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Probabilistic framework for assessing the arsenic exposure risk from cooked fish consumption

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Abstract Geogenic arsenic (As) contamination of groundwater is a major ecological and human health problem in southwestern and northeastern coastal areas of Taiwan. Here, we present a probabilistic framework for assessing the human health risks from consuming raw and cooked fish that were cultured in groundwater As-contaminated ponds in Taiwan by linking a physiologically based pharmacokinetics model and a Weibull dose–response model. Results indicate that As levels in baked, fried, and grilled fish were higher than those of raw fish. Frying resulted in the greatest increase in As concentration, followed by grilling, with baking affecting the As concentration the least. Simulation results show that, following consumption of baked As-contaminated fish, the health risk to humans is $<10^{-6}$ excess bladder cancer risk level for lifetime exposure; as the incidence ratios of liver and lung cancers are generally acceptable at risk ranging from 10^{-6} to 10^{-4}, the consumption of baked As-contaminated fish is unlikely to pose a significant risk to human health. However, contaminated fish cooked by frying resulted in significant health risks, showing the highest cumulative incidence ratios of liver cancer. We also show that males have higher cumulative incidence ratio of liver cancer than females. We found that although cooking resulted in an increase for As levels in As-contaminated fish, the risk to human health of consuming baked fish is nevertheless acceptable. We suggest the adoption of baking as a cooking method and warn against frying As-contaminated fish. We conclude that the concentration of contaminants after cooking should be taken into consideration when assessing the risk to human health.

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Introduction

Arsenic (As) is distributed throughout the air, groundwater, soil, and sediment. The US Food and Drug Administration (US FDA) discovered that As-associated species are present in food at various concentrations; the majority of As exists in seafood around 90 % and the remaining 10 % in other types of food (US FDA 1993). Seafood often has a high content of organic As, generally constituting 65–90 % of total As. Inorganic As in seafood constitutes 2–10 % of total As. The most common As-associated species in seafood are monomethylarsenate (MMA) and dimethylarsenate (DMA) for organic As and arsenite (As^{3+}) and arsenate (As^{5+}) for inorganic As (Huang et al. 2003). Arsenic exists in its inorganic form in groundwater, comprising 95 % or more of total As (NRC 1983).

Inorganic As is classified as carcinogenic to humans by the US Environmental Protection Agency (US EPA) and the International Agency for Research on Cancer (IARC). Arsenic also is the top ranking on the Agency for Toxic Substances and Disease Registry (ATSDR) priority list of hazardous substances (ATSDR 2011). The World Health Organization (WHO) suggested in 1993 that allowable concentrations of As in drinking water be lowered from 50 to 10 μg L^{-1}. Taiwan also took action and lowered its maximum allowable level from 50 to 10 μg L^{-1} in 2000 (WHO 2006).

In recent years, survey data related to groundwater quality provided by the Taiwan Environmental Protection Administration (Taiwan EPA) have indicated that in southwest and northeast coastal areas, a number of observation stations have measured As concentration in groundwater of above 10 μg L^{-1}; a number of stations have even obtained readings of over 50 μg L^{-1} (Taiwan EPA 2012). Researches indicate that blackfoot disease and peripheral vascular diseases in southwest coastal areas of Taiwan are highly correlated with high levels of As in local groundwater (Chen and Liao 2008; Huang et al. 2003; Chiou et al. 1997; Tseng et al. 1994; Tseng 1989). Chen et al. (1995), Huang et al. (2007), and Tseng (2008) also reported that cardiovascular disease among residents from Taiwan's northeast coastal regions are significantly correlated with high concentration of As in groundwater.

Liang et al. (2013) applied a probabilistic approach to assess As exposure risk in the southwest coastal areas of Taiwan. Their results showed that inorganic As intake from fish and shellfish fell below the provisional tolerable weekly intake (PTWI) threshold values for the 95th percentiles, but exceeded the target cancer risk of one in 1 million (1×10^{-6}). Liang et al. (2011) also recommended an acceptable risk zone for acceptable consumption rates of fish and shellfish adapted from PTWI and acceptable risk measures. They applied a geographic information system (GIS)-based approach to communicate risk levels via human health risk map representations (Liang et al. 2010).

At present, residents of southwest and northeast coastal regions of Taiwan do not use groundwater for drinking water; however, groundwater is still used for the cultivation of fish. Inorganic arsenic in groundwater can enter fish through bioaccumulation and subsequently be accumulated in the human body via fish consumptions. Total As consists of inorganic As and organic As, referred to as As species. Except MMA and DMA, the organic As also includes the following: trimethylasyine (TMA^{+}), trimethylarsine oxide (TMAO), arsenobetaine (AB), and arsenocholine (AC). Conversion between inorganic and organic As changes the binding affinity of As with various proteins, which in turn alters the relative toxicity of As species. Inorganic As, of which As^{3+} and As^{5+} are the most common forms, generally exists in the air, soil, and water, entering the human body through food consumption of food. As^{3+} is highly toxic. Several studies identified the toxicity order of As species are following: $MMA^{3+} > DMA^{3+} > As^{3+} > As^{5+} > TMA^{+} > MMA^{5+} = DMA^{5+} > TMAO > AC >$ AB (Szramek et al. 2004; Shraim et al. 2002; Styblo et al. 2000; Lin et al. 1998).

According to the findings of Devesa et al. (2001a) when cooked, organic As in food, such as AB converts into TMAO at 150 °C; at 160 °C, AB converts to TMA^{+}; at a high temperature of 180 °C, 2 % AB converts into TMA^{+} and 16 % into TMAO. Similarly, AC converts to DMA when the temperature reaches 170 °C. Devesa et al. (2001b) also indicated that when cooked at a constant temperature, prolonged cooking duration led to an increase in the concentrations of TMAO and TMA^{+}. Furthermore, the concentrations of As species differed among various cooking methods for different foods. Devesa et al. (2005) also discovered that under direct

contact with cooking temperature at 250 °C, concentrations of inorganic As and DMA increased. Devesa et al. (2005) showed that the cooking temperature and cooking duration of various cooking methods are the key factors in the conversion of As species.

