Appraising zinc bioaccumulation in abalone *Haliotis diversicolor supertexta* and alga *Gracilaria tenuistipitata* var. *liui* by probabilistic analysis

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Abstract

We appraise a first-order two-compartment model describing zinc (Zn) bioaccumulation in abalone *Haliotis diversicolor supertexta* and their food source, red alga *Gracilaria tenuistipitata* var. *liui* by probabilistic analysis of the biokinetic parameter variabilities. The model was parameterized using field and laboratory data, and predictions were quantitatively compared with field-measured tissue Zn concentrations obtained from selected abalone farms. Based on the reliable information from the published literature, we assigned the lognormal distribution model to characterize model inputs. Input variables included bioconcentration factor (BCF) of abalone, biomagnification factor of abalone, BCF of algae and depuration rate constants of abalone (k_2) for Zn from water and food. Compared with the field data, most of the measurements fall within the predicted 25th and 75th percentile range, indicating applying Monte Carlo technique to the first-order two-compartment model generated probabilistic estimates of Zn concentrations in abalone and algae that were consistent with field observations. Sensitivity analysis reveals that the input critical parameters that most influence the model output are BCF and k_2 of abalone. Our results suggest that the probabilistic approach allows a range of possible outcomes and their likelihood; it better informs both aquacultural risk assessors and risk managers. The degree of conservatism in the deterministic bioaccumulation models can also be evaluated against this distribution.
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Keywords: Abalone; Algae; Bioaccumulation; Probabilistic analysis; Zinc

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1. Introduction

Abalone, *Haliotis diversicolor supertexta*, is the most abundant abalone species in Taiwan. *H. diversicolor supertexta* is commercially important for fisheries and aquaculture in Taiwan (Chen, 1989). *H. diversicolor supertexta* is appreciated for its delicacy and high market value. The aquaculture of *H. diversicolor supertexta*, thus, is a promising business (Chen, 1989; Singhagraiwan and Doi, 1993).

Zinc (Zn) is an essential micronutrient found at high levels in the algae and in the tissues of fish/shellfish (Hogstrand et al., 1998; Genter and Lehman, 2000). Zinc is available to abalone from both the dissolved phase (e.g., gill uptake) and the diet (e.g., red alga *Gracilaria tenuistipitata* var. *liui* ingestion). If waterborne Zn levels are elevated, however, toxicity can occur and have severe effects on the health of abalone, which will reduce market prices and cause closure of abalone farms (Hahn, 1989; Conroy et al., 1996; Knauer et al., 1997).

Previous investigations indicated that Zn has been detected in many rivers and that maximum Zn concentrations in contaminated aquacultural waters are reported to range from 60 to 300 $\mu g\ l^{-1}$ in different areas of Taiwan (Lee et al., 1996; Lin and Liao, 1999). Because few previous studies have evaluated Zn toxicity to *H. diversicolor supertexta*, we did not have an a priori estimate of internal lethal body burdens. Mechanisms of Zn toxicity in abalone have not been investigated extensively.

Vermeire et al. (2001) pointed out that probabilistic modeling has received increasing support as a promising technique for characterizing uncertainty and variation in estimates of exposure to environmental contaminants. To date, however, only a limited number of risk assessments regarding aquacultural management have incorporated probabilistic analyses. A predictive assessment is needed to evaluate the potential for Zn bioaccumulation, toxic effects to abalone and risks to human health. The determination of biokinetic parameters is an essential component in the risk assessment of potential harmful chemicals. A well-established one-compartment uptake-depuration model may provide realistic estimates of the biokinetic parameters such as uptake and depuration rate constants through a laboratory exposure experiment (Lin and Liao, 1999). The reliability of the predictive model, however, is determined by the precision of the model inputs. In order to assess how model predictions are affected by the uncertainties in biokinetic parameter values, we use the probability density function (pdf) to characterize biokinetic variables.

This paper applied a first-order two-compartment model to evaluate the importance of biokinetic variability in Zn bioaccumulation in abalone. The two-compartment model describes the processes of uptake and elimination of Zn in abalone between water and its food source, red alga *G. tenuistipitata* var. *liui*. The following three elements are used to perform a probabilistic analysis in the predictive bioaccumulation modeling: (1) to characterize the uncertainty on the selected biokinetic parameters influencing bioaccumulation, (2) to produce a Monte Carlo-simulated prediction of Zn concentrations in abalone and algae and to perform a sensitivity analysis to identify critical inputs and (3) to interpret the results in the light of uncertainty and to compare with published field studies obtained from three selected commercial abalone farms.

The probabilistic framework was selected because it overcomes certain limitations in the deterministic approach. In particular, the deterministic approach lacks any precise

evaluation of the uncertainties and the conservation estimates implied in the results. The combination of a predictive bioaccumulation model and an uncertainty analysis would help to understand the biokinetic behavior of waterborne metals in abalone associated with the human health risk resulting from consumption of abalone.

