

# Sublethal Antimony (III) Exposure of Freshwater Swamp Shrimp (*Macrobrachium Nipponense*): Effects on Oxygen Consumption and Hepatopancreatic Histology

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## Abstract

This study was an attempt to realize the effects of antimony on freshwater swamp shrimp (*Macrobrachium nipponense*). An experiment of this nature, which have not previously been carried out in this species. First, median lethal concentrations were determined in acute toxicity tests. The 96-h LC<sub>50</sub> value was 6.748 (5.728-7.950) mg Sb/l for adult *M. nipponense* and 1.635 (1.271-2.103) mg Sb/l for juvenile *M. nipponense*. Juvenile *M. nipponense* were exposed to 4 different sublethal levels of antimony (0.1, 0.4, 0.8, and 1.2 mg Sb/l) over a 7-d test period and a 7-d recovery period. After 30 min (acute), there was an increase in the amount of oxygen consumed in all exposed groups. On days 3, 7, and 14, decreases in oxygen consumption were significant ( $p < 0.05$ ) for the higher-exposure level groups (0.8 and 1.2 mg/l). Light microscopy investigations showed histopathological alterations in the hepatopancreas which correlated with exposure concentrations. The alterations included degenerative changes in the lumen, a reduction in the lumen volume, and injury to epithelial cells in the histoarchitecture of hepatopancreas.

**Keywords:** Antimony, *Macrobrachium Nipponense*, Oxygen Consumption, Hepatopancreas

## 1. Introduction

Antimony (Sb) compounds such as indium antimony (InSb) and gallium antimony (GaSb) are important materials for the manufacture of integrated circuits and optoelectronic devices in the semiconductor industry [1]. Manufacturing processes devoted to the fabrication of GaSb-based semiconductor devices generate large volumes of wastes that contain the toxic metals antimony and gallium. In addition, both metals are listed as hazards by the Environmental Protection Agency in the US [2]. Previous reports indicated that mammalian exposure to trivalent forms of Sb can cause severe liver damage, hemolysis, hematuria, and circulatory disease. And antimony trichloride (SbCl<sub>3</sub>) induced sister chromatid exchanges (SCEs) in V79 cells and apoptosis in human fibroblasts (HFs), a human bronchial epithelial cell line (BES-6), and a Chinese hamster ovary cell line (CHO-K1) [3,4].

Industrial spills can lead to high concentrations of

toxic materials in water, affecting freshwater ecosystems with acute and chronic health effects. Toxicants may significantly damage certain physiological and biochemical processes when they enter the organs of fishes [5]. The liver is an important organ involved in metabolic processes and in detoxification of xenobiotics. In some situations, poisonous materials may accumulate in the liver to toxic levels and cause pathological alterations [6]. Histopathological alterations are recognized and commonly used diagnostic tools in aquatic toxicology studies [7]. Oxygen consumption is widely considered to be a critical factor for evaluating the physiological response and a useful variable for an early warning for monitoring aquatic organisms [8]. Like most aquatic organisms, they maintain their oxygen consumption at a constant level along a gradient of environmental oxygen concentrations, until a critical oxygen concentration is reached, and below which oxygen consumption begins to fall. Under conditions of stress, this critical oxygen concentration is likely to increase, reflecting organisms'

capacity for coping with environmental perturbations [9, 10].

Swamps are among the world's most productive wetland ecosystems, and they are also important as nursery grounds for many ecologically and economically important species. The freshwater swamp shrimp (*Macrobrachium nipponense*) is a common aquatic invertebrate widely distributed in upper and middle sections of rivers throughout the eastern Asia-Pacific area. The purposes of this study were to examine the effects of antimony on acute toxicity and the effects of sublethal antimony concentrations on the oxygen consumption and hepatopancreatic histology of *M. nipponense*, so that this evidence could then be used to evaluate possible adverse effects of this metal on the freshwater swamp shrimp.

