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養殖九孔對水中鋅之急性毒及毒物動力行爲

Acute Toxicity and Toxicokinetics of Waterborne Zinc in Pond Abalone *Haliotis Diversicolor Supertexta*

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摘　要

本研究分析養殖九孔之鋅生物累積動力行爲及急性毒以評估鋅之生物濃縮及毒性特性。以 14 天實驗室暴露試驗推估九孔在餵食與非餵食狀況下對鋅之吸收及排除速率常數(即，k_1 及 k_2)，其中亦決定生物濃縮因子(BCF)。一階單區塊模式可成功地推求出毒物動力常數 k_1 及 k_2。結果九孔在非餵食藻類狀況下暴露在 1 mg L^{-1} 鋅濃度之 k_1 及 k_2 值分別爲 102.04 ± 23.2 ml $g^{-1}d^{-1}$ 及 0.611 ± 0.43 d^{-1}。當九孔在餵食狀況下，k_1 及 k_2 值則分別爲 113.84 ± 24.4 g $g^{-1}d^{-1}$ 及 0.636 ± 0.21 d^{-1}。在餵食狀況下，九孔之軟體組織及殼之 BCF 值分別爲 179 ± 15 及 19 ± 4。由急性毒實驗結果得知九孔之 96-h LC_{50} 值爲 1.2 ± 0.4 mg L^{-1}。LC_{50} 值可由已知之暴露時間，k_2 及 BCF 值，藉由一階單區塊模式預測得知。當暴露時間達無限時，推估九孔之起始 LC_{50} 值爲 0.987 mg L^{-1}。

關鍵詞：九孔，急性毒，生物濃縮，毒物動力，鋅。

ABSTRACT

This work analyzed the Zn bioaccumulation kinetics and acute toxicity in the abalone *Haliotis diversicolor supertexta* for assessing bioconcentration and toxicity in an aquacultural system. A 14-d laboratory exposure experiment estimated uptake and depuration rate constants (k_1 and k_2, respectively) of *H. diversicolor supertexta* via nondietary and dietary processes. Bioconcentration factor (BCF) were determined. A simple first-order one-compartment model was successfully fitted the toxicokinetic parameters k_1 and k_2. The resulting values of k_1 and k_2 of *H. diversicolor supertexta* were 102.04±23.2 ml g^{-1} d^{-1} and 0.611±0.43 d^{-1}, respectively, when the abalone were exposed to 1 mg L^{-1} Zn seawater without the presence of algae. When the abalone were fed with the algae, k_1 and k_2 values were estimated to be 113.84±24.4 g g^{-1} d^{-1} and

0.636±0.21 d^{-1}, respectively. BCFs for the soft tissue and shell are 179±15 and 19±4, respectively in the food-exposed condition. The 96-h LC_{50} for abalone was 1.2±0.4 mg L^{-1}. LC_{50} value could be predicted from the acknowledge of the exposure time, k_2, and BCF followed by the one-compartment model. When the exposure time approaches infinity, the incipient LC_{50} value could be estimated. The incipient LC_{50} value for the abalone was estimated to be 0.987 mg L^{-1}.

Keywords: Abalone, Acute toxicity, Bioconcentration, Toxicokinetics, Zinc.

INTRODUCTION

Abalone is a common gastropod mollusc that inhabits the coastal reefs in tropical and subtropical areas (Hahn, 1989). The herbivorous gastropod, *Haliotis diversicolor supertexta*, is the most abundant abalone species in Taiwan. The red alga *Gracilaria tenuistipitata* var. *liui* is the major forage for culturing the abalone *H. diversicolor supertexta*. These two species are commercially important for fisheries and aquaculture in Taiwan (Chen, 1989). *H. diversicolor supertexta* is also appreciated for its delicacy and high market value; the aquaculture of *H. diversi-color supertexta* thus is a promising business in Taiwan (Singhagraiwan and Doi, 1993). However, the coastal regions of Taiwan where the abalone and algae aquaculture facilities are located are subjected to polluted discharges from rivers. Previous investigations indicated that heavy metals such as zinc (Zn) have been detected in many rivers in Taiwan. The widespread dispersal of Zn through anthropogenic activities has also resulted in an increase in Zn residues throughout the environment.

