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Original Research

A PBTK/TD Modeling-Based Approach Can Assess Arsenic Bioaccumulation in Farmed Tilapia (*Oreochromis mossambicus*) and Human Health Risks

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ABSTRACT

A biologically based risk-assessment model of arsenic (As) exposure evaluated farmed tilapia (*Orechromis mossambicus*) and human health during tilapia consumption in an area of southwestern Taiwan where blackfoot disease (BFD) occurs. The risk assessment addressed exposures to city residents who lived in Taipei, Taichung, and Kaohsiung, as well as subsistence fishers living in the BFD area who consumed tilapia. The models implemented included a physiologically based toxicokinetic and toxicodynamic (PBTK/TD) model to account for As exposure and dose-response profiles in tilapia and a human health exposure/risk model that accounts for target lifetime risk (TR) and hazard quotient (HQ) for humans consuming farmed tilapia. Results demonstrated that 90th percentiles of TR ranged from 1.53×10^{-8} to 1.62×10^{-6} for city residents with farmed tilapia consumption rates of 0.41 to 1.37 g/d. The 90th percentiles ranged from 2.07×10^{-6} to 7.89×10^{-5} for subsistence fishers in the BFD area with farmed tilapia consumption rates of 16.80 to 59.15 g/d. All predicted 90th percentiles of HQ were less than 1 for city residents and subsistence fishers in the BFD area, indicating small contributions from farmed tilapia consumption. Critical variables included whole-fish body weight, water As content, and As level in tilapia muscle. Although bioaccumulation of As seems unlikely to result in toxicity to farmed tilapia and humans consuming tilapia, the theoretical human health risks in the BFD area are alarming, using a probabilistic risk-assessment model based on conservative assumptions.

Keywords: Arsenic Human health risk Model-based risk assessment PBTK/TD Tilapia

INTRODUCTION

Humans are exposed to arsenic (As) from many sources, such as food, water, air, and soil; food is the major exposure source for As. The U.S. Food and Drug Administration (USFDA) (USFDA 1993), in examining this food category, indicated that fish and other seafood account for 90% of total As exposure. Donohue and Abernathy (1999) reported that the total As in marine fish, shellfish, and freshwater fish tissues ranged from 0.19 to 65, 0.2 to 125.9, and 0.007 to 1.46 µg/g dry wt, respectively. Koch et al. (2001) demonstrated that total As in freshwater fish ranged from 0.28 to 3.1 for whitefish (*Coregonus clupeaformis*), 0.98 to 1.24 for sucker (*Catostomus commersoni*), 0.46 to 0.85 for walleye (*Stizostedion vitreum*), and 1.30 to 1.40 µg/g dry wt for pike (*Esox lucius*).

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 the southwestern coastal area of Taiwan, consisting mainly of four towns: Putai, Yichu, Hsuehchia, and Peikangtzu, located at Chiayi and Tainan counties. Recently, a number of studies on acquired and genetic susceptibility to As have been carried out in the BFD-endemic areas of southwestern Taiwan to elucidate the cause of BFD (Chen et al. 2001). Nowadays, most of the people living in these areas do not drink water from groundwater sources because tapwater has been made available in this area. However, groundwater is still used for aquaculture.

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Lin et al. (2001), Singh (2001), and Liao et al. (2003a) conducted a long-term investigation during 1998–2001 in the BFD area and indicated that As was detected in many aquacultural ponds and that As concentrations in aquacultural waters were reported to range from 26.3 ± 16 to 251.7 ± 12.2 µg/L, whereas As concentrations in farmed fish ranged from 0.94 ± 0.3 to 15.1 ± 8.2 µg/g dry wt. The results are much greater than the maximum contaminant level of 10 µg/L for As in drinking water. Farming of tilapia (*Orechromis mossambicus*) is a promising type of aquaculture in the BFD area because of its high market value. The fish are fed with artificial bait, which does not contain As. These fish are maintained in the ponds for at least eight months (March–October) before they go to market. If waterborne As levels are elevated, toxicity can occur and can have severe effects on the health of cultured fish, which will reduce market prices and cause closure of fish farms. The presence of As in the aquacultural environment directly threatens tilapia, and consequently, As poses a risk to human who consume exposed tilapia.

We have attempted to develop a biologically based risk-assessment framework, which is most needed to interpret the significance of the reported exposures of tilapia to waterborne As under a variety of scenarios. A major complication in predicting or estimating risks for aquacultural species is the high degree of uncertainty resulting from the lack of dose-response information and the large environmental variability in exposures among individuals. As a result, formal risk assessments are scarce regarding aquacultural species. We focused on the risk of survival of tilapia exposed to waterborne As. Probabilistic modeling has received increasing support as a promising technique for characterizing the un-

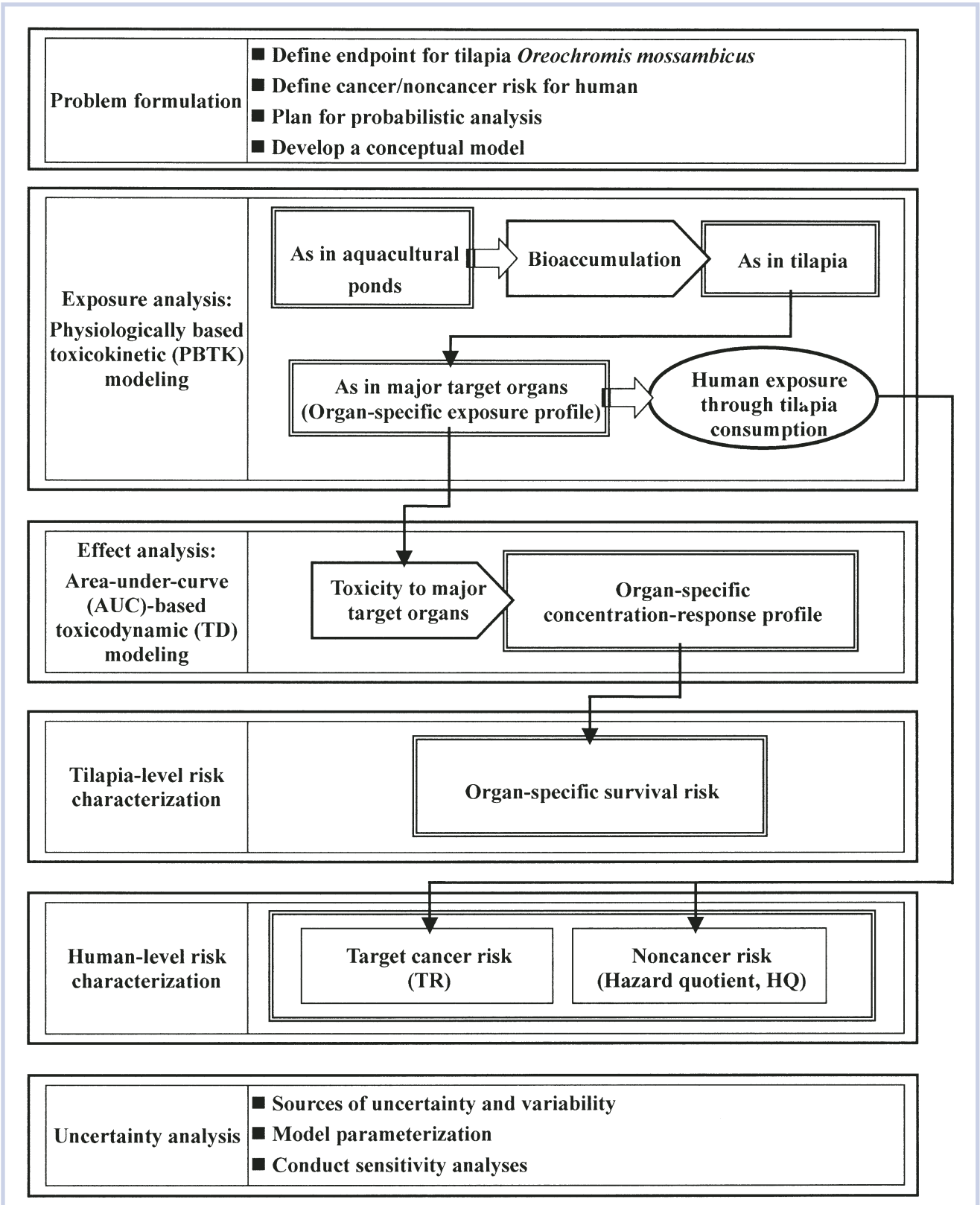

Figure 1. A conceptual model describing the approach phases of a PBTK/TD modeling-based risk assessment for farmed tilapia *O. mossambicus* exposed to waterborne As in the BFD area in Taiwan and human exposure through consumption of contaminated tilapia.

certainty and variation in estimates of environmental exposure assessment regarding aquacultural management (Liao and Ling 2003, 2004; Liao et al. 2003b).