## 2. Materials and Methods

Juvenile and adult of the freshwater swamp shrimp (*M. nipponense*) were obtained from local commercial suppliers. Shrimp were transported to a glass aquarium in our laboratory which was equipped with a water-cycling device; dechlorinated tap water (with a pH of 7.4-8.1, dissolved oxygen (DO) of 7.0-7.7 mg/l, and hardness of 38-45 mg CaCO<sub>3</sub>/l) was used during the entire experiment. The temperature was maintained at 24.0 ± 0.5 °C, and a 12-h light and 12-h dark photoperiod was used. Shrimp were acclimated for 2 weeks and fed an aquarium shrimp mixture every day. Juvenile (4 weeks old, 0.42 ± 0.17 cm in total length) were used for acute toxicity tests and oxygen consumption tests; adult (17 weeks old, 1.2 ± 0.38 cm in total length) were used for the acute toxicity tests and histological examinations in the initial experiments. Antimony trichloride (purity ≥ 99%) was purchased from Sigma (St. Louis, MO, USA). Stock solutions were prepared in deionized water (1000 mg/l test chemical in 0.1% nitric acid).

Laboratory static renewal tests [11] were conducted to determine the median lethal concentrations (LC50) for juvenile and adult *M. nipponense*. Ten shrimp of similar size were randomly sampled and placed in 10-l glass beakers. After 24 h of acclimatization, the shrimp were exposed to different antimony concentrations (0, 0.1, 0.2, 0.5, 1.0, 2.0, 4.0, 8.0, 12.0, 14.0, and 16.0 mg Sb/l) for 96 h or more. The control and each treatment with 4-l testing solution, and all groups were run in duplicate. During the experiment, dead shrimp were removed, and the mortality was recorded. The LC50 of antimony and its 95% confidence limits for *M. nipponense* were calculated using a Basic program from the probit analysis described by [12].

The oxygen consumption analysis was carried out using a former method with slight modification [8]. Groups of 20 juvenile *M. nipponense* were randomly sampled and placed in 10-l glass beakers with 4-l test solution;

triplicate shrimp were then respectively exposed to a test solution of 0.0, 0.1, 0.4, 0.8, and 1.2 mg Sb/l. Sublethal levels of antimony were equivalent to approximately 6%, 24%, 49%, and 73% of the 96-h LC50 value (1.635 mg Sb/l) according to acute toxicity tests. Twice a week, 50% of the water was renewed with standard water containing antimony to maintain constant environmental conditions throughout the entire experimental period. The exposure time was 1 week, followed by a 1-week recovery period in Sb-free water.

Both control and exposed samples were taken after 30 min (for acute exposure), and 3<sup>rd</sup> d, 7<sup>th</sup> d, and 14<sup>th</sup> d for estimation of oxygen consumption. Oxygen consumption tests were customarily carried out by sealing two juvenile *M. nipponense* in a 325-ml respiratory jar capacity with an oxygen electrode (Microprocessor Oximeter, WTW, Heidelberg, Germany). All respiratory jars contained up to 7 mg/l DO before the initial measurement. At each interval, two juvenile *M. nipponense* were put into a respiratory jar with an acclimatization time of 30 min as recorded earlier, and then the oxygen consumption was estimated. We allowed them to deplete the oxygen until death occurred, and the residual dissolved oxygen was measured using a multiple-range temperature and oxygen analyzer and recorder (Yokogawa, Tokyo, Japan). Oxygen consumption (QO<sub>2</sub>, mg O<sub>2</sub>/kg/h) was calculated as follows:

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

where QO<sub>2</sub> is the amount of oxygen (Δppm) consumed in the interval t (h) and BW is the wet body weight (kg) at the start and at the end of the test period.

Adult *M. nipponense* for sublethal tests were randomly placed in 20-l glass aquaria with 10-l test solution. Every aquarium contained 10 shrimp which were exposed to the following concentrations: 0.2, 1.0, and 4.0 mg Sb/l test solutions and a control. Sublethal levels of antimony were equivalent to approximately 3%, 15%, and 60% of the 96-h LC50 value (6.748 mg Sb/l) according to the acute toxicity tests. Twice a week, 50% of the water was renewed with standard water containing antimony to maintain constant environmental conditions throughout the entire experimental period.