Whatever the source, the contamination of ecosystems by metals can be characterized by various mechanisms of transfer between abiotic and biotic compartments. Metals are available to abalone from both the water and the algae they eat. Largely due to the development of realistic approaches in measuring metal bioavailability, there has recently been increasing interest on metal uptake in abalone from ingested food resources. Heavy metals are toxic at high concentrations and have severe effects on the health of organisms, which then become unsalable for human consumption (Conroy *et al.*, 1996). Our work deals with the biomonitoring of Zn in the aquacultural ecosystem.

Zn can be accumulated by humans via the dietary route of consuming fish and shellfish. Fish and shellfish are major contributors to dietary Zn among seafood consumes. Thus it is important to know the levels of Zn in fish and shellfish when estimating risks from seafood consumption. The objectives of this work were to establish the acute toxicity of Zn and to measure the rates of uptake and depuration of Zn in *H. diversicolor supertexta*. We hope these measurements can give information about the bioavailability of the metals and thus about their potential to be transferred through the food chain.

MATERIALS AND METHODS

Living abalone *H. diversicolor supertexta*, and the alga *G. tenuistipitata* var. *liui* were collected from Toucheng situated in the northern Taiwan region. The abalone was sampled by selecting a shell length of 4 cm. The algae samples were selected by including only adult, whole and healthy individuals. Those organisms were then transferred into aquatic tank of approximately 54 L volume,

containing 50 L of artificial sea water. Dissolved oxygen was maintained at close to saturation by aeration. The temperature and salinity were maintained at 25±1.5°C and 35‰ under constant illumination (Yang and Ting, 1986). The pH values remained fairly constant during the assays (7.75±0.24). Every 5 abalone were trapped in a basket and daily fed with *G. tenuistipitata* var. *liui* to imitate the environment in aquacultural ponds. The abalone and algae were acclimatized for 2 weeks before they were exposed to Zn.

Bioconcentration and depuration assays of Zn were examined in two replicated tanks. In two tanks Zn ($ZnCl_2$) was added to the sea water; in one tank the abalone were fed with algae (water/food-exposed), and in the other tank the abalone were kept without food (water-exposed). 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 mg L^{-1}. The tolerance (LT_{50}) of abalone at ≤ 1 mg L^{-1} Zn was longer than 21 d. Thus, the organisms were exposed to 1 mg L^{-1} Zn for 7 d. The algae and the abalone were reared in the contaminated environment for 7 d uptake, then transferred to clean seawater and reared for an additional 7 d of depuration. There were two controls for each experiment.

Water sample of 500 mL and one basket, with 4 individuals of algae and 4 individuals of abalone, were collected at day 0, 1, 2, 4, and 7, starting from the day that those organisms were exposed to the contaminated sea water and from the day the organisms were transferred to clean sea water. The water samples were fixed with 5 mL 1N HNO_3, and the samples of abalone were stored in the dark at -20°C until they were analyzed. The shucked abalone were freeze-dried overnight, and then grounded to fine powder in a grinder (Tai-Hsiang S36-89, Taiwan). A 500 mg portion of the powders was digested in 10 mL concentrated HNO_3 (65% wt) overnight at room temperature. The resulting solution was evaporated and redissolved in 0.1 N HCl (Karez *et al.*, 1994).

Acute toxicity assays were conducted to determine the median lethal concentration (LC_{50} value) for abalone. Abalone was exposed to Zn concentrations ranging from 0.25 – 7 ppm. For each dose of metal, 10 animals were exposed. The mortality was recorded every 1 h for the first 12 h and every 6 h thereafter up to 7 d, and dead animals were removed from the test system. When the abalone did withdraw while the soft tissue was mechanically stimulated, they were pronounced dead. 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 become 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 uptake kinetics study. During the experiments, exposure and control waters were sampled daily from randomly determined replicates for pH, DO, temperature and for analysis of Zn. The water samples were acidified with 5 mL 1 N HNO_3 and then stored at 4°C in the dark for analysis of Zn.

