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Short Communication

Effects of sodium hypochlorite (NaOCl) on Asian tiger mosquito larvae (*Aedes albopictus*): acute toxicity and anal gill histology

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ABSTRACT

The current work is an investigation of the acute toxicity and periodic pathological status in fourth instar larvae anal gills of Asian tiger mosquito, *Aedes albopictus* exposed to various sodium hypochlorite (NaOCl) levels over a 24-h test period. The static renewal test method of acute lethal concentrations determination was used, and water temperature was maintained at 24.0 ± 0.5 ºC. NaOCl data obtained during acute toxicity tests were determined using the probit analysis method. The median lethal concentrations (8-h, 12-h, and 24-h LC_{50}) of NaOCl for *A. albopictus* larvae were estimated as 6046.848, 3075.075, 671.423 mg/L, respectively. *A. albopictus* larvae were exposed to 100, 200, and 400 mg/L of NaOCl for lethal toxic tests. Anal gills of exposed *A. albopictus* larvae frequently exhibited shrinkage of epithelium, rupture of tracheoles, and poor-organized cuticle and pathological changes became more pronounced with increasing concentrations.

KEYWORDS: sodium hypochlorite, *Aedes albopictus*, LC_{50}, tracheole, bioindicator.

INTRODUCTION

Sodium hypochlorite (NaOCl) has long been used as a traditional fungicide [1]. However, it has been extensively used as a disinfectant during the COVID-19 outbreak [2]. NaOCl is also widely used as an essential chemical for the industrial manufacture of electronic devices, including integrated circuits, electroplate, and photoelectric appliances [3]. NaOCl and other hazardous wastes are released into the environment by manufacturing processes, such as etching and cleaning operations [4]. Meador and Carlisle [5] suggested that high chloride levels are harmful to many stream fish species of the United States. However, fish are less sensitive to chloride exposure than small zooplankton [6]. The pollutant levels in aquatic systems can directly influence the diversity and abundance of mosquito species [7], but there is limited knowledge on the adverse effects of chloride on mosquito.

Aedes albopictus, the Asian tiger mosquito (Culicidae), is native to the tropical and subtropical areas of Southeast Asia. However, *A. albopictus* has spread to South Korea, East Africa, Europe and United States through the transport of goods and international travel during the past few decades [8]. *A. albopictus* is an epidemiologically important vector for the transmission of many viral pathogens, including the yellow fever virus, Zika virus, and Chikungunya fever [9]. Furthermore, *A. albopictus* is considered to be a potential vector for dengue fever and dengue hemorrhagic fever transmission among humans [10].

Malpighian tubules, rectum, and anal gills could be major sites of acid/base homeostasis as has been

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shown in mosquito larvae. In hypertonic solutions of NaCl, water is extracted from the anal gills and the body of the larva shrinks [11]. Furthermore, some papers reported that high levels of chemicals can induce adverse effects in the anal gills of mosquito larvae, and these biological effects may serve as indicators of environmental pollution [12].

The purpose of this study was to assess under laboratory conditions the toxic effects, acute toxicity, and anal gill histopathology in Asian tiger mosquito larvae exposed to various levels of sodium hypochlorite.

MATERIALS AND METHODS

Animal maintenance and chemicals

Asian tiger mosquito larvae (*Aedes albopictus*) were obtained from Tainan county, Taiwan. *A. albopictus* fourth instar larvae were transported to our laboratory and placed in a covered (dark netting) 10-L glass aquarium filled with dechlorinated tap water (with a pH of 7.4~8.1, dissolved oxygen (DO) of 3.0~5.7 mg/L, and hardness of 41~55 mg $CaCO_3$/L). The temperature was maintained at 24.0 ± 0.5 ºC. *A. albopictus* larvae (fourth instar, 0.52 ± 0.06 cm in body length) were used for all toxicity tests. Sodium hypochlorite powder (NaOCl) was purchased from Sigma (St. Louis, MO, USA). Stock solutions were prepared in deionized water. Chloride levels were determined using a chloride detecter (6742, Ezdo, Tainan, Taiwan).