In recent years, there has been a continued effort to improve the scientific basis of both cancer and noncancer dose-response assessments by incorporating information about physiologically based toxicokinetics (PBTK, the detailed mechanisms by which chemicals are distributed from the external environ-

ment to target organs) and toxicodynamics (TD, the detailed mechanisms by which target-organs doses are transformed into adverse biological responses). The use of PBTK models in toxicology research and chemical risk assessment today is primarily related to their ability to make more accurate predictions of target-organ dose for different exposure situations in different animal species. PBTK/TD models have been extensively used in risk assessment and prediction of TK and TD behavior in several aquatic species, e.g., Cd in rainbow trout *Salmo gairdneri* (Thomann et al., 1997), paraoxon in rainbow trout *Oncorhynchus mykiss* (Abbas and Hayton 1997), Zn in abalone *Haliotis diversicolo supertexta* (Liao et al. 2000), and waterborne organic chemicals in brook trout *Salvelinus fontinalis* (Nichols et al. 1998) and in lake trout *Salvelinus namaycush* (Lien et al. 2001).

To aid in the development of realistic estimates of the risk associated with tilapia exposed to waterborne As, a PBTK model was developed to improve the accuracy of human health risk assessment. To achieve this goal, it is critical to characterize the distribution of model responses that are associated with variability and uncertainty in key model parameters. Monte Carlo methods have been applied to numerous PBTK models to help determine the overall impact of parameter variability and uncertainty on risk-assessment predictions (Clewell and Andersen 1996; Clewell et al. 1999).

The objectives of this study were 2-fold: (1) to conduct an environmental risk assessment based on the U.S. Environmental Protection Agency (U.S. EPA) methodology for aquacultural farms to develop As exposure estimates for farmed tilapia in the BFD area and human health through tilapia consumption and (2) to address the uncertainties by using a probabilistic PBTK/TD approach to characterizing risks that yields quantitative risk estimates and their associated uncertainties. A PBTK model was used to estimate As concentrations in target organs of tilapia and apply a human health exposure and risk model to account for the hazard quotient (HQ) and lifetime risk for humans consuming contaminated tilapia. Predicted specific organ concentration and internal lethal body-burden data were combined with a dose-response relationship derived from a TD model to assess the survival endpoint of tilapia. To determine overall uncertainty in predicted risks, the uncertainties resulting from the assessment of exposure and dose response and propagated through the risk-characterization process using a Monte Carlo technique were determined, whereas a sensitivity analysis was performed to identify the critical inputs.

MATERIALS AND METHODS

The PBTK/TD modeling-based risk-assessment analysis is divided into six phases (Figure 1) and is described in the subsequent sections.

Problem formulation

The problem formulation of this study is the phase wherein the assessment endpoint is defined, analyses for associating As contamination with the assessment endpoint are planned, and a conceptual model is developed (Figure 1). The conceptual model is developed starting from the environmental contamination with As, in terms of As concentration in tilapia farms in the BFD area, in that the major As exposure database was adopted from the previous studies conducted by Singh (2001) and Liao et al. (2003a). They collected farmed tilapias (*O. mossambicus*) from two fish ponds in Hsuehchia and three fish ponds in Yichu situated in the BFD area of

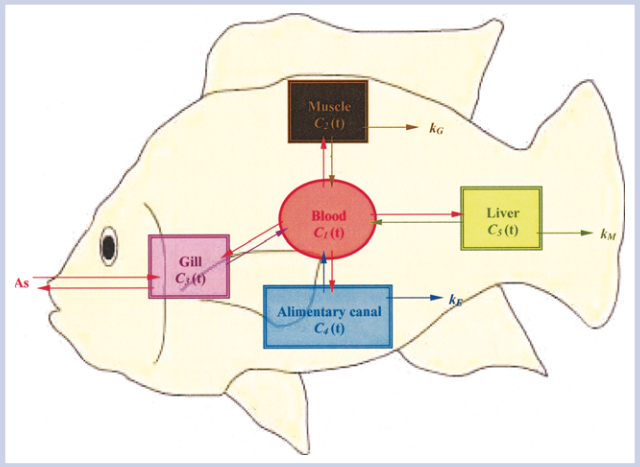

Figure 2. Schematic diagram of PBTK model for As in tilapia, in that a PBTK model structure consists of muscle, gill, alimentary canal, and liver that is interconnected by blood circulation.

southwestern Taiwan in January, August, and November 1999, 2000, and 2001. They measured As concentrations in pond water and major organs and conducted 15-d laboratory exposure experiments to estimate the essential biokinetic and physiological parameters in an As-tilapia system. The selected five tilapia farms had similar feeding strategies and management practices.

The assessment endpoint for tilapia is defined as the organ-specific mortality risk of gill, muscle, and liver associated with As exposure because the muscle, gill, and liver are the most sensitive to the effects of As (Liao et al. 2003a). The target cancer risk and noncancer HQ are used as the assessment endpoints for human exposure through farmed-tilapia consumption. The conceptual model is based on a number of assumptions. These assumptions are necessary mainly because of a lack of data and to keep the model simple yet reasonably functional. Most of the assumptions are stated in the parameter descriptions in the subsequent sections. The major assumptions are as follows. The pathway of As exposure of farmed tilapia occurs only via water ingestion. Farmed tilapia are contaminated with As through groundwater. Exposure to As in city residents in Taipei, Taichung, and Kaohsing and in subsistence fishers in the BFD area occurs only from farmed tilapia obtained from tilapia farms in the BFD area.

Exposure analysis

A PBTK model was used to estimate As concentrations in target organs. The PBTK model structure consisted of muscle (compartment 2), gill (compartment 3), alimentary canal (compartment 4), and liver (compartment 5), which are interconnected by blood (compartment 1) circulation (Figure 2). The essence of almost all PBTK models can be described by a linear dynamic equation (Abbas and Hayton 1997; Thomann et al. 1997; Nichols et al. 1998; Liao et al. 2000; Lien et al. 2001) (see Appendix A for details)

$$\frac{d\{C(t)\}}{dt} = [K]\{C(t)\} + [X]\{u(t)\} \quad (1)$$

where $\{C(t)\}$ is a state variable vector that describes the chemical concentration in each assigned target organ, $\{u(t)\}$ represents an input vector of chemical concentration in ambient

water, [*K*] is a state matrix describes the diffusion exchange rate between target organs, and [*X*] is a constant input matrix that describes the exchange rate into target organs.

