Bull. Environ. Contam. Toxicol. (2004) 72:70–77

DOI: 10.1007/s00128-003-0242-7

Environmental Contamination and Toxicology

Growth Toxicity Bioassays of Abalone *Haliotis diversicolor supertexta* Exposed to Waterborne Zinc

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Received: 2 April 2003/Accepted: 11 September 2003

Abalone is common gastropod mollusk that inhabits the coastal reef in tropical and subtropical areas. The herbivorous gastropod, *Haliotis diversicolor supertexta*, is the most abundant species in Taiwan. The aquaculture of *H. diversicolor supertexta* is one of the most important aquatic products in Taiwan (Chen, 1989). *H. diversicolor supertexta* is 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 are 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 levels in the tissues of gastropod mollusks (Lin and Liao, 1999; Wang and Ke, 2002). Zinc is available to abalone from both the dissolved phase (e.g., gill uptake) and the diet (e.g., algae ingestion). Heavy metal pollution affects the abalone mainly via the ambient water exposure route (Lin and Liao, 1999). If waterborne Zn levels are elevated, toxicity can occur and has severe effects on the health of abalone, which then become unfit for human consumption (Conroy et al., 1996). Previous investigations indicated that Zn has been detected in many rivers in that maximum Zn concentrations in aquaculture waters are reported to range from 60 to 300 μg L^{-1} in different areas of Taiwan (Liao et al., 2002). Our research deals with the bioassay of Zn in the aquacultural ecosystem. The objective of this work was to establish the chronic toxicity of Zn followed by a standardized growth toxicity test (Gomot, 2000) and to determine the growth rates, the no-observed effect concentration (NOEC), the lowest-observed effect concentration (LOEC) and the median effected concentration (EC_{50}) of abalone after 28 d chronic exposure. These data could be further used for environmental risk assessment and establishing aquacultural water quality criteria.

MATERIALS AND METHODS

A total numbers of 240 abalone *H. diversicolor supertexta* with an average shell

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length of 34±1.69 mm were collected from Toucheng, the most important farming areas for the production of abalone on the north coast of Taiwan, on August 4, 2002, for the chronic toxicity test. The abalone were transported in an aerated, iced cooler to the laboratory. Upon arrival, abalone were transferred into six glass aquariums of 54 L volume, which all filled with 50 L artificial seawater. Because we can't make sure whether the available natural seawater is polluted or contaminated, we prepared the artificial seawater to be the exposure media by adding commercially available artificial sea salt (Waterlife, UK) into distilled water and adjusted it to close to the natural seawater condition. The artificial sea salt is for general aquarium use. The temperature and salinity were maintained at 25±1.5°C and 35‰, respectively, under constant illumination (Yang and Ting, 1986). Photoperiod was set to 12:12 light: dark to mimic the natural photoperiod and dissolved oxygen (DO) was maintained at close to saturation by aeration. In order to imitate the environment of the abalone farms, six abalone were held in each basket. Each tank contained 2 baskets. The abalone were acclimatized to the laboratory environmental condition for 2 weeks before initiation of the chronic toxicity test. To eliminate the diet route, abalone were only fed at night with fresh red alga *Gracilaria. tuenuistipitata* var. *liui* that were also taken from the same aquacultural farm in which the algae remained in each aquaria tank and be discarded in the next morning to avoid being a contaminated source of abalone.

