行政院國家科學委員會專題研究計畫　成果報告

台灣烏腳病地區養殖魚類砷累積之生態毒物模擬(3/3)

計畫類別：個別型計畫
計畫編號：NSC93-2313-B-002-005-
執行期間：93 年 08 月 01 日至 94 年 07 月 31 日
執行單位：國立臺灣大學生物環境系統工程學系暨研究所

計畫主持人：廖中明

報告類型：完整報告

處理方式：本計畫可公開查詢

中　華　民　國 94 年 10 月 12 日

行政院國家科學委員會專題研究計畫成果報告

台灣烏腳病地區養殖魚類砷累積之生態毒物模擬(3/3)

計畫編號：NSC 93－2313－B－002－005
執行期限：93/08/01~94/07/31
執行單位：國立台灣大學生物環境系統工程學系
主 持 人：廖中明

摘要

此三年研究計畫主要進行台灣烏腳病地區養殖魚類砷累積之野外調查及生態毒物模擬，經由發展以生物為基礎的藥理動力及動態模式探討吳郭魚體內器官之砷濃度。模式模擬主要標的器官包括魚肉、鰓、腸壁、消化道及肝臟，各器官間由血液傳輸。經由標的器官濃度與動態反應關係顯示砷的吸收、代謝及分布情形，並結合濃度和時間曲線下面積(AUC)為基礎之急性毒暴露模式描述毒理動態。模式驗證過程採用養殖池野外調查資料及吸收排除實驗資料進行比較，結果顯示預測資料與實測濃度吻合，且模式應用可合理地模擬與建構標的器官濃度與致死效應之劑量反應關係。經由模擬結果顯示在短期暴露於砷的過程之中，鰓可以做為一個具有高敏感度的生物指標。此外，經由整合砷的藥理動力及動態模式與濃度曲線下面積(AUC)模式可定量地估測吳郭魚標的器官濃度與動態反應。本研究建立以生物為基礎之風險評估架構可提供未來水域生物在不同暴露情境下的重金屬累積模擬。

關鍵詞：砷，曲線下面積，烏腳病，藥理動力及動態模式，吳郭魚。

Abstract

This proposal focuses on field investigation and ecotoxicological modeling arsenic accumulation in aquacultural fish from blackfoot disease area in Taiwan. A physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) model was developed for arsenic (As) in tilapia *Oreochromis mossambicus*. The PBPK/PD model structure consisted of muscle, gill, gut wall, alimentary canal, and liver, which were interconnected by blood circulation. We integrate the target organ concentrations and dynamic response describing uptake, metabolism, and disposition of As and the associated area-under-curve (AUC)-based toxicological dynamics following an acute exposure. The model validations were compared against the field observations from real tilapia farms and previously published uptake/depuration experimental data, indicating predicted and measured As concentrations in major organs of tilapia were in good agreement. The model was utilized to reasonably simulate and construct a dose-dependent dynamic response between mortality effect and equilibrium target organ concentrations. Model simulations suggest that tilapia gills may serve as a surrogate sensitive biomarker of short-term exposure to As. This integrated As PBPK/PD/AUC model quantitatively estimates target organ concentration and dynamic response in tilapia and is a strong framework for future waterborne metal model development and for refining a biologically based risk assessment for exposure of aquatic species to waterborne metals under a variety of scenarios.

Keywords: Arsenic; Area-under-curve; Blackfoot disease; Physiologically based pharmacokinetics; Pharmacodynamics; Tilapia

Introduction

Humans are exposed to arsenic (As) from many sources such as food, water, air and soil in that food is the major exposure source for As. Chen et al. (2001) indicated that long-term exposure to ingested inorganic As in groundwater has been found to induce blackfoot disease (BFD), a unique peripheral vascular disease that ends with dry gangrene and spontaneous amputation of affected extremities in southwestern coastal area of Taiwan, consisting mainly of four towns, Putai, Yichu, Hsuehchia, and Peikangtzu located at Chiayi and Tainan counties. Nowadays, most of the people living in these areas do not drink water from groundwater because tap water has been made available in this area. However, groundwater is still used for aquaculture.