A steady-state condition is assumed in Equation 1. Solving for the equilibrium As concentrations in muscle (C_2), gill (C_3), and liver (C_5) gives results in Equations 2, 3, and 4 as follows:

$$C_2 = \frac{q_{21} f_d IJCG}{\left[AFHIJ - \left(HIJBq_{21} f_d + FIJCq_{31} f_d + FHJDq_{41} f_d + FHIEq_{51} f_d \right) \right]} \quad (2)$$

$$C_3 = \frac{G\left[q_{31} f_d FIJC + \left(AFHIJ - HIJBq_{21} f_d + FIJCq_{31} f_d + FHJDq_{41} f_d + FHIEq_{51} f_d \right) \right]}{H\left(AFHIJ - HIJBq_{21} f_d + FIJCq_{31} f_d + FHJDq_{41} f_d + FHIEq_{51} f_d \right)} \quad (3)$$

$$C_5 = \frac{q_{51} f_d FICG}{AFHIJ - HIJBq_{21} f_d + FIJCq_{31} f_d + FHJDq_{41} f_d + FHIEq_{51} f_d} \quad (4)$$

where

$$A = f_d\left(q_{12} + q_{13} + q_{14} + q_{15}\right), \quad B = q_{12} f_2^{-1}, \quad C = q_{13} f_3^{-1},$$

$$D = q_{14} f_4^{-1}, \; E = q_{15} f_5^{-1}, \; F = q_{21} f_2^{-1} + k_G W_2, \; G = q_{3w} \alpha_{3w} C_w,$$

$$H = q_{31} f_3^{-1} + q_{3w} f_3^{-1}, \; I = q_{41} f_4^{-1} + k_E W_t,$$

$$J = q_{51} f_5^{-1} + k_M W_5 \;,$$

and C_w is the waterborne As concentration (μg/L); q_{ij} is the diffusive exchange rate between organs *i* and *j* (L/d); f_d is the binding coefficient of As concentration to plasma proteins (g/L); f_i is defined as C_i/C_{di}, which denotes the partition coefficient, or which is referred to as an organ-blood equilibrium distribution ratio for linear binding in specific organ *i* (L/g); C_i is the total As concentration in target organ *i* (μg/g); C_{di} is the dissolved As concentration in the blood leaving target organ *i* (μg/ml); W_i is the time-independent tissue weight of organ *i* (g wet wt); W_t is the whole-fish body weight (g wet wt); q_{3w} is the gill-water exchange rate (L/d); α_{3w} is the gill sorption factor representing enhancement of surface sorption; k_E is the elimination rate of fecal egestion (g/g/d); k_M is the metabolic transformation rate of As in liver (g/g/d); and k_G is the tilapia growth dilution rate (g/g/d). The input variables needed to simulate the As bioaccumulation in major organs of tilapia include tilapia properties (W_i), biokinetic parameters (k_E, k_M, k_G), physiological parameters (q_{ij}, f_d, f_i), and a geochemical variable (C_w).

Effect analysis

Target-organ residues and adjusted response frequencies were used to construct an organ-specific dose-response relationship for mortality effect versus steady state. The As level in tilapia is based on an area-under-the-curve (AUC)–based TD model (Lalonde 1992; Bourne 1995). Liao et al. (2004b) have established a quantitative relationship between target-organ residues and mortality effects in tilapia. It can be seen from the previously published acute toxicity data that the mortality function is estimated from observed mortality percentages in exposure regimes in which mortality is an increasing function of the As concentration in water. In fitting the AUC-based TD model to the observed mortality for specific-interval acute toxicity data, the dose-response profile can be expressed (Liao et al. 2004b) as

$$M = \frac{100 \times C_w^{4.07}}{(96 - \text{h LC50})^{4.07} + C_w^{4.07}} \quad (5)$$

where *M* is the mortality (%); the exponent 4.07 is a fitted average value, referred to as the Hill coefficient; and 96-h LC50 is the 96-h median lethal concentration (mg/L).

Equation 5 can be transformed appropriately to an organ-specific concentration-response relationship using the AUC-based TD model framework to predict the response (Liao et al. 2004b) as

$$M_i = \frac{100 \times C_{f,i}^{4.07}}{(C_{L,50})^{4.07} + C_{f,i}^{4.07}} = \frac{100 \times C_{f,i}^{4.07}}{\left(BCF_i \times LC50(\infty)\right)^{4.07} + C_{f,i}^{4.07}} \quad (6)$$

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*; and $LC50(\infty)$ is the incipient value of $LC50(t)$. We treated BCF_i and $LC50(\infty)$ in Equation 6 probabilistically. Applying the AUC-based TD model, the cumulative distribution function (CDF) of a predicted mortality function for a given organ As concentration, $F(M_i|C_{f,i})$, can be expressed symbolically as a conditional CDF:

$$F(M_i \mid C_{f,i}) = \Phi\left(\frac{100 \times C_{f,i}^{4.07}}{(BCF_i \times LC50(\infty))^{4.07} + C_{f,i}^{4.07}} \right) \quad (7)$$

where $\Phi(\bullet)$ is the cumulative standard normal distribution.

Tilapia-level risk characterization

Risk characterization is the phase of risk assessment wherein the results of the exposure and quantitative effects assessments are integrated to provide an estimate of risk for the

population under study. Risk at a specific As concentration in tilapia target organ i ($C_{f,i}$) can be calculated as the proportion of tilapia expected to have that organ concentration multiplied by the conditional probability of tilapia mortality, given concentration $C_{f,i}$. This results in a joint probability function (JPF), or exceedence profile, which describes the probability of exceeding the concentration associated with a particular degree of effect. This can be expressed mathematically as

$$R(C_{f,i}) = F(C_{f,i})F(M_i \mid C_{f,i}) \tag{8}$$

where $R(C_{f,i})$ is the risk for a specific organ i at concentration $C_{f,i}$, and $F(C_{f,i})$ is the CDF of having organ concentration $C_{f,i}$.

A risk curve was generated from the cumulative distribution of simulation outcomes. Each point on the risk curve represents both the probability that the chosen proportion of tilapia will be affected and also the frequency with which that level of effect would be exceeded. The x-axis of the risk curve can be interpreted as a magnitude of effect (a percentage of given tilapia expected to suffer the adverse effect), and the y-axis can be interpreted as the probability that an effect of at least that magnitude will occur. These probabilities are based on the current exposure data, so at each point on the JPF, we can also interpret this as follows: under current conditions, x% of tilapia will be affected, and this proportion of tilapia would be affected by y% of the current observations.

The overall expected risk for tilapia may be computed as the sum of the risks for all possible $C_{f,i}$s. Specifically, because the As concentrations in a tilapia specific organ are distributed log-normally and the responses follow Equation 8, the overall expected risk for specific organ i, $\mathrm{E}[R_i]$, could be estimated as

$$\mathrm{E}[R_i] = F(\bar{C}_{f,i})F(M_i \mid \bar{C}_{f,i}) \tag{9}$$

where E[•] is the expectation operator, and the CDFs of $F(\bar{C}_{f,i})$ and $F(M_i \mid \bar{C}_{f,i})$ can be expressed, respectively, as

$$F(\bar{C}_{f,i}) = \Phi\left(\frac{\ln \bar{C}_{f,i} - \ln \mu_g}{\ln \sigma_g}\right) \tag{10}$$

and

$$F(M_i \mid \bar{C}_{f,i}) = \Phi\left(\frac{100 \times \bar{C}_{f,i}^{4.07}}{(\mathrm{BCF}_i \times \mathrm{LC50}(\infty))^{4.07} + \bar{C}_{f,i}^{4.07}}\right) \tag{11}$$

The mean concentration of As in a tilapia specific organ i, $\bar{C}_{f,i}$, has the form

$$\bar{C}_{f,i} = \frac{\int_0^\infty \left(\mathrm{LN}_{C_{f,i}}(\mu_g, \sigma_g)\right) C_{f,i} dC_{f,i}}{\int_0^\infty \mathrm{LN}_{C_{f,i}}(\mu_g, \sigma_g) dC_{f,i}} \tag{12}$$

where $\mathrm{LN}(\mu_g, \sigma_g)$ denotes a log-normal distribution with geometric mean μ_g and a geometric standard deviation σ_g. A confidence interval (CI) for expected risk was determined on the basis of the 2.5 and 97.5 percentiles of the simulation results.

Human-level risk characterization

Donohue and Abernathy (1999) and Schoof et al. (1999) reported that the amount of inorganic As in seafood ranges from <3 to 7% of the total As. In this work, it is assumed that inorganic As accounts for 7.4% of the total As in tilapia, as suggested by Huang et al. (2003), who reported that the average inorganic As percentage of farmed tilapia obtained from the BFD area was 7.4%. The target cancer risk to adults is defined as (USEPA 1996)

$$\mathrm{TR} = \frac{C_{f,m}^* \times \left(\mathrm{CSF}_{\mathrm{IRIS}}\left(\frac{\mathrm{BW}}{70\ \mathrm{kg}}\right)^{1/3}\right) \times \mathrm{IR}_f \times \mathrm{EF} \times \mathrm{ED}}{\mathrm{BW} \times \mathrm{AT}_c \times 10^3} \tag{13}$$

where TR is the incremental individual lifetime cancer risk (dimensionless); $\mathrm{CSF}_{\mathrm{IRIS}}$ is the oral carcinogenic slope factor from IRIS (Integrated Risk Information System, provided by the U.S. EPA) database $(\mathrm{mg/kg/d})^{-1}$; IR_f is the annualized fish ingestion rate (g/d); $C_{f,m}^*$ is the inorganic As concentration for 7.4% of the total As in tilapia muscle (μg/g wet wt), EF is the exposure frequency (d/year); ED is the exposure duration (year); AT_c is the averaging time for carcinogens (d); BW is the body weight of Taiwanese adults (kg); and 10^3 is the unit conversion factor.

