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Probabilistic risk assessment of abalone *Haliotis diversicolor supertexta* exposed to waterborne zinc

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"Capsule": *A novel risk assessment method was developed for abalone.*

Abstract

This paper describes a risk assessment approach that integrates predicted tissue concentrations of zinc (Zn) with a concentration–response relationship and leads to predictions of survival risk for pond abalone *Haliotis diversicolor supertexta* as well as to the uncertainties associated with these predictions. The models implemented include a probabilistic bioaccumulation model, which linking biokinetic and consumer-resource models, accounts for Zn exposure profile and a modified Hill model for reconstructing a dose–response profile for abalone exposed to waterborne Zn. The growth risk is assessed by hazard quotients characterized by measured water level and chronic no-observed effect concentration. Our risk analyses for *H. diversicolor supertexta* reared near Toucheng, Kouhu, and Anping, respectively, in north, central, and south Taiwan region indicate a relatively low likelihood that survival is being affected by waterborne Zn. Expected risks of mortality for abalone were estimated as 0.46 (Toucheng), 0.36 (Kouhu), and 0.29 (Anping). The predicted 90th-percentiles of hazard quotient for potential growth risk were estimated as 1.94 (Toucheng), 0.47 (Kouhu), and 0.51 (Anping). These findings indicate that waterborne Zn exposure poses no significant risk to pond abalone in Kouhu and Anping, yet a relative high growth risk in Toucheng is alarming. Because of a scarcity of toxicity and exposure data, the probabilistic risk assessment was based on very conservative assumptions.

Keywords: Abalone; Probabilistic; Risk assessment; Zinc

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* are appreciated for their delicacy and high market value; therefore, the aquaculture of *H. diversicolor supertexta* is a promising business (Chen 1989; Singhagraiwan and Doi 1993). Abalone were readily identifiable and can be sampled easily. Their biological and ecological characteristics are known, they strongly accumulate pollutants, and they can be easily reared both in the laboratory and commercially (Hahn, 1989). However, the coastal regions of Taiwan where the abalone aquaculture facilities are located are subjected to polluted discharges from rivers.

Zinc (Zn) is an essential micronutrient found at high level in the tissues of gastropods mollusks (Lin and Liao, 1999; Richardson, 2001; Wang and Ke, 2002). Zn is available to abalone from both the dissolved phase (e.g., gill uptake) and the diet (e.g., algae ingestion). If waterborne Zn levels are elevated, toxicity can occur and has 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 have been detected in many rivers in that maximum Zn concentrations in aquaculture waters are reported to range from 60 to 300 ng ml^{-1} in different areas of Taiwan (Lin and Liao, 1999; Liao et al., 2002a). Because few previous studies have evaluated Zn toxicity to *H. diversicolor supertexta*, the mechanisms involved in the inhibition of growth remain unknown.

Zinc was chosen for practical as well as theoretical reasons with the availability of reasonable amounts of suitable information as the primary consideration. Generally, as prerequisites for data suitability, we

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Nomenclature

A	Steady-state algae biomass (g l^{-1})
$A(t)$	Algae biomass as a function of time t (g l^{-1})
B	Steady-state abalone biomass (g l^{-1})
$B(t)$	Abalone biomass as a function of time t (g l^{-1})
BCF_a	Bioconcentration factor for algae (ml g^{-1})
BMF_m	Biomagnification factor for abalone (g g^{-1})
BCF_m	Bioconcentration factor for abalone (ml g^{-1})
C_a	Steady-state Zn concentration in algae (μg g^{-1} dry wt.)
$C_a(t)$	Time-dependent Zn concentration in algae (μg g^{-1} dry wt.)
$C_{L,50}$	Internal effect concentration at the site of action that cause 50% mortality (μg g^{-1} dry wt.)
C_m	Steady state Zn concentration in abalone soft tissue (μg g^{-1} dry wt.)
$C_m(t)$	Time-dependent Zn concentration in abalone soft tissue (μg g^{-1} dry wt.)
$\bar{C}_m$	Mean concentration of Zn in abalone soft tissue (μg g^{-1})
C_w	Dissolved Zn concentration in water (μg ml^{-1})
D	Half-saturation for algae ingestion (g $^{-1}$)
$\mathbf{E}[\bullet]$	Expectation operator
$\mathbf{E}[R]$	Expected risk
EC_{10}	Effective concentration that causes 10% effect (μg g^{-1})
EC_{50}	Median effective concentration (μg g^{-1})
f	Biomass conversion rate for algae ingested by abalone (g g^{-1})
$F(C_m)$	Cumulative distribution function (cdf) of having abalone tissue Zn concentration
$F(\bar{C}_m)$	Cdf of having mean Zn concentration in abalone soft tissue
$F(M\|C)$	Cdf of predicted mortality function for a given tissue Zn concentration
$F(M\|C_m)$	Conditional cdf of the mortality at given tissue Zn concentration
$F(M\|\bar{C}_m)$	Conditional cdf of the mortality at given mean Zn concentration in abalone soft tissue
g	Grazing rate of abalone (g g^{-1} day^{-1})
HQ	Hazard quotient
K	Algae carrying capacity (g l^{-1})
k_1	Uptake rate constant from dissolved Zn by abalone (ml g^{-1} day^{-1})
k_{1a}	Uptake rate constant from dissolved Zn by algae (ml g^{-1} day^{-1})
k_2	Depuration rate constant for Zn in abalone (day^{-1})
k_{2a}	Depuration rate constant for Zn in algae (day^{-1})
LC_{50}	Median lethal concentration (mg l^{-1})
$LC_{50}(\infty)$	Incipient median lethal concentration (mg l^{-1})
LN_{C_m}	Lognormal probability density function (pdf) of having tissue Zn concentration
M	Mortality (%)
NOEC	Chronic no-observed effect concentration of toxicant reference value
PEC	Predicted environmental concentration
r_a	Growth rate of algae (day^{-1})
$R(C_m)$	Risk at a specific Zn concentration in abalone
t	Time of exposure (day)
TRV	Toxicant reference value
α	Assimilation efficiency (%)
μ_g	Geometric mean
σ_g	Geometric standard deviation
μ_m	Death rate of abalone (day^{-1})
$\mathbf{\Phi}(\bullet)$	Cumulative standard normal distribution

required exposure and whole-body Zn levels measured by accepted analytical techniques. In this respect, we considered experimental exposure data to be acceptable only when whole-body concentration data were available and when the exposure duration was at least 14 days. Our previous published Zn-abalone database meet this principle. On the other hand, Zn was chosen in this study because this represents a metal of general concern in terms of environmental protection and in future study, it can span the continuum from nutritionally essential to nonessential, such as Pb or Ag.