The Zn contamination level was determined by a preliminary test of exposing abalone to different Zn concentrations of 0.25, 0.5, 1, 2, 4, and 6 mg L^{-1}. The median lethal tolerance (LT_{50}) of abalone at $\leq$ 1 μg mL^{-1} Zn was longer than 21 d. Thus, nominal Zn concentrations for the chronic test were 1, 0.5, 0.125, 0.0625, 0.03125 and 0 μg mL^{-1}. Chemical stock solutions were prepared by dissolving a calculated amount of reagent-grade $ZnCl_2$ in deionized water. New stock solutions were prepared as needed during the chronic toxicity tests. All the chronic tests were repeated 3 times and each concentration was assigned to two replicate tanks for 28 d. The aim of this chronic test was to determine the toxic effects on the abalone growth response that includes growth rate, the mortality, the NOEC, the LOEC, and the EC_{50} that exposed in different Zn concentrations described above. In order to maintain the ideal experimental condition, we removed the faeces every 6 h and collected forage debris 3 h after feeding. We also replaced 20 – 30% Zn solution every 1 – 2 d to avoid regression of ambient water quality. The whole Zn solution was replaced weekly in each tank. The seawater change rate is similar to the real abalone farm. For each dose of Zn, 12 abalone were exposed. The test lasted 28 d. Mortality was monitored at 0, 6 and 12 h through the first day of exposure, then twice daily to the end of the test. Dead abalone will be removed and recorded at each observation.

Every week, the mean shell length of each exposed groups was calculated and compared with that of the controls. Also, every week, for each Zn concentration, we established growth coefficient (mean shell length after 1, 2, 3, and 4 weeks × 100 / mean shell length at the start of the experiment) (Gomot, 2000) of abalone with respect to the initial shell length before exposure to Zn. The values of the growth coefficient for each concentration were plotted with arithmetic coordinates

then with semi-log coordinates with corresponding regression equations. The curves obtained give an estimation of the median inhibiting concentrations (EC_{50}). The shell lengths of the abalone from the different groups were expressed as means ± standard deviation. Growth rates are calculated by fitting abalone shell length data obtained from chronic test to an exponential model (ln shell length $(L) = a + gt$, where a is a constant, g is the growth rate (d^{-1}), and t is the time in d). A standard analysis of variance test (ANOVA: Scheffe' F test) was employed to determine the significance of differences between the means of the different groups. A level of significance of $p \leq 0.05$ was used in all tests. All curve fitting were performed using 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®.

In order to detect Zn concentrations in each test media, exposure water characteristics during the chronic test were measured 3 times weekly in one selected replicated aquarium for each nominal concentration for analysis of Zn. we checked the water level in each aquarium every 6 h, if the water level dropped drastically, we supplied with distilled water to the constant level. The exposure waters were sampled from randomly determined replicates for pH, DO, and temperature once a week and for the analysis of Zn content in every two days. The 10 mL water samples were acidified (pH < 1) with 5 mL 1 N HNO_3 and then stored at -4°C in the dark until they were analyzed. Zn analysis was carried out by using a Perkin Elmer model 5000 atomic absorption flame spectrophotometer equipped with a graphite furnace. The detection limits are 5 μg Zn/L water. Calibration standards and a reagent blank were analyzed with every 10 samples. The calibration curve and the method detection limit for the instrument, as well as the control chart for Zn through UV irradiation of reference material sample (Nass-3, Canadian General Standard Board), were compared and found to be in close agreement (± 5%).

RESULTS AND DISCUSSION

The dose-response relationship of control and treated *H. diversicolor supertexta* populations are shown in Table 1. No mortality was observed at Zn concentrations ≤ 0.25 μg mL^{-1}, whereas one abalone from 0.5 μg mL^{-1} treated population died on day 27 of the experiment. Analysis of variance indicated that abalone population growth rates were significantly ($p < 0.05$) reduced when the Zn concentrations rise above 0.125 μg mL^{-1} (Table 1 and Fig. 1).

The highest Zn concentration that did not have an inhibiting effect (NOEC) on the growth was 0.0625 μg mL^{-1}. All the other concentrations (0.125 – 1.0 μg mL^{-1}) affected the growth. The lowest concentration that slowed growth (LOEC) was 0.125 μg mL^{-1}. We used growth coefficient curves (Fig. 2) incorporated with semi – log plots (e.g., Fig. 3) to establish the regression equations for the four weeks, from which we can calculate EC_{50} values (Table 2). Fig. 2 indicates that at nominal Zn concentrations between 0.125 – 0.5 μg mL^{-1}, a clear reduction was found in the growth.

