A Toxicologically-Based Framework Can Enhance Urban Aquatic Ecosystem Risk Assessment

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Abstract: This study proposes a toxicologically based algorithm to relate arsenic (As) toxicity to the internal effect concentration (IEC) in tilapia *Oreochromis mossambicus*. The relationships among As exposure, uptake, accumulation, and toxicity to tilapia are investigated using toxicokinetic (TK) and toxicodynamic (TD) modeling. A 7-d exposure bioassay reveals that the bioconcentration factor (BCF) value of tilapia is 2.88, indicating that the tilapia is capable of accumulating waterborne As. As acute toxicity is analyzed by determining the median external effect concentration (LC_{50}) at different integration times, indicating that 96-h LC_{50} and $LC_{50}(\infty)$ for tilapia are 28.68 (95% CI: 24.92-32.44) and 12.04 μg mL^{-1}, respectively. To determine the mode of action (MOA) governing the As toxicity, this study employed the damage-assessment model (DAM) to describe LC_{50}s. Result suggests that the DAM characterizes As toxicity well and the intrinsic MOA of As toxicity acts through the reversible reaction between As and specific receptors. This study kinetically links the DAM with IEC-based Hill equation model to derive dose-response relationships between equilibrium As residue and mortality. We suggests that considering MOA in ecotoxicological assessment is useful to improve the construction of environmental quality criteria for protecting rapidly degrading aquatic ecosystems in urban area.

Key-words: Arsenic; Mode of action; Tilapia; Toxicokinetics; Toxicodynamics

1 Introduction

Metals are widespread in the environment as a consequence of both anthropogenic and natural processes. Aquatic ecosystems, including streams, rivers and lakes are the ultimate sink for pollutants. Acompanying negative impacts may arise on the organisms therein. Society is faced with the enormous risk to assess numerous chemicals while protecting different species in ecosystems. Liao et al. [1] pointed out that pond water As concentration in southwestern urban area of Taiwan ranged from 8.1 to 251.7 μg L^{-1} which exceed the criteria for total As in freshwater ecosystems (150 μg L^{-1}) documented by the Criterion Continuous Concentration [2]. Tilapia (*Orechromis mossambicus*) is a dominant species in local inland waters and estuarine regions. Fish are idea indicators of metal contamination because they occupy different trophic levels and are one of the major food sources of humans. The extent and rate that metals concentrate from water into fish continue to raise major environmental concerns. Traditionally, environment concentrations are adopted as the dose to produce a given effect. In recent years, body residues are suggested to assess the hazard of chemicals [3]. The MOA is a common set of physiological signs that characterize adverse response [4]. Escher and Hermens [3] indicated that knowing the MOA is helpful for deducing general principles for developing descriptive and predictive models in ecotoxicology. This study proposed an integrated methodology to enhance the metal risk assessment. Essential bioassays, including bioaccumulation and acute toxicity bioassays are conducted to elucidate the TK/TD behavior of As in tilapia. Mechanistic models are developed based on inherent MOA to quantitatively elucidate the relationships between adverse effects and IEC. This study is done to better assess the health of organisms in rapidly degrading urban aquatic ecosystems.

2. Material and Methods

2.1 Toxicokinetic bioassays

The presented test is designed to examine the accumulation ability of As in tilapia.

Bioaccumulation experiments are conducted in 1 mg L^{-1} sodium arsenite ($NaAsO_2$) for 7 d based on the suggestion of Suhendrayatna et al. [5]. Exposure bioassays are conducted with 42 fish of a specific size class [mean body length = 13.9 ± 1.54 cm (mean ± SD) and mean weight = 16.8 ± 5.2 g wet wt.]. Experiments are assigned to two replicate tanks. The whole As solutions are renewed weekly to avoid the regression of ambient water quality and to keep the constant As concentration. To analyze As accumulation in tilapia, 3 individuals are sequentially removed from solutions after 0, 1, 2, 4, and 7-d of exposure. The fish are rinsed with deionized water and then are anesthetized in pH-neutralized tricaine methane sulfonate (MS-222) (Sigma Chemical Co., St. Louis, MO) solution. Fish samples are cleaned with deionized water and are freeze-dried overnight, and then grounded to fine powder in a grinder (Tai-Hsiang S36-89, Taiwan). A 500 mg portion of the powder is digested in 10 mL concentrated HNO_3 (65% wt.) overnight at room temperature. The resulting solution is evaporated and the residues redissolve 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 is used to analyze As. Analytical quality control is 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 is 94.6 ± 3.6% and the levels of detection are 0.62 μg As L^{-1} for water samples and 0.05 μg As g^{-1} for tissue samples.