Four adult *M. nipponense* per exposure concentration were anesthetized with MS-222 (1:6000) (Sigma, St. Louis, MO, USA) and sacrificed after 2 weeks of exposure. Shrimp hepatopancreases were collected, fixed in 4% buffered formalin, and routinely processed for examination using standard techniques with hematoxylin and eosin (H&E).

All values of oxygen consumption assessment were performed with one-way analysis of variance (ANOVA). Duncan's multiple-range test was used to evaluate the mean difference among individual groups at the 0.05 significance level.

### 3. Results and Discussion

According to the static renewal method for acute toxicity testing, median lethal concentrations (LC<sub>50</sub>) of antimony for swamp shrimp (*M. nipponense*) were calculated. The calculated 48- and 96-h LC<sub>50</sub> values are listed in Table 1. From our investigation, juvenile *M. nipponense* were found to be more susceptible than adults *M. nipponense* to acute antimony toxicity. 96-h LC<sub>50</sub> of antimony to juvenile zebrafish (*Brachydanio rerio*) was 4.65 mg Sb/l, and to juvenile common carp (*Cyprinus carpio*), larval tilapia (*Oreochromis mossambicus*), juvenile red seabream (*Pargus major*), and juvenile sheepshead minnows (*Cyprinodon variegatus*), were 14.05, 18.9, 12.4, and 6.2-8.3 mg Sb/l, respectively [2,13-16]. No toxic effect was seen at 0.2 mg Sb/l in the acute toxicity testing of juvenile *M. nipponense*, which is equivalent to 12% of the 96-h LC<sub>50</sub> value, and is in close agreement with the concept of a safe level (one-tenth of the 96-h LC<sub>50</sub> value) as described by [17]. Less than 0.2 mg Sb/l was proposed as a biologically safe concentration which can be used for establishing tentative water quality criteria for *M. nipponense* of this size.

The results of the oxygen consumption rates for the control and exposed juvenile *M. nipponense* were presented in Table 2. After 30 min (acute), there was an increase in the amount of oxygen consumed in the exposed groups; a maximum increase of 27.3% was observed at the highest exposure concentration (1.2 mg Sb/l). Percentage of oxygen consumption decreased over their respective controls from acute exposure 3<sup>th</sup> d to 7<sup>th</sup> d during the exposure time. No increasing in oxygen consumption, however, was observed in the recovery period (on 14<sup>th</sup> d) compared with the respective same level groups on 7<sup>th</sup> d. In the present study, juvenile *M. nipponense* showed no recovery at higher exposure level groups (0.8 and 1.2 mg Sb/l) after 1-week period in Sb-free water. Former study indicated that exposure to sublethal toxicant concentrations increases respiratory activity, resulting in increased ventilation and increased uptake of the toxin [18]. Later, cytological damage should be related to the decrease in oxygen consumption be-

cause the gills are most likely the first target of waterborne heavy metals, including thickening of branchial epithelium and deep changes in hemolymph patterns in the gills with a concomitant increase in vacuolization and reduced hemolymph spaces causing perfusion stagnation [19]. For example, an increased number of nephrocytes in gill filaments, a blackened appearance of the gills, necrosis of gill cells resulting in narrowed or obstructed hemolymphatic vessels, the appearance of a space between the cuticle and the epithelial cells which contains black electron-dense material, and even fragmentation of nuclei within gill cells could be observed when *P. japonicus* were exposed to different concentrations of Cd for 4 days [20]. A review of the responses of aquatic crustaceans in low ambient dissolved oxygen, mentioned that many crustaceans possess an excellent regulatory ability in their oxygen consumption patterns [21]. We proposed that measurements of oxygen consumption could be used to assess the effects of antimony on sublethal exposure levels of juvenile *M. nipponense*.