The noncancer risk was estimated using the HQ approach, defined as (USEPA 1996)

$$\mathrm{HQ} = \frac{C_{f,m}^* \times \mathrm{IR}_f \times \mathrm{EF} \times \mathrm{ED}}{\left(\mathrm{RfD}_{\mathrm{IRIS}}\left(\frac{\mathrm{BW}}{70\ \mathrm{kg}}\right)^{1/3}\right) \times \mathrm{BW} \times \mathrm{AT}_{\mathrm{nc}} \times 10^3} \tag{14}$$

where HQ is the toxicity HQ (dimensionless); $\mathrm{RfD}_{\mathrm{IRIS}}$ is the oral reference dose from the IRIS database (mg/kg/d); $\mathrm{AT}_{\mathrm{nc}}$ is the averaging time for noncarcinogens (d); and 10^3 is the unit conversion factor. BW, $C_{f,m}^*$, and IR_f are treated probabilistically in Equations 13 and 14.

The outputs of the bioaccumulation model are predictions of As concentrations in an organ of an individual fish at steady state. The exposure duration is defined as the exposure frequency of 365 d/year for 30 years (i.e., 10,950 d). The averaging time and number of fish consumed are required to provide input for an estimate of human health risk from exposure through fish ingestion. An averaging time of 365 d/year for 70 years (i.e., AT_c = 25,550 d) was used to characterize lifetime exposure for cancer risk calculation. An averaging time of 365 d/year for 30 years (i.e., $\mathrm{AT}_{\mathrm{nc}}$ = 10,950 d) was used in characterizing noncancer risk.

The cancer slope factor and reference dose for ingested inorganic As are 1.50 $(\mathrm{mg/kg/d})^{-1}$ and 3×10^{-4} mg/kg/d, respectively, provided by the U.S. EPA IRIS database (http://www.epa.gov/iris) and normalized to account for extrapolation to a different body weight from the standard of 70 kg (Equations 8 and 9), as suggested in the *Exposure Factors Handbook* (USEPA 1997). These values are specified as point estimates following U.S. EPA guidance (USEPA 1989b). It was assumed in accordance with the U.S. EPA (USEPA 1989a) guideline that the ingested dose is equal to the absorbed contaminated dose and that cooking has no effect on the contaminants. The acceptable risk distribution was assigned by constraints on percen-

Table 1. Input variables/parameter values to define distributions for Monte Carlo simulations

Parameters	Symbol	Uncertainty/ variability	Distribution
Biokinetic parameters			
Tilapia growth dilution rate[a]	k_G (g g^{-1} d^{-1})	U	LN (0.0035, 4.93)
Metabolic transformation rate of liver[b]	$k_{M,}$ (g g^{-1} d^{-1})	U	LN (0.0861, 1.24)
Elimination rate of fecal egestion[c]	k_E (g g^{-1} d^{-1})	U	LN (0.0034, 1.14)
Geochemical parameter			
Dissolved As concentration in water	C_w (μg L^{-1})	U	LN (44.24, 2.64)
Tilapia parameters			
Whole-fish body weight[d]	W_t (g wet wt) [e]	V	N (218.91, 131.36)
Body weight of specific target organ *i*[d]	W_i (g wet wt)		
Muscle		V	N (151.24, 91.02)
Liver		V	N (5.30, 3.10)
Dose-response parameters			
Incipient median lethal concentration	LC50(∞) (mg L^{-1})	U	N (25.55, 5.21)
Bioconcentration factor for tilapia of specific target organ *i*	BCF_i (mL g^{-1})		
Muscle		U	LN (16.49, 1.01)
Gill		U	LN (18.62, 1.01)
Liver		U	LN (66.93, 1.00)
Human health parameters			
Body weight for Taiwanese adult	BW (kg)	V	N (59.92, 4.36)
Tilapia ingestion rates for subsistence fishers in BFD area	IR_f (g d^{-1})		
2–6 meals per week		U	LN (22.07, 2.61)
7–14 meals per week		U	LN (36.68, 1.75)

[a]Calculated from $k_G = 0.043\exp(-0.012W_t)$ (Liao et al. 2004a).
[b]Calculated from $k_{M,i} = \ln 2/(\ln 2/k_{2,i})$ in that $k_{2,i}$ is the depuration rate of specific target organ *i* (Liao et al. 2004b).
[c]Adopted from Liao et al. (2004b).
[d]Measured from field tilapia samples (Liao et al. 2003a).
[e]Wet weight = dry weight × 5.35 (81.3% water content) (Lung et al. 2003).

tiles. The lower end of the range of acceptable risk distribution is defined by a single constraint on the 90th percentile of risk distribution that must be equal to or lower than 10^{-6} for carcinogens and equal to or lower than 1 for noncarcinogens.

Uncertainty analysis

Model parameterization. Parameterization of the model involved selecting data sets and deriving input distributions. Current literature was reviewed to develop probability distributions for the random variables appearing in the PBTK/TD model, organ-specific dose-response model, and the human health exposure and risk model adopted. The input variables of Equations 2 to 4, 6, 9, 13, and 14 were adopted from previous published studies (Singh 2001; Liao et al. 2003a, 2003b). Data were sorted by reported statistical measure, e.g., mean, standard deviation, standard error, etc. The data were divided into a minimum of 10 bins as equally as possible. Absolute and relative frequencies were calculated, and distributions were plotted using bin midpoints. The χ^2 and Kolmogorov-Smirnov (K-S) statistics were used to optimize the goodness of fit of distributions. The software program @RISK (version 4.5, Professional Edition, Palisade, Newfield, NY, USA) was used to analyze data and to estimate distribution parameters. The selected distribution type and parameters were based on statistical criteria and comparisons of distribution parameters. The implemented parameter probability distributions are summarized in Table 1 and described in the subsequent sections.

Biokinetic parameters in PBTK model: k_E, k_G, k_M. The PBTK model is composed of terms involving tilapia body size and biokinetics and terms involving physiological metal-specific processes. The PBTK model for As in tilapia has been validated against field observations and experimental data and has indicated that the predicted and measured As levels in