Lin et al. (2001) and Liao et al. (2003) conducted a long-term investigation during 1998 – 2001 in BFD area indicated that As have been detected in many aquacultural ponds in that As concentrations in aquacultural waters are reported to range from 26.3±16 to 251.7±12.2μg l^{-1}, whereas As concentrations in cultured fish are ranged from 0.94±0.3 to15.1±8.2μg g^{-1} dry wt. The results are much greater than the maximum contaminant level (MCL) for As in drinking water of 10μg l^{-1}.

Physiologically based pharmacokinetic models (PBPK) have been developed in several aquatic species. However, no PBPK model in an aquatic animal has related the toxicological effect to the target organ concentration, which would increase the significance of the model both for examination of the mechanism of toxicity of a chemical and for risk assessment. The objectives of the present paper are threefold: (1) to characterize the pharmacokinetics and pharmacodynamics of As after water exposure of tilapia, (2) to validate the PBPK/PD model against experimentally determined data by water exposure of tilapia to As and field measurements from real tilapia farms, and (3) to couple dynamically a PBPK/PD

model and an area-under-curve (AUC)-based acute toxicity model to construct concentration-response relationships.

Materials and Methods

Sample collection and chemical analysis

Tilapia (*Oreochromis mossambicus*) were collected from two fish ponds in Hsuehchia and three fish ponds in Yichu situated in the BFD area in the southwestern Taiwan region between August/November 1999 and January 2000. After the fish were stocked in the ponds, we used nylon nets to collect the samples of stocked tilapia from the fishponds. Fish samples were kept at 0°C cooler and were transported to the laboratory as quickly as possible. Thus, five sets of fish samples from Yichu and Hsuehchia had been collected. Each time three 500 ml water samples per pond were collected. One-liter polyethylene bottles cleaned with 10% nitric acid and then rinsed with deionized water were used as containers for the collection of water samples. The collected water was filtered through a 125 μm nylon mesh to remove large suspended particles and macro-invertebrates immediately after collection, and then acidified by adding 5 ml 1N HNO_3 before As analysis.

A Perkin-Elmer Model 5100PC atomic absorption spectrometer 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 l^{-1} for water samples and 0.05 μg As g^{-1} for tissue samples.

Acute Toxicity Bioassays

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 experimental design and calculations for the acute toxicity were based on well-known procedures given by Finney (1978) and Sparks (2000). The tests were carried out in 54 l rectangular fiberglass aquaria of well-aerated and reconstituted dilution water (pH 7.8 – 8.0). The tested fish were collected from fish ponds in Taiwan Fisheries Research Institute, Lukang, Chunghwa. Six fish of a specific size class (mean body length = 17.67±1.65 cm (mean ± SD) and mean body weight = 148.72±6.5 g wet wt) were randomly selected and transferred into each test aquarium. Dissolved oxygen in each tank was maintained at close to saturation by aeration (7.19±0.03 mg l^{-1}). The temperature in each aquarium was maintained at 24.3±0.32°C using submerged heaters. The photoperiod was 16 h light:8 h dark with an intensity of 1400±100 lux.

The sodium arsenite ($NaAsO_2$) stock solution was prepared with deionized water. The fish were visibly free of any deformities, lesions or disease and acclimated in tap water for one week prior to experiment. The nominal concentrations of As tested were 0 (control), 1, 2, 4, 10, 30, 50, and 80 mg l^{-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 mg l^{-1}. Gross mortality of fish to each concentration was recorded every 1 h for the first 12 h and every 2 h thereafter for 7 d, and dead fish being removed every 1 – 2 h. Tilapia were not fed throughout the test. Control and each test concentrations were tested in duplicate. The water quality management protocol was the same as deployed as the exposure experiments. No mortality occurred in the controls.

