行政院國家科學委員會專題研究計畫　期中進度報告

台灣烏腳病地區養殖魚類砷累積之生態毒物模擬(2/3)

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計畫主持人：廖中明

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摘要

第二年研究工作針對砷於吳郭魚(*Oreochomis mossambicus*)各器官內之毒理動力及劑量反應關係進行調查，並結合以 AUC 為基礎之急性毒模式及藥理動態模式以建立各器官之平衡砷濃度與死亡率之關係。7 天暴露實驗顯示，魚體器官組織中生物濃縮因子值最高者為胃，最低為肌肉，其值分別為 9.56 及 1.04，各器官生物濃縮因子大小排序如下：胃>腸>肝>鰓>肉，顯示吳郭魚對砷具有累積能力。急性毒實驗顯示 24 及 96 小時魚體半致死濃度分別為 69.06 及 28.86 mg L^{-1}。以 Hill 方程式建立之劑量反應關係顯示肌肉、鰓與肝有較顯著之效應曲線，其半數致死時各器官砷濃度分別為 26.6、62.5 及 78.5 μg g^{-1}乾重。本研究建議選取吳郭魚鰓之砷濃度作為風險評估之依據。

關鍵詞：砷，吳郭魚，急性毒，生物累積，烏腳病。

Abstract

The objective of our work in the second year is to determine the organ-specific toxicokinetics and dose-responses of arsenic (As) burdens in tilapia. We linked kinetically an AUC-based acute toxicity model and a pharmacodynamic model to derive dose-response relationships between equilibrium organ-specific As concentrations and mortality effects. The 7-d exposure test revealed that the highest bioconcentration factor (BCF) is found in the stomach and the lowest one is in the muscle, and the values are 9.56 and 1.04, respectively. The order of BCF was stomach > intestine > liver > gill > muscle. The 7-d acute toxicity bioassay showed that 24 and 96-h LC_{50} for tilapia exposed to As are 69.06 and 28.68 mg L^{-1}, respectively. Dose-response relationships followed the refined Hill equation indicated that that the muscle, gill and liver have a relative steep sigmoid dose-response profile in that IEC_{50} are 26.6, 62.5 and 78.5 μg g^{-1} dry wt, respectively. We suggested that the gill could be used as a surrogate in site-specific risk assessment.

Keywords: Arsenic; Tilapia; Acute toxicity; Bioaccumulation; Blackfoot disease

Introduction

Arsenic (As) is potentially a toxic trace element and is widespread in the environment as a consequence of both anthropogenic and natural processes. Chen et al. (2001) indicated that long-term exposure to ingested inorganic As in artesian well water has been found to induce blackfoot disease (BFD) in southwestern coastal area of Taiwan. 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. Han et al. (1998) reported that the consumption of contaminated fish/shellfish has been as an important route of human exposure to trace elements in Taiwan. Farming tilapia (*Orechromis mossambicus*) is a promising practice in the BFD area because of its high market value.

Currently, a lack of reliable data exists on uptake and effects of As in tilapia, and little is known about the actual uptake and elimination processes. The objectives of this paper are twofold: (1) to provide knowledge of the organ-specific toxicokinetics and distributions of As in tilapia and (2) to construct dose-response relationships of As in target organs of tilapia by kinetic coupling of an acute toxicity model and a pharmacodynamic model.

Materials and Methods

Exposure Experiments

The present laboratory study was designed to examine the accumulation ability of arsenic in the muscle, gill, stomach, intestine, liver, and whole body of tilapia. We conducted an uptake experiment in As concentration of 1 mg L^{-1} for 7 d based on the suggestion by Suhendrayatna et al. (2001).

The As solution was replaced daily to avoid the regression of ambient water quality. The measured As concentration was 0.94 ± 0.072 mg L^{-1}. In order to analysis the As uptake by the fish, 3 fish were sequentially removed from each tank after 0, 1, 2, 4, and 7 d of exposure. An adequate portion of the gill, intestine, liver, stomach, and muscle of each individual was collected. The dissected tissues samples were freeze-dried overnight, and then grounded to fine

Table 1. Estimates for uptake rate constant (k_1), elimination rate constant (k_2) and bioconcentration factor (BCF), during a 7-d As-exposure period for *O. mossambicus*