Data analysis. Toxicokinetic parameters was estimated by fitting data to the integrated form of the kinetic equation for constant water exposure using iterative nonlinear regression [6][7],

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

where C_f is the As concentration in tilapia (μg g^{-1}), k_1 is the uptake rate constant (mL g^{-1} d^{-1}), k_2 is the depuration rate (d^{-1}) constant and t is the time in d. The BCF is calculated as: BCF = $k_1/(k_2+k_g)$, representing the net accumulation ability that is the result of the competition between uptake and depuration associated with growth dilution and C_w is the measure waterborne As concentration (μg mL^{-1}). Equation 1 provides a TK-based approach to predict As residues in constant exposure scenarios. Nonlinear option of the Statistica® software (StatSoft, Tulsa, OK, USA) is applied to perform all curve fittings. The Statistica® is also used to calculate the coefficient of determination (r^2) and statistical analyses (analysis of variance and Student's t-test). Statistical significance is judged if p values are less than 0.05.

2.2 Acute toxicity bioassays

Laboratory static bioassays are conducted to determine the 24-h, 48-h, 72-h, 96-h, 120-h, and 144-h LC_{50}s for tilapia exposed to As. The experimental design and calculations are based on procedures given by Finny [8]. Six fish of a specific size class [mean body length = 12.67 ± 5.65 cm (mean ± SD) and mean body weight = 13.72 ± 3.5 g wet wt.] are randomly selected and transferred into each test aquarium. Experimental settings and managements are followed the protocol of TK bioassays. The nominal As concentrations are 0 (control), 1, 2, 4, 10, 30, 50, and 80 mg L^{-1} [9]. Gross mortality of fish in each concentration is recorded every 1 h for the first 12 h and every 2 h thereafter to the end of the experiment, and the dead fish being removed every 1 – 2 h. Tilapia are not fed through the test. Control and each test concentrations are tested in duplicate. No mortality occurrs in the controls. LC_{50} values are determined using mean assayed As concentrations and cumulative mortality and then are estimated by maximum likelihood estimates of linear functions relating log As concentration to probit transformations of percent mortality [8]. All of the observations are used in probit analysis.

2.3 Mode of action

Toxicity models had been proposed to describe inherent interactions between chemicals and receptors and to depict the time course of toxicity. These models assumed a first-order TK process to predict the $C_{L,50}$ from LC_{50} data. $C_{L,50}$ is difficult to be determined directly yet could be estimated by mechanistic models . Lee et al. [10] proposed thecDAM to depict the MOA of compounds with rapid reversible binding to the target site as well as to those that act with irreversible binding. In this model, the constant threshold of adverse effect is a buildup of a critical level of tissue damage. DAM assumes that the time-dependent toxic response was determined by both of the chemical accumulation (TK) and damage accumulation (TD) in an organism. The damage-based $LC_{50}(t)$ and $C_{L,50}(t)$ can be expressed as [10],

$$LC_{50}(t)=\frac{D_{L,50}/k_a}{\left(\frac{e^{-k_r t}-e^{-k_2 t}}{k_r-k_2}+\frac{1-e^{-k_r t}}{k_r}\right)}BCF^{-1}, \quad (2)$$

and

$$C_{L,50}(t)=\frac{D_{L,50}/k_a}{\left(\frac{e^{-k_r t}-e^{-k_2 t}}{k_r-k_2}+\frac{1-e^{-k_r t}}{k_r}\right)}\left(1-e^{-k_2 t}\right), \quad (3)$$

where $D_{L,50}/k_a$ is a coefficient reflects the compound equivalent toxic damage level required for 50% mortality (μg d g^{-1}). With sufficient $LC_{50}(t)$ data, it is possible to estimate the best-fit values of the $D_{L,50}/k_a$, k_r in Eqs. (2) and (3) by using a nonlinear regression technique. We further depicts the relationship between the $LC_{50}(t)$ and the time-dependent cumulative damage [$D(t)$] in the DAM as LC_{50} (t) = $D_{L,50}C_w/D(t)$, it can be rearranged to a new expression as,

$$\frac{D(t)}{D_{L,50}}=\frac{C_w}{LC_{50}(t)}, \quad (4)$$

where $D(t)/D_{L,50}$ is the relative accumulation damage level to induce the 50% mortality. Whenever the predicted result excesses the value of 1.0, the mortality of 50% will occur in a given exposure scenario. Equation (4) provides a prediction tool to assess the lethality to organisms in a given time interval.