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. Statistical comparisons between LC_{50}s were based on the standard error of the difference. When it became apparent no statistically significant differences in LC_{50}s between bioassay replicates ($p > 0.05$), the replicates were pooled and a single LC_{50} was calculated for As. Chi-square tests were performed to test the homogeneity of mortality between replicates.

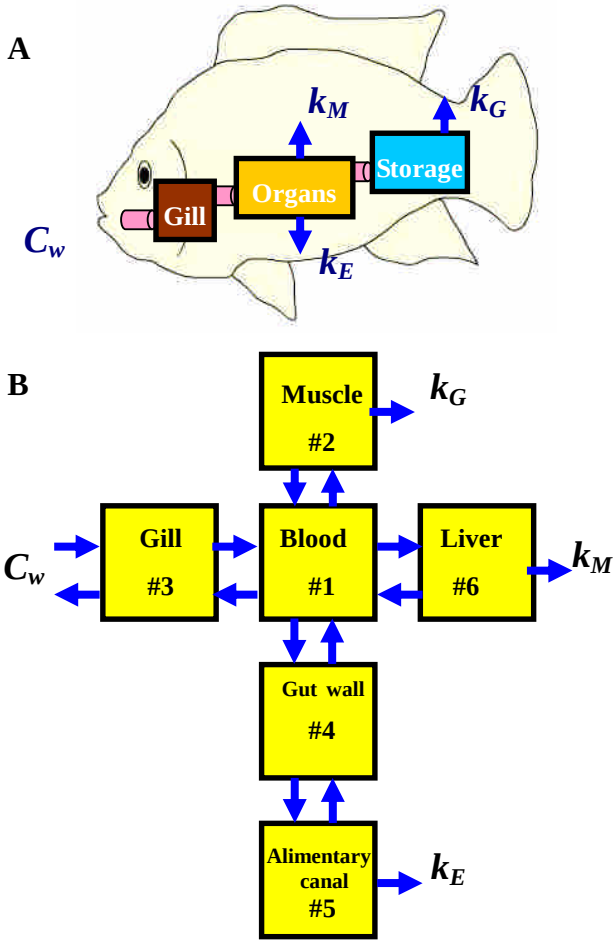

Fig. 1. Schematic diagram of physiologically based pharmacokinetic (PBPK) model for As in tilapia: (A) a compartment model showing the anatomical units of tilapia and (B) a PBPK model structure consisted of muscle, gill, gut wall, alimentary canal, and liver that interconnected by blood circulation.

PBPK model

The following assumptions were made to develop the PBPK model (Fig. 1): (i) there is a six-compartment pharmacokinetic model of blood-gill-muscle-gut wall-alimentary canal-liver, representing actual anatomical units of tilapia, (ii) it is assumed that the gill acts as a continuous-stirred tank reactor or well-mixed compartment into and out of which water flows, with chemical and oxygen being transferred to the tilapia based on diffusive mass transfer., (iii) a flow rate $Q_{ij} \geq 0$ gives the blood flow from the *j*th blood compartment to the *i*th organ compartment for $i \neq j$ with $1 \leq i, j \leq n$ in that all transport occurs through blood flows, (iv) there is a complete equilibrium of chemical between the blood phase and the tissue phase of each compartment and assumed that there is an inert soluble chemical with blood-chemical partitioning/binding coefficient f_i presents in amount of chemical partitioned to compartment tissue *i*, (v) there is a local mass balance of chemical substance in that for each compartment the amount of chemical substance entering is equal to the amount leaving, and (vi) there is a local mass balance of blood flow.

The essence of almost all PBPK models can be described by a linear dynamic equation

$$\frac{d\{C(t)\}}{dt} = [K]\{C(t)\} + [B]\{q(t)\}, \quad (1)$$

where $\{C(t)\}$ is a state variable vector which describes the chemical concentration in each assigned target organ, $\{q(t)\}$ represents an input vector of chemical concentration in ambient water, $[K]$ is a state matrix describes the diffusion exchange rate between target organs, and $[B]$ is a constant input matrix describes the exchange rate into target organs.