The controlled group and adult *M. nipponense* treated with 0.2 mg Sb/l showed well-developed hepatopancreatic tubules with star-shaped lumen, and with closely arranged basal laminae surrounding each tubule (Figure 1). Alterations in the structural organization of tubules were observed after 1.0 mg Sb/l treatment. Degenerative changes in the lumen, detachment of the basal lamina, and impairment of the structural integrity were observed (Figure 2). Severe hepatopancreatic lesions were found in *M. nipponense* exposed to 4.0 mg Sb/l. Disorderly and disrupted hepatopancreatic tubules and reduced lumen became more prominent (Figure 3). The crustacean heap-

**Table 1. Median lethal concentrations (LC<sub>50</sub>) of antimony to *M. nipponense*.**

Life stage	LC <sub>50</sub> (mgSb/L)	
	48 h	96 h
Juvenile	2.240	1.635
	(1.649-3.046)	(1.271-2.103)
Adult	10.083	6.748
	(8.736-11.378)	(5.728-7.950)

**Table 2. Effect of sublethal exposure to antimony on oxygen consumption (mg O<sub>2</sub>/kg/h) of juvenile *M. nipponense*.**

	Control	0.1 mgSb/L	0.4 mgSb/L	0.8 mgSb/L	1.2 mgSb/L
acute	0.653±0.043 <sup>a</sup>	0.660±0.027 <sup>a</sup>	0.674±0.042 <sup>a</sup>	0.808±0.071 <sup>b</sup>	0.831±0.064 <sup>b</sup>
day 3	0.641±0.089 <sup>a</sup>	0.651±0.063 <sup>a</sup>	0.647±0.045 <sup>a</sup>	0.507±0.065 <sup>b</sup>	0.421±0.033 <sup>c</sup>
day 7	0.658±0.043 <sup>a</sup>	0.644±0.059 <sup>a</sup>	0.628±0.071 <sup>a</sup>	0.545±0.077 <sup>b</sup>	0.504±0.021 <sup>b</sup>
day 14	0.659±0.041 <sup>a</sup>	0.652±0.050 <sup>a</sup>	0.641±0.058 <sup>a</sup>	0.573±0.046 <sup>b</sup>	0.539±0.063 <sup>b</sup>

All values are given as the mean±SD; n = 3.

Values in the same row with different superscripts differ significantly at p < 0.05.

