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Acute Toxicity of the Pesticides Chlorpyrifos and Atrazine to the Chinese Mitten-handed Crab, *Eriocheir Sinensis*

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Pesticides are widely used to minimize the damage by insect in agriculture. However, they also have significant adverse effect on the natural environment, for example, they have obvious effect on the non-target aquatic organisms through surface runoff and sub-surface drainage channels from the treated area, including those commercially cultured important fish and crustaceans (Varó et al. 2000; Key et al. 2003).

Chlorpyrifos [O,O-diethyl-O-(3,5,6-trichloro-2-pyridil) phosphorothioate] is an organophosporus insecticide used extensively in agriculture throughout the world. Evidence shows that it is highly toxic to some groups of aquatic organisms (Marshall and Roberts 1978; Barron and Woodburn 1995; Varóet al. 2000). Atrazine (6-chloro-N-ethyl-N'-isopropyl-1,3,5-triazine-2,4-diamine), a triazine chemical, is widely used as a selective herbicide to control grass and broadleaf weeds in growing of rice, sorghum, corn, beans, peas, rangeland, sugarcane and orchards. Studies on toxicity of atrazine to fish and crustacean have been conducted (Ward and Ballantine 1985; Solomon et al. 1996).

However, the toxicity of these two pesticides to the Chinese mitten-handed crab, *Eriocheir sinensis* H. Milne Edwards, an economically important cultured species especially in Asian countries like China, has not been investigated. We conducted this study to evaluate the acute toxicity of chlorpyrifos and atrazine to adult, juvenile and embryonic *E. sinensis*. Such a study will help us better understand the effects of these pesticides on this crab species.

MATERIALS AND METHODS

E. sinensis were collected from a local farm and brought to the bioassay laboratory. They were acclimatized for 7 days in 300-liter polyethylene containers. The temperature was adjusted to 23±1°C, and the photoperiod was 12 hr L:D cycle. During this period, crabs were fed with fresh clam, *Anodonta fluminea* Heude. The water in the tank was replaced daily and was aerated mechanically. The mortality of the stock crabs was lower than 1%. Juvenile crabs in intermolt were classified as Juvenile I (0.50±0.08g) and Juvenile II (1.07±0.11g) based on their weights. Juvenile crabs and adult crabs (71.3±8.1g) were kept respectively in

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10L and 100L aquaria for 7 days as acclimatizing conditions. The dechlorine tap water was used to acclimatize crabs and conduct tests. Embryos (Stage prezoea with the characteristics of oval eyes and rapid heartbeats) were examined and excised from the gravid females with a brush and a pair of forceps under a stereomicroscope. Embryos from several females were pooled for the following tests.

The organophosporus chlorpyrifos (97% purity, Baolin Chem. Ltd. Co.) and herbicide atrazine (97% purity, Ruize Pesticide Ltd. Co.) were dissolved in acetone. The acute median lethal concentration (LC50) for chlorpyrifos was determined in the laboratory using the semi-static method. The following nominal concentrations were used based on the preliminary data, chlorpyrifos: 19.7, 29.6, 44.4, 66.7, 100μg/L for Juvenile I, 35.4, 50.0, 70.7, 100.0, 141.4, 200μg/L for Juvenile II and 99.2, 121.4, 148.4, 181.5, 222.0, 333.0μg/L for Adult Crabs, respectively; atrazine: 5.0, 7.1, 10.0, 14.1, 20.0mg/L for juvenile crabs, 9.8, 14.8, 22.2, 33.3, 50.0mg/L for adult crabs, respectively. The container receiving only acetone served as the control. Ten animals were used in each of the two replicates. Used solutions were discarded with a siphon and replaced by fresh ones of the same concentration at 24-hour intervals and the whole process lasted 96 hours. No food was supplied during the experiments. Mortalities were monitored every 24 hours of exposure, and temperature (°C), pH and dissolved oxygen (mg/L) of the test solutions were measured daily.

