Coupling Toxicokinetics and Pharmacodynamics for Predicting Survival of Abalone (*Haliotis diversicolor supertexta*) Exposed to Waterborne Zinc

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Received 1 November 2001; revised 10 July 2002; accepted 16 July 2002

ABSTRACT: We developed a mortality model, by coupling an acute toxicity model and a pharmacodynamic model, to predict survival of abalone (*Haliotis diversicolor supertexta*) exposed to waterborne zinc (Zn). We conducted a laboratory 14-day exposure experiment to obtain biokinetic parameters of depuration rate constant (k_2) and bioconcentration factor (BCF). A one-compartment uptake–depuration model was used to fit the exposure data to estimate BCF and k_2 values. The acute toxicity model was developed based on the receptor theory and was verified with $LC_{50}(t)$ data obtained from a 7-day acute toxicity test. A highly significant correlation ($r^2 = 0.98$) was found between predictions and $LC_{50}(t)$ data for the acute toxicity model, indicating a successful description of 7-day $LC_{50}(t)$ data of Zn in abalone. The predicted time course of lethal body burden of Zn in abalone was compared with measured data, showing that the average percent error was 14.04 ± 3.02%. A refined pharmacodynamic model was expressed as the Hill equation, which in terms of waterborne Zn and $LC_{50}(t)$ data was used to fit observed mortality percentages to determine the Hill coefficient ($r^2 = 0.98$). The proposed mortality model in terms of whole body burden and lethal body burden at site of action was then employed to predict the time-varying mortality of abalone exposed to various Zn concentrations in pond water. Our results demonstrate that 96-h LC_{50} and incipient LC_{50} for *H. diversicolor supertexta* exposed to Zn are 1.1 and 1.05 mg L^{-1}, respectively. Our predictions also demonstrate that equilibrium lethal body burden at site of action is about 198 μg g^{-1}, whereas the mortalities never reach 50% when *H. diversicolor supertexta* exposed to Zn is $\leqslant$ 1 mg L^{-1}.
 Environ Toxicol 17: 478–486, 2002; Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/tox.10082

Keywords: abalone; mortality; pharmacodynamics; toxicity; toxicokinetics; zinc

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 (Hog-

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strand 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., algae ingestion). If waterborne Zn levels are elevated, however, toxicity can occur and have severe effects on the health of abalone, which then become unsalable for human consumption (Hahn, 1989; Conroy et al., 1996; Knauer et al., 1997). Abalone/algae uptake and depuration are the most relevant processes affecting the fate of waterborne Zn in aquacultural ecosystems. Abalone/algae uptake is the first step in the bioaccumulation of waterborne Zn in aquacultural food webs.

Based on toxicological principles, toxic chemicals may elicit their toxicity by a nonspecific/specific reversible/irreversible disturbance of the cell membrane caused by their accumulation within the aquatic organisms (Much, 1996). McCarty and Mackay (1993) suggested that the lethal body burden (or critical body residue) at the cell membrane is well correlated with the whole body concentrations. Moreover, it has been shown that toxic chemicals exhibiting the same mode of action are associated with a specific range of internal body burden. These findings lead to the application of the internal lethal body burden as a surrogate parameter for the risk assessment of chemicals. Mancini (1983), Connolly (1985), Menzel (1987), and Bartell et al. (1988), among others, in modeling environmental fate, bioaccumulation, and toxicity have recognized the need for body burden–effect relationships and advocated a body burden–based approach in environmental toxicity and risk assessment.

Bast (1996) indicated that the adverse effects of metals depend on the site of exposure in that the toxicity of heavy metals is largely due to their reactions with sulfhydryl groups. McCarty and Mackay (1993), Abbas and Hayton (1997), Maxwell et al. (1988), and Gearhart et al. (1990) pointed out that the inhibition of acetylcholinesterase (AChE) in nervous tissue and other target organs is generally considered the critical effect leading to the acute toxicity of many metals. Gearhart et al. (1990) and Abbas and Hayton (1997) further indicated that AChE inhibition and mortality from toxic chemical exposure have been shown to be dependent on both exposure concentration and duration for a variety of aquatic organisms.

Based on toxicological principles, the mechanisms through which the dose at the target site elicits the ultimate adverse response are described by pharmacodynamics and are referred to as the action of the effective dose at the target site. The most useful models to describe the relationship between dose or plasma concentration and pharmacological response can be derived from the law of mass action (Lalonde, 1992). Pharmacological response can be assumed to reflect the combination of inhibited molecules (e.g., AChE) with receptors. Therefore, in that the response is proportional to the concentration of receptors that are occupied at a particular time the receptor (occupancy) theory is a basis for pharmacodynamics. Lalonde (1992) indicated that based on the receptor theory, many pharmacodynamic concepts and principles have their roots in a rather broad range of scientific endeavors. The mathematical relationships are relatively simple and can be derived by application of receptor theory principles that have been widely recognized for several decades (Ariens and Simonis, 1964a, 1964b).