2. Materials and methods

2.1. Model structure

We used a first-order two-compartment model to describe uptake and elimination processes of abalone exposed to Zn in an abalone-farming pond. Fig. 1 shows the processes considered in this study. The scenarios (Fig. 1) that we considered were (i) the exchange of Zn between abalone and dissolved Zn was modeled as a first-order process, with additional Zn accumulation from ingested algae; (ii) abalone ingest only algae and other suspended particles uptakes are negligible; (iii) tissue concentration of Zn per unit biomass of abalone increases as a result of direct uptake from water and through assimilation of algae; and (iv) tissue concentration tend to decrease as a result of elimination from the whole body.

The first-order two-compartment model for the gain and loss of Zn accumulation in abalone and algae features constant biokinetic rates and constant water concentration.
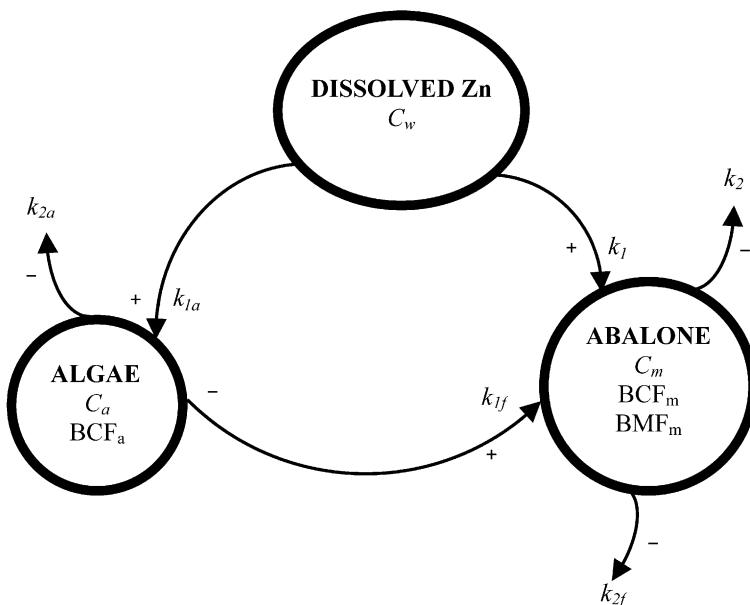

Fig. 1. Schematic showing the first-order two-compartment model of Zn bioaccumulation in abalone *H. diversilor supertexta* and their food red alga *G. tenuistipitata* var. *lilu* (see Eqs. (1) and (2) for full explanation).

Accordingly, the dynamic behavior corresponding to the graphic model of Fig. 1 would be represented as

$$\frac{dC_m(t)}{dt} = k_1 C_w + k_{1f} C_a(t) - (k_2 + k_{2f}) C_m(t), \tag{1}$$

$$\frac{dC_a(t)}{dt} = k_{1a} C_w - (k_{2a} + k_{1f}) C_a(t), \tag{2}$$

where $C_m(t)$ is the time-dependent Zn concentration in abalone soft tissue ($\mu g\ g^{-1}$), $C_a(t)$ is the time-dependent Zn concentration in algae ($\mu g\ g^{-1}$), t is the time of exposure (day), C_w is the dissolved Zn concentration in water ($\mu g\ g^{-1}$), k_1 is the uptake rate constant from dissolved phase by abalone ($ml\ g^{-1}\ day^{-1}$), k_{1f} is the uptake rate constant from algae by abalone ($g\ g^{-1}\ day^{-1}$), k_2 is the depuration rate constant for Zn in abalone (day^{-1}), k_{2f} is the elimination rate constant for Zn from food in abalone (day^{-1}), k_{1a} is the uptake rate constant from dissolved Zn by algae ($ml\ g^{-1}\ day^{-1}$) and k_{2a} is the depuration rate constant for Zn in algae (day^{-1}).

We consider the steady-state condition in Eq. (2) and solve for C_a gives,

$$C_a = \frac{k_{1a}}{k_{2a} + k_{1f}} C_w = BCF_a C_w, \tag{3}$$

where $BCF_a \equiv C_a/C_w = k_{1a}/(k_{2a} + k_{1f})$ is the bioconcentration factor (BCF) for algae ($ml\ g^{-1}$). By substituting Eq. (3) into Eq. (1), and solving for $C_m(t)$ gives,

$$C_m(t) = C_m(t = 0)e^{-k_e t} + \frac{k_u}{k_e} C_w(1 - e^{-k_e t}), \tag{4}$$

where k_u and k_e represent the overall uptake and overall elimination rate constants and have the form as,

$$k_u = k_1 + k_{1f} BCF_a, \tag{5}$$

$$k_e = k_2 + k_{2f}. \tag{6}$$

The Zn concentration in abalone is calculated as $C_m = k_u/k_e C_w = BAF_m C_w$ when equilibrium is achieved in Eq. (4) in that we define $BAF_m \equiv k_u/k_e$ and has a form as,

$$BAF_m = \frac{k_u}{k_e} = \frac{BCF_m}{1 + k_{2f}k_2^{-1}} + BMF_m BCF_a, \tag{7}$$

where BAF_m is the bioaccumulation factor (BAF) for abalone ($ml\ g^{-1}$), $BCF_m = k_1/k_2$ is the BCF for abalone ($ml\ g^{-1}$) and $BMF_m = k_{1f}/k_e$ is the biomagnification factor (BMF) for abalone.