An evaluation of acute toxicity to *E. sinensis* embryos was made in 24-well Corning multiwell plates (Key et al, 2003). One embryo of Stage prezoea and 2mL of pesticide solution were added into each well, and then the covered plates were incubated in an orbital shaker (60 rpm, 20°C, 25‰ salinity) in a 24-hr dark cycle. Test solutions were prepared using artificial seawater (Instant sea salt, Salt Industry Co., Ningbo, China) filtered with 0.2 μm filters and a required amount of stock solution. The blank control and carried control were seawater with or without acetone respectively. Toxic nominal concentrations were: 3.1, 6.3, 12.5, 25.0, 50.0, 100.0μg/L for chlorpyrifos and 1.25, 2.50, 5.00, 10.00, 20.0mg/L for atrazine. After 96 hours, surviving embryos were allowed to hatch.

Median lethal concentrations (LC50) with 95% confidence limits were determined by a probit analysis (Finney 1971). A Kruskal-Wallis nonparametric one-way ANOVA was performed followed by the Dunn's method to determine if the effects of each insecticide on embryo hatching time were significant ($p<0.05$).

RESULTS AND DISCUSSION

The water quality did not vary significantly during the test. The mean temperatures were 23.0±1°C and dissolved oxygen remained above 7.2±0.4mg/L in the juvenile and adult tests. The mean pH of the test solutions was 7.03±0.26. Temperature (°C), pH and dissolved oxygen (mg/L) of the embryo test solutions were 19.9±0.1°C, 8.12±0.05, and 8.5±0.1mg/L, respectively. Although the actual chlorpyrifos concentrations in the water could not be measured during the tests, it

was suggested that a constant chlorpyrifos concentration in test solution could be kept during the exposure by the method of daily renewal (Varó et al. 2000).

Table 1. LC50 values of chlorpyrifos to *E. sinensis* in renewal acute toxicity tests

Rep. no.	LC50 (95% confidence limit) (μg/L)			
	Adults	Juveniles II	Juveniles I	Embryos
1	142.2 (122.0-162.6)	75.9 (60.3-94.2)	22.9 (17.4-27.5)	54.7 (7.4-109.0)
2	143.9 (130.2-162.6)	78.50 (64.1-95.7)	24.4 (16.7-30.2)	28.2 (6.2-61.4)

In adult *E. sinensis*, mortality induced by the chlorpyrifos exposure first occurred within 24 hours in the two highest concentrations and all individuals died within 96 hours. In Juvenile II *E. sinensis*, mortality induced by the chlorpyrifos exposure first occurred within 24 hours in the three highest concentrations, and complete mortality occurred after the 48 hours of exposure in the highest concentration. In Juvenile I *E. sinensis*, however, the mortality did not occur until 24 hours of exposure in the two highest concentrations, and complete mortality occurred after 72 hours of exposure in the highest concentration. Although the embryos' mortality from the chlorpyrifos exposure occurred within 24 hours, significant death occurred only after 96 hours, and thus only 96 hr LC50 values could be calculated, while LC50 values for the crabs in the other stages were calculated over all the intervals. These results indicated that the toxicant activity in embryos appeared more slowly than that in other life history stages.

For adult crabs, a 24 hr of LC50 for chlorpyrifos was obtained (460.9 μg/L) but with a large 95% confidence limit (316.36-100544.09 μg/L). The LC50 values for crabs decreased with exposure time (Figure 1). This indicated that the sensitivity of crabs to chlorpyrifos increased gradually during the exposure. In the 96 hour chlorpyrifos toxicity test, adult crabs (LC50: 143.02 μg/L) were significantly less sensitive to chlorpyrifos than juveniles and embryos. Juveniles I crabs were relatively sensitive to chlorpyrifos regarding the lethal toxicity (Table 1). For different life history stages, the sequence of sensitivity to chlorpyrifos was: Juveniles I > embryos >Juveniles II >adults. This result was, however, different from that found by other authors (Lund and Fulton 1999; Key et al. 2003) who suggested that embryo grass shrimp were the least sensitive to this compound and other pesticides. They attributed the low embryo toxicity to the embryonic coat, developed phase I monooxygenases and the immature embryonic nervous system. In the present study, the relatively high level of sensitivity of embryo *E. sinensis* to chlorpyrifos may be due to the permeability of embryonic coat. The embryo *E. sinensis* are in the Stage prezoea charactered by oval eyes, body movement and rapid heartbeat, which are similar to those of the embryo grass shrimp of Stage VI used elsewhere (Lund and Fulton 1999; Key et al. 2003).

