

# Oxygen Consumption, Ammonia-N Excretion, and Growth Rate in Juvenile Green-Neon Shrimp (*Neocaridina denticulata*) Exposed to Chlordane and Lindane

Da-Ji Huang<sup>1</sup> and Hon-Cheng Chen<sup>2\*</sup>

<sup>1</sup>Institute of Zoology, National Taiwan University, Taipei, Taiwan 106, ROC

<sup>2</sup>Institute of Fisheries Sciences, National Taiwan University, Taipei, Taiwan 106, ROC

## ABSTRACT

The purpose of this study was to investigate the chronic toxicity of chlordane and lindane as well as their sublethal concentration effects on green-neon shrimp (*Neocaridina denticulata*), a common habitant of freshwater systems of eastern Asia and Hawaii. In this study, the effects of experimental concentrations of 1 and 10 ng/L of chlordane and 0.1 and 1 µg/L of lindane on the growth rate, the duration of the molt cycle, oxygen consumption (QO<sub>2</sub>), ammonia-N excretion (ENH<sub>4</sub><sup>+</sup>), and the O:N ratio were examined. Results indicated that growth rates of the were lower than those of the control group, and the molt cycle duration times had also been altered. In all treatments, we also observed that QO<sub>2</sub> increased in the first few days, then it gradually returned to normal during the latter part of the experiment from days 14 to 28. Furthermore, a decrease in ENH<sub>4</sub><sup>+</sup> and an increase in the O:N ratio were observed in chlordane-treated groups, while lindane showed significantly increased ENH<sub>4</sub><sup>+</sup> and a decreased O:N ratio compared to the control. Accordingly, the results of this study demonstrate that lower concentrations of both chlordane and lindane toxicity to *N. denticulate*, even though their effects varied.

**Key words:** chlordane, chronic toxicity, lindane, *Neocaridina denticulata*

## INTRODUCTION

Chlordane and lindane, both organochlorine pesticides (OCPs), have been extensively used over the last 2 decades. OCPs are some of the most dangerous pesticides because of their toxicity, stability, high liposolubility, and long biological half-life. OCPs exhibit high degrees of bioaccumulation and biomagnification through the food chain, and these chemicals are known to have carcinogenic, teratogenic, and endocrine-disrupting effects on humans and wildlife (Klaassen, 2001). Therefore, many developing and

developed countries, due to its serious toxicity have prohibited the use of these compounds for many years, even though OCPs are still being detected in ecosystems (Chen *et al.*, 1999). Therefore, the influence of OCP residues on the development of wildlife has become a major concern.

Toxicity is the capacity of a chemical agent to adversely affect the activity of a living organism: its growth, health, life span, and/or reproductive capacity. Adverse effects also include behavioral changes in individual organisms and ecological changes that affect collective

\*Corresponding author: Room 605, Institute of Fisheries Science, National Taiwan University, No. 1 Roosevelt Road, Sec. 4, Taipei 106, Taiwan, R.O.C.

Tel: +886-2-3366-2885, Fax: +886-2-2363-6837, E-mail: honcheng@ntu.edu.tw

populations. More commonly, animals are subjected to low-dose toxic chemical stresses arising from exposure to sublethal concentrations, so there is a need to assess the effects of toxic compounds on aquatic organisms. Several studies have used growth rate, oxygen consumption, ammonia-N excretion, etc. as indicators to reflect alterations after exposure to a chemical (Schweer, 2002).

The growth rate is an index associated with stress and common signal in chronic-toxicity studies. The growth of an organism is generally used as a sensitive and reliable endpoint in chronically-toxicological investigations. It has clear potential for providing predictions of the harmful properties of a chemical present in a watercourse (Rosas *et al.*, 2001; Benimeli *et al.*, 2003). Molting is either directly or indirectly involved in the expression of growth, and it may be examined through toxicological testing. Because noticeable growth can only occur as a result of molting, any disruption of molting can result in alterations in growth (Skinner, 1985; Schweer, 2002).