The MOA of As acute toxicity is clarified by testing the DAM with the lab-derived exprimental data to compare observed and predicted LC_{50} values, and then to estimate the time-dependent $C_{L,50}$s as a function of a few constants that are verified with acute toxicity data.

2.4 Toxicodynamic model

The relationships between mortality and As dose in fish are represented by using an empirical three-parameter Hill equation model. We can obtain the time-mortality curves [$M(t)$] as functions of As residue (C_f) and DAM-based $C_{L,50}(t)$ in Eq. (3) as [11],

$$M\ (t)=\frac{M_{max}\times C_f^n(t)}{C_{L50}^n(t)+C_f^n(t)}, \quad (5)$$

where M_{max} is the tilapia maximum mortality exposed to As and n is a slope factor, or is referred to as the Hill coefficient, which reflects the extent of cooperativity among chemical and receptor [12].

We can estimate the best-fit value of Hill coefficient appeared in Eq. (5) by nonlinear regression with sufficient data of percent mortality over a suitable As concentration in water associated with the specific interval of LC_{50} data.

3. Result

3.1 Toxicokinetics

The 7-d water exposure bioassay of As in tilapia had significant correlated with nonlinear regression profiles (r^2 = 0.97, p < 0.05) resulting from the best fit of the first-order bioaccumulation model (Figure 1A). The estimated k_1, k_2, and BCF are 0.39 mL g^{-1} d^{-1}, 0.075 d^{-1}, and 3.88 mL g^{-1}, respectively. The BCF is above 1, showing that the tilapia are capable to accumulate waterborne As. We employed Eq. 1 cooperating with TK parameters to predict the As kinetics when tilapia are exposed to 1, 2, and 4 μg mL^{-1} waterborne As, respectively (Fig. 1B).

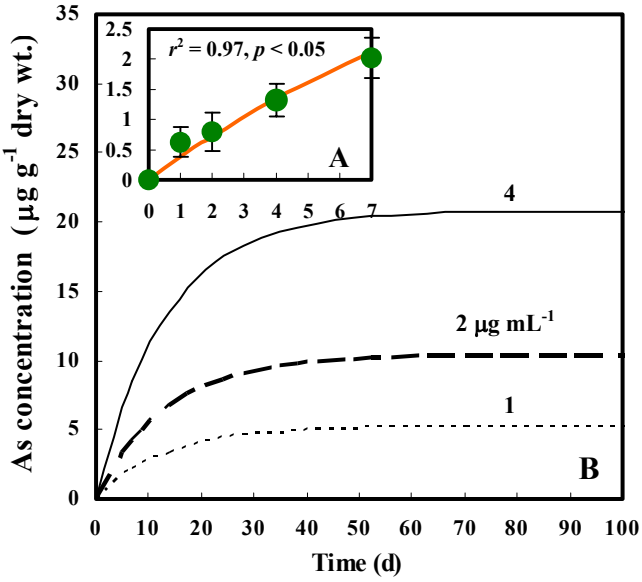

Fig. 1. (A) Bioassays of tilapia exposed to 1 μg mL^{-1} As for 7-d uptake. The solid line is the best-fit regression curve from bioaccumulation model. (B) Predicted As concentration in tilapia when exposed to 1, 2, and 4 μg mL^{-1} As.

3.2 LC_{50} and $C_{L,50}$

The selected time intervals of LC_{50} values with 95% CI of As to tilapia are given in Fig. 2A. LC_{50}s lower progressively as the duration of exposure increases. Our 96-hr LC_{50}s of As to tilapia is 28.68 (95% CI: 24.92 – 32.44) μg mL^{-1}, closed to the range of 96-hr LC_{50} of As to seawater tilapia (26.5; 95% CI: 23.2 – 33.8 μg mL^{-1}) reported by Hwang

and Tsai [9]. Figure 2B elucidates the predicted time trends of $C_{L,50}$s by DAM, showing an decrease from 124.41 to 34.69 μg g^{-1} in the 0.7th h and then it remains