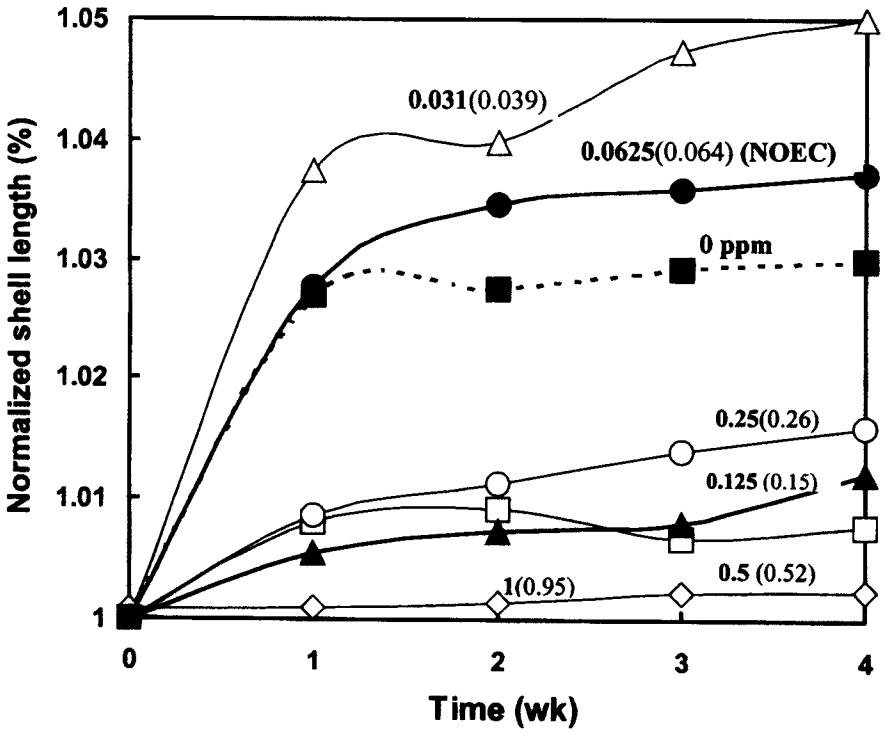

Figure 1. Growth curves of abalone *H. diversicolor supertexta* exposed to different Zn concentrations during 4 weeks test. The measured precise concentrations are shown in parentheses.

Table 1. Growth parameters (mean ± SE) for abalone *H. diversicolor supertexta* exposed to Zn.

Treatment ($\mu g\ mL^{-1}$)	Growth rate ($10^{-3}\ d^{-1}$)	r^2	Mortality (%)
Control	0.865 ± 0.261	0.59	0
0.03125 (0.039±0.008) [a]	1.50 ± 0.33	0.74	0
0.0625 (0.064±0.008)	1.14 ± 0.27	0.70	0
0.125 (0.15±0.013)	0.38 ± 0.04	0.92	0
0.25 (0.26±0.051)	0.53 ± 0.065	0.90	0
0.5 (0.52±0.048)	0.09 ± 0.01	0.96	10
1 (0.95±0.04)	0.20 ± 0.094	0.84	40

[a]The values in parentheses are the measured Zn concentrations (mean ± SD, n=4).