Oxygen consumption and ammonia-N excretion are widely considered to be critical factors for evaluating the physiological responses of crustaceans (Claybrook, 1983; McMahan and Wilkens, 1983; McMahan, 2001). These changes in metabolic substrate usage can be measured by monitoring the oxygen:nitrogen (O:N) ratio of test organisms. The O:N ratio indicates the relationship between the amount of oxygen consumed by an organism and the amount of nitrogen excreted, and shows the relative role protein catabolism plays in the organism's energy budget. Theoretical values of

O:N of between 3 and 16 have been suggested for the catabolism of protein, whereas catabolism of equal quantities of proteins and lipids yields O:N values of between 50 and 60. Greater values of O:N correspond to increases in lipid and carbohydrate catabolism (Mayzaud and Conover, 1988). Studies have concluded that changes in the O:N ratio measured among test organisms can serve as a sensitive indicator which provides for the relatively early detection of reproductive impacts by contaminants (Schweer, 2002).

Invertebrates constitute the vast majority of animal species on earth, and crustaceans represent an important and diverse group. Therefore many invertebrate toxicity test protocols are routinely used in regulatory toxicity testing (Schweer, 2002). The green-neon shrimp (*Neocaridina denticulata*) is distributed in rivers throughout eastern Asia and the Hawaiian islands (Shy and Yu, 1998, Englund and Cai, 1999). The major characteristic of *N. denticulata* is the appearance of the oval-like endopod on the first pleopod. In males, the endopod of the second pleopods have an appendix masculine that is oval-shaped and has cilia around it. In the female, the endopods of the second pair of pleopods bear an appendix only (Shy and Yu, 1998).

Even though *N. denticulata* is a common shrimp in fresh waters of Taiwan, only a few attempts have so far been made to determine the effects of chlordane and lindane in freshwater systems on *N. denticulata* (Chen *et al.*, 1999). Therefore, the purpose of this study was to investigate the effects of exposure to sublethal concentrations of these two OCPs on oxygen consumption, ammonia-N excretion, duration of the molt cycle, and growth

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rate in juveniles of *N. denticulata*. The results may provide objective information which can be applied to minimize the impacts of chlordane and lindane on aquatic ecosystems.

## MATERIALS AND METHODS

### *Animal maintenance and chemicals*

Green-neon shrimp (*Neocaridina denticulata*) were taken from rivers in Taipei County, northern Taiwan for laboratory testing. They were transferred to a 50-L glass aquarium after being identified. This aquarium was equipped with a water-cycling device; the pH was maintained at 7.4~7.8; the dissolved oxygen concentration was greater than 7.3 mg/L; and the hardness was 38~45 mg CaCO<sub>3</sub>/L. The temperature was maintained at 25 ± 1 °C, and a 12-h light-dark photoperiod was used. Under these conditions, shrimp were fed twice a day and acclimated for 2 weeks before testing. Newly hatched shrimp (7 d old, 1.5 mm in body length, and 0.5 mg in body weight) were used for the growth tests; juveniles (6 ± 0.2 mm in body length, and 3 ± 0.4 mg in body weight) were used for the molting, oxygen consumption, and ammonia-N excretion tests.

Chlordane and lindane were purchased from Sigma (St. Louis, MO, USA). Stock solutions of chlordane (10 mg/L) and lindane (100 mg/L) were prepared in acetone. It was reported that the values of the 96-h LC<sub>50</sub> for chlordane and lindane are 127.03 ng/L and 9.36 µg/L for *N. denticulata*, respectively (Huang and Chen, 2004). Hence, experimental concentrations of chlordane and lindane were 1 and 10 ng/L, and 0.1 and 1 µg/L, respectively. In the control group, only acetone was added.

Toxicity tests with water renewal every 48 h were based on the *Standard Guide for Conducting Acute Tests with Shrimps* (EPA/ROC, 1998).

### *Growth rate*

Groups of 50 newly hatched shrimp were randomly sampled and placed in 10-L glass beakers, with exposure times of 120 d. Body length and body weight were measured using an electronic ruler and electronic balance at 7-d intervals. Body length was measured as the distance from the base of the eyestalk to the tip of the telson (Hung et al., 1993).

### *Molt (duration of the molt cycle)*

Juvenile shrimp were individually placed in 100-ml glass beakers containing 50 ml of test medium for each concentration of each chemical and the control. The incidence of molting among all specimens was checked every day, and the shed exoskeletons were carefully removed. The experiments ended after the shrimp had completed their 6th molt.

### *Oxygen consumption, ammonia-N excretion, and the O:N ratio*

Groups of 15 juvenile shrimp were randomly sampled and placed in 10-L glass beakers. Control and exposed samples were taken at intervals of 30 min (acute), and 3, 7, 14, and 28 d for estimation of oxygen consumption and ammonia-N excretion.