In this present work, we develop a systematic and quantitative risk assessment framework, which is most needed to interpret the significance of the reported exposures. 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 the aquacultural species. We focus on the risk of survival and growth of abalone exposed to waterborne Zn because evidence for this type of adverse effect has been presented in numerous studies (Lin and Liao, 1999; Liao and Lin, 2001; Liao et al., 2002b; Tsai et al., 2003). Thus, knowledge of the potential risks associated with exposure to Zn is essential for the effective formulation of conservation and management plans.

The objectives of this study are twofold: (1) to conduct an environmental risk assessment based on the USEPA methodology, and (2) to address the uncertainties by using a probabilistic approach to risk characterization that yields quantitative estimates of the risks themselves and also of their associated uncertainties. We use a bioaccumulation model, which links biokinetics and consumer-resource dynamics, to estimate the tissue Zn concentrations in abalone. We combine predicted tissue concentration and internal lethal body burden data with a dose–response relationship derived from experimental studies on abalone allowing us to assess survival endpoint. A hazard quotient featuring measured water Zn level and chronic no-observed effect concentration is used to assess the growth endpoint. To determine overall uncertainty in predicted risks, the uncertainties resulting from the assessments of exposure and dose–response are propagated through the risk characterization process using Monte Carlo analysis.

2. Materials and methods

2.1. Study sites

The major Zn exposure data was obtained from the previous studies conducted by Lin and Liao (1999), Liao et al. (2002a,b, 2003). They chose three appropriate management practices on abalone farms for three different study sites situated at Toucheng, Kouhu, and Anping, respectively, in north, central, and south Taiwan region. They measured Zn concentrations in pond water, algae and soft tissue of abalone and conducted laboratory exposure experiments to estimate the essential biokinetic and physiological parameters in an abalone–Zn system. The selected nine abalone farms had similar feeding strategies and the biomass of algae and abalone were monitored throughout each growing season by the farm owners.

2.2. Exposure profile

We used a first-order two-compartment model considered from the viewpoint of consumer-resource dynamics based on the approach developed by Liao et al. (2002a) to estimate Zn concentrations in abalone and algae. The scenario (Fig. 1) that we considered is (1) the exchange of Zn between abalone and dissolved Zn was modeled as a first-order process, with additional Zn accumulation from ingested algae, (2) abalone ingest only algae and other suspended particles, bacteria and detritus uptakes are negligible, (3) tissue concentration of Zn per unit biomass of abalone increases as a result of direct uptake from water and through assimilation of contaminated algae, and (4) tissue concentration tends to decrease as a result of elimination from the whole body and growth dilution.

The first-order two-compartment model for the gain and loss of Zn accumulation in abalone and algae features constant biokinetic and physiological rates and constant water concentration. Accordingly, the dynamic behavior corresponding to the graphic model of Fig. 1 would be represented as (Liao et al., 2002a),

$$\frac{\mathrm{d}A(t)}{\mathrm{d}t} = r_a\left(1-\frac{A(t)}{K}\right)A(t) - g\left(\frac{A(t)}{A(t)+D}\right)B(t), \tag{1}$$

$$\frac{\mathrm{d}B(t)}{\mathrm{d}t} = fg\left(\frac{A(t)}{A(t)+D}\right)B(t) - \mu_m B(t), \tag{2}$$

$$\frac{\mathrm{d}C_m(t)}{\mathrm{d}t} = \left(k_1 + \alpha g\left(\frac{A(t)}{A(t)+D}\right)\mathrm{BCF}_a\right)C_w - \left(k_2 + fg\left(\frac{A(t)}{A(t)+D}\right)\right)C_m(t), \tag{3}$$

$$\frac{\mathrm{d}C_a(t)}{\mathrm{d}t} = k_{1a}C_w - \left(k_{2a} + \alpha g\left(\frac{B(t)}{A(t)}\right)\mathrm{BMF}_m\right)C_a(t), \tag{4}$$

where r_a is the growth rate of algae (day^{-1}), K is the algae carrying capacity (g l^{-1}), $A(t)$ is the algae biomass as a function of time t (g l^{-1}), $B(t)$ is the abalone