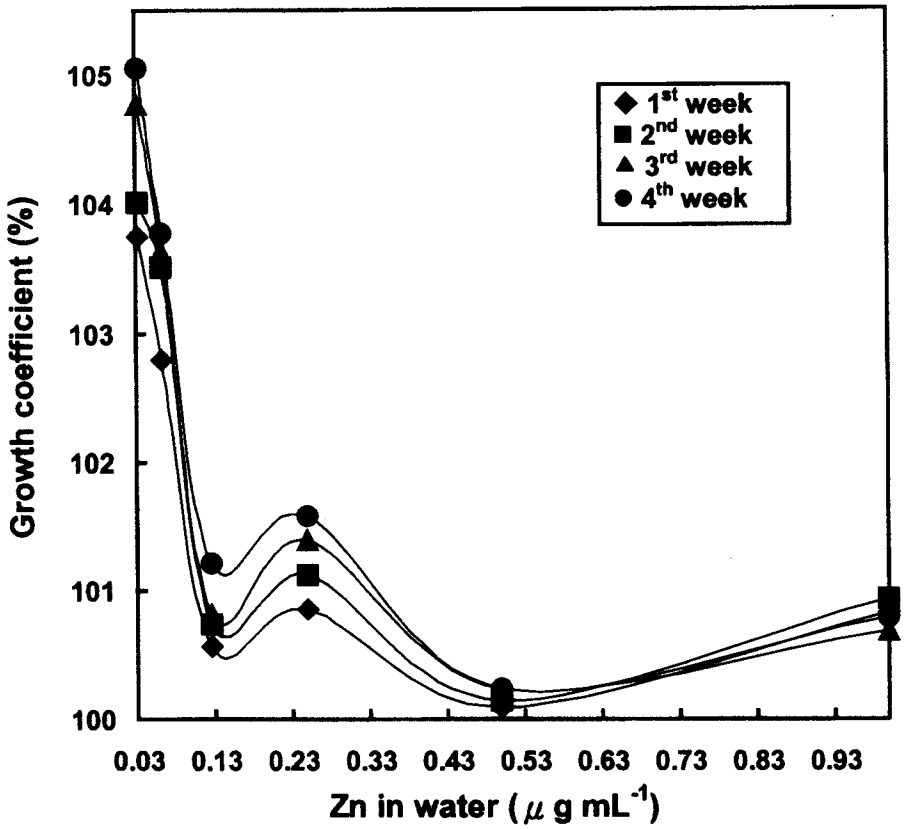

Figure 2. Growth curves of *H. diversicolor supertexta* at the 1st, 2nd, 3rd, and 4th week versus the nominal Zn concentrations in water.

Fig. 3 illustrates an example for expressing the regression relationship between the growth coefficient and log-transformed Zn concentration in the 4th week. Table 2 indicates that calculated EC_{50} values did not change significantly over time and ranged from 0.109 – 0.137 μg mL^{-1}.

Table 2. Estimated effects of regressive equations of Zn on growth for *H. diversicolor supertexta* after one to four weeks.

Time (week)	Regression equation	r^2	EC_{50} (μg mL^{-1})
1	Y=-0.0214X+1.063	0.69	0.109
2	Y=-0.0207X+1.065	0.70	0.137
3	Y=-0.0286X+1.083	0.76	0.110
4	Y=-0.0299X+1.088	0.80	0.117

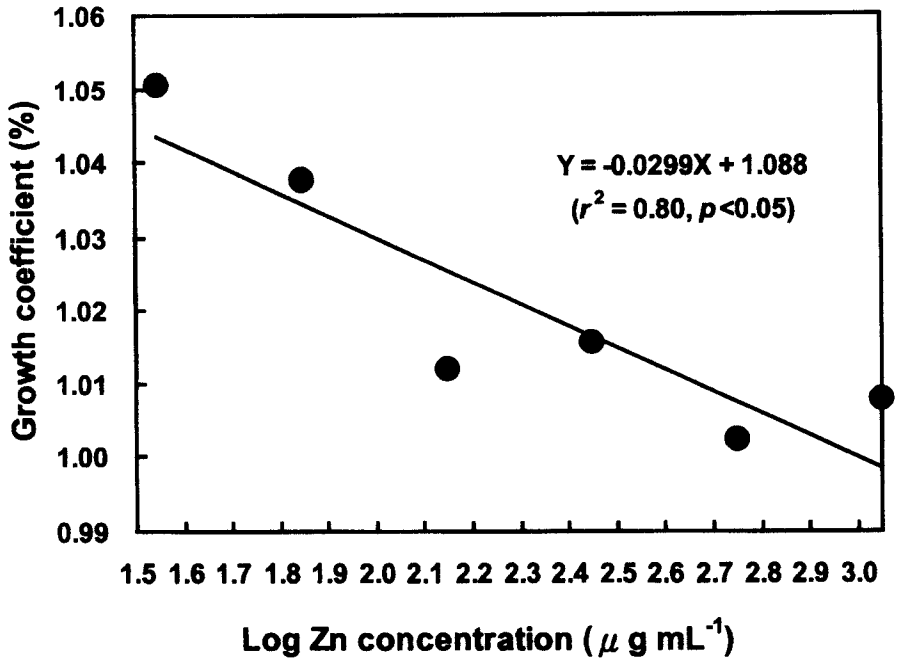

Figure 3. Growth coefficients of abalone *H. diversicolor supertexta* versus log nominal Zn concentrations in the 4th week.

