{HBL}	5.40[a]	LN (6.36, 1.06)[c]
log K_{CaBL}	LN (3.53, 1.03)[b,c]	LN (3.40, 1.08)[c]
log K_{NaBL}	LN (3.09, 1.04)[b,c]	LN (2.57, 1.17)[c]
Bioaccumulation parameter[d]		
k_1 (ml g^{-1} d^{-1})		0.31 ± 0.086
k_2 (d^{-1})		0.028 ± 0.11
BCF (ml g^{-1})		11.07

[a] Adopted from Niyogi and Wood (2004)

[b] Adopted from Liao et al. (2007)

[c] Lognormal distribution with a geometric mean and a geometric SD

[d] Adopted from Liao et al. (2004)

Model simulations were carried out by introducing the affinity constants, the bioaccumulation parameters, the toxicological parameters, and the field data of water chemistry characteristics into Eq. 3 to calculate the response time-dependent effective concentration. Two tilapia farms with known water chemistry characteristics located at Pudai and Chiangchun counties in the BFD-endemic areas were selected to implement the proposed model (Table 2).

Statistical analyzes

Monte Carlo simulation was performed to obtain 2.5th and 97.5th percentiles as the 95% confidence interval (CI). TableCurve 2D (Version 5, AISN Software Inc., Mapleton, OR, USA) was used to optimal fit the published data to obtain the optimal statistical models. WHAM (Windermere humic aqueous model) Version 6 (WHAM VI, Centre for Ecology and Hydrology, Lancaster, UK) was performed to calculate the activities of the competing cations considered in the present study. The default inorganic arsenic form in WHAM is arsenate ($\mathrm{AsO_4}^{3-}$). Crystal Ball$^{\circledR}$ software (Version 2000.2, Decisioneering Inc., Denver, Colorado, USA) was employed to implement the Monte Carlo simulation.

Results

BLM-based dose-response profile

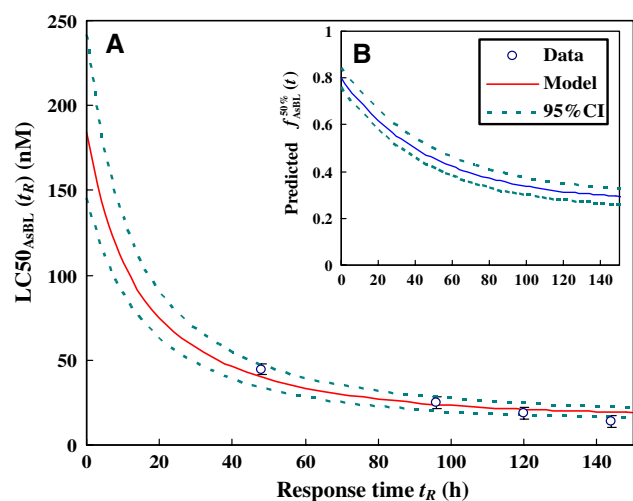The time-dependent fraction of the total number of arsenic binding sites occupied by arsenic at X% effect, $f_{\mathrm{AsBL}}^{X\%}(t_R)$,