Lin et al. (2005a), Liu et al. (2005), Huang et al. (2003), Liao et al. (2008, 2009), and Ling and Liao (2007) investigated the concentrations of various As species in aquacultural fish along the southwest and northeast coasts of Taiwan, estimating the risks faced by local residents from the consumption of As-contaminated fish. However, in these literatures, data related to concentrations of As species in aquacultural fish were derived from analysis of raw fish muscle. Estimation results might therefore have resulted in an overestimation or underestimation of the actual values. To accurately estimate the risk to human health from the consumption of As-contaminated fish, this study examined the variations of As species concentration under varying cooking temperatures, cooking duration, and cooking methods. The risks associated with consuming cooked As-contaminated fish could be estimated.

For more precise estimation of the risk to human health, this study adopted a physiologically based pharmacokinetics (PBPK) model to investigate changes in the As concentration over particular time intervals, through its distribution to various tissues and organs via blood circulation. Mechanisms related to absorption, distribution, metabolism, and excretion were taken into consideration while estimating the total As concentration in various organs following consumption of cooked fish.

The objectives of this study were (1) to assess the changes in As species in cooking As-contaminated fish; (2) to quantitatively estimate the As species distributions in human-specific target organs by linking an PBPK model and a Weibull dose–response model following consumption of cooking As-contaminated fish; and (3) to determine which cooking method resulted in more human health risk and suggest an optimal cooking method.

Materials and methods

Problem formulation

As-associated species are distributed at various contents throughout various fish tissue. Organic As is obtained through metabolism or bioaccumulation, whereas inorganic As comes mostly from bioaccumulation. The accumulation of As-associated species in fish varies according to pollution concentration, fish tissue, fish age, water temperature, and water salinity. Smaller fish accumulate relatively higher amounts of As compared to larger species (Liu et al. 2006). Han et al. (1998) analyzed polluted industrial areas of Taiwan, discovering that total concentrations of As in tilapia (*mossambica*) were 0.225–1.805 μg g^{-1} (dry wt.), while concentrations in milkfish (*Chanos chanos*) were 0.365–1.805 μg g^{-1} (dry wt.). Huang et al. (2003), Liao et al. (2003, 2006), Lin et al. (2004, 2005, 2008), and Liu et al. (2005, 2006) investigated arseniasis-endemic areas in Taiwan, showing that total As concentrations were 0.184–11.8 μg g^{-1} (dry wt.) in aquacultural tilapia, 0.2–15.2 μg g^{-1} (dry wt.) in milkfish, and 2.38–4.18 μg g^{-1} (dry wt.) in mullet (*Mugil cephalus*) (Table 1).

Inorganic As is more toxic than organic As in total As to humans. Furthermore, after ingesting the inorganic As, the inorganic As will convert into toxicant organic As such as MMA and DMA. This study is therefore based on the research of Huang et al. (2003) estimating the inorganic As concentration by assuming that inorganic As in fish constitutes 7.4 % of total As. To explicitly account for the probabilistic method, we adopted a Monte Carlo (MC) simulation. The distribution of concentrations of inorganic As in raw fish was best fitted from the data (Table 1) and the selected lognormal (LN) distribution with the optimal Kolmogorov–Smirnov (K–S) and chi-square (χ^2) goodness of fit. The estimated concentration of inorganic As in raw fish was a LN distribution with a geometric mean (gm) of 0.83 μg g^{-1} and geometric standard deviation (gsd) of 3.28 (LN(0.83 μg g^{-1}, 3.28)).

This study adopted baking, frying, and grilling methods described in Devesa et al. (2001c) and Ersoy et al. (2006) to investigate the conversion of As-associated species in As-contaminated fish cooked with various methods. Concentration of As can be varied according to temperature and duration of the three cooking methods (Table 2). Distributions of the cooking temperatures and durations via various cooking methods were fitted to the Devesa et al. (2001) and Ersoy et al. (2006) investigations, and the selected normal (N) distribution (N (mean, sd)) had the optimal K–S and χ^2 goodness of fit. Results provided the cooking temperature and cooking duration distribution

Table 1 Total arsenic concentration of cultivated seafood in Taiwan

Seafood species	Tissue	Total arsenic (μg g^{-1})	References
Milkfish (*Chanos chanos*)	Whole body (dry wt.)	0.37–1.81[a]	Han et al. (1998)
	Whole body (dry wt.)	15.20 ± 5.10[b]	Lin et al. (2004)
	Whole body	0.3 ± 0.1	Linet al. (2005)
		0.2 ± 0.0	
		1.6 ± 0.3	
		0.4 ± 0.1	
		0.4 ± 0.1	
		1.2 ± 0.6	
		3.4 ± 0.3	
		0.3 ± 0.0	
		0.3 ± 0.1	
		0.5 ± 0.2	
		0.3 ± 0.0	
		0.2 ± 0.0	
	Whole body	0.21 ± 0.02–3.35 ± 0.32	Lin and Liao (2008)
Tilapia (*Mossambica*)	Whole body (dry wt.)	0.23–1.81	Han et al. (1998)
	Whole body (dry wt.)	0.85 ± 0.83	Liu et al. (2005)
	Whole body (dry wt.)	0.67 ± 0.00	Huang et al. (2003)
		0.36 ± 0.12	
		1.30 ± 0.22	
		3.29 ± 0.69	
		2.92 ± 0.66	
		0.55 ± 0.07	
		0.22 ± 0.08	
		0.41 ± 0.02	
		0.67 ± 0.16	
		0.35 ± 0.07	
		0.79 ± 0.04	
		0.61 ± 0.23	
		0.44 ± 0.01	
		0.31 ± 0.01	
		0.506 ± 0.02	
		0.91 ± 0.16	
		1.05 ± 0.27	
		0.88 ± 0.01	
		0.52 ± 0.07	
		0.18 ± 0.00	
		0.86 ± 0.15	
	Muscle (dry wt.)	3.96 ± 1.56 (Hsuehchia)	Liao et al. (2003)
		3.13 ± 2.26 (Yichu)	
	Whole body (wet wt.)	2.20	Liao et al. (2006)
		5.80	
		11.80	
Mullet (*Mugil cephalus*)	Muscle (dry wt.)	2.38 ± 0.78[c]	Liu et al. (2006)
		4.18 ± 1.12[d]	