The oxygen consumption examination was based on the method described by Chinni *et al.* (2000). After being weighed, animals were placed into an oxygen-consumption detection bottle (WTW KF12, Weilheim, Germany) with a microprocessor oximeter (WTW OXI196). Oxygen consumption (QO<sub>2</sub>, µg O<sub>2</sub>/g/h) was calculated as follows:

$$QO_2 = \Delta ppm \times 1/BW \times V \times 1/t;$$

where  $QO_2$  is the amount of oxygen ( $\Delta ppm$ ) consumed in the interval  $t$  (h),  $BW$  is the wet body weight (g) of the individual, and  $V$  is the volume (ml) of the oxygen-consumption detection bottle. Oxygen consumption tests were estimated for a period of 6 h. Additionally, the water within the bottle at the start as well as at the end of oxygen consumption analysis was immediately sampled to examine the level of ammonium-N before its transformation to nitrite or nitrate because of oxidation and activities of some microorganisms. Examination of ammonium-N within the water was detected using an ammonia electrode (Mettler-Toledo, type-15 230 3000, Urdorf, Switzerland). Ammonium-N

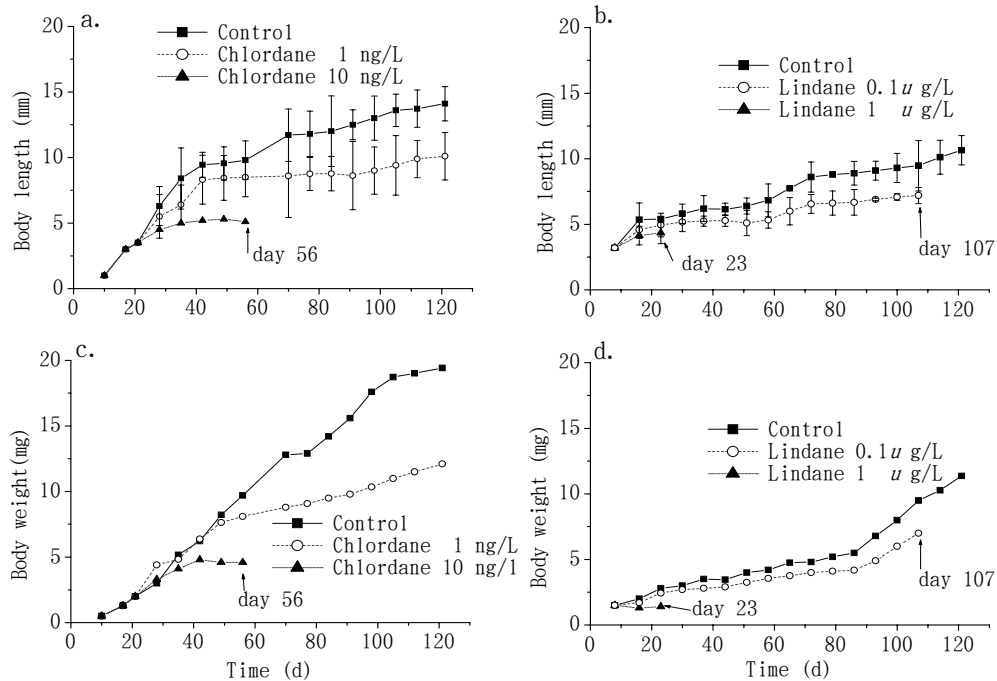
excreted ( $ENH_4^+$ ,  $\mu g NH_4^+/g/h$ ) by animals was calculated as follows:

$$ENH_4^+ = \Delta ppb \times 1/BW \times V \times 1/T;$$

where  $ENH_4^+$  is the amount of ammonium ( $\Delta ppb$ ) generated in the total experimental time  $T$  (h). In addition, the oxygen: nitrogen (O:N) ratios were also calculated as the ratio of atoms of oxygen consumed to atoms of nitrogen excreted in the above intervals.

### Statistical analysis

All values of growth rate, molting rate, oxygen consumption, and ammonia-N excretion tests were analyzed by analysis of variance using Microcal™ vers. 6.0. (Northampton, MA, USA). Experimental and control values were compared using Student's  $t$ -test (paired assay,  $p < 0.05$ ).