Bioconcentration is assumed to follow a well-established first-order one-compartment model as $dC_b / dt = k_1 C_w - k_2 C_b$ in that the solution at the constant C_w is $C_b(t) = C_b(t=0) + BCFC_w(1 - e^{-k2t})$ (referred to as UD model), where C_b is the chemical concentration in abalone (μg g^{-1}), C_w is the chemical concentration in water (μg mL^{-1}), k_1 is the

uptake rate constant (ml g^{-1} d^{-1}), k_2 is the depuration rate constant (d^{-1}), and BCF is the equilibrium bioconcentration factor: BCF = $k_1/k_2 = C_b/C_w$. The k_1 and k_2 can be estimated by fitting the UD model to measured Zn concentration data from the 14-d exposure experiments.

LC_{50} can be predicted from the acknowledge of the exposure time, depuration rate constant, BCF followed by the UD model subjected to zero concentration of metals at the site of action: $LC_{50}(t) = (C_{L,50}/BCF)(1-e^{-k_2 t})^{-1}$ (referred to as UDT model), where $C_{L,50}$ is the Zn concentration at the site of action that causes 50% mortality (μg g^{-1}). When the exposure time approaches infinity, the incipient lethal levels (i.e., LC_{50} (∞)) can be determined as: $LC_{50}(\infty) = C_{L,50}$ / BCF. Therefore, incipient lethal levels can be predicted by using nonlinear regression fitting of the UDT model to $LC_{50}(t)$ data with the kinetic input parameter k_2 based on bioconcentration/depuration bioassays and the estimation of $C_{L,50}$ / BCF could be obtained.

Zn analysis was carried out by atomic absorption spectrophotometry using a Perkins Elmer model 5000 atomic absorption flame spectrophotometer equipped with a graphic furnace. All curve fitting were performed using the nonlinear regression option of the Statistica® software (StatSoft, Tulsa, OK, USA). The coefficient of determination (r^2) and statistical analyses (analysis of variance and Student's t test) were also calculated by the Statistica®.

RESULTS AND DISCUSSION

The exposure experiments of Zn by abalone in soft tissue (Fig. 1) have the nonlinear regression equations of the UD model best fits as, for food-exposed: $C(t) = 111+180.40\,(1-e^{-0.636t})$, ($r^2$=0.99) and for water-exposed: $C(t) = 111+166.01\,(1-e^{-0.611t})$, ($r^2$=0.98). Fig. 2 shows best fits of the UD model to the bioconcentration data of Zn by

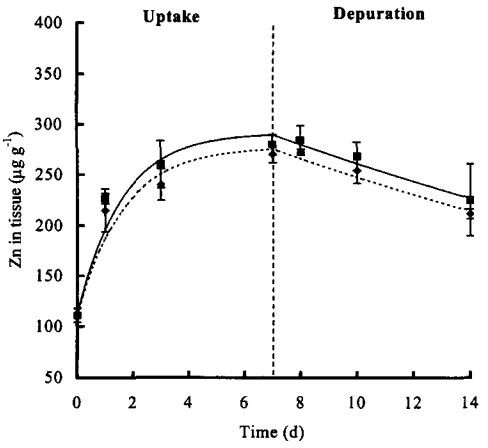

Figure 1. Optimal fit of the UD model to toxicokinetics of Zn in the soft tissue of Haliotis diversicolor supertexta. Measurements (Mean±SE) are shown with symbols (■ : food-exposed, ◆ : water-exposed) and model predictions are shown in line (solid line: food-exposed, dotted line: water exposed).

abalone in shell as, for food-exposed: $C(t) = 14.4+19.17\,(1-e^{-0.325t})$, ($r^2$=0.99) and for water-exposed: $C(t) = 14.4+95.09\,(1-e^{-0.046t})$, ($r^2$=0.99).