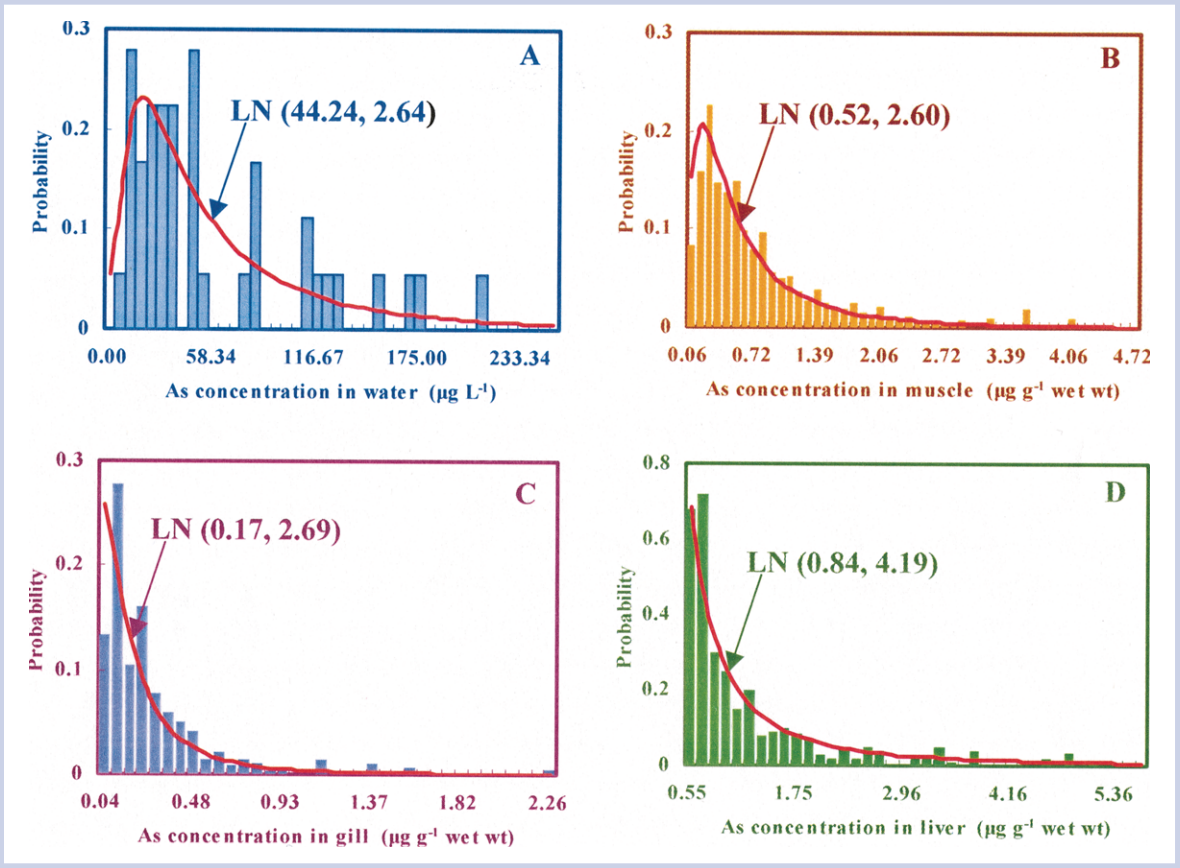

Figure 3. Probability density distributions of predicted As concentrations in (**A**) water, (**B**) muscle, (**C**) liver, and (**D**) gill by exposure analysis compared with frequency distribution by measurements. LN(α,β) denotes a log-normal distribution with a geometric mean α and a geometric standard deviation β.

major organs of tilapia were in good agreement (Singh 2001; Liao et al. 2004a). The estimated physiological parameters of partition coefficients and exchange rates among target organs in the PBTK model were reported only as average values (see Appendix B). As a result, the risk curves and CIs reported here do not incorporate this source of uncertainty. We polled lab- and field-derived biokinetic data obtained from different sources, and the selected log-normal distributions had an acceptable χ^2 fit and a K-S fit, in that optimizations using either statistics yielded a geometric mean and geometric standard deviation (Table 1).

Tilapia properties. Distributions of weight of specific target organ were fitted to the measurements, and the selected normal distributions had the optimal K-S goodness of fit (Table 1).

Geochemical parameter: C_w. Distributions of water As concentrations in tilapia ponds (C_w) were fit to the polled field observations obtained from five assigned tilapia farms from the BFD area, and the selected log-normal distributions had the optimal K-S and χ^2 goodness-of-fit (Table 1).

Parameters in TD model: BCF_i, $LC50(\infty)$. In applying dose-response relationships derived from experimental study, we must consider the limitations of the data and account for the inherent uncertainty that arises from a number of sources, including the limited number of observations and the limited sample size within treatment sets. To account for this uncertainty, the distributions for the input variables of BCF_i and $LC50(\infty)$ in the AUC-based TD model were derived from the dose-response function in Equation 7. A log-normal distribution for BCF_i and a normal distribution for $LC50(\infty)$ (Table 1) was determined and incorporated the distributions into the Monte Carlo simulation to obtain 2.5 and 97.5 percentiles as the 95% CI for the reconstructed dose-response profile. Uncertainty and/or variability was not considered for the reported Hill coefficient because the Hill coefficient from the published study was reported only as an average value.

Human health exposure/risk model parameters: $C^*_{f,m}$, BW, IR_f. Data on tilapia consumption patterns were adapted from two sources: (1) Lung et al. (2003), which was estimated by dividing the annual consumption quantities of tilapia in three major cities, Taipei, Taichung, and Kaohsiung, by the number of residences (age >5 y) in each assigned city, in that the average weight of Taiwanese adults was 65 kg, and (2) M.C. Lin (Nanhua University, Chiayi, Taiwan, Republic of China, unpublished data), which was based on a questionnaire on tilapia daily consumption rate for 57 subsistence fishers in the BFD area. The estimated average tilapia consumption rates in Taipei, Taichung, and Kaohsing were 1.37, 3.58, and 0.41 g/d, respectively. M.C. Lin (unpublished data) provided data on tilapia daily consumption rates for subsistence fishers in the BFD area: 16.8 to 50.05 and 29.4 to 59.15 g/d, for 2 to 6 and 7 to 14 meals/week, respectively, in that the edible percentage of farmed tilapia is 35% (Lung et al. 2003). We approximated these data using a log-normal distribution and transformed them appropriately to ensure that the data did not differ from a normal distribution before parametric analysis. Results give tilapia consumption rate distributions of LN(22.07 g/d, 2.61) and LN(36.68 g/d, 1.75), for 2 to 6 and 7 to 14 meals/week, respectively, for subsistence fishers in the BFD area. Distribution of the average weight of Tai-

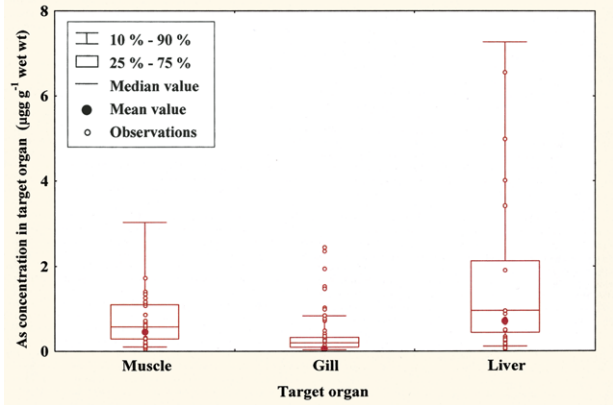

Figure 4. Box-and-whisker plot representations of distributions of As concentration in tilapia muscle, gill, and liver. The observations and predicted mean values are also shown. Box-and-whisker plots are used to represent the uncertainty in As level estimates.

wanese adults was fitted to the data obtained from the Department of Health, Taiwan (http://www.doh.gov.tw) (ages range from 19–65 years), and the selected normal distribution had the optimal K-S goodness of fit (Table 1).

Monte Carlo analysis. To quantify this uncertainty and its impact on the estimation of expected risk, a Monte Carlo simulation was implemented that included input distributions for the parameters of the derived dose-response function, as well as for estimated exposure parameters. To test the convergence and the stability of the numerical output, we performed independent runs at 1,000, 4,000, 5,000, and 10,000 iterations with each parameter sampled independently from the appropriate distribution at the start of each replicate. Largely because of limitations in the data used to derive model parameters, inputs were assumed to be independent. The result shows that 10,000 iterations are sufficient to ensure the stability of results. Sensitivity analysis identified the most significant parameters that were included in the uncertainty and variability analysis. The sensitivity of each variable relative to one another was assessed by calculating rank correlation coefficients between each input and output during simulations and then estimating each input contribution to the output variance by squaring the output variance and normalizing to 100%. The Monte Carlo simulation and sensitivity analysis were implemented using Crystal Ball software (version 2000.2, Decisioneering, Denver, CO, USA).