[a] Min–max; [b] Mean ± SD; [c] aquacultural; [d] wild

Table 2 Total arsenic concentration in raw and cooked seafood

Cooked methods	Temperature (°C)	Duration (min)	Seafood species	Location	Total arsenic concentration (μg g^{-1})		References
					Raw	Cooked	
Bake	180	20	Sea bass fillets (wet wt.)	Turkey	0.372 ± 0.042[a]	0.324 ± 0.199	Ersoy et al. (2006)
	100	12	Dory (dry wt.)	Spain	9.0–14.1[b]	13.2 ± 0.1	Devesa et al. (2001)
	160	25			9.0–14.1	17.2 ± 0.2	
	94	25	Hake (dry wt.)	Spain	4.0–9.2	8.8 ± 0.1	Devesa et al. (2001)
	100	20			4.0–9.2	7.73 ± 0.01	
	120	17			4.0–9.2	8.3 ± 0.2	
Fry	110	16	Sole (dry wt.)	Spain	47.6	17.5 ± 0.1	Devesa et al. (2001)
	100	11	Dory (dry wt.)	Spain	9.0–14.1	12.5 ± 0.5	Devesa et al. (2001)
	110	20			9.0–14.1	11.97 ± 0.01	
	180	4	Sea bass fillets (wet wt.)	Turkey	0.372 ± 0.042	2.66 ± 0.084	Ersoy et al. (2006)
Grill	99	5	Dory (dry wt.)	Spain	9.0–14.1	8.8 ± 0.1	Devesa et al. (2001)
	106	8			9.0–14.1	11.6 ± 0.3	
	108	7			9.0–14.1	10.4 ± 0.3	
	100	12	Hake (dry wt.)	Spain	4.0–9.2	4.2 ± 0.1	Devesa et al. (2001)
	116	12			4.0–9.2	4.0 ± 0.1	
	131	12			4.0–9.2	4.1 ± 0.1	
	90	5	Sardine (dry wt.)	Spain	9.8–13.9	10.21 ± 0.01	Devesa et al. (2001)
	90	5			9.8–13.9	8.1 ± 0.1	
	120	5			9.8–13.9	14.6 ± 1.0	
	180	20	Sea bass fillets (wet wt.)	Turkey	0.372 ± 0.042	0.398 ± 0.068	Ersoy et al. (2006)

[a] Mean ± SD, [b] Min–max

of N(125.7 °C, 36.0) and N(19.8 min, 5.0) for baking, N(114.0 °C, 26.6) and N(9.1 min, 4.9) for frying, and N(127.1 °C, 36.4) and N(12.3 min, 6.8) for grilling, respectively. Frying required a lower temperature and less duration than baking or grilling. Baking and grilling required similar temperatures, but baking also required a longer cooking duration.

Exposure analysis: concentration estimation

This study applied a multiple linear regression model to simulate changes of total As concentration in cooked As-contaminated fish and adopted a PBPK model to evaluate the distribution of accumulated total As in the human body after consumption of contaminated fish (Fig. 1).

Devesa et al. (2001) and Ersoy et al. (2006) verified the changes of total As concentration in cooked and raw fish under various cooking methods (baking, frying, grilling) based on different cooking temperatures and durations (Table 2). The multiple linear regression models for baking, frying, and grilling methods have the formula,

$$\text{Baking: } Y = -7.14 + 0.02 \times X_{\text{Temp}} + 0.20 \times X_{\text{Time}} + 1.44 \times X_{\text{Conc}}, \quad (1)$$

$$\text{Frying: } Y = 19.77 - 0.10 \times X_{\text{Temp}} + 0.05 \times X_{\text{Time}} + 0.16 \times X_{\text{Conc}}, \quad (2)$$

$$\text{Grilling: } Y = -22.07 + 0.09 \times X_{\text{Temp}} + 0.25 \times X_{\text{Time}} + 1.85 \times X_{\text{Conc}}, \quad (3)$$

where Y is the total As concentration in cooked fish (μg g^{-1}), X_{Temp} is cooking temperature (°C) (Table 2), X_{Time} is cooking duration (min) (Table 2), and X_{Conc} is the total As concentration of raw As-contaminated fish (μg g^{-1}) (Table 1).

This study assumed that when the absorption rate was constant, it showed zero-order kinetics; As concentration change rate proportional to As concentration denotes first-order kinetics of distribution and excretion. At lower As concentrations, first-order