Table 1 summarizes the toxicokinetic parameters for *H. diversicolor supertexta* contaminated by Zn from food and water. BCF for soft tissue of abalone fed with algae (179) is higher than that of the abalone kept without algae (167); whereas for shell, BCF of food-exposed (19.17) is lower than that of water-exposed (95.09) (Table 1).

A simple first-order one-compartment model thus was successfully fitted (i.e., significant regression) by the nonlinear technique to the uptake curve of the 14-d exposure tissue Zn concentration data in that coefficients of determination generally were high (r^2>0.95) (Figs. 1 and 2). Results suggest that the fitted first-order equation is an appropriate model for the data set. Estimates of k_2 (Table 1) were determined from the depuration-phase experiments (Figs. 1 and 2) in that all of these regression were significant, with r^2 values that ranged from 0.70 – 0.73. The k_2 values deter-

Table 1. Toxicokinetic parameters (Mean±SE) for the UD model describing Zn bioconcentration/depuration process in *Haliotis diversicolor supertexta* contaminated from food and water.

	k_1[a]	k_2 (d^{-1})	BCF
Soft tissue			
Food-exposed	113.84±24.4	0.636±0.209	179±15
Water-exposed	102.04±23.2	0.611±0.432	167±16
Shell			
Food-exposed	6.23±24.4	0.325±0.209	19.17±4
Water-exposed	4.37±23.2	0.046±0.432	95.09±6

[a]Units for k_1: food-exposed: g g^{-1} d^{-1}; water-exposed: ml g^{-1} d^{-1}.

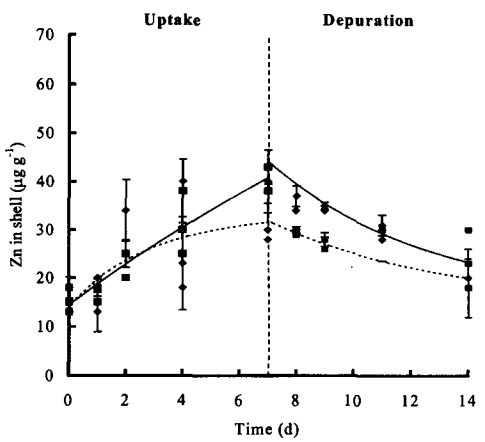

Figure 2. Optimal fit of the UD model to toxicokinetics of Zn in the shell of *Haliotis diversicolor supertexta*. Measurements (Mean±SE) are shown with symbols (■ : food-exposed, ◆ : water-exposed) and model predictions are shown in line (solid line: food-exposed, dotted line: water exposed).

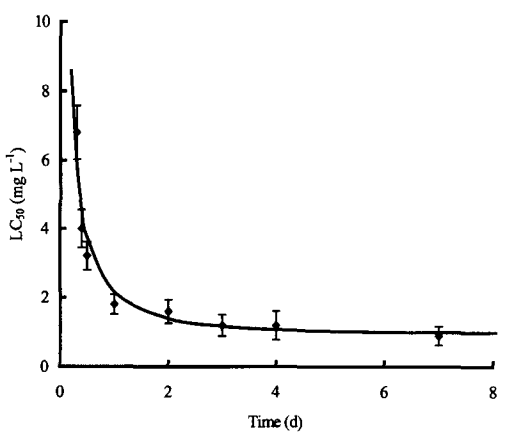

Figure 3. Optimal fit of the UDT model (solid line) to $LC_{50}(t)$ data of Zn in *Haliotis diversicolor supertexta* presented as Mean±SE.

mined in depuration experiments were also statistically significant from their corresponding k_2 values derived from curve fitting the first-order one-compartment model to the uptake phase.

Analysis of variance revealed that k_1s of abalone fed with algae were not different from those of the abalone kept without algae (F=0.0078, P>0.05 for soft tissue; F=0.012, P>0.05 for shell), indicating uptake of Zn from food by the abalone is unimportant compared with uptake from water. Variance analysis of Zn in soft tissue and shell shows that Zn in shell is less than that in soft tissue (r^2=0.69, F=30.12, P<0.05). Zn in shell was proportional to that in tissue in that the ratio of Zn in tissue to that in shell was 10:1 on a dry weight basis. In addition, Zn in abalone fed with algae were not significantly different from those in the abalone kept without algae (F=1.90, P>0.05 for soft tissue; F=0.45, P>0.05 for shell), indicating the effect of starvation can be neglected during the experiments.