The input variables needed to model the Zn bioaccumulation in abalone and algae include abalone bioconcentration factor BCF_m, abalone biomagnification factor BMF_m, algae bioconcentration factor BCF_a and biokinetic parameters of abalone elimination rate constant for Zn form food k_{2f} and abalone depuration rate constant for Zn k_2.

2.2. Input probability distributions

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 bioaccumulation model adopted. Source data of biokinetic parameters appeared in Eqs. (1) and (2) would be obtained from a published study by Chen (1984, 1989), Lee et al. (1996), Lin and Liao (1999) and Chen and Lee (1999). Data were sorted by reported statistical measure, e.g., mean, minimum, maximum, etc. Table 1 summarizes the estimated biokinetic parameters (BCF_m, k_{2f} and k_2) that were determined from a 14-day laboratory uptake-depuration experiment and field observations for BMF_m and BCF_a obtained from real abalone farms. Wherever possible, we tried to account for uncertainty in these estimates. Table 1 gives the published biokinetic data as the mean (μ) with one deviation (σ) represented as 1 S.E. or 95% confidence limits (95% c.l.).

The goal of the distribution selection process for each input variables was to identify a mathematical distribution expressing the range of variation and likelihood of values within the range. The normal distribution function is rarely used to account for the uncertainty in estimates because smooth symmetric variation of mean values is unexpected and data might be insufficient for estimating a mean and standard deviation since individual measurements reflecting interindividual variation about that mean, due to environmental or physical factors. A second problem in applying the normal distribution to any quantity that varies over a wide range is that such a wide normal distribution requires a certain fraction of the parameters to have negative values. This problem combined with the frequently observed skewed shape of the distribution, led to the use of the logarithmic transformation of parameter data to obtain the lognormal distribution.

Table 1
Estimates of input biokinetic parameters obtained from published data and geometric mean and geometric standard deviation for each parameter modeled as a lognormal distribution for the model simulation

Biokinetic variable[a]	Deterministic parameter values	Ref.	Parameter of the lognormal distributions employed	
			Geometric mean	Geometric S.D.
k_2 (day^{-1})	0.611 ± 0.53[b]	Lin and Liao (1999)	0.437	4.13
k_{2f} (day^{-1})	0.636 ± 0.26[b]	Lin and Liao (1999)	0.602	1.53
BCF_m (ml g^{-1})	166 ± 16[c]	Lin and Liao (1999)	165.22	1.10
BMF_m (g g^{-1})	1.53 ± 0.25[c] $(1.09, 1.87)$[d]	Chen (1984, 1989), Lin and Liao (1999), Chen and Lee (1999)	1.51	1.19
BCF_a (ml g^{-1})	524 ± 149[c] $(329, 698)$[d]	Lee et al. (1996), Lin and Liao (1999), Chen and Lee (1999)	501.00	1.39

[a] k_2 is the depuration rate constant for Zn in abalone, k_{2f} is the elimination rate constant for Zn from food in abalone, BCF_m is the bioconcentration factor for abalone, BMF_m is the biomagnification factor for abalone and BCF_a is the bioconcentration factor for algae.
[b] Mean $\pm$ 95% confidence limit.
[c] Mean $\pm$ S.E.
[d] Values are (minimum, maximum).

Moreover, the lognormal distribution is often considered the default in environmental analysis (Finley et al., 1994; Thompson et al., 2000). Its extensive use may be explained by the fact that environmental processes involve products of several variables, which suggests applicability of the central limit theorem upon logarithmic transformation (Johnson et al., 1995; Balthis et al., 1996; El-Shaarawi and Viverros, 1997; Vermeire et al., 2001; Jager et al., 2001). The data are divided into a minimum of 10 bins as equally as possible, based on a normal distribution in that the mean and S.E. or 95% c. l. are shown in Table 1.

We used the chi-square (χ^2) and the Kolmogorov−Smirnov (K−S) statistics (Zar, 1999) to optimize the goodness-of-fit of distributions. Data and distribution parameters were analyzed and estimated using the Statistica® software package (StatSoft, Tulsa, OK, USA). The Statistica® software generated p-values for the χ^2 statistics and provided critical values of d_{max} for the K−S statistics to estimate α values from 0.01 to 0.50. For optimization, $p \geq 0.05$ considered good, $p = 0.05 - 0.10$ was acceptable and $p < 0.10$ was poor. We determined that the lognormal distribution model fits the observed data favorably and were transformed appropriately to ensure the data did not differ from a normal distribution before parametric analysis. All input variables that modeled as the lognormal distributions from which geometric mean (gm: μ_g) and geometric standard deviation (gsd: σ_g) for each variable was calculated (Table 1). Table 1 indicates that parameter variability (σ_g) of the estimate in k_2 is broader than that in k_{2f}, BCF_m, BMF_m and BCF_a in that variability determines the contribution of an input to the variance of model predictions.