The purpose of this study was to develop a mortality model by coupling an acute toxicity model and a pharmacodynamic model in order to predict survival of abalone (*Haliotis diversicolor supertexta*) exposed to waterborne Zn.

We conducted a laboratory 14-day exposure experiment to obtain two biokinetic parameters: the depuration rate constant (k_2) and the bioconcentration factor (BCF). We used a one-compartment uptake–depuration model to fit the exposure data in order to estimate BCF and k_2 values. We also conducted a laboratory 7-day acute toxicity bioassay to verify the proposed acute toxicity mode. The predicted time course of lethal body burden of Zn in abalone was compared with measured data. A refined pharmacodynamic model expressed as the Hill equation in terms of waterborne Zn and median lethal concentration [$LC_{50}(t)$] data was used to fit the observed mortality percentages to determine the Hill coefficient. Finally, the proposed mortality model in terms of whole body burden and lethal body burden at the site of action was then employed to predict the time-varying mortality of abalone exposed to various Zn concentrations in pond water.

MATERIALS AND METHODS

Uptake/Depuration Experiment

Living abalone (*H. diversicolor supertexta*) were collected from Toucheng, in the north Taiwan region, for the laboratory exposure experiment because this place was the most Zn-contaminated area. All the abalone farms there used seawater from polluted coastal areas. Abalone with a shell length of 4 cm were selected for the experiment. The algae samples selected were mature, whole, and healthy. Two hundred abalone were transferred into 4 aquatic tanks approximately 54 L in volume containing 50 L of artificial seawater. To imitate the environment of the abalone farms, the abalone were held in baskets. Each tank contained 10 baskets. Four abalone per basket were used for analysis. To assure that at least 4 abalone would be alive at the end of the experiment, we put one extra abalone in each basket. Dissolved oxygen was maintained at close to saturation by aeration throughout the experiment. The temperature and salinity were maintained at 25 ± 1.5°C and 35% under constant illumination (Yang and Ting, 1986). The pH value remained fairly constant during the experiment (7.75 ± 0.24). Abalone were acclimated for 2 weeks before they were exposed to Zn.

Bioconcentration and depuration assays of Zn were examined in two replicate tanks. In two tanks Zn ($ZnCl_2$) was added to the seawater. The Zn contamination level was determined by a preliminary test exposing abalone to different Zn concentrations of 0.25, 0.5, 1, 2, 4, and 6 μg mL^{-1}. The tolerance (LT_{50}) of abalone at ≤ 1 μg mL^{-1} Zn was longer than 21 days. Thus, the organisms were exposed to 1 μg mL^{-1} Zn for 7 days. The abalone were reared in the contaminated environment for 7 days of uptake, then transferred to clean seawater and reared for an additional 7 days of depuration. There were two controls for each experiment.

A 500-mL water sample and one basket with 4 individual abalone were collected on days 0, 1, 2, 4, and 7, starting from the day those organisms were exposed to the contaminated seawater and from the day the organisms were transferred to clean seawater. The water samples were fixed with 5 mL of 1 N HNO_3, and the samples of abalone were stored in the dark at -20°C until they were analyzed.

Abalone soft tissue was freeze-dried overnight and then ground into a fine powder in a grinder (Tai-Hsiang S36-89, Taiwan). A 500-mg portion of the powder was digested in 10 mL of 65% concentrated HNO_3 (v/v) overnight at room temperature. The resulting solution was evaporated and redissolved in 0.1 N HCl (Karez et al., 1994).

Acute Toxicity Bioassay

An acute toxicity bioassay was conducted to determine the median lethal concentration (LC_{50}) and internal lethal body burden of Zn in the abalone. Abalone were exposed to Zn concentrations ranging from 0.25 to 7 mg L^{-1} in a 7-day LC_{50} test. For each dose of metal, 10 animals were exposed. The mortality was recorded every hour for the first 12 h and every 6 h thereafter up to 7 days, and dead animals were removed from the test system. If the abalone did not withdraw when the soft tissue was mechanically stimulated, they were considered dead. During the experiments exposure and control waters were sampled daily from randomly determined replicates for pH, DO, and temperature and for analysis of Zn.

The LC_{50} values were determined from maximum likelihood estimates of linear functions relating log Zn concentration to probit transformations of percent mortality (Finney, 1971). The LC_{50}s were determined using mean assayed Zn concentrations and cumulative mortality. Statistical comparisons between LC_{50}s were based on the standard error of the difference. When it became apparent that there were no statistically significant differences in LC_{50}s between bioassay replicates, the replicates were pooled, and a single LC_{50} was calculated for Zn. A split-plot ANOVA design was used to analyze the data from the acute toxicity bioassays. The 95% confidence limits on estimates of LC_{50} were based on fiducial limits (Finney, 1971).