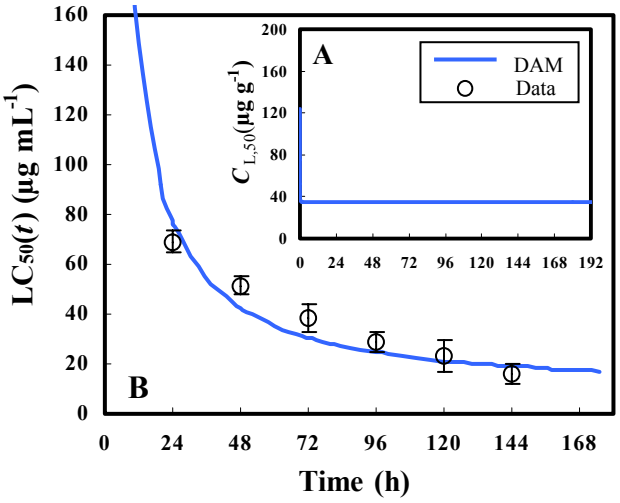

Fig.2. (A) Optimal fits of $LC_{50}(t)$ data by the DAM. (B) Predicted $C_{L,50}(t)$ values.

constantly to the end of the simulation. The incipient $C_{L,50}$s are calculated to be 36.68 (Table 9).

3.3 Lethal potential assessment

This study employed Eq. (4) to assess the probability of 50% lethality. Results indicate if the tilapia are exposed to As concentration ≤ 27.2 μg mL^{-1}, the 50% mortality never occurs (Fig. 3). The predicted 50% mortalities occur in 120-h and

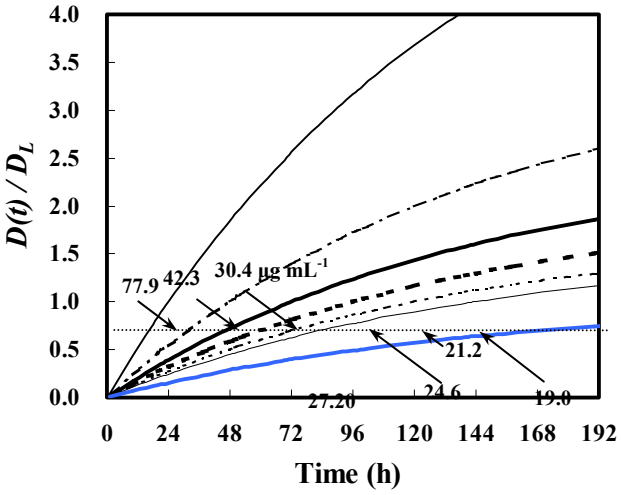

Fig. 3. DAM predicted $D(t)/D_{L,50}$ [= $C_w/LC_{50}(t)$] values in that when the curves excess the value of $D(t)/_{L,50}$ = 1.0, the 50% mortality will occur in the period *t*.

144-h are 21.2 and 19.00 μg mL^{-1}, respectively. Both of the values fall within the 95% CI of the observed LC_{50} data (Fig. 2). Although the predicted concentrations in 24, 48, 72, and 96-h do not fall within the ranges of observed data, but they are closed to the measured values and could reflect the time trend of decreasing LC_{50}s. This study reveals that the damage-based scheme provides an effective tool to assess the chemical toxicity to fish.

3.4 Does-response relationship

Dose-response relationships between equilibrium As concentrations in tilapia and mortality are derived using Eq. (5). This study substitutes $C_f(t)$ obtained from the bioaccumulation model and the damage-based $C_{L,50}(t)$ [Eq. (9)] into Eq. (23) to obtain the time-mortality profiles. Figure 5 shows that the predicted mortalities are highly agreed with the experimental values in lower exposure conditions (0-4 μg mL^{-1}) The predicted mortalities never reached 50% when tilapia are exposed to waterborne As < 10 μg mL^{-1}. The predicted mortalities are slightly higher than observed

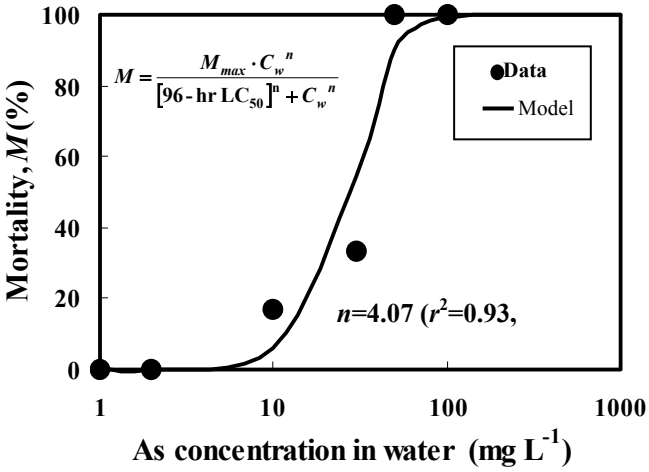

Fig. 4. Optimal fit of the Hill equation model to accumulative mortality of tilapia verse waterborne As concentration in the 96th hr acute toxicity bioassay.