Fig. 2 illustrates pdfs of the optimized lognormal distribution with gm and gsd ($\ln(\mu_g, \sigma_g)$) for five of the model inputs. The histograms of source data with frequency functions of the normal distribution ($N(\mu, \sigma)$) are also shown. Therefore, we suggest that random variables characterizing uncertainty about any model input biokinetic variable x has a lognormal pdf with gm ($\sigma_{g,x}$) and gsd ($\mu_{g,x}$) as $x \sim f_x(x) \equiv \ln(\mu_{g,x}, \rho_{g,x})$ in that

$$f_x(x) = \frac{1}{\sqrt{2\pi} x \ln\sigma_{g,x}} \exp\left(-\frac{(\ln x - \ln\mu_{g,x})^2}{2(\ln\sigma_{g,x})^2}\right),\tag{8}$$

and the cumulative distribution function (cdf) can be expressed as

$$F_x(x) = \Phi\left(\frac{\ln x - \ln\mu_{g,x}}{\ln\sigma_{g,x}}\right),\tag{9}$$

where $\Phi(\cdot)$ is the cdf of the standard normal random variable.

2.3. Sensitivity analysis and validation

We are interested in the long-term equilibrium rather than the dynamics over a single growing season. We used Eqs. (3) and (4) to predict Zn concentrations in abalone and algae. Because the idea of the present model was to incorporate uncertainty into the model by selecting model parameters from lognormal probability distributions rather than experimentally derived values or field observations, we used a Monte Carlo technique to deal with the uncertainty (Vose, 2000). To test the convergence and the stability of the numerical output, we performed independent runs at 1000, 4000, 5000 and 10,000 iterations with each
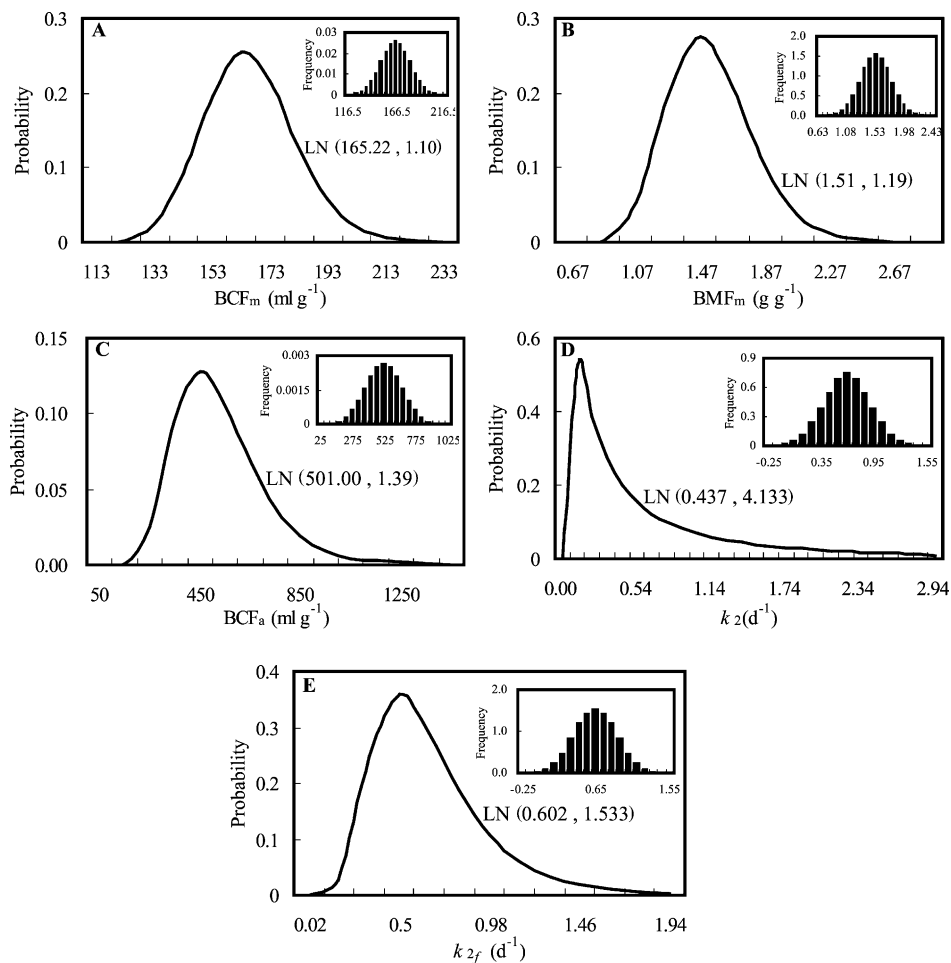
Fig. 2. Probability density functions of optimized lognormal distribution with geometric mean (μ_g) and geometric standard deviation (σ_g) as $\ln(\mu_g, \sigma_g)$ for five model inputs. The histogram of source data with frequency functions of the normal distribution are also shown.

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 coefficient of variation (the ratio of standard deviation to mean for each number of iterations) was computed, with the conclusion that 5000 iterations are sufficient to ensure the stability of results. In this case, the numerical error on the 95th percentile is equal to 2%. Sokal and Rohlf (1995) also indicated that more than 1000 replicate simulations gives K−S 95% confidence limits of approximately ± 4% on output distributions and should be sufficient to ensure reliable results.