Before fitting the probit regressions, mortality adjustments were made for each replicate and observation period by subtracting the average control mortality from the exposure mortality. The maximum value during successive time periods was imposed in determining the adjusted values.

For determination of internal lethal body burden, dead abalone were removed from the aquaria, rinsed with demineralized water, quickly frozen in liquid nitrogen, and stored at -20°C until they were analyzed.

Chemical and Statistical Analyses

A Perkin Elmer model 5100PC atomic absorption flame spectrophotometer equipped with an HGA-300 graphite furnace atomizer was used to analyze Zn. Analytical quality control was achieved by digesting and analyzing identical amounts of rehydrated (90% H_2O) standard reference materials (DORM-2, NRC-CNRC, Canada). Recovery rates ranged from 95% to 97%, and the levels of detection were 0.5 μg Zn/g of tissue and 5 μg Zn/L of water.

All curve fittings were performed using the nonlinear regression option of the Statistica® software (StatSoft, Tulsa, OK, USA). Statistica® was also used to calculate the coefficient of determination (r^2) and to perform all statistical comparisons.

Acute Toxicity Model

We used the time-integrated concentration (TIC) toxicity model (Liao and Lin, 2001) to determine time-dependent median lethal concentrations [$LC_{50}(t)$],

$$LC_{50}(t) = \frac{A_{UC,a}}{BCF}\left(\frac{k_2}{k_2 t + e^{-k_2 t} - 1}\right) + LC_{50}(\infty), \qquad (1)$$

where BCF (L kg^{-1}) is the steady-state bioconcentration factor, defined as the ratio between the uptake and depuration constants [i.e., k_1 (mL g^{-1} d^{-1}) and k_2 (d^{-1})] or between the whole-body burden of Zn [C_a (μg g^{-1})] and waterborne Zn concentration [C_w (μg mL^{-1})], as BCF = $k_1/k_2 = C_a/C_w$; $LC_{50}(\infty)$ is the incipient LC_{50} value (mg L^{-1}); and $A_{UC,a}$ is the area under the whole-body burden of Zn concentration in the abalone-versus-time curve (μg d g^{-1}).

$A_{UC,a}$ in Eq. (1) can be derived from the solution of a first-order one-compartment uptake-depuration model as,

$$A_{UC,a} \equiv \int_0^t C_a(t)dt = \int_0^t \mathrm{BCF} C_w(1 - e^{-k_2 t})dt,$$

$$= \left(\frac{\mathrm{BCF}}{k_2}\right) C_w(k_2 t + e^{-k_2 t} - 1),$$

$$= \text{constant}, \qquad (2)$$

in that the first-order one-compartment uptake–depuration model is described as $dC_a(t)/dt = k_1C_w - k_2C_a(t)$ and its solution is subject to the initial condition of $C_a(t = 0) = 0$ is $C_a(t) = \text{BCF}C_w(1 - e^{-k_2t})$. With sufficient $\text{LC}_{50}(t)$ data, it is possible to calculate best-fit values of two toxicological parameters [i.e., $A_{UC,a}$ and $\text{LC}_{50}(\infty)$] that appeared in Eq. (1) using a nonlinear regression technique.

The area under the dose concentration–versus–time curve (the so-called area under the curve, or AUC) is commonly applied to estimate the total amount of substance eliminated from the body over a certain time period when the pharmacokinetic model has been applied (Bourne, 1995; de Vries, 1996). The AUC concept introduced there suggests that over a certain time period, the total amount of inhibited molecules in the target tissue equals the amount of toxic compound that has been removed from the target tissue.

The TIC toxicity model in Eq. (1) is based on a direct relationship between adverse effects and the extent of inhibited molecules in the target tissue, that is, mortality is assumed to occur at a fixed percentage of inhibited molecules. The TIC toxicity model assumes that the concentration of inhibited molecules in the target tissue is constant, that is, the percentage of lethal inhibited molecules is related to a critical amount of occupied target sites.

Substitution of C_w in the one-compartment uptake–depuration model by $\text{LC}_{50}(t)$ in Eq. (1) and regarding $C_a(t)$ as the internal lethal body burden at the site of action that cause 50% mortality, $C_{L,50}(t)$ leads to the following expression for $C_{L,50}(t)$ as (Liao and Lin, 2001),

$$C_{L,50}(t) = A_{UC,a}\left(\frac{k_2(1 - e^{-k_2t})}{k_2t + e^{-k_2t} - 1}\right) + \text{BCF}(1 - e^{-k_2t})\text{LC}_{50}(\infty). \quad (3)$$

Eq. (3) shows that the internal lethal body burden in abalone can be expressed as functions of biokinetic parameters k_2 and BCF and toxicological parameters $A_{UC,a}$ and $\text{LC}_{50}(\infty)$.