We also found that the resistance to chlorpyrifos in *E. sinensis* of all the life

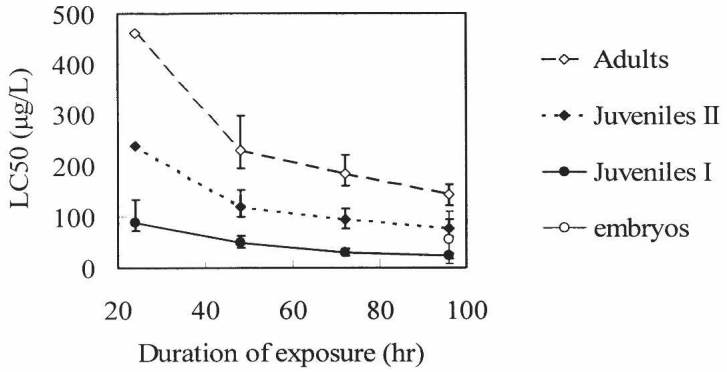

Figure 1. LC50 values and 95% confident limit for the three different groups of the crab *E. sinensis* exposed to chlorpyrifos.

history stages except for the embryos was body weight (age) dependent (Table 2), which are consistent with the results of adult grass shrimp exposed to chlorpyrifos (Lund and Fulton 1999). The aquatic organisms are exposed to the aquatic environment and absorb harmful compounds from the ambient water through the gills. The younger animals have relatively more surface area than adults, which may allow greater uptake of harmful compounds into body. On the other hand, the adult crabs maybe have a better metabolic system than the juvenile crabs, which can allow the adult crabs to effectively metabolize and eliminate pesticides.

Table 2. Linear regression analysis of weight and mortality of *E. sinensis* exposed to chlorpyrifos.

Duration of exposure（hr）	Mortality rate= a*lg (Body weight)+b	R^2
24	y = 166.08x + 162.52	0.9670
48	y = 78.98x + 87.26	0.9535
72	y = 63.25x + 63.33	0.9482
96	y = 52.35x + 50.46	0.9518

Previous studies on toxicity of organophosporus to aquatic fauna show great variability in both toxicity of different pesticides and sensitivity of different species (UNEP 1991). The toxicity of chlorpyrifos to *E. sinensis* has not yet been reported. In other studies with grass shrimp using the similar protocol, toxicity of chlorpyrifos to crustacean was significantly higher than many other insecticides such as malathion, azinphosmethyl, fipronil and endosulfan. Different levels of chlorpyrifos sensitivity were determined for some fish and macrocrustaceans. The most sensitive species of crustaceans recorded was *Mysidopsis bahia* with a 96 hr LC50 value of 0.035μg/L (Marshall and Roberts 1978), while the most sensitive species of marcocrustaceans was the *Procambarus acutus acutus* which had a 96 hr LC50 value of 2.00 μg/L (Carter and Graves 1973). Data for acute toxicity of chlorpyrifos to other crabs (*Oziotelphusa senex senex*) reported by

Radhakrishnaiah and Renukadevi (1990) indicated that they were in the same order of magnitude as those assessed in the present study of *E. sinensis*.

No mortality of juvenile and adult crabs induced by the atrazine exposure occurred during the whole exposure period. For embryos, the mortality did not exceed 27.1% at the blank control and carried control. Although embryo mortality from the atrazine exposure was calculated as 43.8% at 5mg/L, there was no significant difference between the embryos exposed to atrazine and the controls. Thus, the LC50 values for juvenile, embryonic and adult crabs were reported to be higher than 20, 20 and 50 mg/L, respectively. The LC50 values for atrazine indicated that *E. sinensis* of all the four life history stages considered in this study were relatively insensitive to atrazine with respect to the lethal toxicity. Unlike chlorpyrifos, the reported data suggested that atrazine had little acute toxicity to most macrocrustaceans. Of those found in the literature, *Eurytemora affinis* are the most sensitive species of crustacean to the atrazine exposure with a 96 hr LC50 value of 0.125 mg/L (Forget-Leray et al. 2005). Ward and Ballantine (1985) working on fiddler crab *Uca pugilator* found a 96 hr LC50 of >29 mg/L for atrazine, whereas Portmann and Wilson (1971) determined the 48 hr LC50 values for the same pesticide of >10 mg/L for shore crab (*Carcinus maenas*). These results were close to our data derived in the present study of *E. sinensis*.