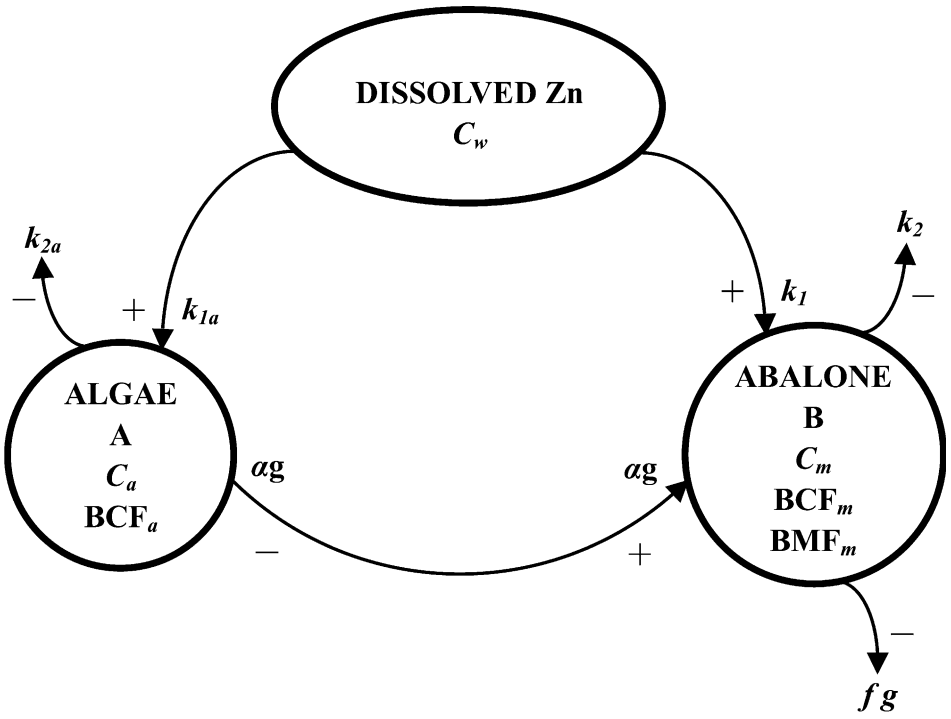

Fig. 1. Schematic showing the first-order two-compartment bioaccumulation model that linking biokinetic and consumer-resource dynamic models to describe Zn accumulation in abalone *H. diversilor supertexta* and their food red algae *G. tenuisipitata* var. *lilu* [see Eqs. (1)-(4) for full explanation].

biomass as a function of time t (g l^{-1}), D is the half-saturation for algae ingestion (g l^{-1}), f is the biomass conversion efficiency of ingested algae (g g^{-1}), g is the grazing rate of algae by abalone (g g^{-1} day^{-1}), and μ_m is the abalone death rate (day^{-1}), $C_m(t)$ is the time-dependent Zn concentration in abalone soft tissue (μg g^{-1} dry wt.), t is the time of exposure (d), C_w is the dissolved Zn concentration in water (μg ml^{-1}), k_1 is the uptake rate constant from dissolved phase by abalone (ml g^{-1} day^{-1}), α is the assimilation efficiency of abalone (%), BCF_a is the bioconcentration factor for Zn in algae (ml g^{-1}), k_2 is the depuration rate constant for Zn in abalone (day^{-1}), $C_a(t)$ is the time-dependent Zn concentration in algae (μg g^{-1} dry wt.), k_{1a} is the uptake rate constant from dissolved Zn by algae (ml g^{-1} day^{-1}), k_{2a} is the depuration rate constant for Zn in algae (day^{-1}), and BMF_m is the biomagnification factor for Zn in abalone (g g^{-1}).

We consider the steady-state condition in Eqs. (1)–(4) and assume $K >> A$ as suggested by Liao et al. (2002a), and solve for C_a and C_m gives

$$C_a = \frac{k_{1a} C_w}{k_{2a} + \alpha g \left(\frac{B}{A}\right) \mathrm{BMF}_m}, \tag{5}$$

$$C_m = \frac{\left(k_1 + \alpha g \left(\frac{A}{A+D}\right) \mathrm{BCF}_a\right) C_w}{k_2 + fg\left(\frac{A}{A+D}\right)}, \tag{6}$$

where B and A can also be obtained, respectively, as

$$B = \frac{r_a A}{g\left(\frac{A}{A+D}\right)}, \tag{7}$$

$$A = \frac{\mu_m D}{fg - \mu_m}. \tag{8}$$

The input variables needed to model the Zn bioaccumulation in abalone and algae include consumer-resource parameters (f, g, μ_m, r_a, α, D), biokinetic parameters (k_1, k_2, k_{1a}, k_{2a}, BCF_a, BMF_m), and geochemical variable of C_w.

2.3. Concentration–response profile

Tissue residues and adjusted response frequencies were used to construct a concentration–response relationship for mortality versus Zn whole-body burden in abalone based on a pharmacodynamic Hill model (Lalonde, 1992; Bourne, 1995). Liao et al. (2002b) have established a quantitative relationship between Zn tissue residues and mortality effects in abalone. Based on the acute toxicity data, mortality function is estimated from observed mortality percentages in exposure regimes in which mortality is an increasing function of the Zn concentration in water. In fitting the Hill model to the observed mortality for specific-interval acute toxicity data, the dose–response profile can be expressed as (Liao et al., 2002b),

$$M = \frac{100 \times C_w^{3.70}}{(\text{24-h } LC_{50})^{3.70} + C_w^{3.70}}, \quad (9)$$

where M is the mortality (%), the exponent 3.70 is an average value of the fitted Hill coefficient and 24-h LC_{50} is the 24-h median lethal concentration (mg l^{-1}).

Eq. (9) can be transformed appropriately to a tissue residue–response relationship using the Hill model framework to predict the response as (Liao et al., 2002b),

$$M = \frac{100 \times C_m^{3.70}}{(C_{L,50})^{3.70} + C_m^{3.70}}$$

$$= \frac{100 \times C_m^{3.70}}{(\text{BCF}_m \times LC_{50}(\infty))^{3.70} + C_m^{3.70}}, \quad (10)$$

where $C_{L,50}$ is the internal effect concentration at the site of action that cause 50% mortality, BCF_m is the bioconcentration factor of abalone (ml g^{-1}), and $LC_{50}(\infty)$ is the incipient value of $LC_{50}(t)$. We treated BCF_m and $LC_{50}(\infty)$ in Eq. (10) probabilistically. Applying the Hill model, the cumulative distribution function (cdf) of predicted mortality function for a given tissue Zn concentration, $F(M|C)$, could be expressed symbolically as a conditional cdf,

$$F(M|C) = \Phi\left(\frac{100 \times C^{3.70}}{(\text{BCF}_m \times LC_{50}(\infty))^{3.70} + C^{3.70}}\right) \quad (11)$$

where C is the given Zn concentration, and $\Phi(\bullet)$ is the cumulative standard normal distribution.