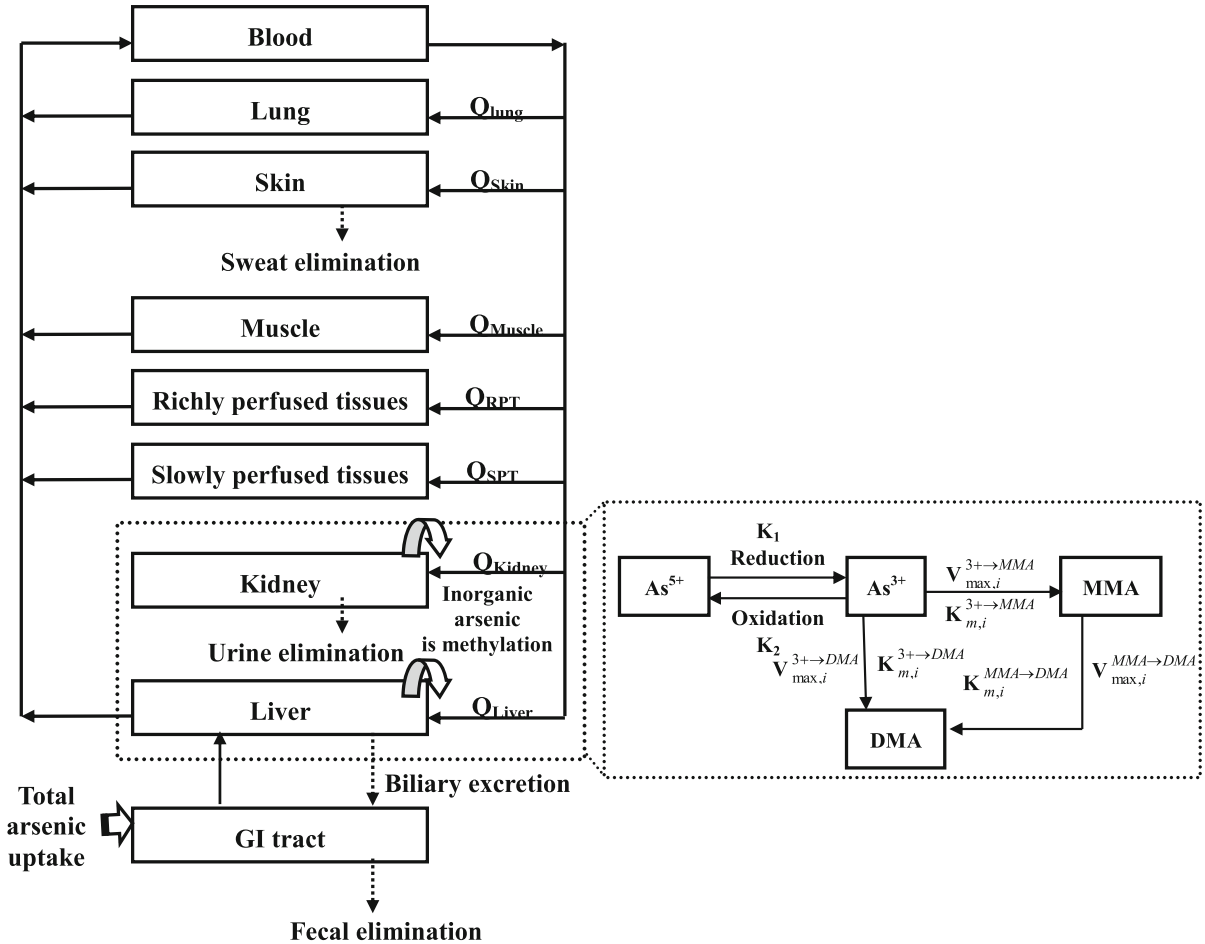

Fig. 1 Schematic of the proposed PBPK model showing target tissue compartments interconnected by blood flow and biotransformation of arsenic showing oxidation/reduction of inorganic arsenic and methylation of As^{3+} in kidney and liver

kinetics was displayed, whereas zero-order kinetics were displayed at a high As concentration:

$$\text{Zero-order kinetics: } \frac{dA_x}{dt} = V_x \times \frac{dC_x}{dt} = K_{\text{absorb}}, \quad (4)$$

$$\text{First-order kinetics: } \frac{dA_x}{dt} = V_x \times \frac{dC_x}{dt} = Q_x\left(C_a - \frac{C_x}{P_x}\right) - Q_E \times C_x, \quad (5)$$

where A_x is the total amount of As species in human organ x (μmol), V_x is the volume of organ x (L), C_x is the As species concentration in organ x (μmol L^{-1}),C_a is the As species concentration in blood (μmol L^{-1}), K_{absorb} is the As species absorption dose per unit time (μmol h^{-1}), Q_x is the blood flow through organ x per unit time (L h^{-1}), Q_E is excretion flow from organ x per unit time (e.g., sweat, urine, biliary, and fecal excretion) (L h^{-1}), and P_x is the ratio of As species concentration in organ x and blood at equilibrium (unit less).

Furthermore, As^{3+} and As^{5+} can be converted into MMA and DMA through methylation after entering the body. This is characterized as nonlinear enzyme kinetics and can be described with Michaelis–Menten kinetics as,

$$\frac{dC_x^{\text{MMA}}}{dt} = \frac{V_{m,x}^{3+\rightarrow\text{MMA}} \times C_x^{3+}}{K_{m,x}^{3+\rightarrow\text{MMA}} + C_x^{3+}}, \quad (6)$$

where C_x^{MMA} is the MMA concentration in organ x (μmol L^{-1}), $V_{m,x}^{3+\rightarrow\text{MMA}}$ is the maximum methylation conversion rate from As^{3+} to MMA in organ x (μmol h^{-1}), and $K_{m,x}^{3+\rightarrow\text{MMA}}$ is the Michaelis constant of As^{3+} methylation into MMA in organ x (μmol L^{-1}).

Following catalysis, chemical compounds first bind with enzymes to achieve a transition state and are subsequently converted into metabolites. Michaelis–Menten kinetics can be applied to describe the amount of chemical metabolic change per unit time.

Exposure analysis: PBPK modeling

A PBPK model was used to estimate As species distributions in specific human target organs through cooked As-contaminated fish consumption. The PBPK model structure features lung (compartment 2), skin (compartment 3), muscle (compartment 4), richly perfused tissues (compartment 5), slowly perfused tissues (compartment 6), kidneys (compartment 7), liver (compartment 8), and GI tract (compartment 9), which are interconnected by blood (compartment 1) circulation (Fig. 1). This study applied the human physiological parameters adopted from Liao et al. (2008, 2009): tissue volume, density, blood perfusion rate, blood flow fraction, total percentage elimination, and tissue/blood partition coefficient (see Table S1 in Supplementary material). Furthermore, the liver and kidneys are the major organs metabolizing inorganic As to MMA and DMA. Michaelis–Menten kinetics was applied to describe the cross-correlation between the liver and kidneys, which was assumed to be first-order kinetics based on metabolic rate constant of methylation of inorganic As based on Liao et al. (2009) (see Table S2 in Supplementary material). The essence of almost all PBPK models can be described by a dynamic equation,

$$\frac{\mathrm{d}A_x^{5+}}{\mathrm{d}t} = \left(-K_1 \times C_x^{5+} + K_2 \times C_x^{3+}\right) \times V_x, \tag{7}$$

$$\frac{dA_x^{3+}}{dt} = (K_1 \times C_x^{5+} - K_2 \times C_x^{3+}) \times V_K - \frac{V_{\max,x}^{3+\to MMA} \times C_x^{3+}}{K_{m,x}^{3+\to MMA} + C_x^{3+}} - \frac{V_{\max,x}^{3+\to DMA} \times C_x^{3+}}{K_{m,x}^{3+\to DMA} + C_x^{3+}}, \tag{8}$$

$$\frac{dA_x^{MMA}}{dt} = \frac{V_{\max,x}^{3+\to MMA} \times C_x^{3+}}{K_{m,x}^{3+\to MMA} + C_x^{3+}} - \frac{V_{\max,x}^{MMA\to DMA} \times C_x^{MMA}}{K_{m,x}^{MMA\to DMA} + C_x^{MMA}}, \tag{9}$$

$$\frac{dA_x^{DMA}}{dt} = \frac{V_{\max,x}^{3+\to DMA} \times C_x^{3+}}{K_{m,x}^{3+\to DMA} + C_x^{3+}} + \frac{V_{\max,x}^{MMA\to DMA} \times C_x^{MMA}}{K_{m,x}^{MMA\to DMA} + C_x^{MMA}}, \tag{10}$$

where A_x is the dose of As species in the organ (μmol h^{-1}), C_x is the concentration of As species in the organ (μmol L^{-1}), K_1 and K_2 are reaction rate constants for As^{5+} and As^{3+} (h^{-1}), $V_{\max,x}$ is maximum reaction rate for the methylation of As species (μmol h^{-1}), $K_{m,x}$ is the Michaelis–Menten constant for As species (μmol L^{-1}). Following integration of As absorption, distribution, metabolism, and excretion mechanisms in human, the accumulation of As^{3+}, As^{5+}, MMA, and DMA in the human skin, lungs, liver, and kidneys was simulated. The first-order differential equations are listed in Table S3 (see Supplementary material).