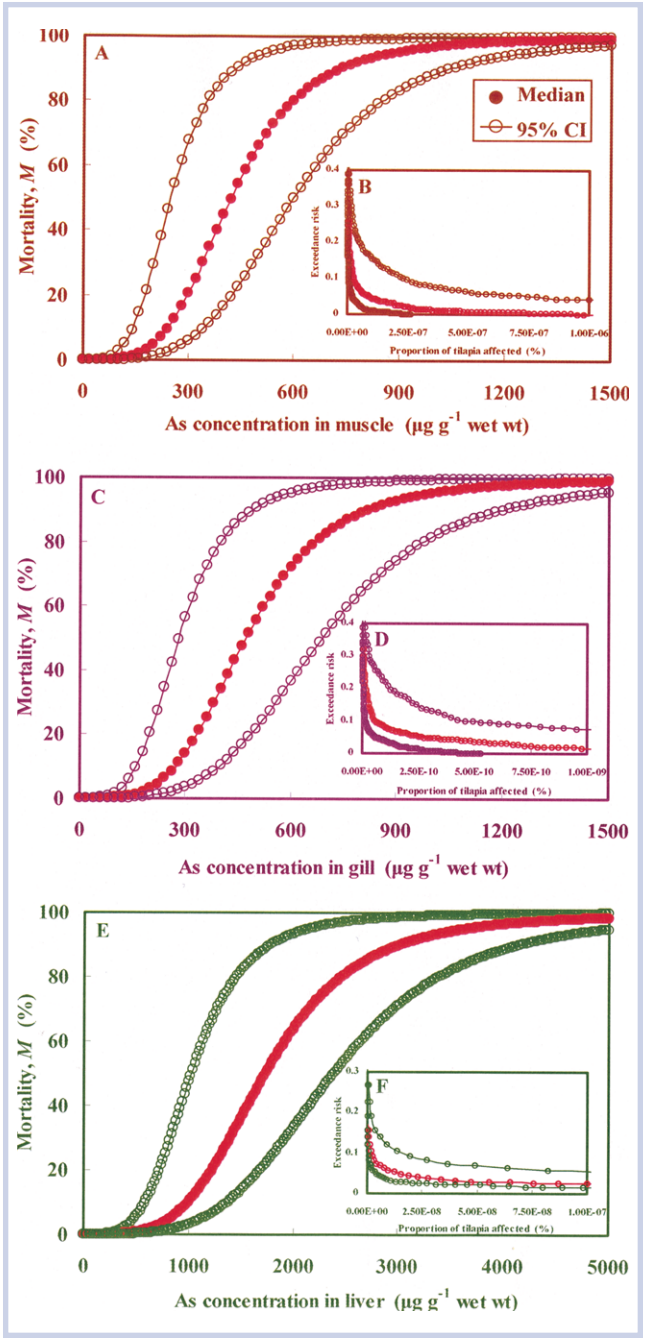

Figure 5. Reconstructed concentration-response profiles with 95% CI by an AUC-based TD model showing the relationships between tilapia mortality and As concentrations associated with exceedence risk (mortality) functions, with 95% CI for tilapia in (**A**, **B**) muscle, (**C**, **D**) gill, and (**E**, **F**) liver.

RESULTS AND DISCUSSION

Exposure assessment

Figure 3 illustrates the predicted probability density functions (PDFs) of As contents in tilapia subject to the measured PDFs of pondwater As concentrations from tilapia farms in the BFD area. Probabilistic simulations of the PBTK model produced skewed distributions of predicted As concentrations in tilapia muscle, gill, and liver. Percentile predictions of As contents in target organs could be determined from CDFs corresponding to PDFs shown in Figure 3. Figure 4 shows the box plots of interquartile and 50th percentile predictions associated with whisker plots indicating 10- and 90-percentile predictions of As contents in tilapia muscle, gill, and liver. The comparison of 90-percentile values of PDFs showed that tilapia exposure to As caused the relative skewness and spread in modeled output that varied among specific organs. Results demonstrated that the distribution of As concentration in tilapia liver was more highly skewed with a long tail at higher concentration (Figure 3D) and that estimated tilapia liver As concentration has a higher uncertainty as quantified by the variance (Figure 4).

Compared with the field observations (Figure 4), measured As contents in tilapia muscle and liver were generally within the predicted 10 to 90 percentiles, whereas the model underestimated the As levels in tilapia gill. The predicted mean concentration of As in tilapia muscle, gill, and liver from probabilistic simulations of the PBTK models were 0.45, 0.06, and 0.71 μg/g wet wt, respectively (Figure 4). The median target-organ

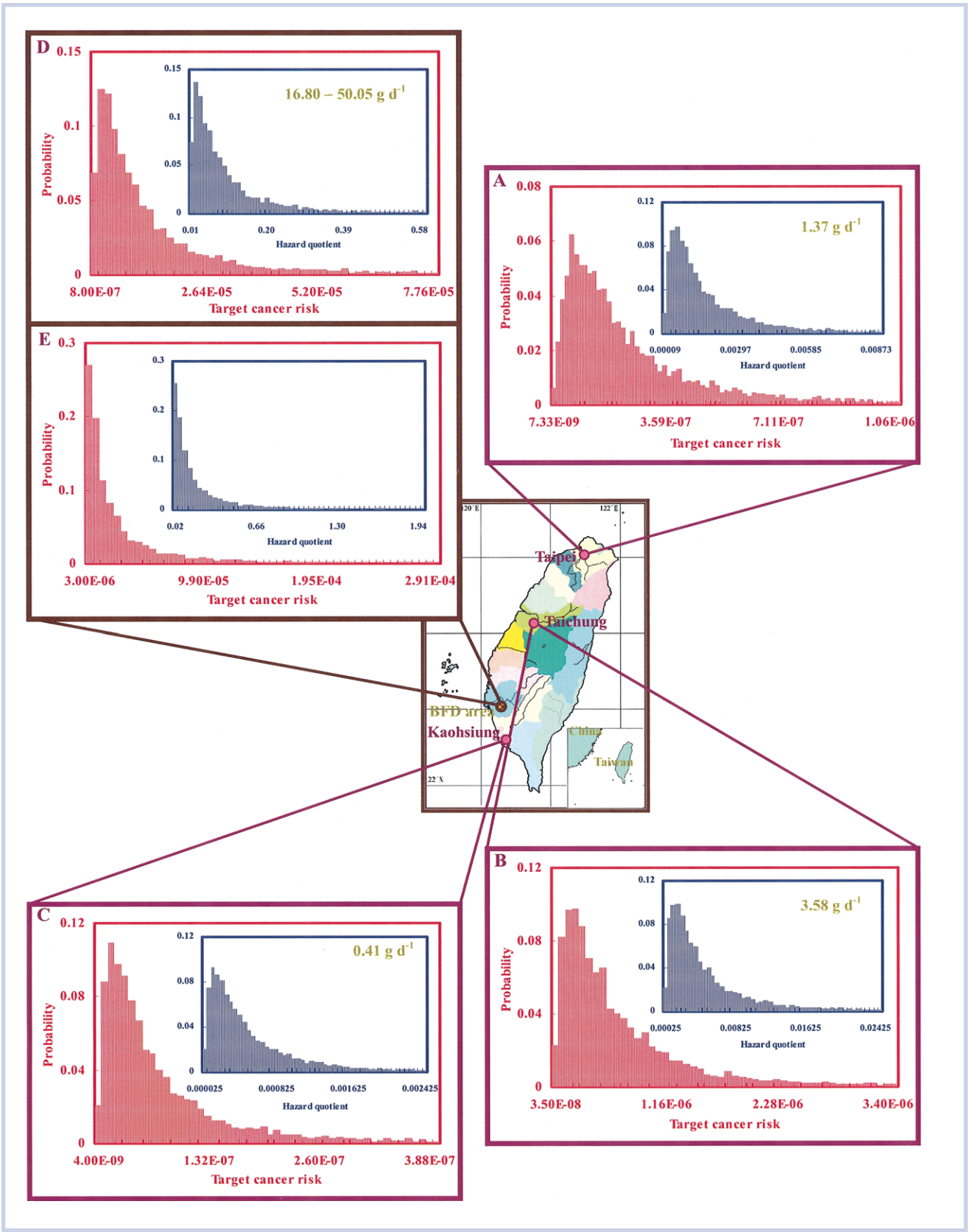

Figure 6. An overall display of probability density distributions of predicted hazard quotient and target cancer risk for city residents in (**A**) Taipei, (**B**) Taichung, and (**C**) Kaohsiung as well as (**D**, **E**) subsistence fishers in the BFD area under different ingestion rates of tilapia consumption.