In view of the one-compartment uptake–depuration model, $\text{LC}_{50}(t)$ can also be predicted from knowledge of the exposure time, depuration rate constant (k_2), and BCF, taking into account $C_{L,50}$ as a constant,

$$\text{LC}_{50}(t) = \frac{C_{L,50}}{\text{BCF}(1 - e^{-k_2t})}. \quad (4)$$

When the exposure time approaches infinity, Eq. (4) gives a relation among $\text{LC}_{50}(\infty)$, $C_{L,50}$, and BCF as: $C_{L,50} = LC_{50}(\infty) \times BCF$. Eq. (4) can be referred to as the whole-body burden (WBB) toxicity model. According to the WBB toxicity model, the $C_{L,50}$ will reach its incipient value when the initial body burden has reached an equilibrium with the external constant aqueous concentration.

Pharmacodynamic Model

In pharmacodynamic modeling, the relationship between dose effect and dose concentration is commonly expressed by the Hill equations or is referred to as the sigmoid $E_{\max}$ model (Lalonde, 1992; Bourne, 1995) as,

$$\text{Effect } (E) = \frac{E_{\max} \times C^n}{\text{EC}_{50}^n + C^n}, \quad (5)$$

where C is the dose concentration in the receptor, $E_{\max}$ is the maximum dose effect, EC_{50} is the concentration that causes an equal effect to 50% of the $E_{\max}$, and n is a slope factor or is referred to as the Hill coefficient. The Hill equation in Eq. (5) is based on the receptor theory (Lalonde, 1992). With sufficient data over a suitable concentration range, it is possible to calculate best-fit values of the three parameters in Eq. (5) by nonlinear regression.

Because the receptor theory is a basis for pharmacodynamics, a pharmacodynamic model that describes the internal lethal body burden of Zn in abalone versus time could be described by Eq. (3), which is also based on the receptor theory. We combine Eqs. (3) and (5) incorporated with the whole-body burden derived from the one-compartment uptake–depuration model to construct a time-varying mortality function. The concept of the pharmacodynamic model in this present research could be restated as follows: there is a direct internal lethal body burden–versus–mortality relationship that can be derived from the Hill equation. Generally, as the internal whole-body burden increases, mortality will increase. Typically, this increase is not an unlimited linear increase but takes on a lazy S shape as the mortality approaches some maximum value.

The mathematical model for representing time-varying mortality, $M(t)$, for abalone in pond water subject to waterborne Zn concentration can be obtained by refining the Hill equation in Eq. (5) as: $E \equiv M(t)$, $C \equiv C_a$ and $\text{EC}_{50} \equiv C_{L,50}(t)$. Thus the time-dependent mortality ($M(t)$) can be redefined as,

$$M(t) = \frac{M_{\max} \times C_a^n}{C_{L,50}^n(t) + C_a^n}. \quad (6)$$

where $M_{\max}$ is abalone maximum mortality of those exposed to waterborne Zn.

We substitute C_a from the solution of the one-compartment uptake–depuration model and $C_{L,50}(t)$ in Eq. (3) into Eq. (6) to rewrite a new form representing the time-varying mortality as functions of the Zn concentration in pond water (C_w) and the toxicokinetic parameters k_2, BCF and $\text{LC}_{50}(\infty)$ as,

TABLE I. Estimated LC_{50} values with 95% confidence limits (c.l.) for selected time intervals and average internal lethal body burden [$C_{L,50}(t)$] values (±*SE*) for *Haliotis diversicolor supertexta* exposed to Zn at different exposure times *t*

Exposure Time, t (h)	$LC_{50}(t)$ (mg L^{-1})	95% c.l.	Average $C_{L,50}(t)$ (μg g^{-1})
24	1.8	1.52–2.08	215 ± 21
48	1.6	1.26–1.94	ND[a]
72	1.2	0.89–1.52	240 ± 16
96	1.1	0.83–1.59	254 ± 11
120			270 ± 8
144			272 ± 3
168	0.9	0.77–1.18	275 ± 5

[a] Not determined.

$$M(t) = \frac{M_{\max} \times [\mathrm{BCF} \times C_w(1 - e^{-k_2 t})]^n}{\left[A_{UC,a}\left(\frac{k_2(1 - e^{-k_2 t})}{k_2 t + e^{-k_2 t} - 1}\right) + \mathrm{BCF}(1 - e^{-k_2 t})\mathrm{LC}_{50}(\infty)\right]^n \cdot + [\mathrm{BCF} \times C_w(1 - e^{-k_2 t})]^n} \quad (7)$$

Based on the acute toxicity test, however, mortality functions were estimated from observed mortality percentages in exposure regimes in which mortality was an increasing function of the Zn concentration in water. Therefore, in fitting the Hill equation to the observed mortality for the specific-interval acute toxicity data, the mortality functions used have to be expressed as the functions of waterborne Zn concentration (C_w) and $LC_{50}(t)$ data as,

$$M(t) = \frac{M_{\max} \times C_w^n}{\mathrm{LC}_{50}^n(t) + C_w^n}. \quad (8)$$

With sufficient data on percent mortality of a suitable Zn concentration in water associated with the specific interval of LC_{50} data, we can estimate best-fit values of the Hill coefficient in Eq. (8) using nonlinear regression.