Acute toxicity

Laboratory static renewal tests [13] were conducted to determine the median lethal concentration (LC_{50}) for *A. albopictus* larvae (fourth instar). Ten animals of similar size were randomly sampled and placed in 50-ml glass tubes which were covered with a dark net. After 2 h of acclimatization, *A. albopictus* larvae were exposed to different NaOCl concentrations (0, 500, 1000, 2500, 5000, and 10000 mg/L) for 24 h or more. Test solutions were renewed six times per day with freshly prepared chloride-spiked water throughout the exposure period. The control and each treated group were run in duplicate. During the experiment, dead animals were removed, and mortality was recorded after 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 24 h of exposure. The LC_{50} of every test chemical and their 95% confidence limits for *A. albopictus* larvae were calculated using a Basic program from the probit analysis described by Finney [14].

Sublethal toxicity

In the sublethal study, groups of five larvae of similar size (fourth instar of *A. albopictus* larvae) were randomly sampled and placed in 50-mL glass tubes, which were covered with a dark net. Larvae were then exposed to test solutions of 0.0, 100, 200, and 400 NaOCl mg/L in triplicate [15]. After 24 hours of exposure, six animals per exposure level were anesthetized with MS-222 (Sigma Chemical, St. Louis, MO, USA) and examined under a dissecting microscope (SMZ 745, NIKON, Tokyo, Japan).

RESULTS

Larval mortality was recorded for all exposure durations and various concentrations of NaOCl. Results demonstrated a positive relationship between the mortality rates of the exposed *A. albopictus* larvae and the concentrations of NaOCl in the test solutions (Table 1). No mortality was observed in the control groups during the experiments, and no mortality was observed in the group of *A. albopictus* larvae exposed to 500 mg/L during entire experiment. 8-h LC_{50} of the *A. albopictus* larvae was determined to be 6046.848 (3375.699-10831.65) NaOCl mg/L; 12-h LC_{50} of the *A. albopictus* larvae was determined to be 3075.075 (1339.603~7058.878) NaOCl mg/L; and 24-h LC_{50} of the *A. albopictus* larvae was 671.423 (139.784~3225.044) NaOCl mg/L (Figure 1).

Sublethal levels (100, 200, and 400 mg/L) of NaOCl were equivalent to approximately 15%, 30%, and 60% of the 24-h LC_{50} value (671.423 mg/L) for 12-h toxicity testing. Therefore, at these exposure levels no mortality was recorded during the entire experimental period. The current microscopic analysis clearly indicates that NaOCl caused histopathological alterations in anal gill region of the fourth instar larvae of *A. albopictus*. *A. albopictus* larvae from the untreated groups displayed four shuttle-shaped anal gills and well-organized anal gill cells with accentuated tracheoles (Figure 2A). *A. albopictus* larvae exposed to 100 NaOCl mg/L for 12 hours showed normal shuttle-shaped anal gills with slight shrinkage of

Table 1. Effect of median lethal concentrations (LC_{50}) of NaOCl on Asian tiger mosquito larvae (*Aedes albopictus*).

8 h-LC_{50} (mg/L)	16 h-LC_{50} (mg/L)	24 h-LC_{50} (mg/L)
6046.848	3075.075	671.423
(3375.699~10831.65)	(1339.603~7058.878)	(139.7839~3225.044)

The 95% confidence limits are given in parentheses.