One step in trying to establish the range of conditions under which a model may be considered reliable is to identify components of the model that are important to model

results. If parameters of great importance to model output are well established experimentally or in the field, then the reliability of the model is strengthened. Alternatively, if the most important parameters are those that are less well-established, then future research should be focused on those parameters to improve the predictive capabilities of the model. To this purpose, a sensitivity analysis was performed in order to determine which pdfs have the greatest effect on model results. Therefore, the final results were analyzed statistically to identify the key parameters causing the model to reproduce the observed behavior.

The theory behind this sensitivity analysis is based on the separation between the baseline and shifted (mean or variance shifts) cumulative distribution functions (cdfs), and a K−S two-sample test is utilized to assess the separation (Hornberger and Spear, 1980; Spear and Hornberger, 1980). The statistic d_{max} is determined as the maximum vertical distance between the cdf curves for baseline and shifted functions. The large values of d_{max} indicate that the parameter is important for simulating the model. The cdf curves also highlight if the values of the parameters causing the model outputs were at the lower or upper bounds or around the midpoint of the range.

Model predictions were compared to measurements of Zn concentration in algae and soft tissue of abalone at three different abalone farms on Toucheng, Kouhu, and Anping, in northern, central, and southern Taiwan region, respectively, to evaluate the predictive ability of the model. Lin and Liao (1999) chose three appropriate management practices on abalone farms for each study location and measured Zn concentrations in pond water, algae and abalone, respectively. Three abalone, three algae samples, and three 500-ml water samples per farm were collected. The nine abalone farms had similar feeding strategies and the biomass of algae and abalone were monitored throughout each growing season by the farm owners.

Simulations were run with each reported water Zn concentration measurements (cdfs of water Zn concentration were also lognormal-transformed from field data), using the inputs listed in Table 1. No other studies containing suitable data were identified, thus, extremely limited field data are available for model validation.

3. Results

Fig. 3 shows the pdfs and cdfs of Zn in abalone and algae for abalone farms of Toucheng, Kouhu and Anping. Probabilistic simulations of the bioaccumulation models produced skewed distributions of predicted Zn concentrations. Percentile predictions of Zn in abalone and algae in each abalone farm could be determined from cdfs illustrated in Fig. 3. Table 2 gives the percentile predictions of Zn concentrations in abalone and algae for three selected abalone farms. We also used box and whisker plot to represent the uncertainty in estimates of Zn concentration in abalone and algae (Fig. 4). Compared with the field observations, median estimates of Zn in abalone and algae were less than

Fig. 3. Simulation results showing probability density functions and cumulative distribution functions of Zn concentrations in *H. diversilor supertexta* and *G. tenuistipitata* var. *lilu* for three selected abalone farms: (A) Toucheng, (B) Kouhu and (C) Anping. (▲) Measured mean concentration in *H. diversilor supertexta*. (▽) Measured mean concentration in *G. tenuistipitata* var. *lilu*.
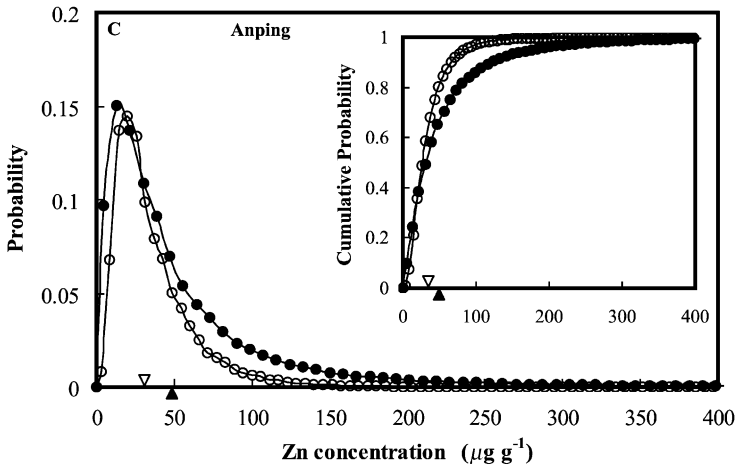
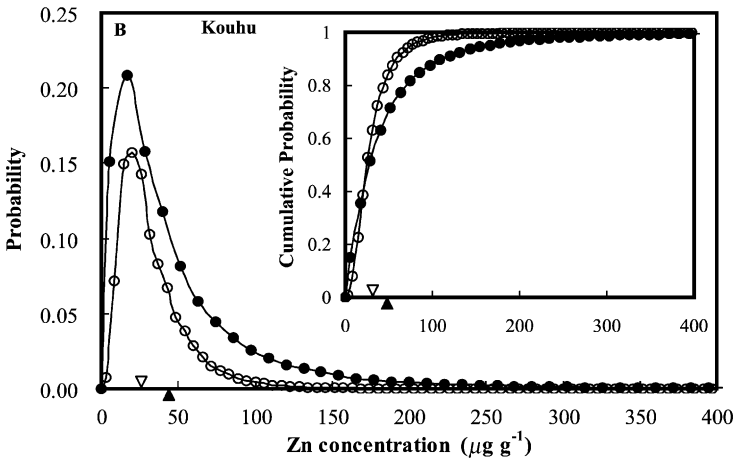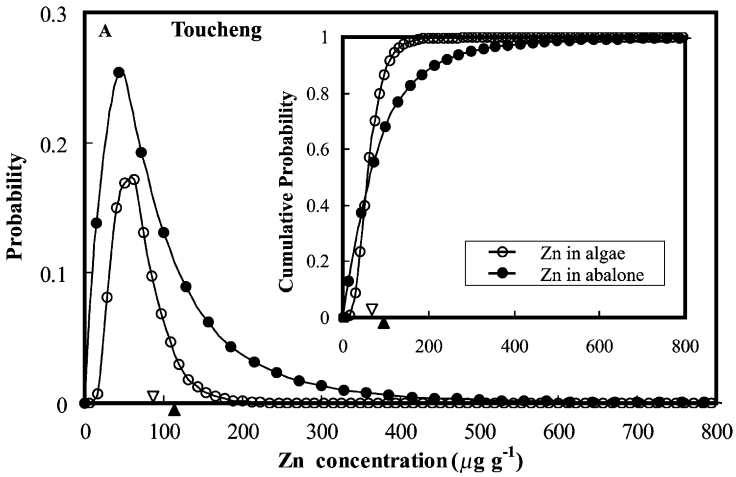Figures showing probability and cumulative probability distributions of Zn concentration ($\mu g\ g^{-1}$) for three sites: A (Toucheng), B (Kouhu), and C (Anping), comparing Zn in algae (open circles) and Zn in abalone (filled circles).