2.4. Analysis of uncertainty

Uncertainty arises from estimation of both exposure and effects. In order to quantify this uncertainty and its impact on the estimation of expected risk, we implemented a Monte Carlo simulation that includes 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, 4, 5, and 10 thousand 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 independently. The result shows 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 Kolmogorov–Smirnov (K–S) 95% confidence limits of approximately ±4% on output distributions and should be sufficient to ensure reliable results. The simulation was implemented using @RISK (Version 4.5, Professional Edition, Palisade Crop., USA).

2.5. 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 bioaccumulation model and dose–response model adopted. Source data of input variables appearing in Eqs. (5)–(8) and Eq. (10) were obtained from published studies by Chen (1984, 1989), Lee et al. (1996), Lin and Liao (1999), Chen and Lee (1999) and Liao et al. (2002a,b, 2003). 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. We used the chi-square (χ^2) and the K–S statistics (Zar, 1999) to optimize the goodness-of-fit of distributions. We employed @RISK to analyze data and to estimate distribution parameters. The @RISK generated p values for the χ^2 statistics and provided critical values of $D_{\max}$ for the K–S statistics to estimate significant levels from 0.01 to 0.50. For optimization, $P \geqslant 0.5$ considered good, $P = 0.5$–0.10 was acceptable and $P < 0.10$ was poor. The selected distribution type and parameters were based on statistical criteria, comparisons of distribution parameters, and visual interpretation of histograms. The implemented parameter probability distributions are summarized in Table 1 and described in the subsequent sections.

2.5.1. Consumer–resource parameters: f, g, μ_m, r_a, α, D

A normal distribution was determined to provide the best fit for the consumer-resource parameters of f, g, μ_m, r_a, α (Table 1). The determination of half-saturation biomass for algae ingestion (D), can be approximately obtained by fitting the Holling type II function of Eqs. (1) and (2) with known values of grazing rate of abalone (g), death rate of abalone (μ_m) and growth rate of algae (r_a) given in Table 1, to the biomass observations for algae and abalone collected from three different abalone farms. Distributions were fitted to the polled data, and the log-normal distribution optimizing the K–S fit was selected for D (Table 1).

2.5.2. Biokinetic parameters: k_1, k_2, k_{1a}, k_{2a}, BCF_a, and BCF_m

Distributions were fitted to polled lab- and field-derived biokinetic data obtained from different sources and the selected log-normal distributions had the acceptable χ^2 fit and K–S fit in that optimizations using either statistics yielded geometric mean (gm) and geometric standard deviation (gsd) (Table 1).

Table 1
Input variables parameters values used to define distributions for Monte Carlo simulations (See Nomenclature for symbol descriptions)

Parameters	Uncertainty/ Variability	Distribution
Consumer–resource parameters		
f (g g^{-1})	V	N (3.500, 0.810)[a]
g (g g^{-1} d^{-1})	V	N (0.250, 0.050)
μ_m (d^{-1})	V	N (0.286, 0.170)
r_a (d^{-1})	V	N (0.038, 0.013)
α (%)	V	N (0.346, 0.146)
D (g l^{-1})	V	
Toucheng		LN (313.64, 1.200)[b]
Kouhu		LN (343.04, 1.300)
Anping		LN (205.82, 1.100)
Lab-derived biokinetic parameters		
k_1 (ml g^{-1} d^{-1})	U	LN (99.011, 1.288)
k_2 (d^{-1})	U	LN (0.390, 4.746)
k_{1a} (ml g^{-1} d^{-1})	U	LN (97.030, 1.292)
k_{2a} (d^{-1})	U	LN (0.556, 1.535)
Field-derived biokinetic parameters		
BCF_a (ml g^{-1})	U	LN (635.362, 8.656)
BMF_m (g g^{-1})	U	LN (2.252, 2.064)
Dose–response parameters		
BCF_m (ml g^{-1})	U	LN (264.053, 1.928)
$LC_{50}(\infty)$ (mgl^{-1})	U	N (1.080, 0.127)
Geochemical parameter		
C_w (μg ml^{-1})	U	
Toucheng		LN (0.127, 1.310)
Kouhu		LN (0.055, 1.700)
Anping		LN (0.059, 1.770)

[a] N (m, S.D.) represents normal distribution with mean (m) and standard deviation (S.D.).
[b] LN (gm, gS.D.) represents lognormal distribution with geometric mean (gm) and geometric standard deviation (gS.D.).

2.5.3. Geochemical parameter: C_w

Distributions of water Zn concentrations in abalone pond (C_w) were fitted to the polled field observations obtained from three assigned abalone farm locations and the selected log-normal distributions had the optimal K–S and χ^2 goodness-of-fit (Table 1).

2.5.4. Dose–response parameters: BCF_m and $LC_{50}(\infty)$

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 limited sample size within treatment sets. To account for this uncertainty, we constructed distributions for the input variables of BCF_m and $LC_{50}(\infty)$ of Hill dose–response function in Eq. (10). We determined log-normal and normal distributions for BCF_m and $LC_{50}(\infty)$, respectively (Table 1), and incorporated the distributions into the Monte Carlo simulation to obtain 2.5th- and 97.5th-percentiles as the 95% confidence interval for reconstructed dose–response profile. Uncertainty and/or variability were not considered for the reported Hill coefficient. This was unfortunate but unavoidable since the Hill coefficient from the published study was reported only as an average value. As a result, the risk curves and confidence limits reported here do not incorporate this source of uncertainty.

2.6. Risk characterization

Risk characterization is the phase of risk assessment where the results of the exposure and quantitative effects assessments are integrated to provide an estimate of risk for the population under study. In this case, it entails combining the exposures, measured as soft tissue concentrations from the various abalone farms, with the quantitative concentration–response relationship between tissue residue and associated mortality determined from the experimental studies.