Furthermore, weight, metabolism, and other such physiological states change with age. This study took into consideration physiological changes due to age, including weight, organ volume, heart blood flow output, and the blood flow of various organs. The ratio of body weight to organ weight was adjusted by the percentage of body weight and applied to the dynamic equations to obtain PBPK models under various physiological states (see Table S1 in Supplementary material).

Effect analysis: Weibull dose–response model

This study assumed that As-contaminated fish was consumed in low doses over a long period, to describe dose–response relationships in human target organ at various time with the Weibull model,

$$g(t, \varepsilon(C)) = \varepsilon(C) k_2 t^{k_2 - 1} \exp(-\varepsilon(C) t^{k_2}), \tag{11}$$

$$\varepsilon(C) = k_0 C^{k_1} + k_3, \tag{12}$$

where $g(t, \varepsilon(C))$ indicates the incidence ratio of specific cancer when the human body is exposed under concentration C (μg g^{-1}) at age t (year), C indicates exposure concentration (μg g^{-1}), $\varepsilon(C)$ is the C-dependent shape parameter, t is age (year), and k_0, k_1, k_2, and k_3 are the disease-specific best-fitted parameters (see Table S4 in Supplementary material). The best-fitted parameters of k_1 and k_2 may regard as the connection degree of the cumulative incidence ratio with As concentration and age, respectively. The cumulative incidence ratio $P(t, \varepsilon(C))$ for human exposed to As concentration C at age t can then be obtained by integral of Eq. (13) as,

$$P(t, \varepsilon(C)) = \int_0^t g(t, \varepsilon(C) dt) = 1 - \exp(-\varepsilon(C) t^{k_2}) = 1 - \exp(-(k_0 C^{k_1} + k_3) t^{k_2}). \tag{13}$$

This Weibull model, based on epidemiological data, simulates the dose–response curve following consumption of As-contaminated fish and performs a time-series observation of the response for human diseases under exposure at concentration $C(\mu g\ g^{-1})$. This was used to predict the cumulative incidence ratio of particular diseases in various organs at various exposure concentrations and ages. The diseases in this study are categorized as non-carcinogenic and carcinogenic; the former includes hyperpigmentation and keratosis, while the latter includes lung, liver, and bladder cancers. The software program Crystal Ball® (Version 7.3, Decisionerring, Inc., Denver, CO, USA) was used to analyze data and to estimate distribution parameters.

Risk characterization

Many studies have evaluated the health risks related to As, and a number of studies have analyzed changes in the As concentration in fish before and after cooking (Devesa et al. 2001c; Ersoy et al. 2006). However, no literature has investigated the human health risks of consuming cooked fish contaminated with As. This study evaluated changes in the As concentration in fish after cooking with various methods and calculated the accumulated As species concentration in various human target organs after the conversion of As species from consumed fish. Factors such as age and body weight were taken into consideration during analysis of dose–response relationships of various organs. Risk at a specific As concentration in human target organs in a joint probability function or accedence profile describes the probability of exceeding the concentration associated with a particular degree of effect. This can be expressed mathematically as,

$$P(R_c) = P(\varepsilon(C)) \times P(t, \varepsilon(C)), \tag{14}$$

where $P(\varepsilon(C))$ is the risk for human-specific organ after consumption of As-contaminated fish from the southwest coast of Taiwan, $P(t, \varepsilon(C))$ is the cumulative distribution function of having organ concentration C.

For known or suspected carcinogens, acceptable risk is set at a level considered to pose a minimal risk of cancer; this usually refers to no more than one-in-a-million (1×10^{-6}) excess lifetime cancer risk due to exposure. Therefore, risk values $<1 \times 10^{-6}$ are considered to be essentially zero and hence acceptable (WHO 2004).

Uncertainty analysis

In exposure and effect analyses, there were several sources of uncertainty. Due to inherent natural variability, model variables can be defined in terms of a probability density functions that were derived from a limited set of observations. The data, however, may not be representative of the entire population, and sample statistics may not be accurate estimates of the true values of the population parameters. This leads to cause uncertainties in the parameter estimation procedures. To explicitly account for this uncertainty/variability and its impact on the estimation of expected risk, a MC simulation was adopted. To test the convergence and the stability of the numerical output, we performed independent runs at 1, 4, 5, and 10 thousand iterations with each parameter sampled independently from the appropriate distribution at the start of each replication. Largely, because of limitations in the data used to derive model parameters, inputs were assumed to be independent. The result showed that 10,000 iterations were sufficient to ensure the stability of results. The MC simulation was implemented using Crystal Ball® (Version 2000.2, Decisionerring, Inc., Denver, CO, USA).

Results and discussion

Variations analysis of As concentration in cooked fish

Devesa et al. (2001) and Ersoy et al. (2006) indicated that the total As concentration of As-contaminated fish increased after baking, frying, and grilling, based on cooking temperature and cooking duration. Frying required lower temperatures and less cooking duration than baking or grilling; baking and grilling required similar temperatures, but the baking method also required longer cooking duration. Based on Han et al. (1998), Huang et al. (2003), Liao et al. (2003, 2006), Lin et al. (2004, 2005, 2008), and Liu et al. (2005, 2006) studies, the total As concentration in aquacultural fish in Taiwan was simulated to be LN(0.83 $\mu g\ g^{-1}$, 3.28) before cooking.

This study estimated the total As concentration in cooked fish via various cooking methods through the adoption of multiple linear regression models (Fig. 2). Results show that the total As concentration distribution was LN(8.75 $\mu g\ g^{-1}$, 1.34), LN(2.93 $\mu g\ g^{-1}$, 2.56),

and LN(0.68 μg g^{-1}, 3.74) for fried, grilled, and baked fish, respectively. Frying resulted in the greatest increase in the total As concentration in cooked fish, with grilling the second highest. Baking also led to an increase in the total As concentration, but to a lesser degree. For baking, the longest cooking duration resulted in the lowest concentration of inorganic As. Therefore, high-temperature and long-duration baking methods caused total As concentrations in fish to increase but to a lesser degree, while quicker duration frying methods at lower temperature resulted in the greatest increase of total As concentration in fish.