**Figure 1.** Growth rate of body length (mean  $\pm$  S.D.,  $n = 10$ ) and body weight measured after exposure to chlordane (a, c) and lindane (b, d). Mortality rates of 100% were observed on days 56, 107, and 23 for the groups exposed to 10 ng/L chlordane, 0.1  $\mu g/L$  lindane, and 1  $\mu g/L$  lindane, respectively.

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### RESULTS

#### *Growth rate*

Growth rates were measured as the decrease in body weight and body length (compared to the control group) (Fig. 1) as affected by different treatments. Mortality rates of 100% were observed at 56, 107, and 23 d for the groups subjected to 10 ng/L chlordane, and 0.1 and 1 µg/L lindane, respectively. The higher the concentration of pesticides the shrimp were exposed to, the lower the growth rates we observed.

#### *Molting*

Results from the experiment on molting rates are shown in Table 1. From the data sets of control shrimp, we observed that the molt cycle duration continued for the entire experimental period. After being treated with chlordane and lindane, the exposed shrimp especially the group exposed to 1 µg/L lindane ( $p < 0.05$ ) showed a decline in the duration before their first molt compared to the controls. However, molt cycle durations from the 3rd to the 6th molts of the treated shrimp tended to be longer than those of the control, and the results for the 6th molt of shrimp

exposed to 0.1 and 1 µg/L lindane treatments showed statistically significant differences from the control ( $p < 0.05$ ).

#### *Oxygen consumption, ammonia-N excretion, and the O:N ratio*

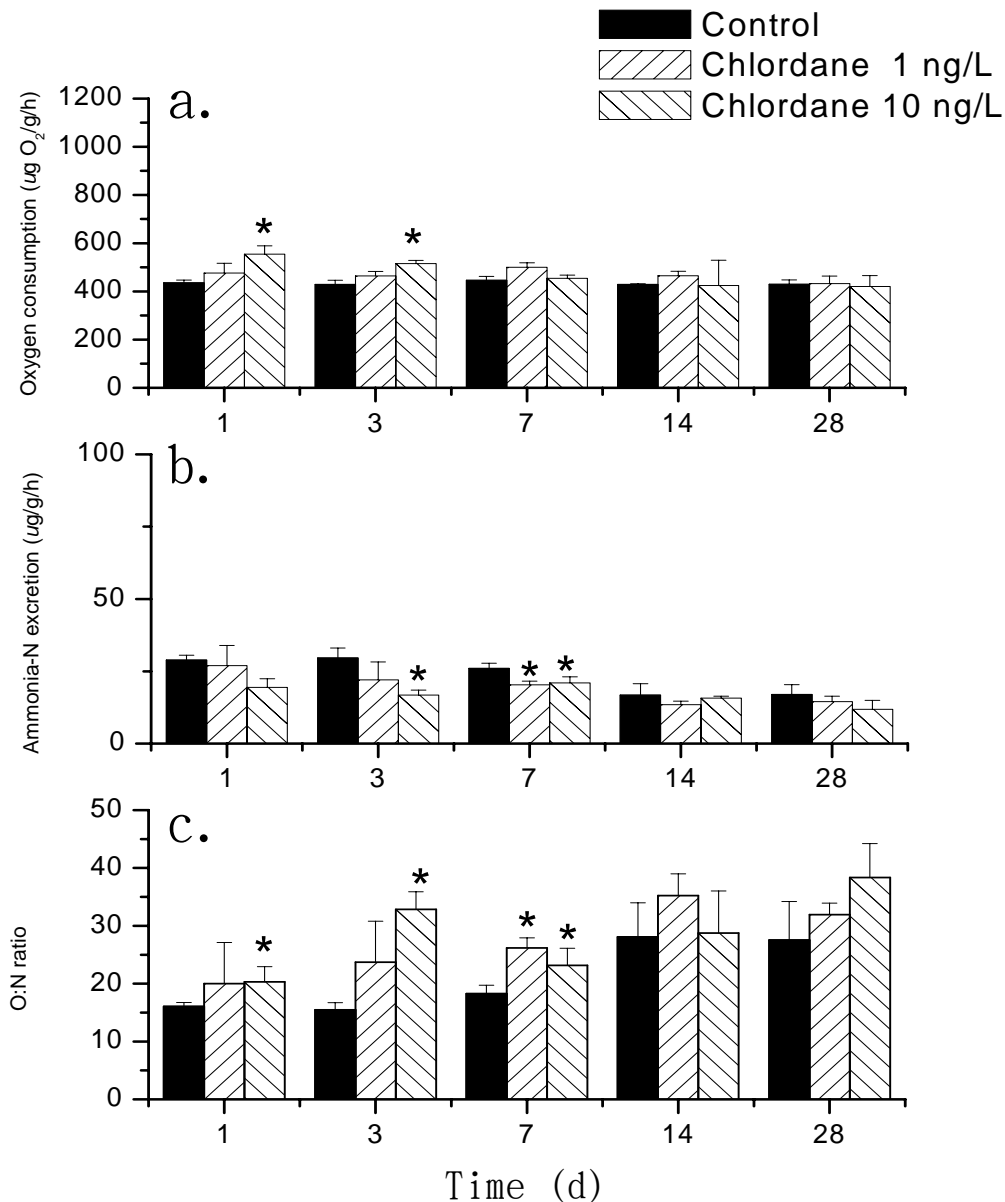
Results in Figs. 2 and 3 show the oxygen consumption ( $QO_2$ ), ammonia-N excretion ( $ENH_4^+$ ), and O:N ratio. The  $QO_2$  values of shrimp treated with 10 ng/L chlordane were significantly higher than those of control shrimp after 1 and 3 d of exposure ( $p < 0.05$ ). We also observed that  $QO_2$  values of shrimp treated with 0.1 and 1 µg/L lindane were higher than those of the control after exposure for 7 d and for 1, 3, and 7 d, respectively ( $p < 0.05$ ). After exposure for 14 d, the  $QO_2$  from the chlordane- and lindane-treated shrimp had gradually recovered. Results for  $ENH_4^+$  showed that the level in the 1 ng/L chlordane-treated shrimp was significantly lower than that of the control group after 7 da of exposure ( $p < 0.05$ ), and the level in 10 ng/L chlordane-treated shrimp was significantly lower than that of the control group after both 3 and 7 d of exposure ( $p < 0.05$ ). After exposure to