Table 2 Site-specific water chemistry features from official published measured ion concentrations for tilapia farms in Putai, Chiangchun and laboratory conditions
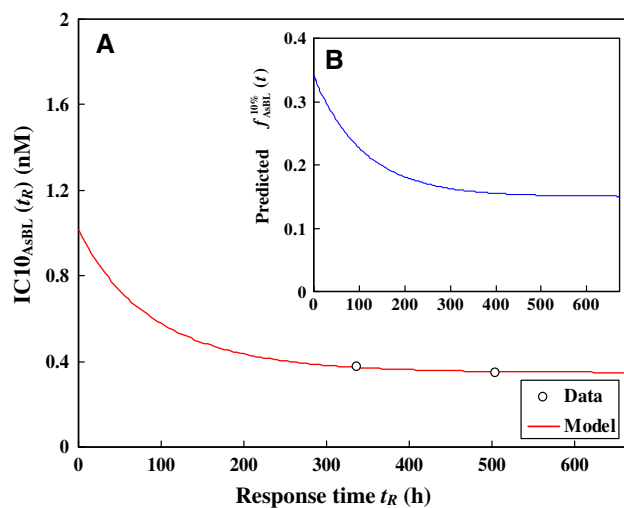
	pH	Temperature (°C)	DO (mg l^{-1})	Water ionic concentrations (mg l^{-1})					
				Ca^{2+}	Mg^{2+}	Na^+	K^+	Cl^-	NO_3^-
Laboratory	8.12 ± 0.06	22.14 ± 1.11	8.16	24.8	1.0	4.9	2.7	0.26	0.318
Putai	7.53 ± 0.05	26.67 ± 1.21	–	311	939.6	8012	322.4	16371.43	0.038
Chiangchun	7.33 ± 0.14	29.00 ± 0.92	–	51.66	25.48	168.8	13.62	129.01	0.038

could be estimated by fitting Eq. 2 to published LC50(t_R) data from Liao et al. (2004) and IC10(t_R) data from Tsai and Liao (2006) associated with known affinity constants and experimental data of water chemistry characteristics shown in Tables 1 and 2 (Figs. 1a, 2a). The relationship between $f_{AsBL}^{X\%}(t_R)$ and response time (t_R) has the form as $f_{AsBL}^{50\%}(t_R) = 0.27 + 0.53\exp(-t_R/48)(r^2 = 0.89)$ for acute toxicity (Fig. 1b), and $f_{AsBL}^{10\%}(t_R) = 0.15 + 0.19\exp(-t_R/110)(r^2 = 0.77)$ for chronic toxicity (Fig. 2b). The concentration of unoccupied tilapia gill BL sites, [BL$^-$], can also be estimated from the relationship of $A_0 = [b][BL^-]BCF^{-1}$ with fitted A_0 value ($A_0 = 46$ and 1.96 nM for acute and chronic toxicity, respectively), and has a value of 4.38×10^{-10} and 1.98×10^{-11} for acute and chronic toxicity, respectively (Table 3). The affinity parameter in the BLM scheme-based ECX equation, [a], can be calculated from the relationship of $[a] = BCF \times [BL^-]^{-1}$, resulting in $[a] = 2.52 \times 10^7$ and 5.59×10^8 M^{-1} for acute and chronic toxicity, respectively (Table 3).

We fitted Eq. 4 to published response time-specific dose response curves, describing the relationship between mortality and arsenic activity in water (Fig. 3a), to obtain the

Fig. 2 a Fitting the proposed IC10$_{AsBL}$(t_R) model Eq. 3 to published experimental IC10(t_R) data. **b** A relationship between the predicted $f_{AsBL}^{10\%}(t_R)$ and response time (t_R)

Table 3 The parameter estimations for the presented model fitted to the LC50$_{AsBL}$(t_R) and IC10$_{AsBL}$(t_R) of the arsenic-tilapia system

	Laboratory	Putai	Chiangchun
Acute toxicity			
[b]	1.16	251.37	8.09
[a][a] (M^{-1})	2.52×10^7	–	–
[BL$^-$][a] (mol g^{-1})	4.38×10^{-10}	–	–
$f_{AsBL}^{50\%}(\infty)$[a]	0.27	–	–
LC50$_{AsBL}(\infty)$ (nM)	17.01	3,682.20	118.48
Chronic toxicity			
[b]	1.10	82.78	3.57
[a][a] (M^{-1})	5.59×10^8	–	–
[BL$^-$][a] (mol g^{-1})	1.98×10^{-11}	–	–
$f_{AsBL}^{10\%}(\infty)$[a]	0.15	–	–
IC10$_{AsBL}(\infty)$ (nM)	0.35	26.13	1.13