AUC-based toxicity model

The AUC-based toxicity model employed in determining the time-dependent median lethal concentrations (LC50(t)) can be expressed as (Liao and Lin, 2001),

$$\mathrm{LC}_{50}(t) = \frac{AUC_f}{\mathrm{BCF}}\left(\frac{k_2 + k_G}{(k_2 + k_G)t + e^{-(k_2+k_G)t} - 1}\right) + \mathrm{LC}_{50}(\infty) \quad (2)$$

where AUC_f is the area under the whole body burden of As in tilapia versus time curve (μg d g^{-1}).

AUC_f in Eq. (2) can be derived from the solution of a first-order one-compartment uptake-depuration model. Growth rate (k_G) can be calculated by fitting tilapia body weight data obtained from Reyes-Sosa and Castellanos-Molina (1995), Xie et al. (1997), and Takeuchi et al. (2002) to an exponential model (ln body weight (W) = $a + k_G t$, where a is a constant, g is the growth rate (d^{-1}), and t is the time in d), resulting a relationship of $k_G = 0.043\exp(-0.012W)$ ($r^2 = 0.96$).

PD model

In pharmacodynamic modeling, the relationship between dose effect and dose concentration is commonly expressed by the Hill equation. Incorporating with internal lethal body burden derived from TIC toxicity model to construct a dose-response profile, the mathematical model for the dose-response profile between mortality and As levels in different target organs of tilapia can be obtained by refining the Hill equation as

$$M_i = \frac{M_{\max} \times C_{f,i}^n}{C_{L,50}^n(\infty) + C_{f,i}^n} = \frac{M_{\max} \times C_{f,i}^n}{(\mathrm{BCF}_i \times \mathrm{LC}_{50}(\infty))^n + C_{f,i}^n} \quad (3)$$

where M_i is mortality for tilapia in 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 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.

Modeling and statistical analysis

We performed all model exercises in Matlab version 5.2 (The Mathworks, Natick, Massachusetts, USA). All steady-state analytical solutions and eigenvalues 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.

The curve fitting was performed using the nonlinear regression option of the Statistica® software (StatSoft, Tulsa, OK, USA). We also employed Statistica® to determine the coefficient of determination (r^2) and to perform statistical analyses including analysis of variance and Student's t test. Statistical significance was determined if p values were less than 0.05.

Table 1. Tilapia properties and biokinetic parameters used for PBPK model simulation

Symbol	Estimated value Mean ± SD	Description
Tilapia properties[a]		
W_t	208±32	Dry weight of whole fish (g)
V_1	0.02±0.002	Blood volume (L)
W_2	154.75±18.24	Dry weight of muscle (g)
W_3	10±1.02	Dry weight of gill (g)
W_4	5±0.75	Dry weight of gut wall (g)
W_5	12±1.25	Dry weight of alimentary canal (g)
W_6	5±0.42	Dry weight of liver (g)
Biokinetic parameters		
k_D	0	Food ingestion rate (g g^{-1} d^{-1})
k_E	2.0×10^{-3}	Egestion rate[b] (d^{-1})
k_G	6.7×10^{-3}	Growth rate[c] (d^{-1})
k_M	8.8×10^{-2}	Metabolic rate of As in liver[d] (d^{-1})

[a] Measured from field tilapia samples.

[b] Adopted from previously published uptake/depuration bioassay (Liao et al., 2004).

[c] Calculated from k_G=0.043exp(-0.012W_2) for an average tilapia muscle dry weight of W_2=154.75 g wet wt.

[d] Calculated from k_M=ln2/(ln2/k_2) in that k_2=8.8×10^{-2} d^{-1} for liver (Liao et al., 2004).