RESULTS

Data for Biokinetic Parameters $LC_{50}(t)$ and $C_{L,50}(t)$

The 14-day water exposure experiment of Zn in soft tissue of abalone had the nonlinear regression equation resulting from the best fit of the first-order one-compartment uptake–depuration model, $C_a(t) = 111 + 166.02(1 - e^{-0.611t})$ ($r^2 = 0.98$), indicating that the whole-body Zn concentration profile for time was better fit by the one-compartment model. As a result, the biokinetic parameters of the depuration rate constant (k_2) and the uptake rate constant (k_1) with a 95% confidence limit (c.l.) were estimated to be $k_2 = 0.611 \pm 0.43$ d^{-1} and $k_1 = 102.04 \pm 23.2$ mL g^{-1} d^{-1}, respectively, whereas BCF was calculated to be 167 ± 16 L kg^{-1}. The background Zn concentration in abalone can also be calculated to be 111 ± 2.7 μg g^{-1} (mean ± 95% c.l.).

The selected time intervals of 24-h, 48-h, 72-h, and 96-h LC_{50} values with 95% c.l. and average internal lethal body burden ($C_{L,50}$) for *H. diversicolor supertexta* exposed to Zn are given in Table I. Table I shows that the average 96-h LC_{50} is 1.1 mg L^{-1}, whereas the average internal lethal body burdens ranged from 215 to 275 μg g^{-1} for *H. diversicolor supertexta* exposed to Zn during the 7-day acute toxicity bioassay.

Fitting Toxicity Models to $LC_{50}(t)$ Data

The optimal fits of the WBB and TIC toxicity models to the $LC_{50}(t)$ data listed in Table I are presented in Figure 1. The input parameter and estimated values for $LC_{50}(\infty)$ and $A_{UC,a}$/BCF are given in Table II. Table II also lists the statistical data associated with the optimal fits of the WBB and TIC toxicity models. Figure 1 indicates that the TIC toxicity model describes the $LC_{50}(t)$ data in a much more accurate way and corresponds with the observed toxicity. Both coefficients of determination (r^2 value) indicate that the qualitative differences between the fits of the TIC toxicity model ($r^2 = 0.98$) and the WBB toxicity model ($r^2 = 0.94$) are small (Table II and Fig. 1).

The estimated incipient LC_{50} values by the TIC toxicity model (1.17 mg L^{-1}) seem accurate because they are in reasonable agreement with the observed LC_{50} value at t = 7 days of 1.0 (0.77–1.18 95% c.l.) mg L^{-1}. The fit of a

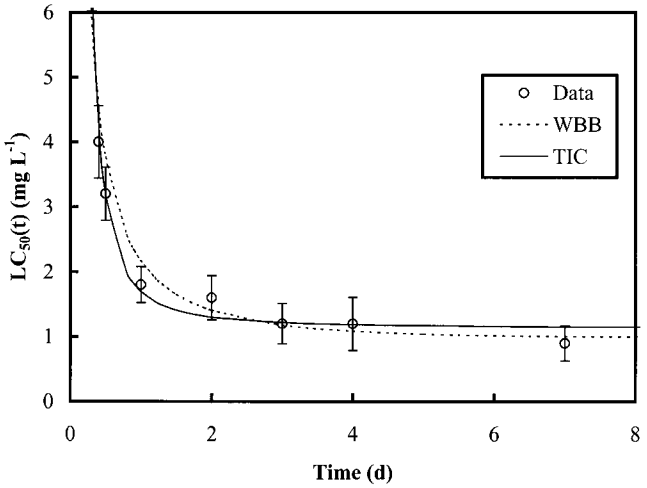

Fig. 1. Optimal fits of the TIC and WBB toxicity models to the $LC_{50}(t)$ data obtained from a 7-day laboratory acute toxicity bioassay for *Haliotis diversicolor supertexta* exposed to Zn. Error bars show 95% confidence limits from the mean.

TABLE II. Input parameters, parameter estimates, and coefficient of determination (r^2) for the whole-body burden (WBB) and time-integrated concentration (TIC) toxicity models fitted to the $LC_{50}(t)$ data listed in Table I

Acute Toxicity Model	Input Parameter k_2 (d^{-1})	Parameter Estimates	r^2
WBB	0.611[a]	$LC_{50}(\infty) = 0.99$ mg L^{-1}	0.942
TIC	0.611[a]	$A_{UC,a}$/BCF = 0.13 (mg d L^{-1})/(L kg^{-1}) $LC_{50}(\infty) = 1.17$ mg L^{-1}	0.981

[a] Determined from 14-day exposure experiment: $k_2 = 0.611 \pm 0.43$.

toxicity model may be strongly determined by the input parameters. Therefore, uncertainties in the k_2 value, which is an input parameter in both the TIC and WBB toxicity models (Table II), affect the validation of the models. Generally, the experimental $LC_{50}(t)$ data for *H. diversicolor supertexta* exposed to waterborne Zn support the validity of the TIC and WBB toxicity models, despite the uncertainties in the input parameter k_2 value.