Exposure assessment

A life-stage PBPK model was applied to estimate the absorption, distribution, metabolism, and excretion of As species after the entry of As into the human through As-contaminated cooked fish consumption. Conversion of inorganic As into organic As through methylation was also considered (Fig. 1) during simulation of As^{3+}, As^{5+}, MMA, and DMA accumulation in various organs. Figure 3 shows that As^{3+}, As^{5+}, MMA, and DMA accumulated at the highest concentrations in the liver, followed by the kidneys, lungs, and skin. DMA displayed the highest accumulated concentration of As species in organs, followed by As^{5+}, As^{3+}, and MMA (Fig. 3).

Ichikawa et al. (2010) reported that accumulations of inorganic As in the tissue of mouse liver following ingestion of cooked Hijiki contaminated with inorganic As for 2 days had the highest value compared to the accumulation in the kidneys, lungs, and skin. Zagana et al. (2008) also reported that tissue accumulations of inorganic As in mice liver had higher accumulation capacity after intraperitoneal administration than in kidneys and lungs. Lin et al. (2005b) pointed out that the accumulated concentration of DMA in rabbit liver after dosing for 1 day was higher than As^{3+} or MMA. By studying postnatal mice, Jin et al. (2010) reported that after drinking contaminated water for 21 days, the concentrations of DMA exceeded those of inorganic As and MMA in the liver; after 35 days, the cumulative concentrations of DMA and MMA in the liver were similar, exceeding those of inorganic As.

Dose–response assessment

This study applied Weibull model to analyze the correlation of changes in the As concentration with the cumulative incidence ratios of hyperpigmentation, keratosis, lung cancer, liver cancer, and bladder cancer in Taiwanese males and females. Average life expectancy was estimated at 60, as shown in Fig. 4. Results showed that following an increase in the As concentration, hyperpigmentation possessed the highest cumulative incidence ratio in both males and females. Furthermore, cancer cumulative incidence ratios indicated that in men, liver cancer was more likely to occur than bladder cancer or lung cancer, while in women, lung cancer had the highest incidence ratio, followed by bladder cancer and liver cancer.

Women had a lower incidence ratio of liver cancer than men, but the incidence ratio of lung cancer and bladder cancer was higher than for men. These results correspond to those of Wu et al. (1989) regarding epidemiological investigation of areas in which black foot disease was prevalent along coastal regions of southwest Taiwan (Fig. 4).

Human health risk assessment via As-contaminated cooked seafood

According to the exposure analysis in this study, frying causes a high As concentration in fish. Therefore, we estimated the human health risk based on frying, considering it the worst-case scenario. With the level of acceptable risks set at 10 % (as assumed in this study), the results of non-carcinogenic analysis showed that after consumption of fried fish (Fig. 5), the incidence ratio of hyperpigmentation in men was higher than that of women. Men exhibited a 13 in 1 million incidence ratio of hyperpigmentation, while women exhibited a 1 in 1 million incidence ratio. The incidence ratio of keratosis was also higher in men, which was 5 and 4 in 1 million for men and women, respectively. The incidence ratios of hyperpigmentation and keratosis for men and women from consumption of baked As-contaminated fish were <1 in 1 million, indicating that it is unlikely to pose a significant risk to human health.

Carcinogenic analysis indicated that males had a higher risk of liver cancer than females. The incidence ratio was 3,180 in 1 million for men and 910 in 1 million for women after consumption of fried fish. The risk of lung cancer was higher in females than in males, with 2,500 women in 1 million developing lung cancer, compared to 890 in 1 million for men, from the consumption of fried As-contaminated fish. In