Risk at a specific Zn concentration in abalone, C_m, can be calculated as the proportion of the abalone expected to have that tissue concentration multiplied by the conditional probability of abalone mortality, given concentration C_m. 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. Graphic display of the JPF also provides a means of assessing how alterations in ambient concentrations due to management efforts or natural attenuation would affect the risk assessment. This can be expressed mathematically as

$$R(C_m) = F(C_m)F(M|C_m), \tag{12}$$

where $R(C_m)$ is the risk at a specific concentration C_m, $F(C_m)$ is the cdf of having tissue concentration C_m, and $F(M|C_m)$ is the conditional cdf of the mortality, given tissue concentration C_m.

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 abalone 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 the given abalone 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 say "under current conditions, x% of abalone will be effected and that this proportion of abalone would be affected by y% of the current observations."

The overall expected risk for abalone may be computed as the sum of the risks for all possible C_ms. Specifically, since the tissue Zn concentrations in abalone is distributed log-normally, and the responses follow Eq. (11), the overall expected risk, $\mathbf{E}[R]$, could be estimated as

$$\boldsymbol{E}[R] = F\left(\bar{C}_m\right)F\left(M|\bar{C}_m\right), \tag{13}$$

where **E**[•] is the expectation operator and $\bar{C}_m$ is the mean concentration of Zn in abalone and can be written as

$$\bar{C}_m = \frac{\int_0^{\infty}\left(\mathrm{LN}_{C_m}\left(\mu_g,\sigma_g\right)\right)C_m dC_m}{\int_0^{\infty}\mathrm{LN}_{C_m}\left(\mu_g,\sigma_g\right)dC_m}, \tag{14}$$

and the cdfs of $F(\bar{C}_m)$ and $F(M|\bar{C}_m)$ can be expressed, respectively, as

$$F\left(\bar{C}_m\right) = \Phi\left(\frac{\ln\bar{C}_m - \ln\mu_g}{\ln\sigma_g}\right), \tag{15}$$

and

$$F\left(M|\bar{C}_m\right) = \Phi\left(\frac{100\times\bar{C}_m^{3.70}}{(\mathrm{BCF}_m\times\mathrm{LC}_{50}(\infty))^{3.70}+\bar{C}_m^{3.70}}\right), \tag{16}$$

where the lognormal probability density function (pdf),$\mathrm{LN}_{C_m}\left(\mu_g,\sigma_g\right)$, can be expressed as

$$\mathrm{LN}_{C_m}\left(\mu_g,\sigma_g\right) = \frac{1}{\sqrt{2\pi}C_m\ln\sigma_g}\exp\left[-\frac{1}{2}\left(\frac{\ln C_m - \ln\mu_g}{\ln\sigma_g}\right)^2\right], \tag{17}$$

where μ_g and σ_g denote the geometric mean and geometric standard deviation of the lognormal distribution exposures, respectively.

A confidence interval for expected risk was determined on the basis of the 2.5th and 97.5th percentiles of the simulation results.

To assess growth endpoint, we use hazard quotient (HQ) to determine the potential growth risk as (USEPA, 2000),

$$\mathrm{HQ} = \mathrm{PEC}/\mathrm{NOEC}, \tag{18}$$

where PEC is the predicted environmental concentration expressed as pond water Zn concentration that inhibiting abalone growth and NOEC is the chronic no-observed effect concentration of toxicant reference value (TRV) expressed by most sensitive chronic endpoint, e.g., 28-day growth. If PEC were equal to a TRV then the HQ would be 1.0. Thus for HQ values greater than 1.0, some potential for inhibiting growth can be inferred. HQ values less than 1.0 indicate that the potential for inhibiting growth is low. Tsai et al. (2003) have conducted a growth toxicity bioassay for *H. diversicolor supertexta* exposed to Zn. A 28-day chronic toxicity study demonstrated that the highest Zn concentration that did not have an inhibiting effect (NOEC) on the abalone growth was 0.0625 μg ml^{-1}.

3. Results and discussion

3.1. Exposure assessment

Fig. 2 illustrates the predicted pdfs of Zn contents in abalone and algae subject to the measured pdfs of pond water Zn concentrations from the three selected abalone farm locations. Probabilistic simulations of the biokinetic–consumer–resource bioaccumulation model produced skewed distributions of predicted Zn concentrations in abalone and algae. Percentile predictions of biomass of abalone and algae associated with Zn contents in abalone and algae could be determined from the cdfs that are derived from the corresponding pdfs shown in Fig. 2. Fig. 3 shows the box plots of interquartile and 50th-percentile predictions associated with whisker plots indicating 10th- and 90th-percentile predictions of Zn contents and biomass of abalone and algae in different abalone farms.

Compared with the field observations, median estimates of Zn in algae and abalone as well as the biomass of abalone and algae were generally less than measured mean values (Fig. 3). Measured algae biomasses are within the predicted interquartile, whereas the model underestimates the abalone biomasses (Fig. 3A). Three of six field observation data sets for each selected abalone farm of Zn in algae and abalone are within the predicted 25th- and 75th-percentile range, and five of them all fall within the 10th- and 90th- percentile range (Fig. 3B). Thus, applying the Monte Carlo technique to the proposed exposure model generated probabilistic estimates of Zn concentrations in algae and abalone as well as biomass that were favorably consistent with field data. Relative to minimum and maximum field data, however, lower and upper probabilistic percentile predictions were more conservative. It is evident that the modeling framework and the distributional parameters and assumptions in the model are appropriate for estimating algae and abalone exposed to waterborne Zn.