The statistical information given in Table II suggests that the use of constant whole-body burden for each individual mode of action as an interpretive and regulatory tool in the environmental risk assessment of waterborne metals might be limited to mode of actions.

Prediction of Internal Lethal Body Burden

The comparison of the predicted internal lethal body burden at the site of action that causes 50% mortality ($C_{L,50}$ values ranged from 239.76 to 307.66 $\mu g\ g^{-1}$ for a 7-day exposure) by the TIC toxicity model based on the input parameters (Table III) with observed $C_{L,50}$ concentrations for abalone (Table I) is depicted in Figure 2. Calculations indicate that the TIC toxicity model predicts the $C_{L,50}$ concentrations with averaged relative difference ranging from 11.52% to 19.32% (Fig. 2), where the relative difference or errors was determined as: $error = |(C_{pred} - C_{obs})/C_{obs}| \times 100$, in which C_{obs} is the observation data and C_{pred} is the predicted concentration. The average percent error was found to be 14.04 ± 3.02%, indicating the deviation is well within normal experimental accuracy.

The $C_{L,50}$ concentration predicted by the WBB toxicity model can be calculated based on values listed in Table III, and the resulting value is 276.33 mg kg^{-1}. Although the prediction of the WBB toxicity model may be in reasonable agreement with the $C_{L,50}$ concentrations in some exposure regions, the WBB toxicity model does not explain the time-dependent $C_{L,50}$ values. In addition, according to the TIC toxicity model, mortality occurs at a critical time-integrated concentration of the toxic agent in the target tissue, whereas in the WBB toxicity model the time to death of abalone can be determined by the aqueous exposure concentration of the chemicals, indicating that for a given AUC (i.e., $A_{UC,a}$), different exposure concentrations followed by different times to death are associated with different internal lethal body burdens. Thus, the TIC toxicity model reflects the dependency of $C_{L,50}$ values on both exposure concentration and time.

TABLE III. Input parameters for the internal lethal body burden [$C_{L,50}(t)$] predictions shown in Figure 3

Input Parameter	WBB Toxicity Model	TIC Toxicity Model
BCF (L kg^{-1})	167[a]	167[a]
k_2 (d^{-1})		0.611[b]
$LC_{50}(\infty)$ (mg L^{-1})	0.99	1.17
$A_{UC,a}$ (mg d L^{-1})		21.77

[a] Determined from 14-days exposure experiment: BCF = 167 ± 16.
[b] Determined from 14-days exposure experiment: $k_2 = 0.611 \pm 0.43$.

Fitting the Hill Equation to Observed Mortality

The optimal fits of the modified Hill equation [Eq. (8)] to the observed percent mortality of *H. diversicolor supertexta* versus waterborne Zn concentration of the 24-h acute toxicity test is presented in Figure 3, resulting in the estimated Hill coefficient, $n = 3.70$ ($r^2 = 0.98$, $df = 5$). We employed Eq. (7) to predict the time-varying mortality of *H.*

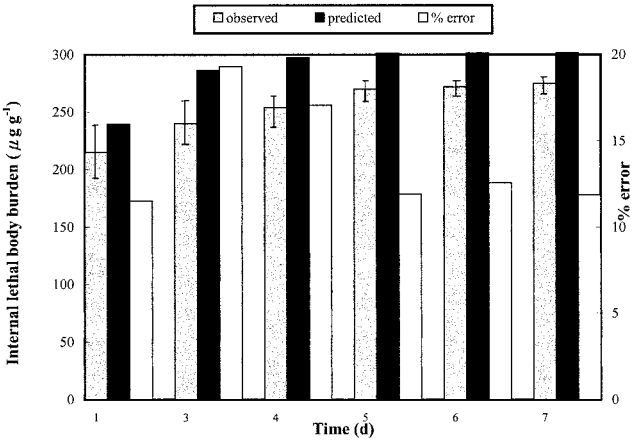

Fig. 2. A comparison between measurements and predictions of internal lethal body burden of Zn in *Haliotis diversicolor supertexta.* The average relative differences (% error) are also shown. Error bars show 1 standard error from the mean.