Table 2
Percentile predictions of Zn concentration ($\mu g\ g^{-1}$) in algae and abalone in abalone farms located at Toucheng, Kouhu and Anping

Abalone farm	Field observations[a]			Percentile predictions				
	Mean	S.E.	(Min, max)	10th	25th	50th	75th	90th
Toucheng								
Water	131.04	31.99						
Abalone	111.00		(93.45, 121.45)	10.21	24.89	61.95	121.87	218.42
Algae	91.04		(52.84, 111.08)	30.00	41.29	58.33	79.86	104.52
Kouhu								
Water	60.71	21.60						
Abalone	46.41		(38.21, 53.47)	4.57	11.74	26.96	58.03	110.23
Algae	25.44		(21.78, 32.39)	9.65	15.05	24.89	38.98	59.04
Anping								
Water	69.59	32.23						
Abalone	49.77		(44.95, 58.21)	4.39	13.71	31.07	64.86	120.87
Algae	31.93		(20.77, 45.98)	9.93	16.25	26.77	43.35	66.11

The field observations are also shown as mean (min, max), whereas measured water Zn concentrations ($\mu g\ l^{-1}$) are shown as mean with 1 S.E. ($n = 9$).

[a] Adapted from Lin and Liao (1999).

measured mean values (Table 2). Five of six field observations of three selected abalone farms are within the predicted 25th and 75th percentile range, and all fall within the 10th and 90th percentile range (Table 2). Therefore, applying the Monte Carlo technique to the first-order two-compartment model generated probabilistic estimates of Zn concentrations
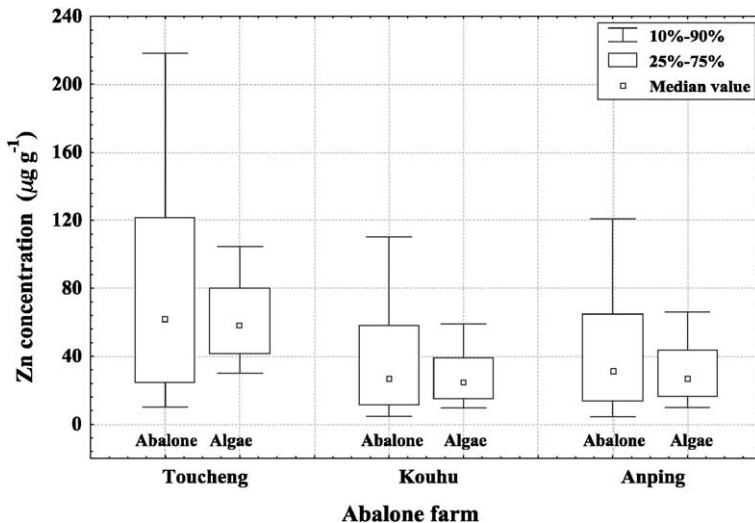