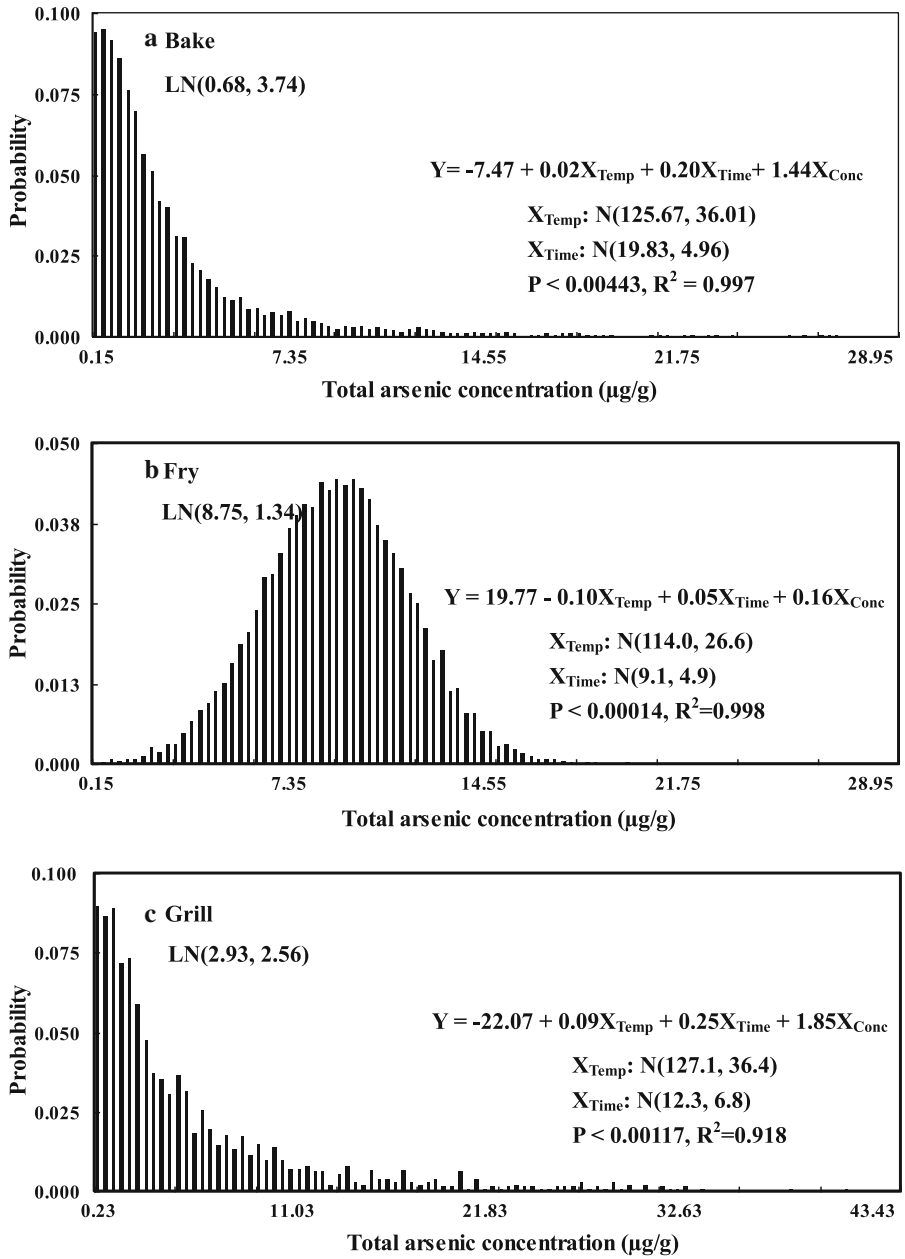

Fig. 2 Intake of total arsenic for three cooking methods: **a** baking, **b** frying, and **c** grilling with cooking temperature and cooking duration to define distributions into regression model to define distributions for Monte Carlo (MC) simulations

addition, the results showed that the risk of bladder cancer was <1 in 1 million in both men and women. This is considered an acceptable level of risk (Fig. 5). For the consumption of baked As-contaminated fish, the risk of bladder cancer was also <1 in 1 million in both men and women. The results show that <1 person in 1 million would develop bladder cancer from consumption of cooked As-contaminated fish. The incidence ratios of liver cancer and lung cancer in both men and women were generally acceptable for the consumption of baked As-contaminated fish, with the risk ranging from 1×10^{-6} to 1×10^{-4}. Simulation results show that the consumption of baked As-contaminated fish is not likely to pose a significant risk

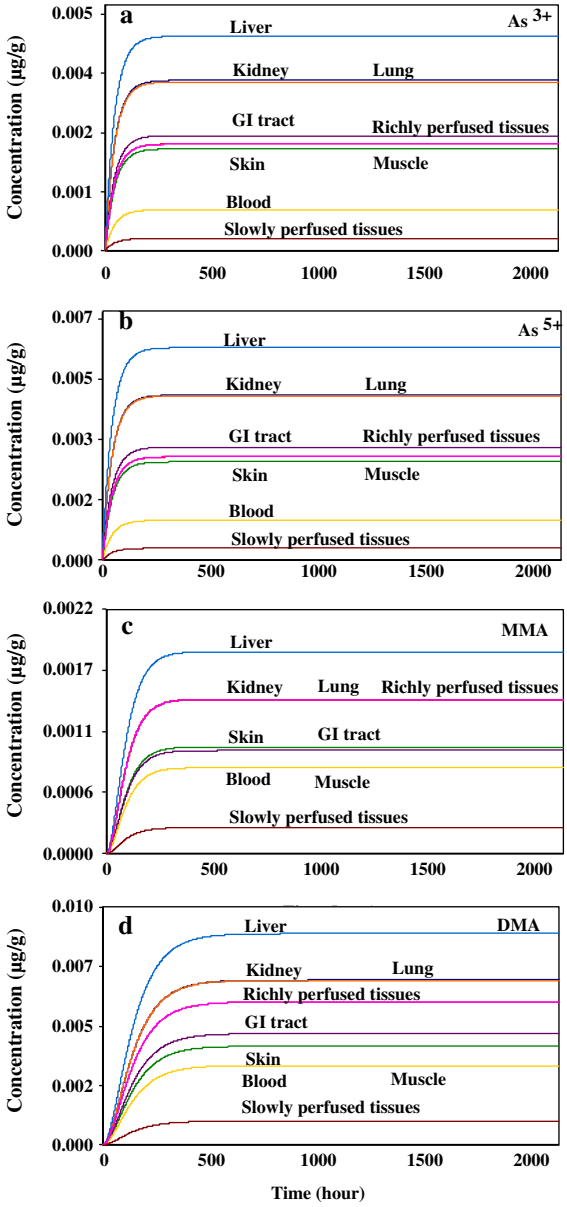

Fig. 3 Simulations of human exposure to arsenic contamination in cooked seafood of **a** As^{3+}, **b** As^{5+}, **c** MMA, and **d** DMA concentration variation for frying in long term

to human health. These results consider only the health risks associated with the consumption of cooked As-contaminated fish. If other exposure sources, such as consumption of other As-contaminated food or water, inhalation or skin contact might result in an elevated risk of hyperpigmentation, keratosis, lung cancer, liver cancer, or bladder cancer.

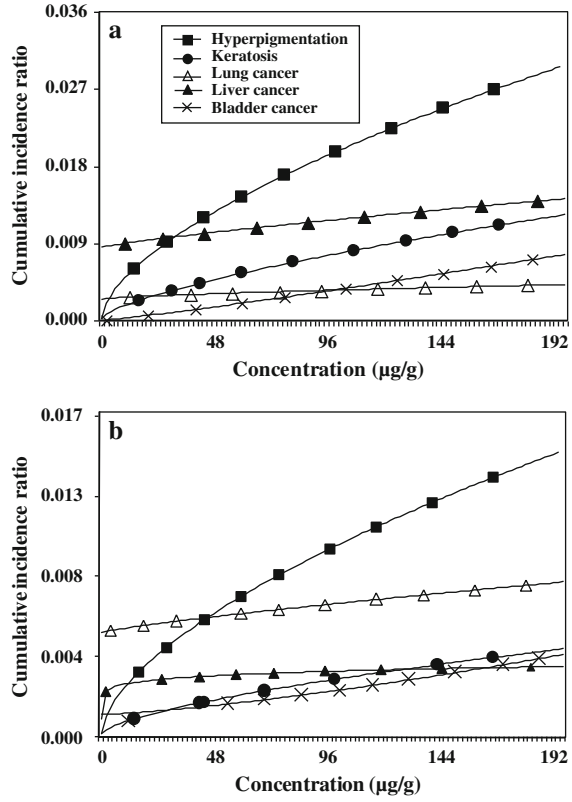

Fig. 4 Weibull dose–response function predicted cumulative incidence ratios as a function of arsenic exposure concentrations for **a** male and **b** female for hyperpigmentation, keratosis, lung, liver, and bladder cancers