3.2. Estimation of concentration–response function

The Hill model and a 5000 Monte Carlo simulation provided an adequate fit for the data (χ^2 goodness-of-fit, $P>0.5$). Based on this concentration-response function (Fig. 4), the calculated median effective concentration (EC_{50}) is 285 μg g^{-1} dry wt. with 95% confidence interval of approximately 214 and 351 μg g^{-1} dry wt. of whole-body abalone on a dry-weight basis. A threshold value can also be estimated on the basis of the fitted dose–response model. The EC_{10} value calculated from the fitted dose–response model is 158 μg g^{-1} dry wt. of whole-body abalone with a 95% confidence interval of 121 and 194 μg g^{-1} dry wt. USEPA (USEPA, 2000) recommended that EC_{10} can be used as a surrogate threshold of regulatory endpoint in probabilistic

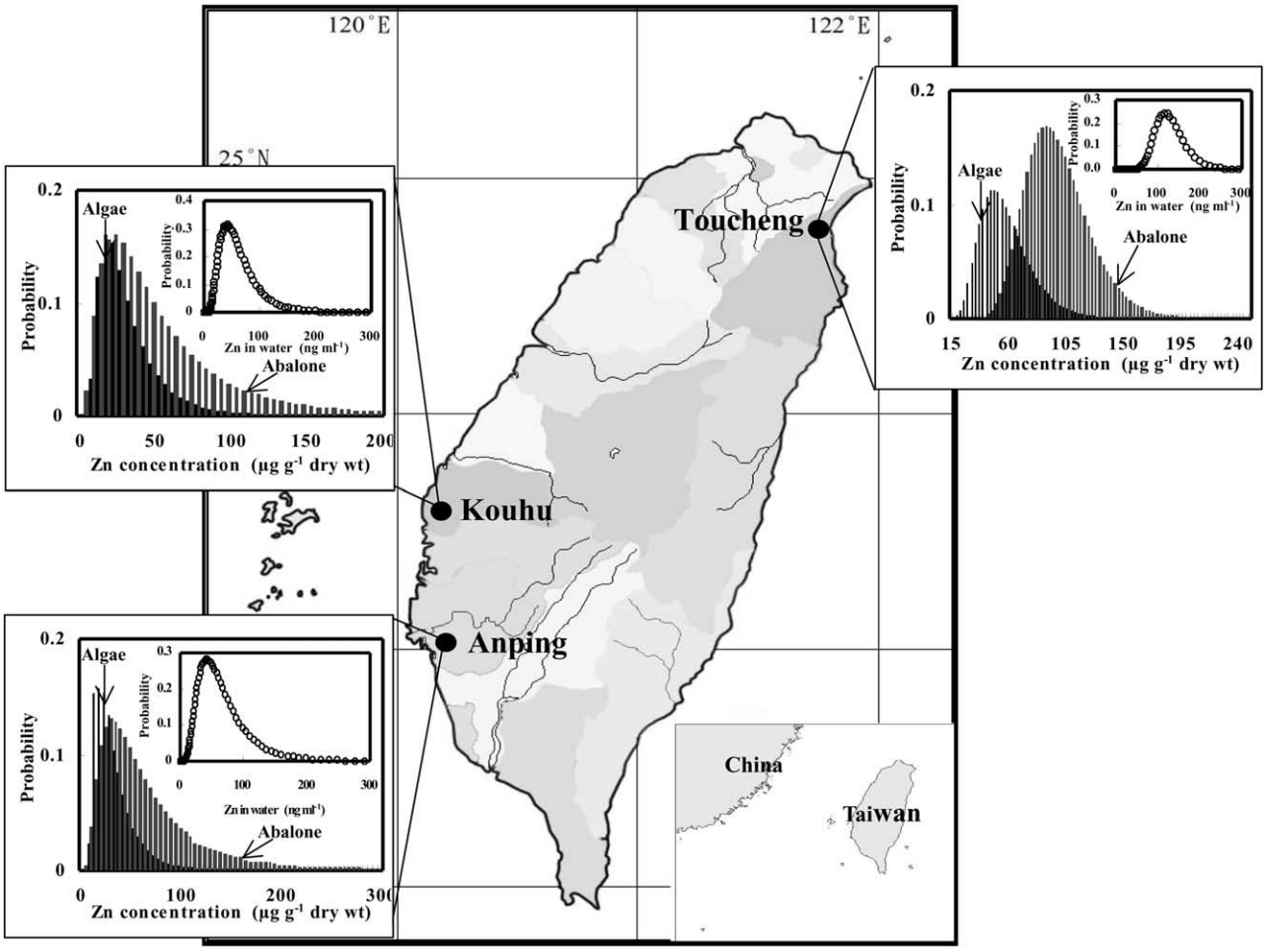

Fig. 2. Overall display of probabilistic distributions of predicted Zn concentrations in abalone *H. diversilor supertexta* and red algae *G. tenuisipitata* var. *lilu* subject to measured water Zn concentration at three selected abalone farm locations.

ecological risk assessment. In view of Fig. 3B, the distributions of Zn concentration in soft tissue of abalone almost entirely below the threshold value of derived EC_{10} (158 μg g^{-1} dry wt.), suggesting that waterborne Zn dose not appear to pose a significant health risk to aquacultural abalone under field conditions at current environmental concentrations.

3.3. Risk estimates

The point estimates of expected exceedence risk of mortality for abalone in three different abalone farm locations are given in Table 2 in that we define risk as the percentage of the abalone that is expected to suffer mortality. The point estimates were calculated from Eq. (13) and ignore the uncertainty associated with estimate of concentration–response parameter of Hill coefficient. Confidence intervals calculated from the 2.5th and 97.5th quantiles of the Monte Carlo simulation results are also shown in Table 2. These ranges represent the uncertainty in expected risk estimates with both the inherent variability and uncertainty in exposure and concentration–response parameter estimates are considered. The confidence intervals for the expected risk are relatively narrow. Table 2 suggests that the relative high risk for abalone farms in Toucheng is alarming since nearly half (46%) of abalone are expected to fail to survive because of their exposure to waterborne Zn.