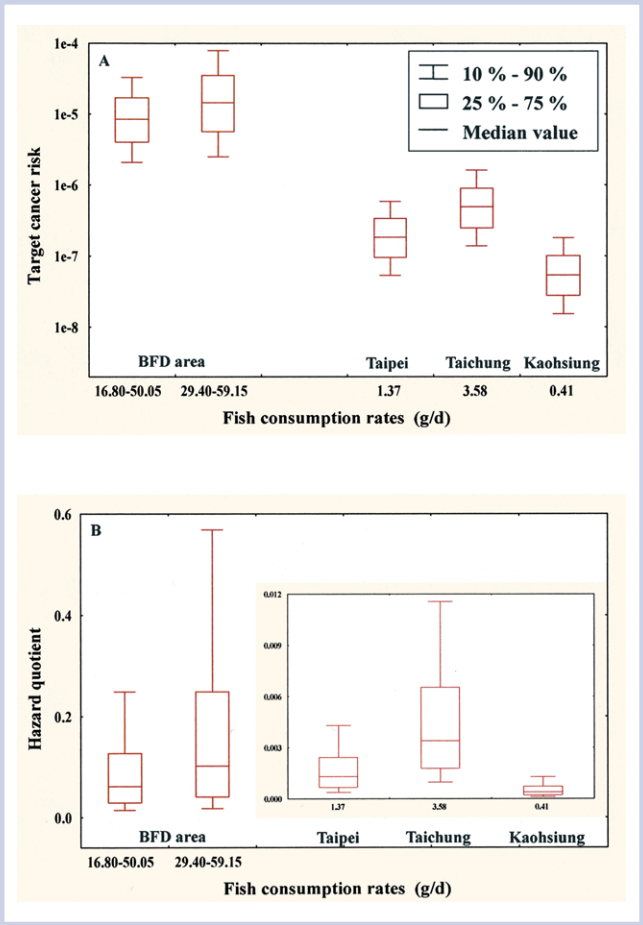

Figure 7. Box-and-whisker plot representations of (**A**) target cancer risks and (**B**) hazard quotients for city residents in Taipei, Taichung, and Kaohsiung as well as subsistence fishers in the BFD area with different tilapia consumption rates.

concentrations generated by probabilistic method were more widely spaced. Thus, applying the Monte Carlo technique to the proposed PBTK model generated probabilistic estimates of As concentration in tilapia that were in good agreement with field data. Relative to minimum and maximum field data, however, lower and upper probabilistic percentile prediction were more conservative. It is evident that the modeling framework and the distributional parameters and assumptions in the model are appropriate for estimating As bioaccumulation in farmed tilapia.

Organ-specific concentration–response relationships assessment

The Hill model and 10,000 iterations of a Monte Carlo simulation provided an adequate fit for the data (χ^2 goodness of fit, $P > 0.5$). It can be seen from Figure 5 that the calculated effective concentration inducing a 10% mortality (EC10) value is 243 µg/g wet wt, with a 95% CI of approximately 144 and 345 µg/g wet wt of tilapia muscle from the fitted concentration-response model.

The median and 95% CI range were 272 and 162 to 398 µg/g wet wt for tilapia gill and 1000 and 591 to 1375 µg/g wet wt for liver, respectively. The U.S. EPA (USEPA 2000) recommended that EC10 could be used as a surrogate threshold for a regulatory endpoint in probabilistic ecological risk assessment. The distributions of As concentration in target organs of tilapia were entirely below the threshold values of derived EC10 (Figure 4), suggesting that waterborne As dose not appear to pose a significant health risk to farmed tilapia under field conditions at current environmental concentrations.

Risk estimates

Tilapia-level risk. We define the tilapia-level risk as the percentage of farmed tilapia that is expected to suffer mortality. The expected exceedence risks of mortality for tilapia muscle, gill, and liver were calculated to be 0.3, 0.1, and 0.3, respectively, indicating that 70 to 90% of farmed tilapia were less sensitive to waterborne As in BFD areas. Risk curves indicated the estimated probabilistic effects for tilapia muscle, gill, and liver (Figures 5A, 5C, 5E, respectively). The plotted probabilities calculated from the outcome of the Monte Carlo simulation followed a JPF (Equation 8) describing the exceedence cumulative probability associated with a particular degree of effect (Figures 5B, 5D, 5F), taking into account the uncertainty in model parameters. Figures 5B, 5D, and 5F demonstrate that the probabilities that 10% or more of the tilapia muscle, gill, and liver (risk = 0.10) affected ranged from 10^{-9} to 10^{-6}%, i.e., the probability is 10% that only 10^{-9} to 10^{-6}% of tilapia organs will be damaged, indicating no significant adverse effect for tilapia major organs exposed to waterborne As from selected tilapia farms in the BFD area.

Human-level risk. Figures 6A to 6E show histograms for the predicted PDFs of HQ and TR, respectively, for human consumption of farmed tilapia by city residents living in Taipei, Taichung, and Kaohsiung, as well as subsistence fishers in the BFD area. The relative skewness and spread in modeled output varied with TRs and HQs. A box-and-whisker plot represents the uncertainty in comparing HQs and TRs. Box plots of interquartile and 50-percentile predictions associated with whisker plots, indicating 10- and 90-percentile values for varied human consumption of farmed tilapia by city residents and subsistence fishers, respectively, are shown in Figures 7A and 7B. The distributions of TRs and HQs for subsistence fishers in the BFD area with consumption rates of 7 to 14 meals/week were more highly skewed, with a long tail at higher uncertainty and variability of inorganic As uptake from tilapia muscle (Figures 6D and 6E).

Under most regulatory programs, an HQ exceeding 1 and a TR between 10^{-4} and 10^{-6} indicate potential risk. Figure 7A shows that for three major city residents, only the probability distribution for Taichung residents had a 90-percentile TR above 10^{-6}, indicating potential health risks associated with inorganic As uptake from tilapia muscle. For subsistence fishers in the BFD area, the probabilities of TR fell within the range of 10^{-6} to 10^{-4} with a consumption rate of 16.80 to 59.15 g/d, indicating higher potential health risks associated with inorganic As uptake from farmed tilapia (Figure 7A). Han et al. (1998) indicated that cancer risk estimates for consumption of inorganic As in fish from the BFD area ranged from TR = 10^{-5} to 10^{-4} for fish consumption rates of 10 to 70 g/d in that they assumed inorganic As constitutes 10% of total As in seafood. For predicted HQ distributions (Figure 7B), 90-percentile HQ was found to be 0.0001 to 0.0116 for three major-city residents and 0.0144 to 0.5689 for subsistence fishers in the BFD area with daily consumption rates, suggesting small contributions from tilapia consumption. Han et al. (1998) reported that HQs caused by consuming fish containing As ranged from 0.136 to 0.340 for fish consumption rates of 10 to 70 g/d.

Table 2. Probabilistic sensitivity analysis

Input	Correlation	Contribution to variance rank (%)[a]
As in tilapia muscle		
Whole-fish body weight	0.6211	49.3951
As in water	0.5760	42.4876
Weight of tilapia muscle	−0.2088	5.5818
Metabolic transformation rate of tilapia liver	−0.1010	1.3066
Weight of tilapia liver	−0.0980	1.2289
As in tilapia gill		
As in water	0.9840	99.8576
Whole-fish body weight	0.0261	0.0700
Weight of tilapia liver	−0.0235	0.0568
Metabolic transformation rate of tilapia liver	0.0108	0.0121
Weight of tilapia muscle	0.0051	0.0035
As in tilapia liver		
As in water	0.7746	78.6743
Weight of tilapia liver	−0.3204	13.4561
Whole-fish body weight	0.2005	5.2736
Metabolic transformation rate of tilapia liver	−0.1280	2.1487
Weight of tilapia muscle	−0.0584	0.4472
Dose–response relationship		
As in tilapia muscle	1.0000	99.9688
Bioconcentration factor for tilapia	−0.0172	0.0297
Incipient median lethal concentration	−0.0039	0.0015
Human exposure: target cancer risk		
As in tilapia muscle	0.8353	73.8596
Tilapia ingestion rates	0.4969	26.1402
Body weight for human	0.0016	0.0003
Human exposure: hazard quotient		
As in tilapia muscle	0.8406	74.4810
Tilapia ingestion rates	0.4897	25.2800
Body weight for human	−0.0476	0.2390

[a] Contribution to variance calculated as sum of squared rank correlation coefficients normalized to 100%.