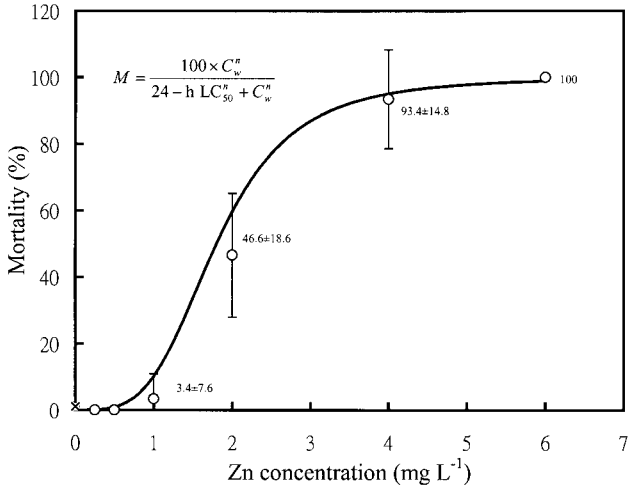

Fig. 3. Optimal fit of sigmoid E_{max} model (i.e., Hill equation) to observed percent mortality of *Haliotis diversicolor supertexta* verse waterborne Zn concentrations in the 24-h toxicity bioassay. Error bars show 1 standard deviation from the mean.

diversicolor supertexta exposed to various Zn concentrations in pond water ranging from 1 to 50 mg L^{-1} with the estimated Hill coefficient [Fig. 4(A)]. The internal lethal body burden at the site of action that caused 50% mortality [$C_{L,50}(t)$] and whole-body burden [$C_a(t)$] are also shown in Figure 4(B) and 4(C).

Figure 4A depicts a clear dose–response relationship between waterborne Zn concentration and mortality, although abalone did not start dying in concentrations that eventually produced 99% mortality until after the first 4 days. Our predictions show that the mortalities never reached 50% when abalone were exposed to waterborne Zn $\leqslant$ 1 mg L^{-1}, whereas after the first day abalone reached 99% mortality when exposed to Zn concentrations of 5, 10, and 50 mg L^{-1}. Internal lethal body burden decreased rapidly during the first week [Fig. 4(B)], and the $C_{L,50}(t)$ decreased by about 70%–80% after the first 2 days. The estimated time-independent internal lethal body burden at the site of action was about 198 μg g^{-1} at $C_a(t = 0) = 0$ [Fig. 4(B)].

The temporal pattern of predicted whole-body burdens in abalone varied with different waterborne Zn concentrations, as shown in Figure 4(C). The equilibrium whole-body burdens for abalone exposed to 1, 2, 3, 5, 10, and 50 mg L^{-1} waterborne Zn were, respectively, 166.6, 333.3, 499.9, 1666.3, and 8331.5 μg g^{-1}.

DISCUSSION

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 fish/shellfish have not been investigated extensively.

Hogstrand et al. (1996) and Galvez et al. (1998) indicated that Zn specifically disrupts calcium uptake by the gills, leading to hypocalcemia, which may end with the death of the fish within a few days, depending on the Zn