Risk curves shown in Fig. 5 indicate the estimated probabilistic of effects of differing magnitude for abalone for each selected abalone farm locations. The plotted probabilities, calculated from the outcome of the Monte Carlo simulation followed a JPF shown in Eq. (12) describing the exceedence cdfs (Fig. 5) associated

Table 2
Expected risk of mortality endpoint. Confidence intervals were computed from 2.5th- and 97.5th-percentiles of 5000 Monte Carlo simulations

Study site	Expected risk	95% confidence interval
Toucheng	0.461	0.455–0.478
Kouhu	0.364	0.356–0.388
Anping	0.288	0.286–0.293

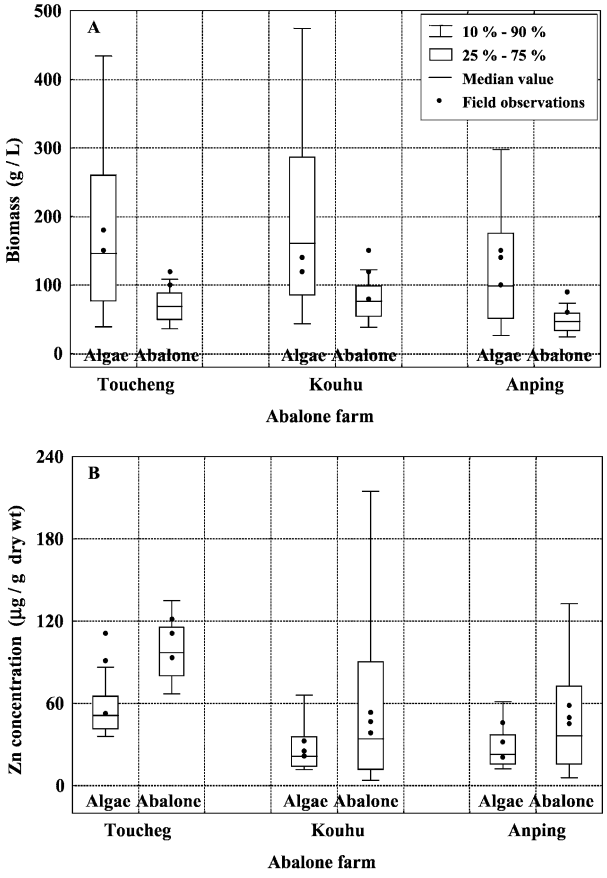

Fig. 3. Box and whisker plot representations of (A) biomass and (B) Zn concentration in abalone *H. diversilor supertexta* and red algae *G. tenuisipitata* var. *lilu* at three selected abalone farm locations. Box and whisker plots are used to represent the uncertainty in biomass and Zn level estimates.

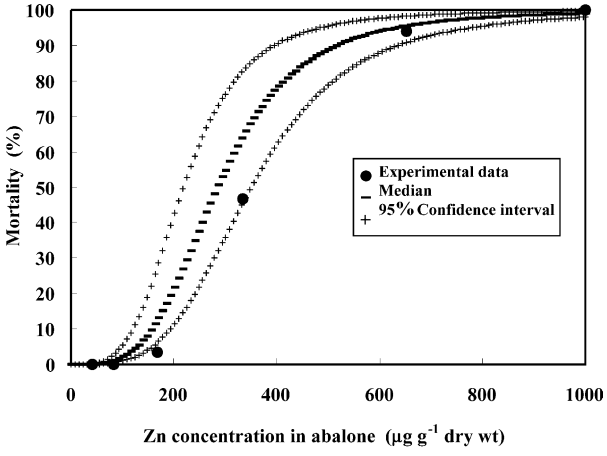

Fig. 4. Reconstructed concentration-response curve with 95% confidence interval for relationship between abalone mortality and Zn concentration in abalone.

with a particular degree of effect (Fig. 4), take into account the uncertainty in estimating risk derived from variability and uncertainty in model parameters. For Toucheng abalone farm, the probability that 25% or more of the abalone is affected (risk = 0.25) is approximately 4%, with 95% confidence interval of 2 and 9%, i.e., the probability is 0.25 that at least 4% of abalone from Toucheng abalone farms will not survive; whereas the probability is 0.5 that at least 2% of abalone will suffer mortality. Furthermore, for Kouhu and Anping abalone farms, the probability is 0.25 that at least 1% mortality for abalone.

Fig. 6 shows that for Kouhu and Anping abalone farms, a 90% probability or less experiencing a HQ less

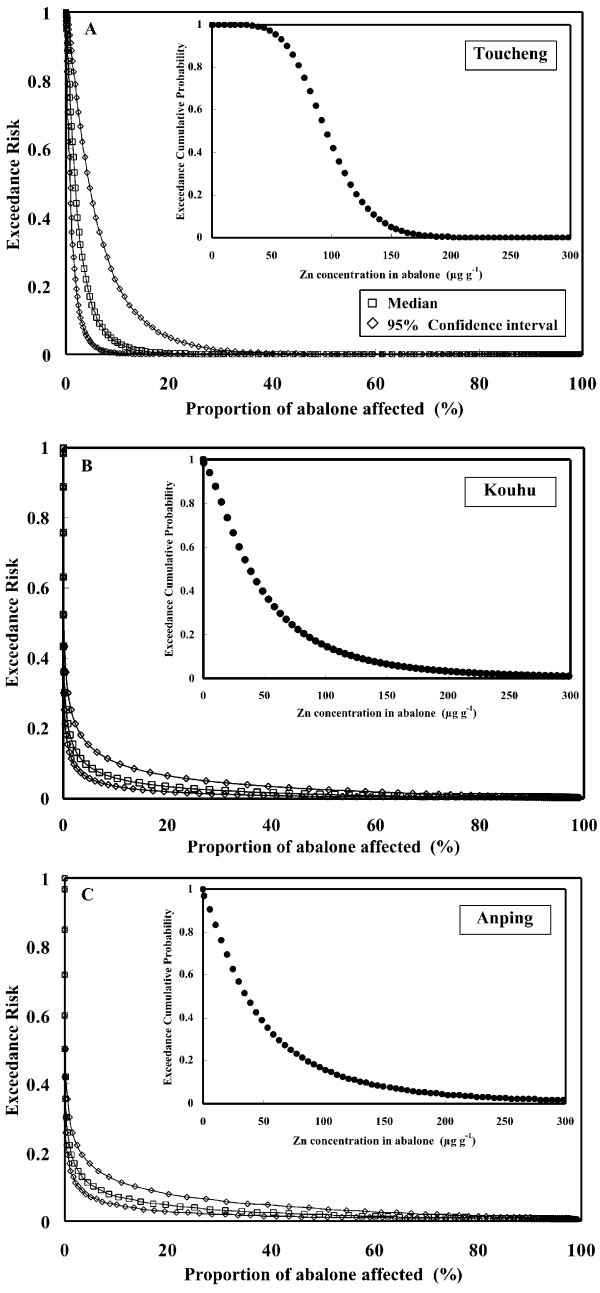

Fig. 5. Exceedence risk (mortality) functions with 95% confidence interval for selected abalone farms at (A) Toucheng, (B) Kouhu, and (C) Anping, respectively. Inset shows the exceedence cumulative dis-