**Table 1.** Duration of the molt cycle of juvenile *Neocaridina denticulata* in the control and treated groups shown with units of day (mean ± S.D.). The number of shrimp ( $n$ ) is shown in parentheses. Mean values of the treated groups with an asterisk (\*) significantly differ from those of the control groups ( $p < 0.05$ ).

treated	Time to molted					
	1st	2nd	3rd	4th	5th	6th
Control	6.3±2.8 (n=10)	7.0±0.9 (n=10)	7.9±1.9 (n=10)	8.6±1.8 (n=10)	9.1±1.5 (n=10)	9.0±1.8 (n=10)
Chlordane 1 ng/L	4.8±1.3 (n=7)	7.2±0.8 (n=7)	7.4±1.1 (n=7)	8.6±3.1 (n=7)	9.2±1.9 (n=6)	9.3±1.9 (n=6)
Chlordane 10 ng/L	5.3±2.0 (n=8)	8.3±0.9 (n=7)	7.3±2.5 (n=7)	9.3±2.3 (n=6)	9.7±1.2 (n=6)	10.0±2.0 (n=6)
Lindane 0.1 µg/L	4.8±3.1 (n=8)	6.8±3.0 (n=7)	8.3±1.3 (n=7)	9.5±2.4 (n=7)	11.0±2.6 (n=7)	11.3±1.3* (n=7)
Lindane 1 µg/L	2.7±0.9* (n=6)	5.7±1.2* (n=4)	8.6±1.3 (n=4)	9.8±3.2 (n=4)	12.0±3.3 (n=4)	12.2±2.1* (n=3)