Table 2. Physiologically based parameters used for PBPK model simulation

Symbol	Estimated value[a]	Description
Q_{3w}	0.01	Gill-water exchange rate (L d^{-1})
Q_{12}	2.5	Blood-muscle exchange rate (L d^{-1})
Q_{13}	0.2	Blood-gill exchange rate (L d^{-1})
Q_{14}	7.5	Blood-gut wall exchange rate (L d^{-1})
Q_{15}	0.5	Blood-alimentary canal exchange rate (L d^{-1})
Q_{16}	3.6	Blood-liver exchange rate (L d^{-1})
Q_{45}	1.4	Gut wall-alimentary canal exchange rate (L d^{-1})
α_{3w}	8	Gill sorption factor
f_d	0.2	Fraction As dissolved in blood (g L^{-1})
f_2	5.2	Partition coefficient of muscle (L g^{-1})
f_3	0.04	Partition coefficient of gill (L g^{-1})
f_4	8.3	Partition coefficient of gut wall (L g^{-1})
f_5	4.6	Partition coefficient of alimentary canal (L g^{-1})
f_6	5.2	Partition coefficient of liver (L g^{-1})

[a]Calibrated from experimentally determined data adopted from Liao et al. (2004) and field observations conducted in the present research.

Results

Parameterization and application of PBPK model

The data for biokinetic parameters and tilapia properties were adopted from our research (Liao et al., 2003; Liao et al., 2004). Table 1 gives the weights of target organs and key biokinetic parameters used in model implementation.

Blood exchange flow rates to the various organs were not available in the literature for the tilapia. The estimates of the exchange flow rates shown in Table 2 therefore are based on the data of Thomann et al. (1997) where the exchange rates to various organs were carefully calibrated and were taken as being proportional to exchange flow on a Ld^{-1} basis in the same proportional as that seen in rainbow trout.

Dynamical coupling of AUC toxicity and PBPK/PD models

The selected time intervals of 24-h, 48-h, 72-h, 96-h, 120-h, and 144-h LC_{50} values with 95% CI of As to tilapia are shown in Fig. 2. LC_{50} lowers progressively as the duration of exposure increases. A limited number of studies have investigated As toxicity to tilapia. Our 96-h LC_{50}s of As to tilapia is 28.68 (95% CI: 24.92 – 32.44) mg L^{-1}, and 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 As to freshwater tilapia (71.7; 95% CI: 67.8 – 76.4 mg L^{-1}) reported by Hwang and Tsai (1993).

A dose-response relationship between equilibrium As concentration in each target organ of tilapia and mortality was derived using Eq. (3) and estimate of Hill coefficient (n) obtained from optimal fitting by nonlinear regression. The optimal fits to the observed percent mortality of tilapia versus waterborne As concentration of the 96-h acute toxicity test resulting in the estimated Hill coefficient, $n = 4.07$ ($r^2 = 0.93$, $p < 0.05$). We substitute $C_t(t)$ of organ-specific As concentrations and $C_{L,50}(t)$ to obtain a PD model describing the time-mortality profiles as functions of toxicokinetic parameters (k_2+k_G, BCF, and $LC_{50}(\infty)$) at certain waterborne As concentration. Therefore, dynamical coupling of the PBPK and PD models with AUC-based toxicity model can obtain a representation as shown in Fig. 3 in that time-concentration, time-mortality, and concentration-mortality profiles reveal a whole picture of this study.

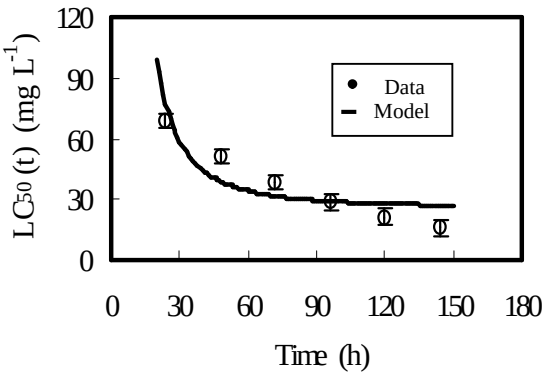

Fig. 2. Optimal fit of the AUC-based toxicity model to the LC_{50}(t) data.