Target organ	k_1 (mL g^{-1} d^{-1})	k_2 (d^{-1})	BCF (mL g^{-1})
Gill	0.31±0.086	0.028±0.11	2.44
Liver	0.61±0.15	0.1±0.11	3.07
Muscle	0.24±0.08	0.13±0.029	1.04
Intestine	0.67±0.16	0.61±0.098	4.19
Stomach	1.53±0.73	0.063±0.2	9.56
Whole body	0.49±0.11	0.075±0.09	5.03

powder in a grinder. A 500 mg portion of the powder overnight at room temperature. The resulting solution as evaporated and the residue redissolved in 0.1 N HCl. was digested in 10 mL concentrated HNO_3 (65% wt)

Acute Toxicity Assays

Laboratory static bioassays were conducted to determine the 24-h, 48-h, 72-h, 96-h, 120-h, and 144-h LC_{50} values for tilapia exposed to As. The nominal concentrations of As tested were 0 (control), 1, 2, 4, 10, 30, 50, and 80 mg L^{-1} (Hwang and Tsai, 1993). Gross mortality of fish to each concentration was recorded every 1 h for the first 12 h and every 2 h thereafter for 96 h, and dead fish being removed every 1 – 2 h. The water quality management protocol was the same as deployed as the exposure experiments.

The LC_{50} values were determined from maximum likelihood estimates of linear functions relating log As concentration to probit transformations of percent mortality (Finney, 1978). The LC_{50} values were determined using mean assayed As concentrations and cumulative mortality.

Data Analysis

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

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

where C_f is the time-dependent As concentration in the target organ of tilapia (μ g g^{-1}), k_1 is the tilapia uptake rate constant (ml g^{-1} d^{-1}), k_2 is the depuration rate (d^{-1}) constant of As and t is the time in d. The organ-specific bioconcentration factor (BCF) can be calculated as: BCF = k_1 / (k_2 + g), representing the net accumulation ability that is the result of the competition between uptake and depuration associated with growth dilution.

Arsenic Acute Toxicity Models

The AUC-based TIC toxicity model employed in determining the internal lethal body burden at the site of action that cause 50% mortality, $C_{L,50}(t)$. When the exposure time approach infinity, a relation among $LC_{50}(\infty)$, $C_{L,50}(\infty)$ and BCF as

$$C_{L,50}(\infty) = \mathrm{LC}_{50}(\infty) \times \mathrm{BCF}. \quad (2)$$

Dose-Response Models

In pharmacodynamic modeling, the relationship between dose effect and dose concentration is commonly expressed by the Hill equation or is referred to as the sigmoid $E_{\max}$ model (Bourne, 1995; de Vries, 1996), We combine Eqs. (1) and (2) incorporated with internal lethal body burden derived from TIC toxicity model to construct dose-response profiles. The mathematical model for dose-response relationships between mortality and As levels in different target organs of tilapia can be obtained by refining the Hill equation in as

$$M_i = \frac{M_{\max} \times C_{f,i}^n}{C_{L,50i}^n(\infty) + C_{f,i}^n} = \frac{M_{\max} \times C_{f,i}^n}{(BCF_i \times LC_{50}(\infty))^n + C_{f,i}^n}, \quad (3)$$

where M_i is mortality for target organ i, $C_{f,i}$ is the internal As concentration in target organ i, BCF_i is the bioconcentration factor for target organ i, $M_{\max}$ is the tilapia maximum mortality exposed to waterborne As. With sufficient data of percent mortality over a suitable As concentration in water associated with the specific interval of LC_{50} data, we can estimate best-fit values of Hill coefficient by nonlinear regression.

Results

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

The highest k_1 occurs in stomach, which is 2 times higher than that of intestine and liver. Muscle is the major biomass yet shows the lowest uptake ability. BCF values of target organs are all above 1, ranging from 1.04 to 9.56, showing the potential to accumulate As when the tilapia was exposed to a given waterborne These toxicokinetic parameters not only can be used to describe the uptake and depuration ability of target organs of tilapia but also can be further used to predict the dose-response relationships of these target organs.

The selected time intervals of LC_{50} values lower progressively as the duration of exposure increases. Our 96-h LC_{50}s of As to tilapia is 28.68 (95% CI: 24.92 – 32.44) mg L^{-1}, which is closed to the range of 96-h LC_{50} of As to seawater tilapia (26.5; 95% CI: 23.2 – 33.8 mg L^{-1}), yet lower than that of to freshwater tilapia (71.7; 95% CI: 67.8 – 76.4 mg L^{-1}) reported by Hwang and Tsai (1993).

The optimal fits of the AUC-based TIC toxicity model to the $LC_{50}(t)$ data are presented in Figure 1. The estimated values for $LC_{50}(\infty)$ and AUC_f/BCF are 25.55 mgL^{-1} and 46.36 (mg d L^{-1})/(L kg^{-1}), respectively. (r^2 = 0.80, $p < 0.05$).

