

Toxicokinetics and Acute Toxicity of Waterborne Zinc in Abalone (*Haliotis diversicolor supertexta* Lischke)

C.-M. Liao,¹ M.-C. Lin²

¹ Department of Agricultural Engineering, National Taiwan University, Taipei, Taiwan 106, Republic of China

² Institute of Zoology, Academia Sinica, Taipei, Taiwan 11529, Republic of China

Received: 30 July 2000/Accepted: 12 February 2001

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. diversicolor 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 unfit for human consumption (Conroy *et al.*, 1996). Our work deals with the biomonitoring of Zn in the aquacultural ecosystem. 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 an 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 $25\pm1.5^{\circ}\text{C}$ and 35‰ under constant illumination (Yang and Ting, 1986). Five abalone were held in a basket and fed daily with *G. tenuistipitata* var. *liui* to imitate the environment in aquacultural ponds. The abalone and algae were acclimated for 2 weeks before they were exposed to Zn.

Bioconcentration and depuration assays of Zn were examined in two replicate tanks. The abalone in one of two tanks were fed with algae (water/food-exposed), and the abalone in the other tank were kept without food (water-exposed) during the experimental exposure period. 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 $\mu\text{g mL}^{-1}$. The tolerance (LT_{50}) of abalone at $\leq 1 \mu\text{g mL}^{-1}$ Zn was longer than 21 d. Thus, the organisms were exposed to 1 $\mu\text{g mL}^{-1}$ Zn for 7 d. The algae and the abalone were reared in the contaminated environment for 7 d uptake, then transferred to clean sea water and reared for an additional 7 d of depuration. There were two controls for each experiment.

A 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 soft tissue of abalone were 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 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 were exposed to Zn concentrations ranging from 0.25 – 7 mg L^{-1} . 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 when the soft tissue was mechanically stimulated, they were considered 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 LC₅₀s were based on the standard error of the difference. When it became apparent that there were no statically significant differences in LC₅₀s between bioassay replicates, the replicates were pooled and a single LC₅₀ was calculated for Zn. A split-plot ANOVA design was used to analyze the data from the acute toxicity bioassays. 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 HNO₃ 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, $dC_b/dt = k_1C_w - k_2C_b$ in that the solution at the constant C_w is, $C_b(t) = C_b(t=0) + BCF C_w (1 - e^{-k_2t})$ (referred to as UD model), where C_b is the chemical concentration in abalone ($\mu\text{g g}^{-1}$), C_w is the chemical concentration in water ($\mu\text{g mL}^{-1}$), k_1 is the uptake rate constant ($\text{mL g}^{-1} \text{d}^{-1}$), 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.

Zn analysis was carried out by atomic absorption spectrophotometry using a Perkin Elmer model 5000 atomic absorption flame spectrophotometer equipped with a graphic furnace. All curve fittings 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 Statistica®.

RESULTS AND DISCUSSION

The exposure experiments of Zn in soft tissue of abalone (Fig. 1) have the nonlinear regression equations resulting from the best fits of the UD model as for food-exposed: $C(t) = 111 + 180.40 (1 - e^{-0.636t})$ ($r^2=0.99$), and for water-exposed: $C(t) = 111 + 166.01 (1 - e^{-0.611t})$ ($r^2=0.98$). Table 1 summarizes the toxicokinetic parameters for *H. diversicolor supertexta* contaminated by Zn from food and water. BCF for the abalone fed with algae is 179 ± 15 ; whereas for the abalone kept without algae, BCF is 167 ± 16 .

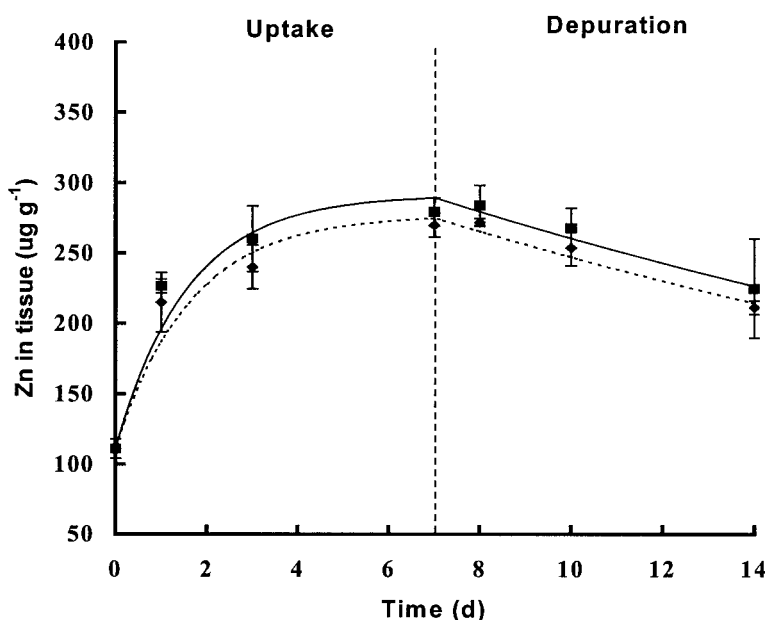


Figure 1. Optimal fit of the UD model to toxicokinetics of Zn in the soft tissue of *Haliotis diversicolor supertexta*. Measurements (Mean±SE) are shown with symbols (■: food-exposed, ◆: water-exposed) and model is shown with lines (solid line: food-exposed, dotted line: water exposed).