Discussion

Internal effect concentration-time responses

The fit of the AUC-based TIC toxicity model may be strongly determined by the input parameters. Therefore, uncertainties in the k_2 and g values, which are input parameters in the AUC-based toxicity model, affect the validation of the model. Generally, the experimental $LC_{50}(t)$ data for tilapia exposed to waterborne As support the validity of the AUC-based toxicity model, despite the uncertainties in the input parameters k_2 and g values. Our analysis suggests that the use of constant whole body burden for each individual mode of action as an interpretive and regulatory tool in the environmental risk assessment of waterborne metals might be limited to mode of actions.

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

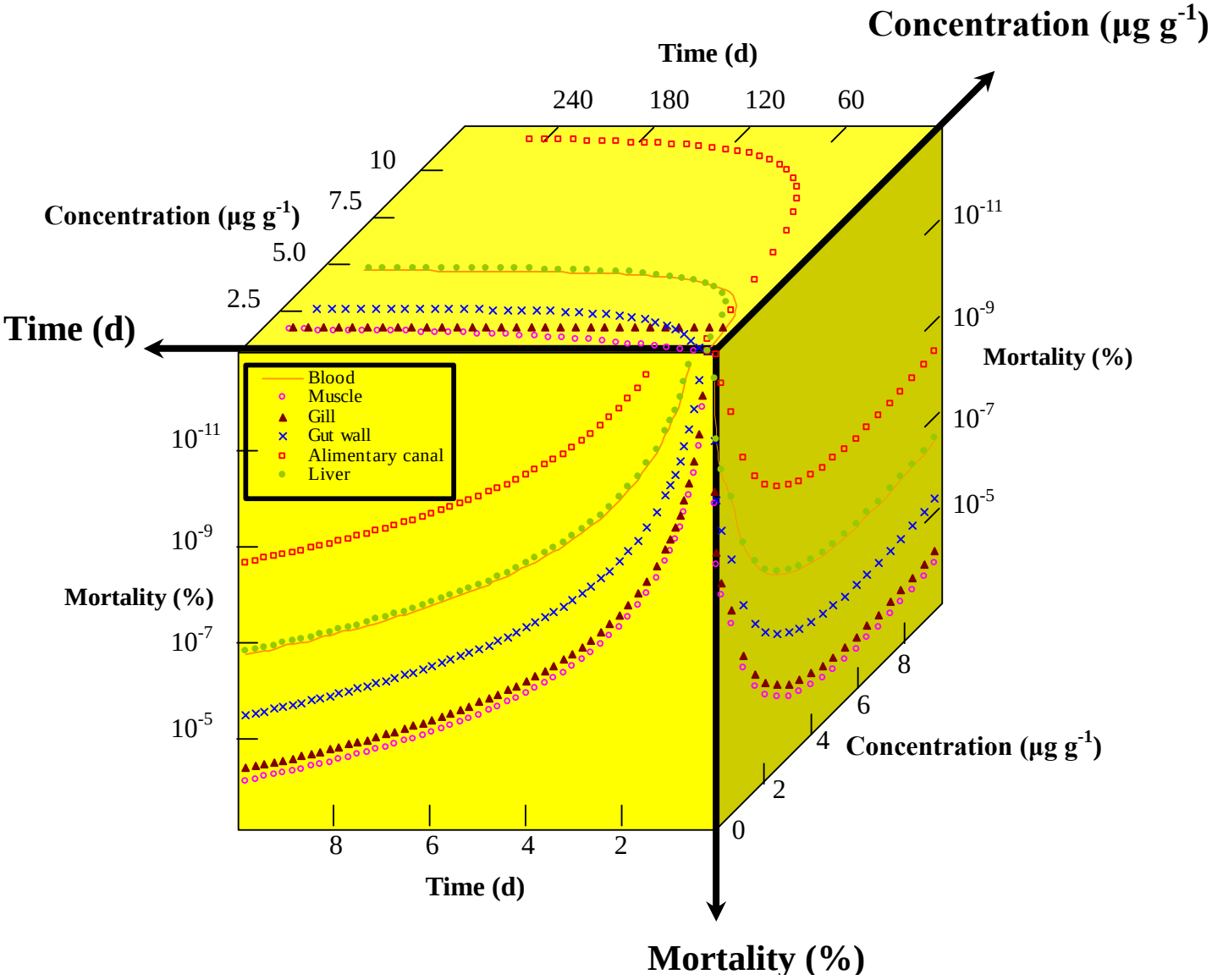

Fig. 3. An integrated PBPK/PD/AUC model simulation for the target organs of tilapia exposed to waterborne As at levels of experimental conditions.

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

PBPK and AUC-based PD models

The utility of a PBPK model derives from the fact that model structure and parameterization are based largely upon real anatomical, physiological, and biochemical attributes. The model structure also reflects the basic assumptions concerning factors that control chemical flux among organs and between the organism and its environment. Correspondence between predicted and measured values provides support for these assumptions as well as estimated values of important physiological parameter inputs. Accepting these assumptions, it then becomes possible to use the model to simulate hypothetical exposure scenarios.

A receptor theory-based PD model representing by a modified Hill equation is used to construct dose-response relationships between organ-specific equilibrium As concentrations in tilapia and their mortality effects (Bourne, 1995; de Vries, 1996). Therefore, by dynamical coupling of an appropriate PBPK model (i.e., the time course of accumulation of the waterborne As) and an AUC-based PD model (i.e., the time course of the adverse biological response by each target site of tilapia to the accumulated As), the complete dose-response profiles and duration of effect can be predicted for aquatic biota exposed to any waterborne metal.

Conclusion