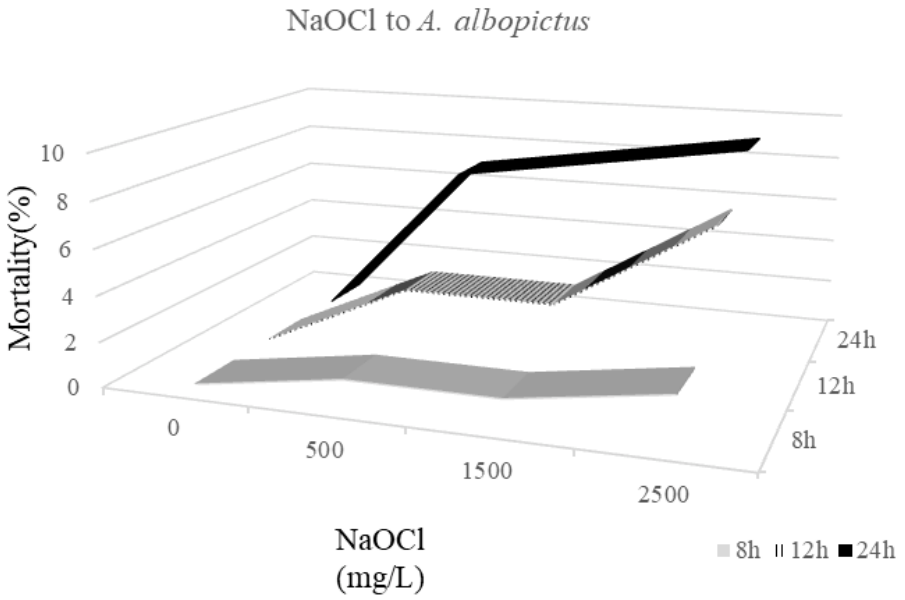

Figure 1. Lethality curves: mosquito larvae (*Aedes albopictus*) exposed to various NaOCl levels (mg/L) within 24 h.

the epithelium and ruptured tracheoles (Figure 2B). The histological analysis of *A. albopictus* larvae exposed to 200 NaOCl mg/L for 12 hours showed shrinkage of the epithelium and some tracheole damage (Figure 2C). *A. albopictus* larvae exposed to 400 NaOCl mg/L for 12 hours displayed bar-shaped anal gills, shrinkage of epithelium, and ruptured tracheoles (Figure 2D). Shrinkage of epithelium became more pronounced with increasing concentrations, leading to deformation of shuttle-shaped anal gills after treatment with 400 NaOCl mg/L for 12 hours. Rupture of anal gill tracheoles was also observed.

DISCUSSION

It is clear that the higher the concentration, the shorter the median lethal concentration of NaOCl to *A. albopictus*. The 48-h LC_{50} value of chloride for water flea, *Daphnia magna*, was estimated to be 3630 mg/L, indicating that *D. magna* is more tolerant to chloride exposure than *A. albopictus* larvae. Further, the 24-h LC_{50} value of chloride for the rotifer, *Brachionus calyciflorus*, was estimated to be 1645 mg/L and this species was found to be more tolerant to chloride exposure than *A. albopictus* larvae [12]. Romi *et al*. [16] revealed growth retardation and high mortality of *A. albopictus* larvae after exposure to 0.5~1.0 copper mg/L, and that the upper end of this range of 1.0 copper mg/L killed all larvae. Reza and Ilmiawati [17] also reported the efficacy of copper at <1 mg/L to kill *A. albopictus* larvae. Comparing the toxicity of sliver was found to be 24-h LC_{50}: 141 mg/L for the same species [18]. The above referenced studies collectively demonstrate that the toxicity of NaOCl to *A. albopictus* larvae is not