Fig. 4. Box and whisker plot representations of Zn concentration in *H. diversilor supertexta* and *G. tenuistipitata* var. *lilu* collected from abalone farms in Toucheng, Kouhu and Anping.
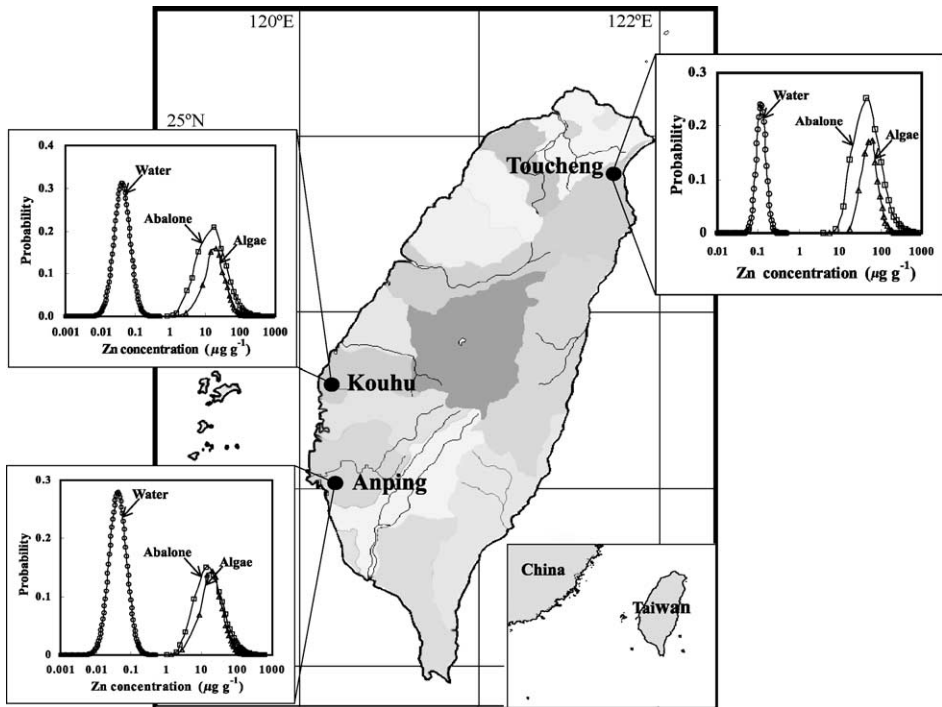
Fig. 5. Overall display of probabilistic distributions of predicted Zn concentrations in *H. diversilor supertexta* and *G. tenuistipitata* var. *lilu* subject to measured water Zn concentration at three selected abalone farms located at north, central and south Taiwan region, respectively.

in abalone and algae that were consistent with field data. Relative to minimum and maximum field data, however, lower and upper probabilistic percentile predictions were more conservative. It is evidence that the modeling framework and the distributional parameters and assumptions in the model are appropriate for estimating bioaccumulation of Zn in abalone and algae.

Although those cdfs for Zn in abalone and algae present in Fig. 3 are equally acceptable, they have different meanings. Risk associated with abalone exposure to Zn in allowable residue concentration in Toucheng abalone farm has a greater likelihood of occurrence than the same risk associated with exposure to Zn in Kouhu and Anping. The comparison of median values of pdfs shows that abalone and algae exposure to Zn will

Table 3
Kolmogorov–Smirnov test for sensitivity analysis of model parameters significant at 95% level (0.152) or greater

Parameter	Description	d_{max}
BCF_m	Bioconcentration factor for abalone	0.194
k_2	Depuration rate constant for Zn in abalone	0.183
k_{2f}	Elimination rate constant for Zn from food in abalone	0.177

cause, on average, the same risk in each selected abalone farms; and all estimated tissue Zn concentrations have a higher uncertainty as quantified by the variance.

The relative skewness and spread in modeled output varied among water, abalone and algae; distributions of Zn concentrations in abalone were more highly skewed, with a long tail at higher concentrations (Fig. 3). Some median tissue concentrations generated by probabilistic method were close to mean values. Measurements of minimum and maximum were less widely spaced, or less conservative, than the 10th and 90th percentile values of probabilistic output. An overall display of distributions of predicted Zn concentrations in abalone and algae by abalone farm locations is illustrated in Fig. 5.

In view of Fig. 2, abalone depuration rate constant k_2 has a higher uncertainty as quantified by the variance (i.e., parameter variability: σ_g), therefore, we assigned a variance shift for k_2, the other four parameters (BCF_m, k_{2f}, BMF_m and BCF_a) are assigned as a mean shift. Based on the shift mappings for cdfs, the parameters and corresponding d_{max} statistics that are significant above the 95% level are listed in Table 3. The parameters listed are mainly those directly controlling the model outputs because the probability model incorporated five stochastic variables in the calculation of Zn concentrations in abalone (Eq. (7)). Simulated Zn concentrations in abalone were most sensitive to abalone bioconcentration factor BCF_m, abalone depuration rate constant k_2, and abalone food elimination rate constant k_{2f} (Table 3). Biomagnification factor for abalone BMF_m and algae bioconcentration factor BCF_a have values of 0.068 and 0.059, respectively, and as such are not significant even at the 90% level (0.102).

Therefore, for Zn accumulation in abalone, the bioconcentration factor and depuration rate constant of abalone are the most influential variables (Table 3). This information implies that the mean value chosen in the deterministic bioaccumulation model for BCF_m and k_2 contribution to Zn accumulation in abalone may not be sufficiently conservative: It will lead to Zn in abalone associated with a probability of exceeding a specific value of Zn, higher than the threshold considered acceptable in the probabilistic context. For example, if the BCF_m mean value corresponds to the 75% percentile of this parameter distribution; the allowable concentrations for Zn in abalone are strongly influenced by BCF_m, and the resulting 75% level of conservatism implied in the mean value is insufficient to ensure a 90% level of conservatism in the specific risk value calculated in the deterministic context.