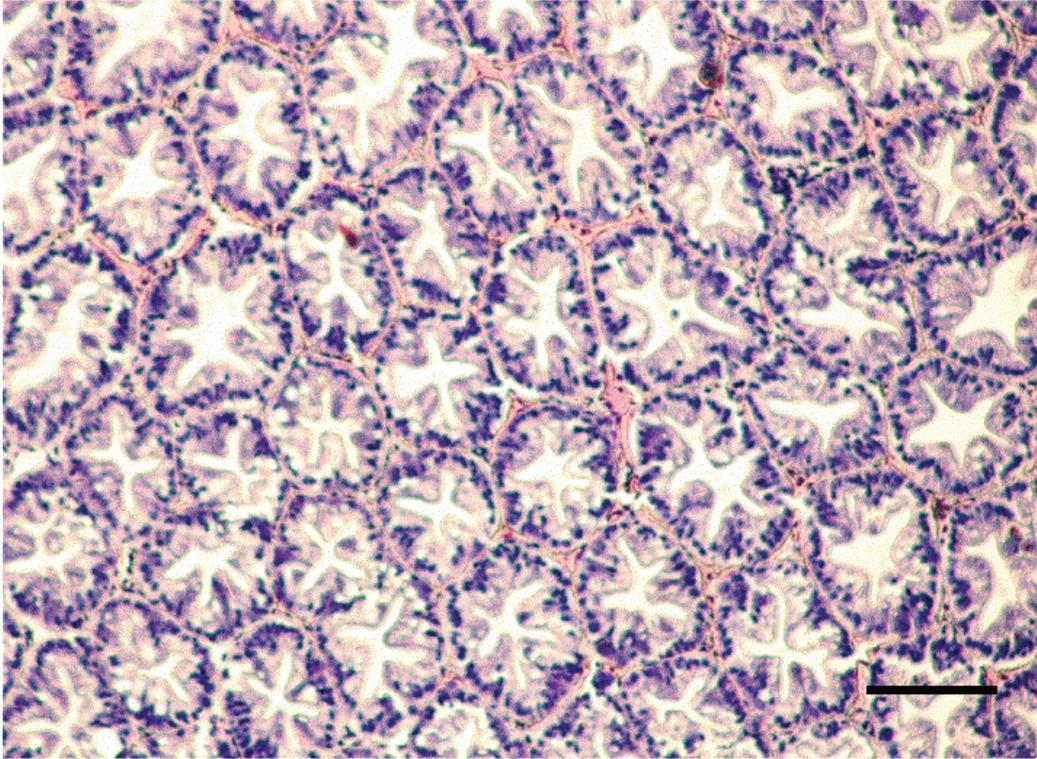


Figure 1. Hepatopancreatic tissue of the adult *M. nipponense* in the control group. (bar = 0.1mm).

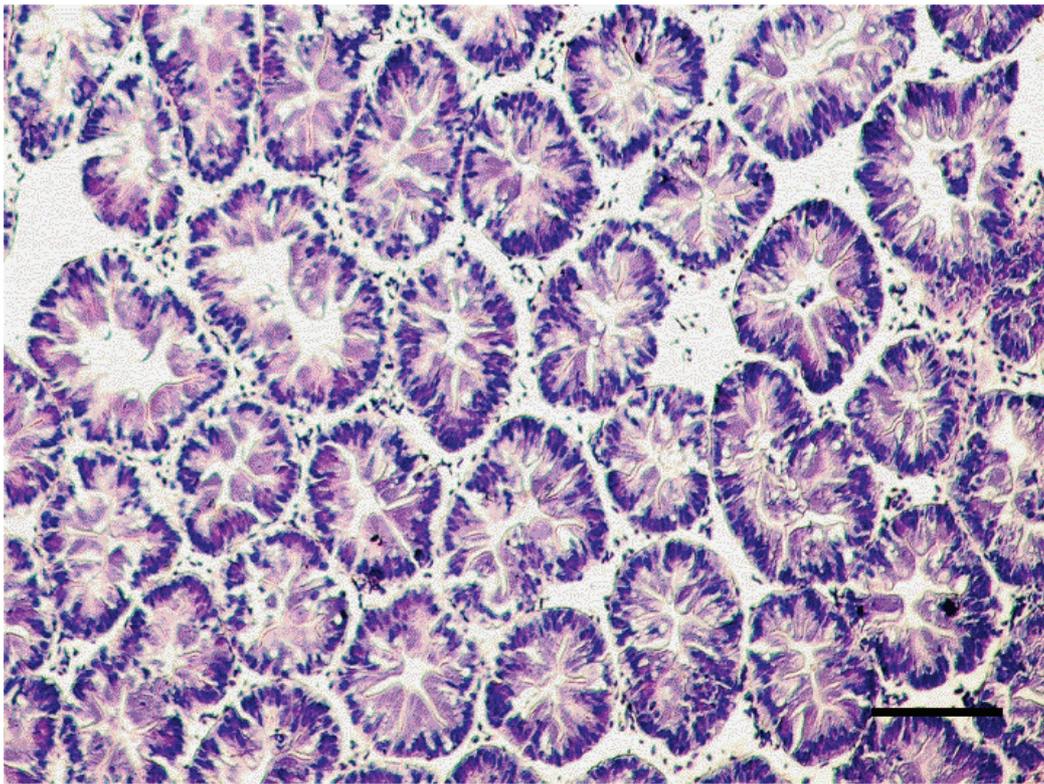
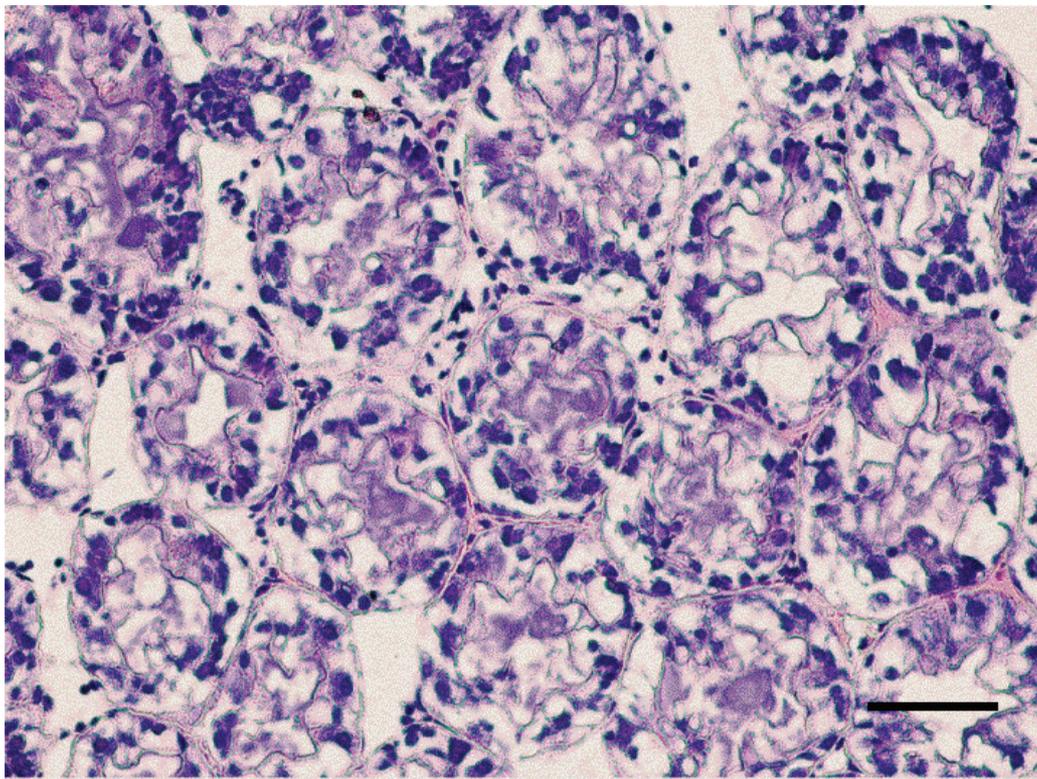