[a] The parameters in Putai and Chiangchun are the same with those in laboratory

Fig. 1 a Fitting the proposed LC50$_{AsBL}$(t_R) model Eq. 3 to published experimental LC50(t_R) data. **b** A relationship between the predicted $f_{AsBL}^{50\%}(t_R)$ and response time (t). Error bars denote standard deviation from mean
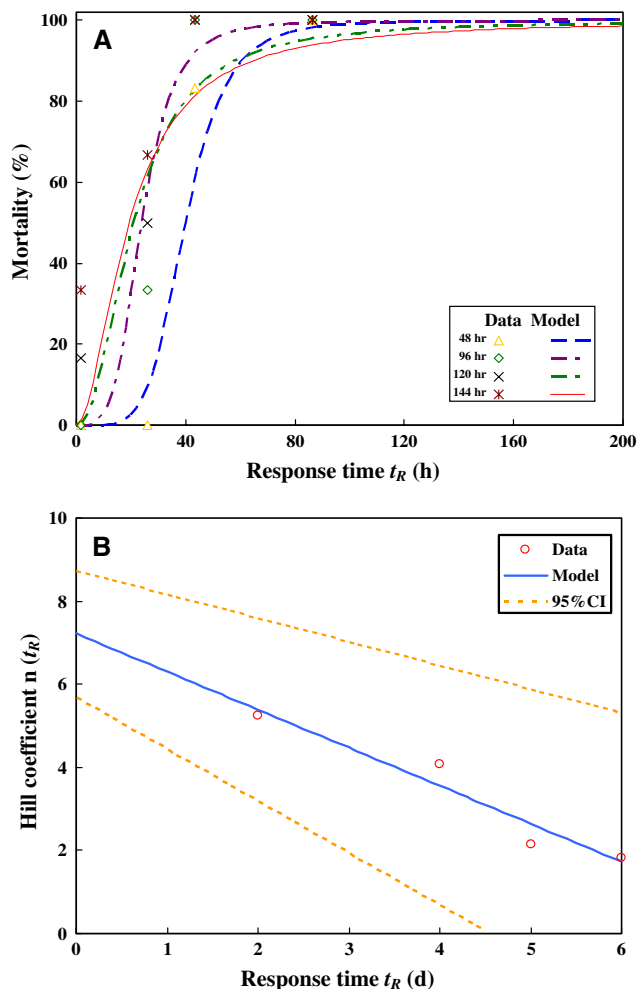
response time-dependent Hill coefficient $n(t_R)$. The relationship between estimated $n(t_R)$ and response time (t_R) has a form as $n(t) = 7.22 + (-0.92t)$ with $r^2 = 0.93$ (Fig. 3b). Based on our fitted arsenic-activity-time-response curves

Fig. 3 a Fitting the proposed BLM-based Hill dose-response model Eq. 4 to published experimental data describing response time-specific tilapia mortality response and waterborne arsenic. **b** Predicted relationship between Hill coefficient and response time (t_R)

(Fig. 3a), the estimated $LC50_{AsBL}(t_R)$ values were 39.98, 23.88, 21.01, and 15.98 nM for response times of 48, 96, 120, and 144 h, respectively. Our results show that there were notably differences in sensitivity to arsenic in difference response time for $n(t_R)$, $R_{max}(t_R)$, $f_{AsBL}^{X\%}(t_R)$, and $LC50_{AsBL}(t_R)$ estimates. In addition, the estimated Hill coefficient n has a value greater than unity, indicating positive cooperativity.