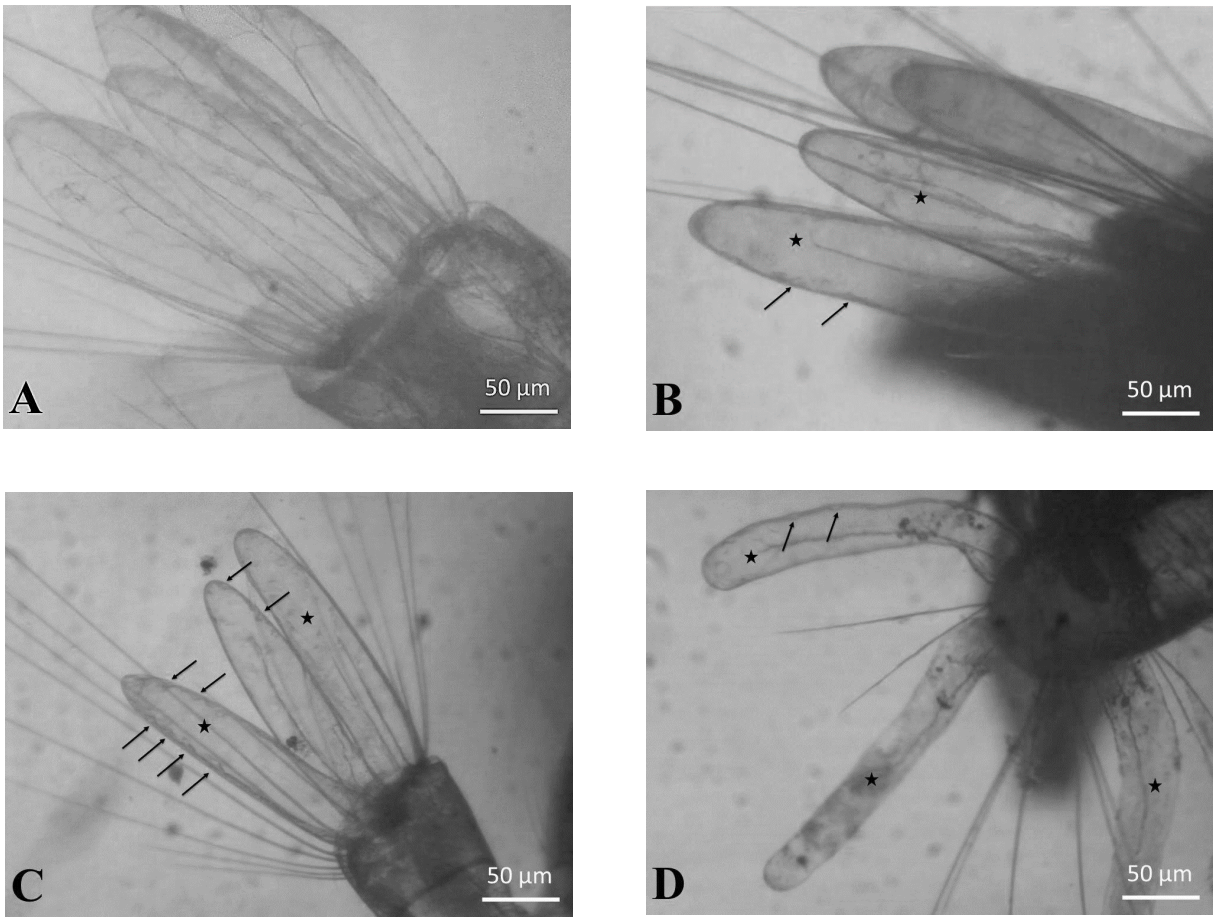

Figure 2. Alterations of mosquito larvae (*Aedes albopictus*) anal gills after 12 h. The control group (A) shows four shuttle-shaped anal gills, and well-organized anal gill cells with accentuated tracheoles. In the 100 NaOCl mg/L group (B), note slightly shrunken epithelium (arrows) and ruptured tracheoles (stars). In the 200 NaOCl mg/L group (C), note shrunken epithelium (arrows) and rupture of some tracheoles (stars). In the 400 NaOCl mg/L group (D), note bar-shaped anal gills, shrunken epithelium (arrows) and ruptured tracheoles (stars). (bar = 50 µm).

stronger than that of copper and nano-particles of silver.

The anal gills of mosquito larvae were surrounded by a thick permeable cuticle and the inner epithelial layer consisted of anal gill cells. Anal gills, also called tracheal gills, are regarded as one of the respiratory organs in mosquito larvae. Our microscopic examination has shown no injury to the anal gill cuticle of dead *A. albopictus* larvae, but shrinkage of the epithelium and tracheole damage was observed in all NaOCl exposure groups, ultimately leading to larval death. A high level of ambient chloride could harm freshwater animals by interfering with the osmoregulatory processes they use to maintain a suitable salt level in their body fluids [19]. Donini *et al*. [20] confirmed that the anal gills of yellow fever mosquito larvae, *Aedes aegypti*, serve as the major site for Na^+, Cl^- and K^+ uptake. Perumalsamy *et al*. [21] has revealed that pellitorine may disturb the Na^+, Cl^-, and K^+ co-transport system mainly by the degeneration of the anal gills of *A. aegypti* larvae. However, little information is available regarding the NaOCL-induced acute toxicity and histopathology in anal gills of *A. albopictus* larvae.

CONCLUSION

In conclusion, the present study may contribute to our understanding of chloride-induced respiratory obstruction and histopathological alterations in the

anal gills of *A. albopictus* larvae. We propose that NOCl in liquid form is a promising candidate due to its potent mosquito larvicide properties in a laboratory setting of our study. Additional studies of oxygen consumption and osmoregulatory analyses of the anal gills are required to follow-up on the present study in order to more fully understand the adverse effects of chloride on *A. albopictus* larvae. *A. albopictus* is also a dominant species in freshwater environments near semiconductor manufacturing districts in Tainan, Taiwan, and these larvae have the potential for serving as a useful biological indicator for evaluating local freshwater quality.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest of personal and financial nature.

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