Values before 96th hr. The predicted maximum mortality reached 26.66 % which is slightly lower to the measured data (33.00 %) in 10 μg mL^{-1}. In extremely high concentrations, the predicted results agreed well with the measured values. In the case of 30 μg mL^{-1}, the predicted mortality approaches the maximum value of 67% in 144th hr

and there are still survivors resist the As toxicity to keep alive in this 384-hr simulation. In the case of 50 μg mL^{-1}, the predicted maximum mortality is 96% in 96th hr and only a few tilapia (4%) keep surviving.

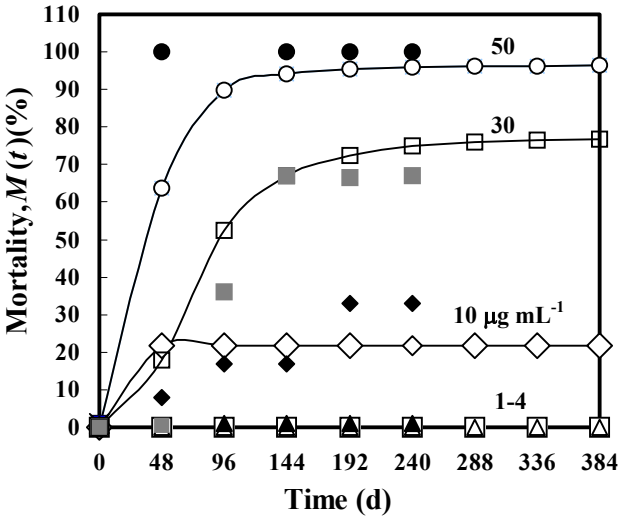

Fig. 5. Prediction of time-mortality of tilapia exposed to waterborne As, ranged from 1 to 50 μg mL^{-1}. Solid symbols are the measured data and corresponding open symbols are the predicted values from Eq. (5). The predicted mortalities are < 1% in 1 to 4 μg mL^{-1}.

4. Discussion

4.1 Toxicokinetics and Toxicodynamics

Study results suggest that the proposed methodology by employing the IEC-based Hill model incorporating with the DAM is capable of describing the time course of As acute toxicity to tilapia; however, minor underestimations of the mortalities occur in sublethal exposures. This might be attributed to limitations of bioaccumulation model because of this model dose not account for the changes of physiological condition in tested organisms induced by chemical toxicities. Results reveal that the assumptions of TK parameters are independent of exposure scenarios have to be addressed in future studies, especially been applied to extreme circumstances.

In this study, a receptor theory-based Hill equation model is modified to construct relationships between equilibrium As concentrations in tilapia and mortality effects. To accurately estimate the Hill equation model parameters, including 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} and EC_{50} will not be proper estimated and the obtained n value will not be correctly estimated [13]. Study results indicate that the 96-hr accumulated mortality data (Fig. 4) is appropriate for parameter estimations and following dose-response predictions.

Although the use of body residues as the chemical dose integrates the uncertainties in bioavailability, assimilation and metabolism of the contaminants. However, Pawlisz and Peters [14] indicated that the relationships between body residues and toxic responses are not always discriminatory. The chemical toxicity may be different between species, creating variation in interpreting the significance of body residues across species. If this is universal, body residues may not be useful in referencing toxicity. 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. Suhendrayatna et al. [15] indicated that inorganic As metabolizes into methylated forms after be taken from water or diet routs by tilapia. Methylated As compounds account for 25-93% of total As in tissues. Methylated As is usually considered as having lower toxicity than inorganic forms. Thus, the extent of metabolism must be determined, and the toxicity of metabolites must be assessed.

4.2 Mode of action

This study points out that the DAM is more applicable to explain the time course of As toxicity and is capable of predicting the IECs. Several reasons support this finding. Firstly, the DAM accounts for the influences of both TK and TD to describe the chemical toxicity, which is more realizable than the one that only depends on TK. Because the DAM demonstrates that elimination is not the sole process for the recovery of organism, since even after chemical is elimination, cellular damage will need to be repaired before the cell will be fully functioned [16]. Secondly, the DAM is a general model which accounts for full, partial, or irreversibility of effects. In spite of the DAM used in this study are applicable for As toxicity description and prediction, nevertheless, to better assist accurate risk assessment posed by metals in aquatic ecosystems, more studies and experimental data are needed to validate the applications of

those models.