Figure 2. Hepatopancreatic tissue of the adult *M. nipponense* exposed to 1.0 mgSb/L for 14 d. Note the degeneration of the lumen; impairment of structural integrity can also be seen. (bar = 0.1mm).



**Figure 3.** Hepatopancreatic tissue of adult *M. nipponense* exposed to 4.0 mgSb/L for 14 d. Note the severe damage to the tubules, pronounced hypertrophy of the epithelia cells were also observed. (bar = 0.07mm).

topancreas is assumed to be functionally equivalent to the mammalian liver and pancreas and is responsible for major metabolic events, including enzyme secretion, absorption and storage of nutrients, molting, and vitellogenesis [22, 23]. The hepatopancreas is essentially composed of branched tubules and different types of epithelial cells (E-, R-, F-, and B-cells) lining the tubules. It is likely that exposure to a harmful chemical, such as copper sulfate, would be reflected in alterations in the structures of tubules and epithelial cells [24]. Histological alterations have been characterized in freshwater shrimp, such as *M. malcolmsonii* and various species of *Macrobrachium*. They are exposed to various chemicals, such as copper, chromium, cadmium, and zinc [25]. Several such structural alterations were noted in the hepatopancreatic tubules of treated *M. nipponense* that had been exposed to antimony in the present study.

#### 4. Conclusions

We determined the acute toxicities of antimony to *M. nipponense* in the form of LC<sub>50</sub> values. After short-term acute exposure, effects of antimony on oxygen consumption, and hepatopancreatic histopathology of *M. nipponense* were also demonstrated. Even exposure of *M.*

*nipponense* to lower levels of antimony can result in such deleterious changes, and our finding suggests that antimony is a potential pollutant in aquatic environments, although limited knowledge on the adverse effects of antimony on aquatic animals has been reported to date.

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