Sensitivity analysis. Table 2 indicates the critical variables in the probabilistic analysis for As in tilapia, dose–response relationship for tilapia target organs, and the human health exposure model. As can be seen in Table 2, the most important input variables for As in tilapia muscle are whole-fish body weight and water As level, which contribute to 49.40 and 42.49% of output variances, respectively. The results show that the key parameter in estimating the tilapia gill and liver concentration profiles is water As content; that contribution to variance ranged from 78.67 to 99.85%. As in tilapia muscle is the key parameter for estimating organ-specific dose–response relationships (Table 2). For the human health exposure/risk model, the key parameter in the TR and HQ is tilapia muscle As content; that contribution to variance is approximately 75%, indicating that human health risks could be reduced by improving the water quality in tilapia farms and consequently, reducing the As accumulated in tilapia.

Implications

Adverse effects related to As exposure were quantified because the weight of available data, including exposure and toxicological data from experimental studies, strongly supports the choice of As in our study of metalloids to carry out the risk assessment. The probabilistic methods used show that field data or experimentally derived parameters may hide significantly different levels of conservatism in relation

to the uncertainty and variability present in each input parameter. Variability and uncertainty in model inputs were addressed using conservative assumptions, a range of tilapia farm scenarios, and probabilistic analysis.

The use of PBTK/TD modeling-based analysis in toxicological risk assessment was related to its ability to make reliable predictions of target-organ residues and associated mortality effects in tilapia. It is appropriate to apply a human health exposure and risk model to account for the HQ and lifetime risk for humans consuming contaminated tilapia. It was assumed that people consumed tilapia muscle only. The TRs for subsistence fishers in the BFD area and Taichung residents are unacceptable, because the 50th percentile at a consumption rate of 29.40 to 59.15 g/d, the 75th percentile at a consumption rate of 16.80 to 50.05 g/d for subsistence fishers, and the 90th percentile with a consumption rate of 3.58 g/d for Taichung residents all exceed 10^{-6}, indicating potential health risks associated with inorganic As uptake from tilapia muscle, i.e., for the carcinogenic effects (inorganic As) for subsistence fishers and Taichung residents, risk is expressed as the excess probability of contracting cancer over a lifetime (70 years).

Our previous published toxicity bioassay data can be used extensively in the emerging field of ecological risk assessment. Nevertheless, probabilistic treatment of the model parameters, in conjunction with sensitivity analyses, should provide a rigorous basis for making sound environmental decisions. The concentration–response relationships can be viewed as integral in an overall scheme of ecological risk assessment involving bioaccumulation modeling. Concentration-response relationships will allow substantial progress in the environmental toxicology of As in farmed tilapia in our earlier work to continue without losing touch with either the exposure-based information or the field-based observations of adverse responses and residue-monitoring data.

CONCLUSIONS

In this paper, we present a novel PBTK/TD modeling-based analysis in risk assessment that integrates the prediction of As concentrations in target organs with a reconstructed dose–response relationship and use a JPF incorporating exposure and concentration-response profiles to characterize the survival risk for farmed tilapia. A human-level exposure model was also used to quantify the risks through tilapia consumption. To aid in the development of realistic estimates of risk associated with tilapia consumption, a PBTK/TD model was developed to improve the accuracy of human health risk assessment. These risk analyses indicate that almost 70 to 90% of farmed tilapia are expected to survive well in tilapia farms in the BFD area.

All predicted 90th percentiles of HQ are less than a value of 1 for city residents living in Taipei, Taichung, and Kaosiung and for subsistence fishers in the BFD area, indicating small contributions from farmed tilapia consumption. For subsistence fishers in the BFD area, probabilities of TR fall within the range of 10^{-6} to 10^{-4} for a consumption rate of 16.80 to 59.15 g/d, indicating higher potential health risks for inorganic As uptake from farmed tilapia. For three major-city residents, 90th percentiles of TRs ranged from 1.80×10^{-7} to 1.62×10^{-6}, indicating low potential health risks associated with inorganic As uptake from tilapia muscle. It is our conclusion that the incorporation of probabilistic analysis into the evaluation of exposure and concentration–response relationships greatly improves the ability to appraise the range of possible exposure scenarios and environmental risk to aquacultural species and human who consume contaminated fish. Probabilistic risk assessment will substantially reduce the compounded conservatism that is inherent in risk assessment that relies on conservative point value estimates for all PBTK, AUC-based TD, and human exposure parameters.

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APPENDIX A

PBTK model

The following assumptions were made to develop the PBTK model: (1) there is a five-compartment pharmacokinetic model of blood-gill-muscle-alimentary canal-liver, representing actual anatomical units of tilapia; (2) it is assumed that the gill acts as a continuously 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; (3) 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 by blood flow; (4) there is complete equilibrium of chemicals between the blood phase and the tissue phase of each compartment, and it is assumed that there is an inert soluble chemical with blood-chemical partitioning/binding coefficient f_i present in amounts of chemical partitioned to compartment tissue i; (5) there is local mass balance of chemical substance, in that for each compartment, the amount of chemical substance entering is equal to the amount leaving; and (6) there is local mass balance of blood flow, in that

$$\sum_{\substack{j=0 \\ j\neq i}}^{n} q_{ij} = \sum_{\substack{j=0 \\ j\neq i}}^{n} q_{ji}$$

for $1 \leq i \leq$ n.

For the compartment of gill that interacts with As in external water, an additional process has to be considered (Thomann et al. 1997). An increased surface sorption to the gill surface was necessary. The exchange of As between internal gill tissue and the blood was therefore set at a lower exchange than the exchange between the gill surface and the water.

It can be seen from Equation 1 that $d\{C(t)\}/dt = \{[K]\{C(t)\} + [X]\{u(t)\}$; using our assumptions, we developed a linear PBTK model (Figure 2) governing the principle features of the bioaccumulation and transport of As in tilapia in five target organs of blood, muscle, gill, alimentary canal, and liver, in that $\{C(t)\} = \{C_1(t)\ C_2(t)\ C_3(t)\ C_4(t)\ C_5(t)\}^T$ (μg g^{-1}), respectively, describes the As concentration in blood, muscle, gill, alimentary canal, and liver; $\{u(t)\} = C_w$ is the As concentration in ambient water (μg L^{-1}), and the state matrix $[K]$ can be written as and input constant matrix

$$\begin{bmatrix} -(q_{12}+q_{13}+q_{14}+q_{15})\frac{f_d}{V_1} & \frac{q_{12}}{f_2V_1} & \frac{q_{13}}{f_3V_1} & \frac{q_{14}}{f_4V_1} & \frac{q_{15}}{f_5V_1} \\ \frac{q_{21}f_d}{W_2} & -(\frac{q_{21}}{f_2W_2}+k_G) & 0 & 0 & 0 \\ \frac{q_{31}f_d}{W_3} & 0 & -(\frac{q_{31}}{f_3W_3}+\frac{q_{3W}}{f_3W_3}) & 0 & 0 \\ \frac{q_{41}f_d}{W_4} & 0 & 0 & -(\frac{q_{41}}{f_4W_4}+k_E) & 0 \\ \frac{q_{51}f_d}{W_5} & 0 & 0 & 0 & -(\frac{q_{51}}{f_5W_5}+k_M) \end{bmatrix} \quad \text{(A1)}$$

$$[X] = \begin{bmatrix} 0 & 0 & \frac{q_{3W}\alpha_{3W}}{W_3} & 0k_D & 0 \end{bmatrix}^T \quad \text{(A2)}$$

APPENDIX B

Table B1. Physiologically based parameters used for PBPK model simulation[a]

Symbol	Description	Estimated value
q_{3W}	Gill–water exchange rate (L/d)	0.01
$q_{12}=q_{21}$	Blood–muscle exchange rate (L/d)	2.5
$q_{13}=q_{31}$	Blood–gill exchange rate (L/d)	0.2
$q_{14}=q_{41}$	Blood–alimentary canal exchange rate (L/d)	5.5
$q_{15}=q_{51}$	Blood–liver exchange rate (L/d)	3.6
α_{3W}	Gill sorption factor (--)	8
f_d	Fraction As dissolved in blood (g/L)	0.2
f_2	Partition coefficient of muscle (g/L)	5.2
f_3	Partition coefficient of gill (g/L)	0.04
f_4	Partition coefficient of alimentary canal (g/L)	20.9
f_5	Partition coefficient of liver (g/L)	5.2

[a] Calibrated from experimentally determined data and field observations adopted from Liao et al. (2003a, 2004a, 2004b).