5. Conclusion

This study supports the suggestion of replacing external concentrations by IEC is a first step toward a measurement for chemical toxicity. Making a connection between accumulated dose, MOA and toxicological effects will permit better interpretation of hazards and can improve the construction of environmental quality criteria programs, aimed at protecting and restoring the rapidly degrading aquatic ecosystems in urban area.

Reference:

[1] Liao CM, Chen BC, Singh S, Lin MC, Liu CW, Han BC. Acute toxicity and bioaccumulation of arsenic in tilapia (*Oreochromis mossambicus*) from a blackfoot disease area in Taiwan. *Environmental Toxicology*, Vol.18, 2003, pp. 252-259.

[2] US EPA. National recommended water quality criteria: 2002. EPA-822-R-02-047. Website: http://www.epa.gov/ost/pc/revcom.pdf.

[3] Escher BI, Hermens JLM. Mode of action in ecotoxicology: their role in body burdens, species sensitivity, QSARS, and mixture effects. *Environmental Science and Technology*, Vol.36, 2002, pp. 4201-4217.

[4] Landis WG, Yu MH. *Introduction to environmental toxicology: impacts of chemicals upon ecological systems*. Florida: Levis Publishers, 1999.

[5] Suhendrayatna, Ohki A, Nakajima T, Maeda S. Studies on the accumulation and transformation of arsenic in fresh organisms II. Accumulation and transformation of arsenic compounds by *Tilapia mossambica*. *Chemosphere*, Vol. 46, 2002, pp. 325-331.

[6] Reinfelder JR, Fisher NS, Luoma SN, Nichols JW, Wang WX. Trace element trophic transfer in aquatic organisms: A critique of the kinetic model approach. *Science of Total Environment*, Vol.219, 1998, pp.117-135.

[7] Clason B, Duquesne S, Liess M, Schulz R, Zauke GP. Bioaccumulation of trace metals in the Antarctic amphipod *Paramoera walkeri* (Stebbing, 1906): comparison of two compartment and hyperbolic toxicokinetic models. *Aquatic Toxicology*, Vol. 65, 2003, pp.117-140.

[8] Finney DJ. *Statistical method in biological assay*. 3rd ed. London: Cambridge University Press, 1978, pp 508.

[9] Hwang PP, Tsai YN. Effects of arsenic on osmoregulation in the tilapia *Oreochromis mossambicus* reared in seawater. *Marine Biology*, Vol.117, 1993, pp.551-558.

[10] Lee JH, Peter FL, Koh CH. Prediction of time-dependent PAH toxicity in *Hyalella azteca* using a damage assessment model. *Environmental Science and Technology*, Vol.36, 2002, pp. 3131-3138.

[11] Liao CM, Tsai JW, Ling MP, Liang HM, Chou YH, Yang PT. Organ-specific toxicokinetics and dose-response of arsenic in tilapia *Oreochromis mossambicus*. *Archive of Environmental Contamination and Toxicology*, Vol.47, 2004, pp.502-510.

[12] Weiss JN. The Hill equation revisited: uses and misuses. *FASEB Journal*, Vol.11, 1996, pp.835-841.

[13] Venitz J. Pharmacokinetic-pharmacodynamic modeling of reversible drug effects. In: Derendorf H, Hochhaus G, eds. *Handbook of pharmacokinetic/pharmacodynamic correlation*. Roca Raton, FL: CRC Press, 1995, pp. 1-34.

[14] Pawlisz AV, Peter RH. A radioactive tracer technique for the study of lethal body burdens of narcotic organic chemicals in *Daphnia magna*. *Environmental Science and Technology*, Vol.27, 1993, pp.2795-2800.

[15] Suhendrayatna, Ohki A, Nakajima T, Maeda S. Metabolism and organ distribution of arsenic in the freshwater fish *Tilapia mossambica*. *Apply Organometal Chemistry*, Vol.15, 2001, pp.566-571.

[16] Schuler LJ, Landrum PF, Lydy MJ. Time-dependent toxicity of fluoranthene to freshwater invertebrates and the role of biotransformation on lethal body residues. *Environmental Science and Technology*, Vol.38, 2004, pp.6247-6255.