Since for most chemicals only data from acute tests are available, acute-to-chronic ratios (ACRs) are applied in environmental risk assessment to estimate a chronic or a subchronic NOEC for sublethal effects based on acute effect data, mostly with survival (median lethal concentration, LC_{50}) as endpoint (Holdway et al., 2001; Wooding et al., 2002). In order to derive the ACR value, we adapted the acute toxicity value from Liao and Lin (2001) from which the acute endpoint was described by 96-h LC_{50}. The chronic toxicity value can be calculated as the geometric mean of the NOEC and LOEC values. The ACR value was calculated by dividing the acute value by the chronic value (Hunt et al., 2002).

An ACR of 13.59 for *H. diversicolor supertexta* exposed to waterborne Zn was obtained by dividing the 96-h LC_{50} value (1.2 μg mL^{-1}) by the most sensitive chronic value (0.088 μg mL^{-1} for 28-d growth). Table 3 summarizes the acute and present growth toxicity test data for *H. diversicolor supertexta* exposed to waterborne Zn. Our ACR value (13.59) is close to the median ACR value of 13.27 for aquatic invertebrates exposed to metals reported by Länge et al. (1998) in that 90% percentile ACR was 180.8 (Min – Max = 13 – 184).

Our results also demonstrate that the growth rates are stimulated in 0.065 and 0.03125 μg mL^{-1}, this result could be referred to as "hormesis," a phenomenon that toxicants stimulate the organism's response at low concentrations (Kooijman,

1997). Because few previous studies have evaluated Zn toxicity to *H. diversicolor supertexta*, the mechanisms involved in the inhibition of growth remain unknown. Gomot (1997) suggested that the high capacity of gastropod mollusk for the accumulation and storage of metals is attributed to the induction of metallothioneins (the metal-binding proteins) that constitute a mean of detoxification. However, the biological function of the metallothioneins is still subject to discussion and the capacities for metal assimilation are very variable depending on the species. Zn and other heavy metals may affect the physiological functions by modifying the locomotor and respiratory activities and modulate the effects of neurotransmitters.

Table 3. Summary of acute and chronic toxicity data for *H. diversicolor supertexta* exposed to waterborne Zn[a].

Parameter	Representation value
Acute endpoint	96-h LC_{50}
Acute value ($\mu g\ mL^{-1}$)	1.2[b]
Most sensitive chronic endpoint	28-d growth
NOEC ($\mu g\ mL^{-1}$)	0.0625
LOEC ($\mu g\ mL^{-1}$)	0.125
Chronic value ($\mu g\ mL^{-1}$)	0.088[c]
ACR	13.59

[a]ACR = acute-to-chronic ratio; LC_{50} = median lethal concentration; LOEC = lowest-observed effect concentration; NOEC = no-observed effect concentration.
[b]Adapted from Liao and Lin (2001).
[c]Geometric mean.

An additional consideration is the possible effect that test endpoint selection in the abalone could have on the generation of acute and chronic toxicity data. We chose to take into account the toxic effect of Zn on growth rather than on just mortality since growth, like reproduction, is better suited to forecasting the responses of populations and communities and to approaching the physiological basis of ecotoxicological implications.

The present chronic value (0.088 $\mu g\ mL^{-1}$) for the economically important abalone *H. diversicolor supertexta* could set as the upper limit and the current study could assist in the development of appropriate Zn water quality criteria for coastal receiving waters. Our resulting data (e.g., ACR = 13.59) have implications for site-specific, seawater, and national water quality criteria for Zn in aquacultural ecosystems.

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