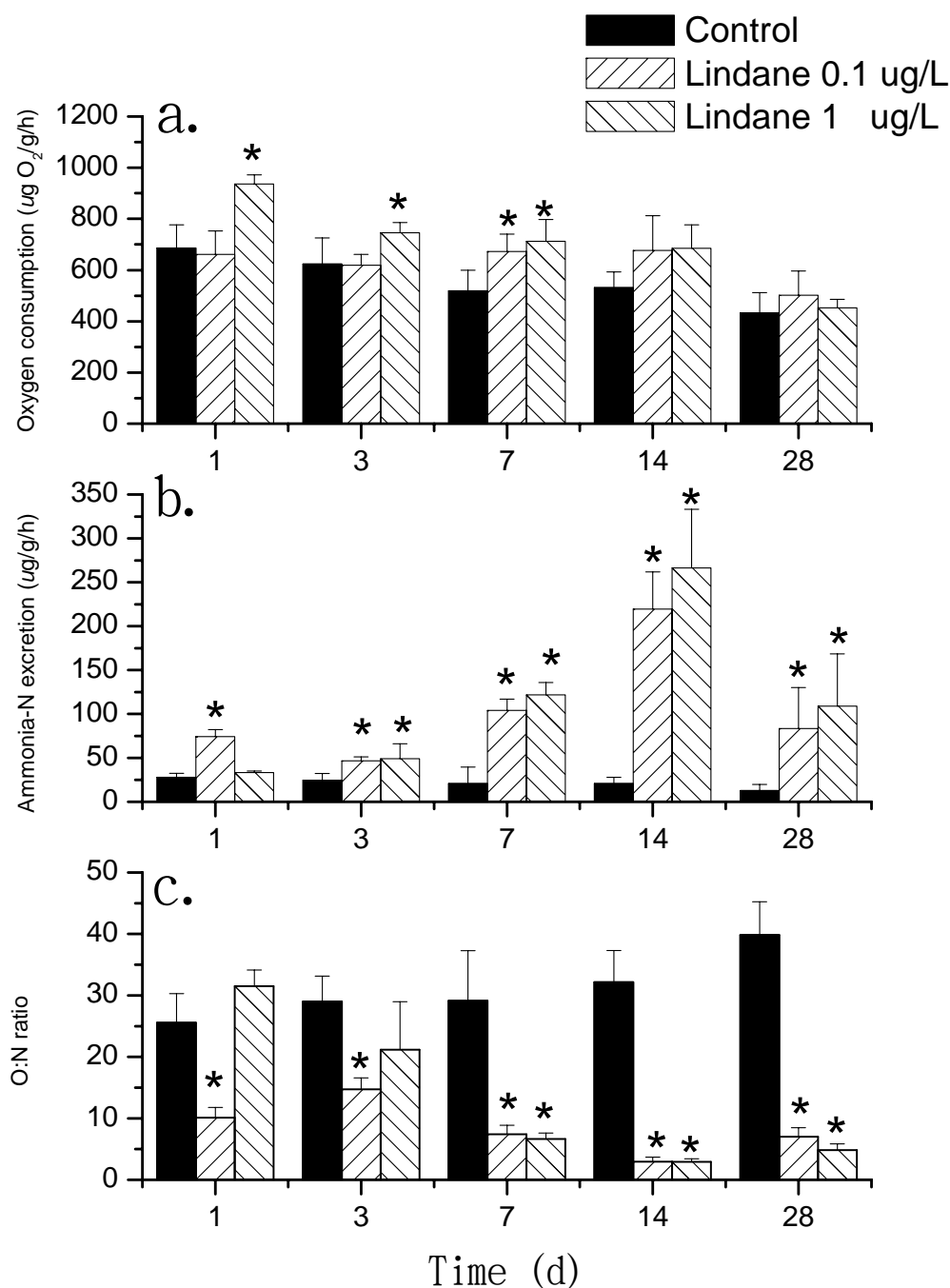
0.1 and 1  $\mu\text{g/L}$  lindane, levels of  $\text{ENH}_4^+$  in the treated shrimp were significantly higher than those of the control on days 1, 7, 14, and 28, and on days 7, 14, and 28, respectively ( $p < 0.05$ ). Values of the O:N ratio in the 1 and 10 ng/L chlordane-treated groups were higher than those of the

control group on day 7 and on days 1, 3, and 7, respectively ( $p < 0.05$ ). Treatment with lindane produced opposite results as those seen in the chlordane groups, as we observed obvious drops in the O:N ratios on days 7, 14, and 28 ( $p < 0.05$ ).



**Figure 2.** Oxygen consumption (a), ammonia-N excretion (b), and the O:N ratio (c) of the control, as well as individuals exposed to 1 and 10 ng/L chlordane in juvenile *Neocaridina denticulata* (mean  $\pm$  S.D.,  $n = 15$ ). Mean values of the treated groups with an asterisk (\*) significantly differ from those of the control groups ( $p < 0.05$ ).