The selected time intervals of 24-h, 48-h, 72-h, and 96-h LC_{50} values for *H. diversicolor supertexta* exposed to Zn are shown in Table 2. Fig. 3 shows the optimal fit of the UDT model to the $LC_{50}(t)$ data (r^2 = 0.92) in that the estimated value of $LC_{50}(\infty)$ is

Table 2. LC_{50} values (Mean±SE) for selected time intervals for *Haliotis diversicolor supertexta* exposed to Zn

Time (h)	LC_{50} (mg L^{-1})
24	1.8 ± 0.28
48	1.6 ± 0.34
72	1.2 ± 0.31
96	1.2 ± 0.41

0.987 mg L^{-1}. The estimated incipient LC_{50} value by the UDT model seem accurate since it is reasonably in agreement with the observed LC_{50} value at t = 21 d for Zn in *H. diversicolor supertexta*. The fit of a model might be strongly determined by the input parameters. Thus, uncertainties in the k_2 value, which is an input parameter in the UDT model, were affect the validation of the model. The experimental $LC_{50}(t)$ data for *H. diversicolor supertexta* exposed to Zn support the validity of the UDT model, despite the uncertainties in the input parameter k_2.

Abalone in the tank without algae absorbed the same quantity of Zn as abalone in the tank with algae (Fig. 1). From this finding we conclude that Zn in the abalone comes from the ambient water and not from the algae. A similar phenomenon was reported by Amiard-Triquet *et al.* (1987) where they demonstrated that the levels of Zn in algae-grazing molluscs, *Gibbula umbillicalis* and *Littorina littorea*, are not different from Zn level in a brown alga, *Fucus serratus*, which is the food species of the molluscs. Consequently, concerning the aquaculture of abalone, it is important to control Zn concentration in the ambient water first.

This study shows that the shell of *H. diversicolor supertexta* accumulated Zn and reflected the composition of the seawater where the organisms lived. Although Zn in the shell was less than that in the soft tissue, the shell is still useful as an indicator. The amount of Zn in the shell was proportional to that in the soft tissue. Data obtained from Bertine and Goldberg (1972) and Walsh *et al.* (1995) also demonstrated that metals were usually higher in the soft parts than that in the solid shell of clams, mussels and shrimps. The presence of contaminants in the shell of *H. diversicolor supertexta* may represent an effective and sensitive means to assess contamination in the soft tissue of this organism and to monitor the aquatic ecosystem. The shell could also act as a toxic waste dump to remove toxic chemicals from the metabolically active tissue and thus effectively eliminate these chemicals from the food chain (Walsh *et al.*, 1994). The relocation of the contaminants to the shell represents an effective detoxification mechanism.

In summary, this study has shown that *H. diversicolor supertexta* was able to accumulate and depurate Zn. We also determined LC_{50} value for Zn. These observations mean that *H. diversicolor supertexta* is suitable for monitoring Zn pollution. Extrapolating the rates of metal uptake and depuration under laboratory to the natural environment, however, is difficult because the metal concentrations in the ecosystms dependent on several biotic factors (e.g., physiology, size, sex, and age of the organisms) and abiotic factors (e.g., pH, other ions in solution, organic matter, etc.) (Elder and Collins, 1991). The combination of measurement of metal concentrations in organisms, the toxicokinetics of uptake and excretion of metals, and the effects of metals on the organisms would be a powerful means of monitoring the impacts of metals on the biota.

Information on the toxicokinetics and acute toxicity of metals in mollusc feeding should be important for the development of realistic bioaccumulation models that provide predictive tools for diagnosing processes most critical in metal accumulation and for delineating metal exposure pathway in the animals.

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