Model applications

The simulation results of the site-specific effective concentration at Pudai and Chiangchun counties were presented in Figs. 4, 5 and Table 3. For acute toxicity, the predicted $LC50_{AsBL}(t_R)$ values ranged from 39.82 (95%CI: 29.90–55.06) to 3.68 (95%CI: 2.98–4.94) μM and from 1.28 (95%CI: 1.03–1.63) to 0.12 (95%CI: 0.10–0.13) μM for Pudai and Chiangchun county, respectively. For chronic
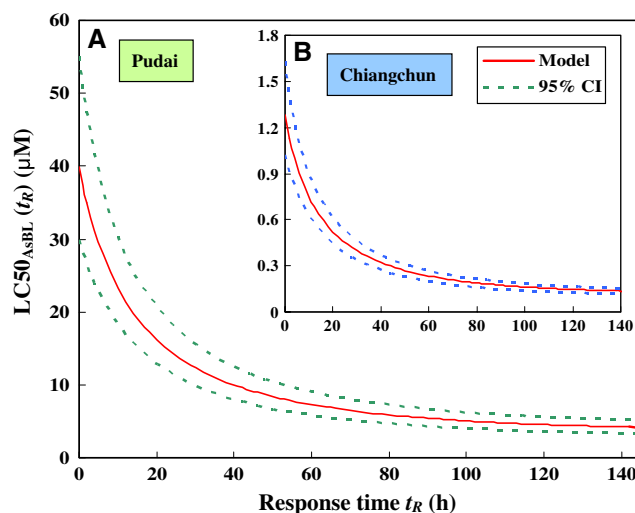
Fig. 4 Predicted site-specific $LC50(t_R)$ of tilapia farms in **a** Pudai and **b** Chiangchun
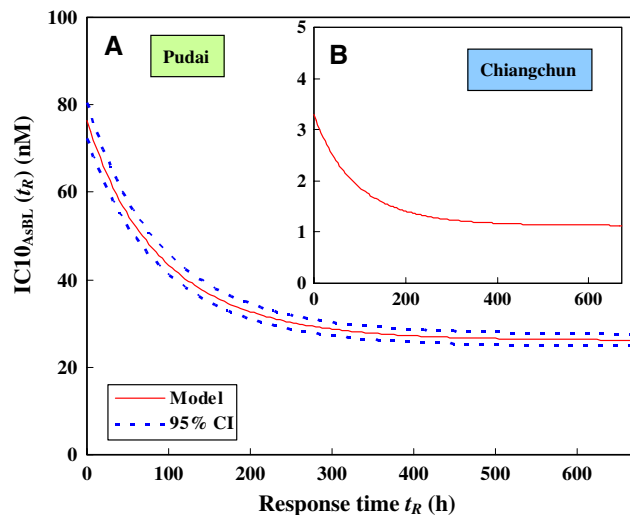
Fig. 5 Predicted site-specific $IC10(t_R)$ of tilapia farms in **a** Pudai and **b** Chiangchun

toxicity, the growth inhibition concentration at 10% effect (IC10) ranged from 76.29 (95%CI: 72.33–80.39) to 26.13 (95%CI: 24.78–27.54) nM for Putai county, and from 3.29 to 1.13 nM for Chiangchun county. These results shows that, for both acute and chronic toxicity, the effective concentrations of arsenic to tilapia in selected aquaculture ponds at Chiangchun are lower than those at Putai.

As mentioned previously, the concentration of metal bound to the BL sites, which actually exert its toxicity on biological surface, depends on the concentrations of all cations that compete with the metal for binding sites on the BL. Equation 2 also showed that effective concentration is in proportion to the stability constants, [b], which represents the binding affinity of metal ions including calcium, magnesium, sodium, and hydrogen to the BL. The

concentrations of metal ions in selected aquacultural ponds of Putai were approximately 6–47 fold higher those of Chiangchun (Table 2), thus effectively reduced the As bioavailability and mitigated its toxicity to the tilapia therein.

These results were in good agreement with previous studies reporting that the toxicity of metals decrease with increasing hardness (Paquin et al. 2000; De Schamphelaere and Janssen 2002). Therefore, the biotic ligand model-based toxicodynamic approach developed in the present study can be used as a strategy for predicting both acute and chronic time variable effective concentrations of arsenic to tilapia under different exposure conditions.