The organ-specific dose-response relationships determined in this study indicate that gill has a more steep sigmoid profile than that of liver; indicating gill is a more sensitive organ than liver response to tilapia exposed to waterborne As. The organ-related difference in the mortality dose-response observed in this study is noteworthy. The gill is an important site of accumulation for many transition metals (Sorensen, 1991) and also many organic pollutants (Landrum et al., 1996). Furthermore, the gill is the primary site of toxicity, metal-induced mortality in freshwater fish occurring through the distribution of branchial ion regulation (Lauren and McDonald, 1987). Under these conditions, the gill appears to be a sensitive biomarker of short-term As exposure robust than the liver, alimentary canal, and gut wall. Parsimoniously, tilapia gill may serve as a surrogate sensitive biomarker of short-term exposure to waterborne As.

The PBPK/PD model is capable of quantifying target organ concentrations and dynamic response in tilapia, and is a useful tool to quantitatively assess risk associated with As exposure as well as helping to design and focus future experimental research. The capability of the model to accurately predict concentration and response is limited by adequately of the model parameters and limitations of the experimental data.

The development and validation of a PBPK/PD model is an iterative process and reflects the current limitations of our understanding of critical biological processes that help identify important data gaps. Additional studies are needed to better understand and to characterize the time course and dose response for inhibition mechanisms, since this PD response is particularly relevant as a potential biomarker for exposure. Our proposed As PBPK/PD model in tilapia linking dynamically of AUC-based toxicology model quantitatively estimates target organ concentrations and dynamic response, and is an effective framework for future metal development in aquatic species, and for establishing a biologically-based risk assessment for metal exposure under a variety of scenarios.

In conclusion, this model contributes to a better understanding of the fundamental processes that regulate the uptake, metabolism, and disposition of As in tilapia. This information is crucial to developing a better understanding of dynamic relationships between concentration exposure and hazard to tilapia and human whom consume the contaminated fish.

References

Bourne, D.W.A., 1995. Mathematical Modeling of Pharmacokinetic data. Technomic Publishing Company, Inc, Lancaster, Penn.