4. Discussion

Based on the available data, the ranges of Zn concentrations in abalone and algae are overestimated within 75th and 90th precentiles, although median Zn concentrations predicted did not exceed observations and most observations were between 25th and 70th percentile predictions. Mean field data and median probabilistic predictions were similar in magnitude and minimum and maximum field data were much more narrowly spaced than the 10th and 90th percentile probabilistic predictions. This resulted from grouping probabilistic extreme minimum and maximum values in field data, which usually leads to unconservatism in exposure and risk estimates. Probabilistic 10th and 90th percentile

estimates yielded slightly more relevant bounds of bioaccumulation in abalone since parameters were sampled independently in the probabilistic simulations. Overpredictions may result from many reasons, in particular, complex partitioning of Zn between water, suspended solid, and nonlinear accumulation of ionic metals in abalone and algae (Knauer et al., 1997). The model dose seems to represent the correct trends of Zn bioaccumulation.

Based on the contribution of key biokinetic parameters to output variance as determined in sensitivity analysis, the most important biokinetic variables are BCF and depuration/elimination rate constants (k_2 and k_{2f}) in abalone. These variables are worth examining closely. Available data seem to provide reasonable measures of central tendency, but replication is limited and variation is not well represented. Because its σ_g is large, k_2 has a certain impact on output variance. By using constant water concentrations and varying BCFs independently, our model reveals that the BCFs for metals in abalone are higher than that in algae, suggesting that BCFs of Zn in abalone may be positively correlated with dissolved concentrations in water. Moreover, sensitivity analysis shows that the contribution of BCF_m to overall accumulation is large in abalone, thus, considering the water route in abalone would appreciably affect estimates of total accumulation. Bertine and Goldberg (1972) and Amiard-Triquet et al. (1987) indicated that the levels of Zn in the algae-grazing mollusk, *Gibbula umbillicalis* and *Littorina littorea*, are not different from Zn level in a brown alga, *Fucus serratus*, that is the food species of the mollusks, indicating Zn in the abalone comes from the ambient water and not from the algae. Lin and Liao (1999) also pointed out that uptake of Zn from water by abalone is more significant compared with uptake from food. Therefore, when considering the aquaculture of abalone, it is a priority to control Zn concentration in the pond water.

The probabilistic methods used show that field data or experimentally derived values may hide significantly different levels of conservatism in relation to the uncertainty and variability present in each biokinetic parameter. Variation and uncertainty in model inputs were addressed using conservative assumptions, a range of abalone farm scenarios, and probabilistic analysis. The analysis does not reflect all source of uncertainty. Voit and Schubauer-Berigan (1998) pointed out that probabilistic analysis dose not account for structural errors in the model or inaccurate distributions of input variables. Although assuming independence among inputs is a common assumption in probabilistic analyses for initial analyses and when data sufficient to derive correlation coefficients are unavailable, biases may result that account for some of the overprediction of 75th percentile in the present study. Indeed extrapolations from laboratory and field data are both uncertain and may underestimate variability. Consequently, in this application, probabilistic analysis may not provide accurate estimates of the distribution of Zn concentrations or risks, for example in 90th percentile concentrations. Furthermore, because model inputs were widely dispersed and lognormal distributed, predictions were right-skewed and spanned large ranges.

Presumably, centers of distribution are more realistic than tails and, thus, the analysis emphasizes 50th to 70th percentile predictions. Fortunately, available field data allow validation over the range of model outputs, including the upper percentile predictions. The model provides preliminary estimates of such statistic and this information is valuable because it can be used to guide future analysis and data collection efforts. In fact, though requiring more resources and skills, a Monte Carlo analysis carried out was very

informative since it revealed the degree of conservatism and took into account the reliability of results.

The possibility to perform a detailed and more precise sensitivity analysis is an advantage that the probabilistic method provides relative to the deterministic one. Sensitivity analysis indicates that to increase the accuracy of the results efforts should focus on a better definition of probability distributions for abalone bioconcentration factor, depuration and food elimination rate constants. Given the scarcity of data, most of the probability distributions were based on limited observations from abalone farms, and this may be a limit to the validity of the case presented.

In summary, this paper illustrates the use of probabilistic distributions for various biokinetic and exposure factors in the context of a mechanistic bioaccumulation model that is amenable to probabilistic analyses for Zn accumulation in abalone and in their food source in the abalone farms.

Our results demonstrate that probabilistic simulations of the bioaccumulation model generated probabilistic distributions of tissue Zn concentrations that are generally consistent with field observations. The lower and upper probabilistic percentiles of Zn concentrations in abalone and algae were conservative relative to minimum and maximum observations in three selected abalone farms. Although these wide prediction ranges are limited in precision, applying such conservative ranges in calculated tissue burdens may be useful for modeling exposures to human consumption in risk assessments. Our model suggests that a simpler statistic-based model, such as empirical regression of BCFs, may generate more accurate predictions of bioaccumulation and be easier to parameterize. The mechanistic bioaccumulation model, however, can be a promising tool for increasing the understanding of heavy metals in aquacultural species as they are tested and refined.

It is our opinion that the incorporation of probabilistic analysis into evaluation of accumulation greatly improves our ability to appraise the range of possible exposure scenarios and environmental risk to aquacultural species and human who consume contaminated fish and shellfish. Probabilistic exposure assessments will substantially reduce the compounded conservatism that is inherent in risk assessments that rely on conservative point value estimates for all biokinetic-and/or toxicological effects-related parameters.

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