Discussion

Model implications

A fundamental assumption of traditional BLM approach is that the non-equilibrium aspects of the transport processes involved are completely neglected (De Schamphelaere and Janssen 2002). In this regard, toxicity is predicted by the BLM based on the steady-state concentration of the metal at the biotic ligand. In reality, however, there is probably insufficient time for equilibrium Santore et al. (2001); Morgan and Wood 2004). In the present study, a response time-dependent BLM was developed to extend the capability of the BLM. Based on our extended BLM concepts, the fraction of the total number of arsenic binding sites occupied by arsenic $\left(f_{AsBL}^{X\%}\right)$, as well as the effective concentration at X% effect (ECX$_{AsBL}$), was dependent upon exposure time, t_R. All parameters revealed in Eq. 3 were calibrated by fitting this equation to the toxicity data obtained from laboratory bioassays, and then applied to predict response time-dependent acute and chronic As toxicity to tilapia exposed to different water chemistry at different sites. It should be noted that the bioaccumulation parameters were determined experimentally and were only valid for specific fish population. With the present methodology, however, our extended BLM can also be applied to other fish population when the bioaccumulation parameters were available. Consequently, the current BLM-based toxicodynamic approach was important in bridging the gap between laboratory toxicity bioassays and field investigations, and may be helpful in enhancing our predictive power for metal effective concentrations at non-equilibrium conditions.

Site-specific ambient water quality criteria

The purpose of the present study is to provide a mechanistic model which can greatly improve our ability for setting permit limits and generating site-specific ambient water quality criteria (AWQC). For a specific water body, the determination of water effect ratios (WERs) is crucial while generating site-specific AWQC (Di Toro et al. 2001; Bielmyer et al. 2007). The WER is defined as the ratio of the effective concentration of the site-specific water to the effective concentration of the reference laboratory water.

Traditionally, a series of toxicity bioassays should be conducted in advance in both site-specific water and reference laboratory water samples. The AWQC obtained from the original laboratory bioassay is then multiplied by the WERs to determine a site-specific AWQC. In practice, however, the overall procedure of the determination of WERs is considered both time-consuming and costly (Paquin et al. 2000; Di Toro et al. 2001). Recently, Natale et al. (2007) proposed a probabilistic approach, which combined the BLM and Monte-Carlo method, to determine site-specific acute toxicity of copper to *Daphnia magna* in selected rivers in Argentina. In this study, both acute and chronic toxicity of As to tilapia were derived from the BLM-based toxicodynamic approach, for the selected aquacultural ponds. By definition, the site-specific WERs can be calculated from Table 3 by dividing LC50$_{AsBL}(\infty)$ and IC10 $_{AsBL}(\infty)$ of Putai and Chiangchun to those of the laboratory. The resulting WERs were 216.47, and 74.66 (Putai) and 6.97, and 3.23 (Chiangchun) for acute and chronic toxicity endpoint, respectively.

As expected, high cations concentrations strongly reduced As bioavailability in aquacultural ponds at Putai, resulting in the higher WERs. The United States Environmental Protection Agency (USEPA 2000) recommended that EC10 could be used as a surrogate threshold of regulatory endpoint in ecological risk assessment. For protection of *O. mossambicus*, a conservative site-specific AWQC value of As was recommended as 26.13 nM in Putai and 1.13 nM in Chiangchun according to the incipient inhibition concentration of growth rate at 10% effect [i.e., IC10$_{AsBL}(\infty)$].

In conclusion, we have developed a biotic ligand model-based toxicodynamic approach which was capable of predicting not only acute toxicity, but also chronic toxicity of arsenic to *O. mossambicus* in site-specific water. Due to its mechanistic foundation, the proposed model provided both spatial and temporal variation of As bioavailability and toxicity. From the simulation results of this study, the site-specific WERs and AWQC were significantly influenced by water chemistry. The main contribution of current methodology is that only modest data sets of physiological and toxicological parameters of aquatic organism, in conjunction with geochemical parameters of ambient water, are required in the generation and adjustment of water quality criteria. The present methodology still need to be tested and should be expanded to include other organisms

and metals. We recommend that future research focus on a more thorough evaluation of the interactions of arsenic with toxic chemicals commonly found in tilapia cultural ponds (e.g., Cu, Zn, Al, PAHs).

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