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 (ml g ⁻¹ d ⁻¹)	k_2 (d ⁻¹)	BCF
Food-exposed	113.84±24.4	0.636±0.209	179±15
Water-exposed	102.04±23.2	0.611±0.432	167±16

Thus, a simple first-order one-compartment model was successfully fitted (i.e., significant regression) by the nonlinear technique to the uptake phase of the 14-d exposure Zn concentration data in that coefficients of determination generally were high ($r^2 > 0.95$) (Fig. 1). Results suggest that the fitted first-order equation is an appropriate model for the data set. Estimates of k_2 were also determined from the depuration-phase experiments (Fig. 1) in that all of these regression were significant, with r^2 values that ranged from 0.70 – 0.73. The k_2 values determined in depuration experiments were also statistically significant from their corresponding k_2 values derived from nonlinear regression fitting the UD

model to the uptake phase.

Analysis of variance revealed that the k_1 s of abalone fed with algae were not different from those of the abalone kept without algae ($F=0.0078$, $P>0.05$), indicating uptake of Zn from food by the abalone is unimportant compared with uptake from water. 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 umbilicalis* and *Littorina littorea*, were not different from Zn levels in a brown alga, *Fucus serratus*, which is the food species of the molluscs. Thus, controlling Zn concentration in the ambient water is one of the most important strategies with respect to the aquaculture of abalone. The selected time intervals of 24-h, 48-h, 72-h and 96-h LC₅₀ values for *H. diversicolor supertexta* exposed to Zn are given in Table 2.

Table 2. LC₅₀ 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

In summary, this study has shown that *H. diversicolor supertexta* was able to accumulate and depurate Zn. We also determined LC₅₀ values 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 conditions compared with the natural environment, however, is difficult because the metal concentrations in the ecosystem is dependent upon 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 pathways in the animals.

Acknowledgments. We thank Dr. Han-Chuan Chiang, Mr. Jein-Wen Chen and Mr. Hong-Ming Liang for deploying experimental equipment and providing technical assistance, and Miss Yu-Min Yeh for her help with statistical analysis. This study was supported by the National Science Council of Republic of China under Grant NSC 88-2313-B-002-070.

REFERENCES

- Amiard-Triquet TC, Metayer C, Amiard JC, Berthet B (1987) In situ and experimental studies of the ecotoxicology of four metals (cadmium, lead, copper, zinc) on algae and grazing gastropod molluscs. *Water Air Soil Pollut* 34:11-30
- Chen HC (1989) Farming the small abalone, *Haliotis diversicolor supertexta*, in Taiwan. In: Hahn KO (ed), *Handbook of Culture of Abalone and Other Marine Gastropods*. CRC Press, FL, USA, pp 143-159
- Conroy PT, Hunt JW, Anderson, BS (1996) Validation of a short-term toxicity test endpoint by comparison with longer-term effects on larval red abalone *Haliotis rufescens*. *Environ Toxicol Chem* 15:1245-1250
- Elder JF, Collins JJ (1991) Freshwater molluscs as indicators of bioavailability and toxicity of metals in surface-water systems. *Rev Environ Contam Toxicol* 122:37-79
- Finney DJ (1971) *Probit analysis*. Cambridge University Press, Cambridge, MA
- Hahn KO (1989) Biotic and abiotic factors affecting the culture of abalone. In: Hahn KO (ed), *Handbook of Culture of Abalone and Other Marine Gastropods*. CRC Press, FL, USA, pp 113-183
- Karez CS, Magalhaes VF, Pfeiffer, WC, Amdo Filho GM (1994) Trace metal accumulation by algae in Sepetiba Bay, Brazil. *Environ Poll* 83:351-356
- Singhagraiwan T, Doi M (1993) Seed production and culture of a tropical abalone, *Haliotis asinina* Linne. The Eastern Marine Fisheries Development Center. Thailand, 31 pp
- Yang HS, Ting YY (1986) Artificial propagation and culture of abalone (*Haliotis diversicolor supertexta* Lischke). *Bull Taiwan Fish Res Inst* 40:195-201