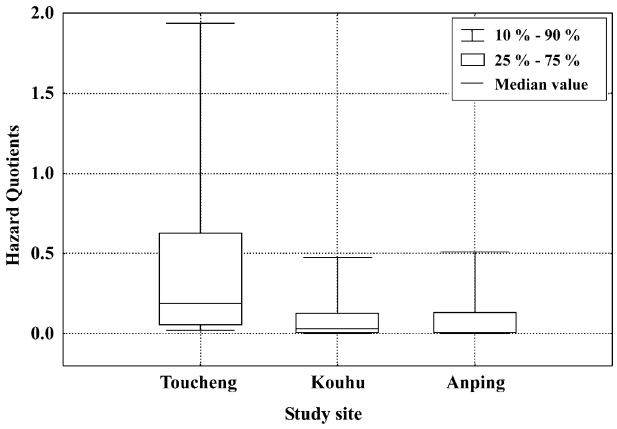

Fig. 6. Box and whisker plot representations of hazard quotients for abalone growth risk estimates.

than 1 for abalone exposed to waterborne Zn, indicating that these probability distributions are acceptable; whereas the 90th-percentile HQ is larger than 1 for Toucheng abalone farms, indicating a conservatism potential for inhibiting abalone growth is inferred.

3.4. Implications

Our analysis indicates no significant risk of survival and growth for abalone in Kouhu and Anping, yet risk of growth particularly pronounced for the Toucheng abalone farms. Our analysis addresses solely the risk associated with Zn exposure, although other metal contaminants were measured in the tissue of abalone (Richardson, 2001; Wang and Ke, 2002). We specifically chose to quantify adverse effects related to Zn exposure because the weight of available data including exposure and toxicological data from experimental studies strongly supports the choice of Zn as our study metal to carry out the risk assessment. Furthermore, if the concentration of some metal contaminants other than Zn is uncharacteristically high for a give abalone population, the possibility of a significantly increased risk unaccounted for by our model should be investigated.

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 input parameters. Variability 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. USEPA (USEPA, 1998) and Voit and Schbauer-Berigan (1998) pointed out that probabilistic analysis does 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.

Presumably, centers of distribution are more realistic than tails and, thus, the analysis emphasizes interquartile predictions (Burmaster and Hull, 1997; Edelmann and Burmaster, 1997). Fortunately, available field data allow validation over the range of model outputs, including the upper percentile predictions. The model provides preliminary estimates of such statistics and this information is valuable because it can be used to guide future analysis and data collection efforts. In fact, through requiring more resources and skills, a Monte Carlo technique carried out was very informative since it revealed the degree of conservatism and took into account the reliability of results. 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.

Risk assessors bring needed expertise to a risk assessment team. Interested parties, e.g., municipal governments, environmental groups, or small-business owners may provide important information to risk assessors. Risk assessors and interested parties describe what they can provide to the risk manager, where problems are likely to occur, and where uncertainty may exist. To give greater emphasis to these interactions, the ecological risk assessment could be modified to include interested parties in the risk planning at the beginning of the process and communicating with interested parties in the risk management following the risk assessment.

The present analysis shows that exposure and concentration–response profiles are important components in evaluating the risk. We have represented a novel approach in that a biokinetic–consumer–resource accumulation model was coupled with dose–response curves to assess abalone survival endpoint. On the other hand, the growth endpoint was assessed by hazard quotients featuring measured water Zn level and no-observed effect concentration. With proper application of risk communication, we can channel this legitimate concern into actions that will result in stricter water quality regulation. The end result of such action will improve the water quality, which will benefit the health of the aquacultural species and the health of the people who eat them.

Our previous published toxicity bioassay data can be used extensively in the emerging field of ecological risk assessment. Three basic categories of factors: exposure, toxicokinetics, and toxicodynamics, interact to determine the responses in bioassays. 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 the substantial progress that environmental toxicology of Zn to abalone has made 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.

4. Conclusions

We present a novel risk assessment technique that integrates predicted soft tissue concentrations of Zn with a reconstructed dose–response relationship and employs a joint probability function incorporating exposure and concentration–response profiles to characterize the survival and growth risks for pond abalone *Haliotis diversicolor supertexta*. Our risk analysis demonstrates that the expected risks of mortality for abalone were estimated as 0.46 (Toucheng), 0.36 (Kouhu), and 0.29 (Anping). The predicted 90th-percentiles of hazard quotient for potential growth risk were estimated as 1.94 (Toucheng), 0.47 (Kouhu), and 0.51 (Anping). Bioaccumulation of Zn seems unlikely to result in toxicity to pond abalone. Using the present risk assessment method, we conclude that waterborne Zn does not appear to pose significant risks of survival and growth to pond abalone in Kouhu and Anping. A relative high growth risk in Toucheng, however, is alarming.

It is our opinion that the incorporation of probabilistic analysis into evaluation of exposure and concentration–response relationship 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 mollusks. Probabilistic risk assessment will substantially reduce the compounded conservatism that is inherent in risk assessment that relies on conservative point value estimates for all biokinetic– consumer–resource–, and toxicological effects-related parameters.

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