The hatching time of stage prezoea exposure to different concentrations of chlorpyrifos was longer than that for the control group (Figure 2). Statistically, the hatching time was significantly higher only for the concentrations of 6.3 and 12.5μg/L. The research with grass shrimp embryos found 96-hr of exposure to all concentrations of chlorpyrifos except for the two lowest levels did increase the hatching time compared to the control group (Lund and Fulton 1999). In the study involving various pesticides, embryos exposed to the pesticides of different concentrations tended to have a significant increase in hatching time (Key et al. 2003).

Interestingly, the hatching time of embryos in Stage prezoea exposure to atrazine showed that the hatching time was shorter for each concentration than the controls except for in the concentration of 5 mg/L. Atrazine of the two highest concentrations tended to significantly increase the hatching time of embryos in Stage prezoea compared to the control. The decreased hatching time maybe influence the survival of newly-hatched larvae due to underdevelopment. The effect of atrazine on hatching of crustacean had not been reported so far. However, Rohr et al. (2004) found the exposure to 400ppb of atrazine decreased streamside salamanders, *Ambystoma barbouri* embryo survival and increased hatching time. The cause of atrazine shortening the hatching time of E. sinensis embryos is not clear yet. Although the hormone (i.e. 20-hydroxyecdysone) play an important role during the embryo development of crustacean and more latest reported findings suspected atrazine as an endocrine disrupting chemical. It is noteworthy that endocrine disruption by atrazine is invoked only in the vertebrate and especially in mammals. Atrazine does not appear to be of concern with respect to the endocrine disruption in aquatic invertebrate organisms based on the current state of

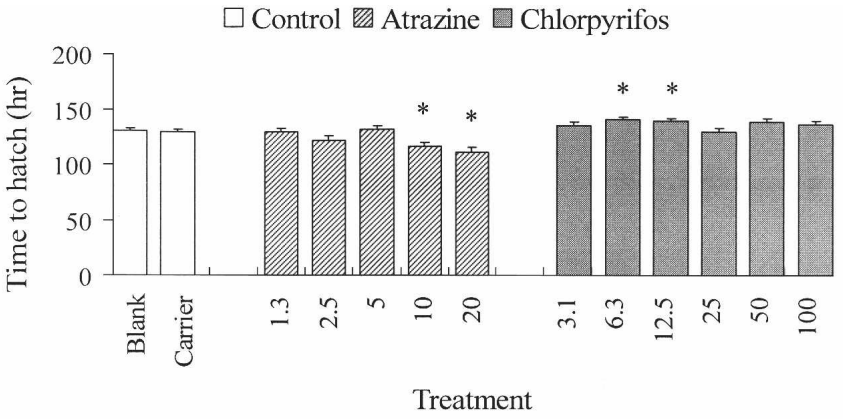

Figure 2. Mean time to hatch (hr) with standard error for *E. sinensis* embryos exposed to chlorpyrifos (μg/L) or atrazine (mg/L). *Significantly different to carrier control. $p<0.05$.

knowledge (Zou and Bonvillain 2004).

In China, environmental data of chlorpyrifos and atrazine are rare. Overseas occasional higher chlorpyrifos detections in agricultural areas, at concentrations between 1 and 30μg/L. Aquatic contamination may extend into the hundreds of μg/L in urban areas. Since it was introduced into China not long ago, serious aquatic organisms kill incidents associated with the use of chlorpyrifos in urban and agricultural areas have not been reported. However, its wide use in agricultural areas every year significantly increases the risk of having such incidents. As for atrazine, it was produced and used in the 1980s in China. Ren and Jiang (2002) found some rivers and reservoirs contaminated by atrazine with the maximum contamination level being 26.1μg/L. With such a level, aquaculture organism fatalities associated with the use of atrazine are unlikely. However, its high water solubility, persistence in the environment and accumulation in biological tissue may eventually carry over to human when contaminated crabs are consumed.

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