Limitations and implications

Many studies to date have discussed total As in foods such as rice, seaweed, beans, olive oil, and potatoes (Mondal et al. 2008, 2010; Perello et al. 2008; Rahman and Hasegawa 2011; Sartal et al. 2012). However, international research on As species-contaminated food before cooking compared with after cooking is scarce. Furthermore, aquaculture is an important industry in Taiwan. Seafood often has high contents of organic As, generally constituting 65–90 % of total As or more. In contrast, the more dangerous inorganic As constitutes 2–10 % of total As in seafood (Huang et al. 2003; NRC 1983; US FDA 1993). Therefore, this study primarily focuses on As species-contaminated fish. This study established multiple linear regression models based on the research of Devesa et al. (2001) and Ersoy et al. (2006) with cooking temperature and cooking duration serving as variables. Factors such as

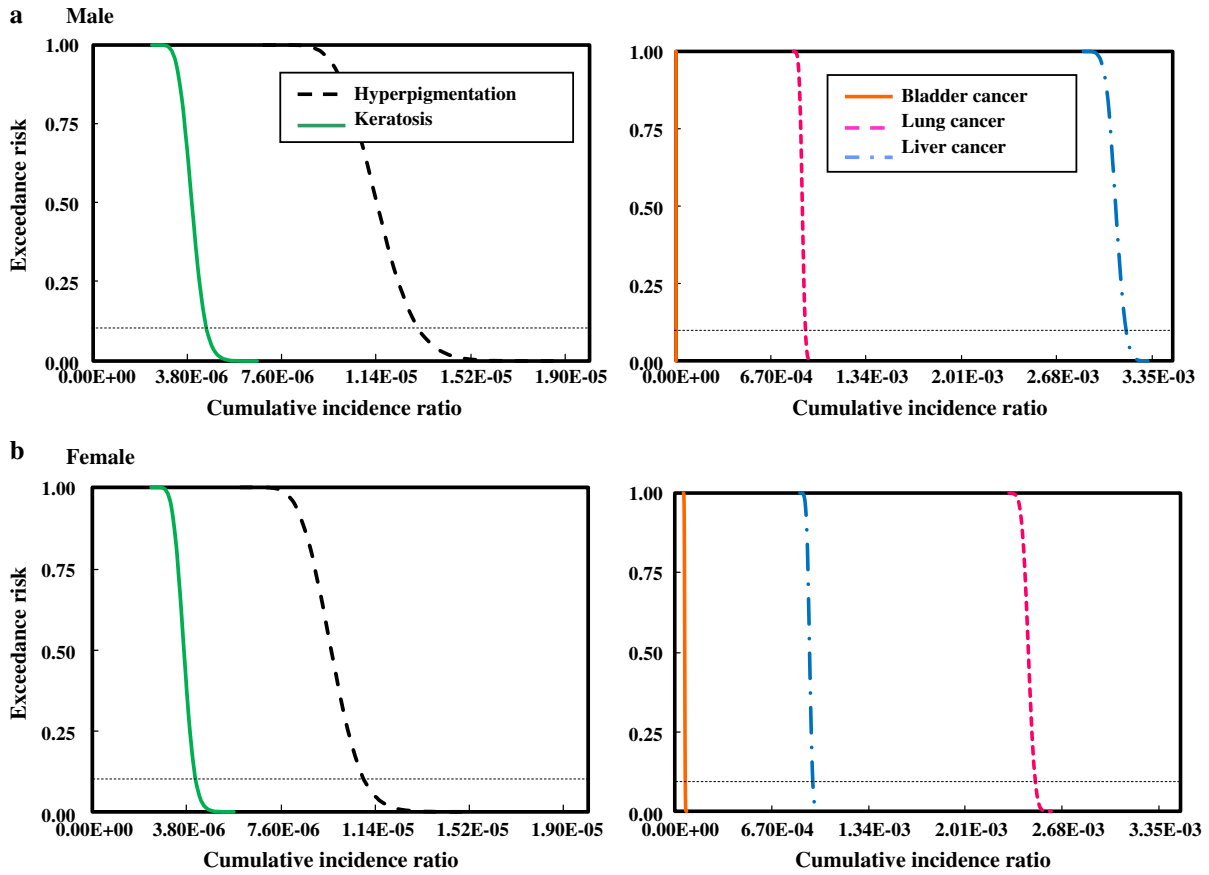

Fig. 5 Exceedance probability risk assessment for **a** male and **b** female intake of fried seafood for hyperpigmentation, keratosis, liver, lung, and bladder cancers

the amount of water added during cooking, seasoning ingredients, and differences between various types of seafood were not taken into consideration during the investigation.

Future studies may consider (1) including the amount of added water as a variable; (2) comparing changes in the concentration of As prior to baking, frying, grilling, heating with a microwave, roasting, and stewing; (3) evaluating changes in the concentration of As in rice (Sun et al. 2012) and other types of seafood, such as shrimps, clams, squid, and oysters, prior to and after cooking; and (4) validating the results against those of recent epidemiological studies. In future research, exposure analysis will be performed on other cooking methods, and regional factors will be included as variables in quantitative analysis. The risk to human health from consuming As-contaminated fish will be estimated by applying risk assessment methods, in the hope of determining the optimal temperature, cooking duration, and amount of water for cooking fish, to reduce the risk of exposure to the accumulation of toxic substances from the consumption of fish.

Conclusions

As is a naturally occurring element widely distributed throughout the earth's crust. The main source of As exposure for humans is through the consumption of As-contaminated groundwater and food. Because piped water is widespread across Taiwan, it is uncommon for residents to consume As-contaminated groundwater; however, such water is commonly used for the cultivation of fish, leading to indirect accumulation of As in fish. If the risk estimation to human health is performed

using concentrations of As in raw fish, results can be underestimated and may not accurately evaluate the effects of As contamination on the health of local residents. To precisely calculate the health risk after consumption, ready-to-eat situations must be applied.

This study adopted multiple linear regression models integrated with a PBPK model and Weibull model, while taking into consideration the correlations of absorption, distribution, metabolism, and excretion with dose, age, and cumulative incidence ratio. We also performed uncertainty analysis through Monte Carlo simulation to estimate the risk of exposure following consumption of As-contaminated fish in Taiwan. Study results discovered that after cooking, the concentration of As in contaminated fish increased. Nevertheless, baking resulted in health risks categorized as acceptable. It is therefore concluded that baking methods should be adopted, and frying should be avoided when cooking As-contaminated fish.

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