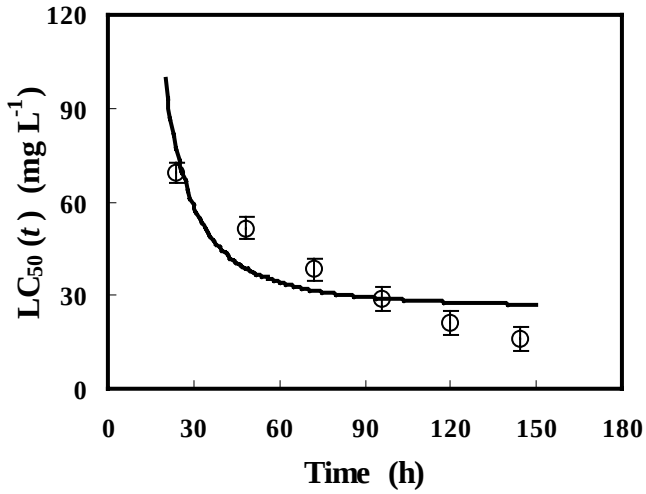

Fig. 1. Optimal fit of the AUC-based TIC acute toxicity model to the $LC_{50}(t)$ data (mean ± 95% CI)

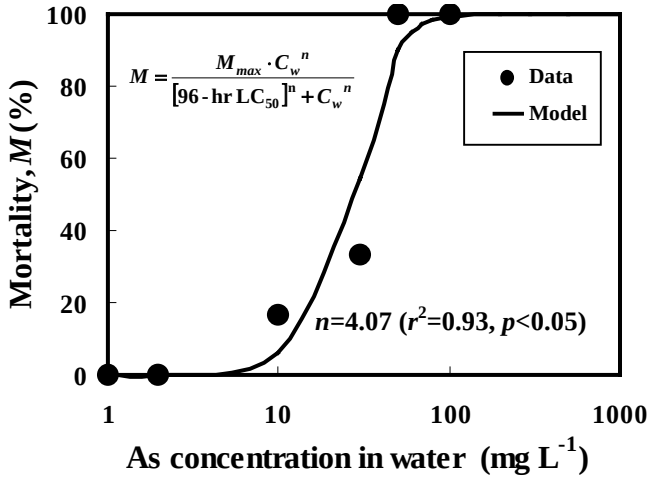

Fig. 2. Optimal fit of Hill equation to observed percent mortality of tilapia verse waterborne As concentrations in the 96-h acute toxicity bioassay.

Dose-Response Relationships

Eq. 3. provides a tool to predict the relationships between organ-specific internal residue and mortality. Figure 3 shows that muscle, gill, and liver have relative steep sigmoid dose-response profiles with mortalities approaching 100%, whereas intestine and stomach have lazier sigmoid dose-response profiles. The median internal effect concentration (IEC_{50}) and IEC_{10} for muscle, gill, and liver are 26.6, 15.5; 62.35, 36.35; and 78.45, 47.74 μg g^{-1} dry wt, respectively. We suggested that the gill can be used as a surrogate to assess the As toxicity to tilapia due to it's higher sensibility to toxic effects and the sampling convenience.

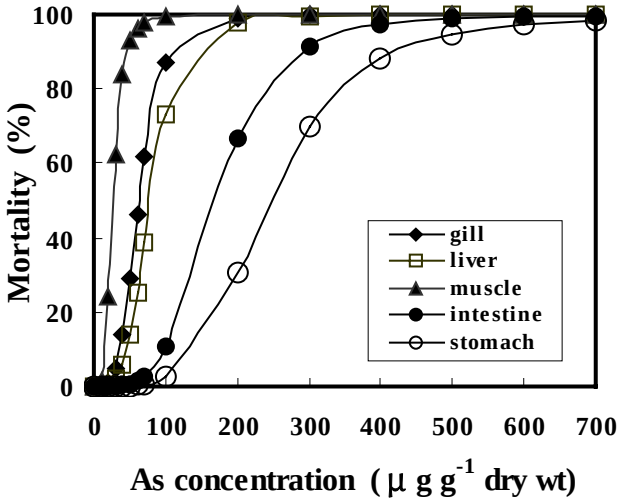

Fig. 3. The derived organ-specific dose-response relationships between equilibrium internal effect concentration of As in target organs and mortality effects for tilapia *O. mossambicus*.

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