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**Figure 3.** Oxygen consumption (a), ammonia-N excretion (b), and the O:N ratio (c) of the control as well as individuals exposed to 0.1 and 1  $\mu\text{g/L}$  lindane in juvenile *Neocaridina denticulata* (mean  $\pm$  S.D.,  $n = 15$ ). Mean values of treated groups with an asterisk (\*) significantly differ from those of the control groups ( $p < 0.05$ ).

### DISCUSSION

Chlordane and lindane were toxic to green-neon shrimp, *N. denticulata*, even with exposures to low toxicity

values of 1 and 10 ng/L chlordane and 0.1 and 1  $\mu\text{g/L}$  lindane. In this study, we describe their effects on the growth rate, duration of the molt cycle,

oxygen consumption, ammonia-N excretion, and the O:N ratio. Growth rates of shrimp treated with both of these OPCs were lower than that of the control. Exposure of newly hatched *N. denticulata* juveniles to 10 ng/L chlordane, and 0.1 and 1 µg/L lindane resulted in 100% mortality within 56, 23, and 107 d, respectively (Fig. 1). Similar results were found in a mysid (*Mysidopsis bahia*) on studies contaminants retard growth rates (McKenney and Celestial, 1996). The growth rate of arthropods is closely related to the molt cycle duration; increases or decreases in the time duration of molts may affect growth rates (Schweer, 2002). The results from this study showed that circumstances of the treated groups differed compared with that of control group. After treatment with chlordane and lindane, a trend appeared in which molt cycle durations from the initial molt decreased, while they increased from the 4th to the 6th molts. And this circumstance was obvious in both lindane-treated groups which showed significant differences from the controls ( $p < 0.05$ ). Molting, one of the key physiological processes of arthropods, is under hormonal control, and it is susceptible to negative effects of contaminants especially endocrine-disrupting chemicals. Molting is primarily regulated by interactions of molt-stimulating hormones (ecdysteroids) and nervous system secretions produced in the cephalothorax with molt-inhibiting hormones produced in the eyestalks (Skinner, 1985; Subramoniam, 2000). Touart (1982) found that the pesticide, diflubenzuron (Dimilin<sup>®</sup>), increased the duration of the molt cycle in the mysid shrimp, *M. bahia*, and probably acted on the mysid endocrine system as a molt inhibitor. Even so, direct

results on the effects of chlordane and lindane on the action mechanism of ecdysteroids of *N. denticulata* were not available in the present study. Furthermore, another consideration concerning the effects of chlordane and lindane on the molt cycle and growth is that the reduced growth rate in *N. denticulata* possibly resulted from a transfer of energy from growth mechanisms as the organism attempted to counteract the stress (McKenney *et al.*, 1991, Khan *et al.*, 1992).

In the present study, we observed that  $QO_2$  increased on the first day after being exposed to 10 ng/L chlordane and 0.1 or 1 µg/L lindane which implies that chlordane and lindane might alter the respiratory rate after acute exposure (Figs. 2, 3). Changes in  $QO_2$  caused by pesticides indicate metabolic alterations, which might affect larval growth. These results agreed with those reported by other authors on the respiratory metabolism of white shrimp, *Penaeus vannamei*, challenged with sublethal doses of pesticides (McKenney *et al.*, 1991; Galindo *et al.*, 1996). However, we found that  $QO_2$  gradually returned to a normal condition from day 7 or 14. Thus, we considered that gills of treated *N. denticulata* were not seriously damaged or destroyed by chlordane (1 and 10 ng/L) and lindane (0.1 and 1 µg/L), and they still could recover their functions in oxygen absorption.  $ENH_4^+$  is the waste product of protein metabolism, and it may be another metabolic waste which indicates that *N. denticulata* was affected by chemical toxicity after considering  $QO_2$  and calculating the O:N ratio. We observed that the situations with  $ENH_4^+$  and the O:N ratio after exposure to chlordane and lindane entirely differed in our