Chen, C.J., Hsueh, Y.M., Tseng, M.P., Lin, Y.C., Hsu, L.I., Chou, W.L., Chiou, H.Y., Wang, I.H., Chou, Y.L., Tseng, C.H., Liou, S.H., 2001. Individual susceptibility to arseniasis. In: Chappell, W.R., Abernathy, C.O., Calderon, R.L. (eds) Arsenic Exposure and Health Effects IV. Elsevier, Oxford, UK, pp 135-143.

de Vries, J., 1996. Toxicokinetic: quantitative aspects. In: Niesink, J.M., de Vries, J., Hollinger, M.A. (eds). Toxicology: Principles and Applications. CRC Press, New York, pp. 136-183.

Finney, D.J., 1978. Statistical Method in Biological Assay. 3rd ed. Cambridge University Press, London, 508 pp.

Hwang, P.P., Tsai, Y.N., 1993. Effects of arsenic on osmoregulation in the tilapia *Oreochromis mossambicus* reared in seawater. Mar Biol 117, 551-558.

Landrum, P.F., Harkey, G.A., Kukkonen, J., 1996. Evaluation of organic contaminant exposure in aquatic organisms: the significance of bioconcentration and bioaccumulation. In: Newman, M.C., Jagoe, C.H. (eds) Ecotoxicology: A Hierarchical Treatment. Lewis Publishers, Tokyo, pp. 85-131.

Lauren, D.J., McDonald, D.G., 1987. Acclimation to copper by rainbow trout, Salmo gairdneri: physiology. Can J Fish Aquat Sci 44, 99-104.

Liao, C.M., Lin, M.C., 2001. Acute toxicity modeling of rainbow trout and silver sea bream exposed to waterborne metals. Environ Toxicol 16, 349-360.

Liao, C.M., Chen, B.C., Singh, S., Lin, M.C., Liu, C.W., Han, B.C., 2003. Acute and bioaccumulation of arsenic in tilapia (*Oreochromis mossambicus*) from a blackfoot disease area in Taiwan. Environ Toxicol 18, 252-259.

Liao, C.M., Tsai, J.W., Ling, M.P., Liang, H.M., Chou, Y.H., Yang, P.T., 2004. Organ-specific toxicokinetics and dose-response of arsenic in tilapia *Oreochromis mossambicus*. Arch Environ Contam Toxicol, 47, 502-510.

Lin, M.C., Liao, C.M., Liu, C.W., Singh, S., 2001. Bioaccumulation of arsenic in aquacultural large-scale mullet Liza macrolepis from blackfoot disease area in Taiwan. Bull Environ Contam Toxicol 67, 91-97.

McCarty, L.S., Mackay, D., 1993. Enhancing ecotoxicological modeling and assessment. Environ Sci Technol 9, 1719-1728.

Reyes-Sosa, C.F., Castellanos-Molina, R., 1995. Nutritional evaluation of gizzard erosion positive brown fish meal starter diets for Nile tilapia, *Oreochromis niloticus*. Aquaculture 138, 323-329.

Sorensen, E.M.B., 1991. Metal Poisoning in Fish. CRC Press, Boston MA, PP. 367-379.

Sparks, T., (ed) 2000. Statistics in Ecotoxicology. Wiley, New York, NY, 320 pp.

Takeuchi, T., Lu, J., Yoshizaki, G., Satoh, S. 2002. Effect on the growth and body composition of juvenile tilapia *Oreochromis mossambicus* fed raw Spirulina. Fisheries Sci 68, 34-40.

Thomann, R.V., Shkreli, F., Harrison, S., 1997. A pharmacokinetic model of cadmium in rainbow trout. Environ Toxicol Chem 16, 2268-2274.

Xie, S., Cui, Y., Liu, J., 1997. Effect of body size on growth and energy budget on Nile tilapia, *Oreochromis mossambicus*. Aquaculture 157, 25-34.