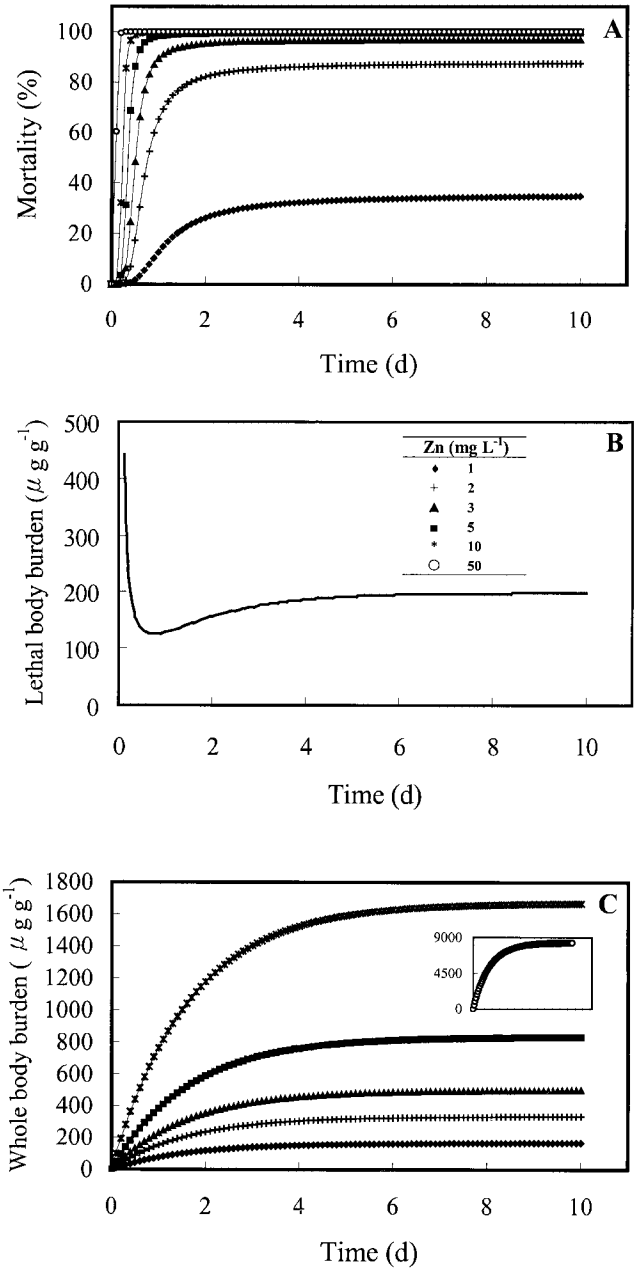

Fig. 4. Predictions of survival of *Haliotis diversicolor supertexta* exposed to waterborne Zn: (A) predicted time-varying mortality of *H. diversicolor supertexta* exposed to waterborne Zn, which ranged from 1 to 50 mg L^{-1}, (B) predicted time-varying lethal body burden of Zn in abalone that causes 50% mortality, and (C) predicted time-varying whole-body burden of abalone exposed to waterborne Zn, which ranged from 1 to 50 mg L^{-1}.

concentration. Some evidence also suggests Zn exposures affect blood morphology and hemostatic mechanisms in freshwater fish by altering the abundance of circulating thrombocytes and small lymphocytes and reducing blood coagulation rates (Seller et al., 1975; Wood and McDonald, 1990). Skidmore (1970) and Wood and McDonald (1990) noted, during a chronic exposure, the precipitation of Zn with mucus secreted by fish and postulated that impaired respiration caused by the physical clogging of the gill filaments resulted in death by asphyxiation. These circulatory and respiratory mechanisms of toxicity seem consistent with the effects of other waterborne metals, such as Al and Co (Jones, 1939; Srivastava and Agrawal, 1979).

Hermens (1989) and McCarty and Mackay (1993) suggested that the concept employed in the WBB toxicity model might not hold for chemicals exhibiting an irreversible adverse effect. A specific model of action, however, could also be complicated and misleading in estimating ecosystem concentrations and comparing these concentrations with LC_{50} data. McCarty and Mackay (1993) also pointed out the constant whole-body burden with respect to time and species for chemicals indicating the same mode of action; thus, the WBB toxicity model differs from the concept of the TIC toxicity model employed. In our study a receptor theory–based pharmacodynamic model represented by a modified Hill equation was used to predict the survival of abalone exposed to waterborne Zn. The proposed mortality function is expressed as a sigmoid function of whole-body burden (in terms of BCF and k_2) and internal lethal body burden at site of action [in terms of BCF, k_2, $LC_{50}(\infty)$, and AUC].

Marr et al. (1998) used a concept similar to the WBB toxicity model to predict the survival of rainbow trout (*Oncorhynchus mykiss*) exposed to Co and Cu. Their results demonstrate the potential for predicting unrealistic mortalities (i.e., $> 100\%$). Our work uses the receptor theory–based TIC toxicity concept to derive the "effective" concentration that produces half the maximum effect attributable to the whole-body burden. Our results strong suggest the applicability of the TIC toxicity concept because they demonstrate that toxicity is indeed dependent on the AUC of body burden in the entire abalone.

More than 30 years ago Wagner (1968) did extensive simulations on the time course of pharmacological response using the sigmoid E_{max} model and simple toxicokinetic models. Based on the sigmoid E_{max} and one-compartment models, Wagner (1968) calculated the AUC as a measure of the total pharmacological response over 24 h and then evaluated how AUC was affected by changing the dosing interval. Therefore, with appropriate toxicokinetic and AUC concept–based pharmacodynamic models, the complete time course of adverse response and duration of effect can be predicted for aquatic biota exposed to any waterborne metals.

Therefore, the estimated AUC value [in our study, $\text{AUC} = A_{UC,a} \equiv \Sigma_0^t C_a(t)dt = \Sigma_0^t \text{BCF}C_w(1 - e^{-k_2 t})dt$] can be linked to the whole-body inhibition percentage in enhancing the ecotoxicological modeling in toxicokinetics, the time course of accumulation of waterborne Zn, and in pharmacodynamics, the time course of the adverse biological response by abalone to accumulated Zn.

Bertine and Goldberg (1972) and Amiard-Triquet et al. (1987) indicated that the levels of Zn in the algae-grazing mollusks *Gibbula umbillicalis* and *Littorina littorea,* are not different from the Zn level in a brown alga, *Fucus serratus,* which is the food species of the mollusks, indicating that Zn in abalone comes from ambient water and not from algae. Therefore, for the aquaculture of abalone, it is a priority to control waterborne Zn concentration in pond water. Our results demonstrate the potential for predicting the survival of abalone exposed to different levels of waterborne Zn.

Our predictions demonstrate that Zn is lethal to *H. diversicolor supertexta* at concentrations > 1 mg L^{-1}, greater than those that normally occur in surface water but within the range of concentrations that may occur at abalone farming sites. Of somewhat more concern, related to potential effects on aquatic biota, is the combined toxicity of waterborne metals. We recommend that future research focus both on mechanisms of Zn toxicity and on a more thorough evaluation of the interactions of Zn with toxic metals commonly found in abalone farming sites (e.g., Al, Cu, Pb, Co, As).

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