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experiment. Decreases in  $\text{ENH}_4^+$  and increases in the O:N ratio were observed in the chlordane-treated groups (Fig. 2b, c). However, lindane showed a totally different effect, in that  $\text{ENH}_4^+$  increased and the O:N ratio significantly decreased compared to the controls (Fig. 3b, c). The main energy source of crustaceans is from protein (free amino acid) degradation, and  $\text{ENH}_4^+$  is produced from proteins. Nevertheless, when a crustacean is in a situation where it requires energy for the long term or the free amino acids in the body are limited, it can transfer its energy source from proteins to lipids in order to enhance its energy (Mayzaud and Conover, 1988). McKenney *et al.* (1991) indicated that exposure of *M. bahia* to the defoliant, DEF (*S, S, S*-tri-*n*-butyl phosphorotrithioate), caused a decrease in  $\text{ENH}_4^+$ , but an increase in the O:N ratio probably because *M. bahia* changes the substance used for its metabolic energy from proteins to lipids in order to enhance its energy for resisting the effect of the toxicity, as occurred in shrimp exposed to chlordane. Mayzaud and Conover (1988) showed that an increasing metabolism rate of crustaceans affected by stress can cause the O:N ratio to drop to below 4. In our lindane-treated groups, we deduced that *N. denticulate* greatly increased its metabolism rate in order to remove the lindane and resist toxicity after the organism was affected by the toxicity of lindane; this possibly caused the rise in  $\text{ENH}_4^+$  and the fall in the O:N ratio. But, even with the effects of lindane, it failed to transfer its energy source to lipids. Thus *N. denticulata* degraded a great deal of protein due to its consumption

of energy in an effort to resist the toxicity of lindane, and this produced a large amount of nitrogen-containing degradation products. After having been affected by lindane for 28 d, the nonstop consumption of protein caused physiological recession, and the quantity of ammonia being discharged was much less compared to that on the 14th day. At present, we are unable to determine which physiological mechanism which was affected by lindane prevented the transfer to lipid metabolism.

The stable development of a population of an organism depends on the health conditions of individuals (Colborn *et al.*, 1993). We found that *N. denticulata* was affected by exposure to low concentrations of chlordane (1 and 10 ng/L) and lindane (0.1 and 1  $\mu\text{g/L}$ ). However, low concentrations of chlordane (< 14.2  $\mu\text{g/L}$ ) and lindane (< 20.6 ng/L) can still be detected in freshwater systems (Abou-Arab *et al.*, 1995; Chen *et al.*, 1999; Galindo *et al.*, 1999). When chemicals such as chlordane and lindane reach wildlife through the food chain, they may cause physiological problems (Colborn *et al.*, 1993). As a result, decreases in wildlife populations may well be expected. This is an urgent situation which requires our attention in order to determine biological safe concentrations, if the reproductive systems and behaviors of *N. denticulata* in our environment are not to be disrupted. We are currently working on related studies to further understand the effects of chlordane and lindane on *N. denticulata*, especially on the structure of the gills, renal gland, and other tissues caused by these two pesticides.

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# 可氯丹( Chlordane )及林丹( Lindane )對多齒新米蝦 ( *Neocaridina denticulata* )所造成之慢性毒性影響

黃大駿<sup>1</sup>、陳弘成<sup>2</sup>

<sup>1</sup>國立臺灣大學動物學研究所

<sup>2</sup>國立臺灣大學漁業科學研究所

## 摘 要

可氯丹(chlordane)及林丹(lindane)為長效性有機氯殺蟲劑。本研究目的在瞭解環境中殘留的可氯丹及林丹對多齒新米蝦( *Neocaridina denticulata* )所造成之慢性毒性影響。將多齒新米蝦曝露於亞致死濃度之可氯丹(1 及 10ng/L)及林丹(0.1 及 1 $\mu$ g/L)下來進行試驗，於曝露後測定多齒新米蝦成長速率、脫殼時間、耗氧(QO<sub>2</sub>)、排氮量(ENH<sub>4</sub><sup>+</sup>)及氧氮比(O : N ratio)改變的情形。實驗結果顯示可氯丹及林丹均會抑制成長及改變脫殼時間，並使曝露初期各組的耗氧量上升；而在實驗後期的第 14 至 28 天，各試驗組的耗氧量則會逐漸回復正常值。此外，可氯丹的試驗組中排氮量降低，氧氮比增加；而林丹的試驗組則呈現排氮量上升，氧氮比減少的情形。由此可知，多齒新米蝦的成長的確受到亞致死濃度之可氯丹(1 及 10ng/L)及林丹(0.1 及 1 $\mu$ g/L)的毒性影響。

**關鍵詞：**可氯丹、林丹、多齒新米蝦、慢性毒