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Effects of Diazinon, Malathion, and Paraquat on the Behavioral Response of the Shrimp *Metapenaeus ensis* to Chemoattractants

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The wide use of various pesticides has provided their entry into the aquatic environment. The lethal effects of pesticides on aquatic animals have been extensively studied (e.g., Sanders and Cope 1966; Naqvi and Hawkins 1989). Pesticides, however, are generally present in levels much below the concentrations which can directly cause mortality. Thus studies on the sublethal effects of pesticides on growth and reproduction are crucial for a comprehensive understanding of their impact on aquatic environments.

Behavioral responses are useful indicators of pollution effects on aquatic animals (Eisler 1979). Foraging behavior of crustaceans depends upon chemoreception (Zimmer-Faust 1989), and impairment of this chemical sense could adversely affect feeding and growth. Chemical stimuli such as amino acids readily elicit crustacean feeding responses, which can easily be characterized and monitored (Atema and Stein 1974; Carr and Derby 1986; Johnson and Atema 1986; Harpaz *et al.* 1987a,b; Tierney and Atema 1988). Atema and Stein (1974) demonstrated that crude oil interferred with feeding behavior of lobster. Similarly, acid exposure inhibited behavioral responses of crayfish to chemical stimuli (Tierney and Atema 1986). Thus such responses can serve as indicators for the sublethal effect of pollutants on crustaceans.

The objective of the present study is to investigate the effects of the pesticides diazinon, malathion and paraquat on the behavioral responses of the shrimp, *Metapenaeus ensis*, to chemical stimuli. In Hong Kong and neighbouring areas of China, diazinon (*O,O*-diethyl *O*-2-isopropyl-4-methyl-6-pyrimidyl thionophosphate) and malathion (*O,O*-dimethyl dithiophosphate of diethyl mercaptosuccinate) are widely used organophosphorus insecticides. Paraquat (1,1'-dimethyl-4,4'-bipyridylium salt)

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is extensively used as a herbicide. *M. ensis* is one of the major shrimps harvested in Southeast Asia (Holthuis 1980). The shrimp has its nursery ground in salt marshes, some of which have been converted to shrimp ponds. Pollutants, such as pesticides, would not only reduce the recruitment of shrimp into the fishery but also directly affect cultured shrimp.

MATERIALS AND METHODS

Metapenaeus ensis were obtained from the local fish markets. Juveniles with a body weight of 2.0 to 4.5 g were chosen for study. Shrimp were transferred to a water table that was divided into compartments with perforated perspex plates. Each shrimp was housed in an individual compartment measuring 9.5 cm x 13.5 cm, and the water depth was kept at about 10 cm. The water was well aerated and its flow was maintained using a circulating and filtering system (EHEIM canister filter model 1034) with a flow rate of 2100 L hr^{-1}. The turnover time of seawater in the water table was about 5 min. Further, about 1/3 of the water was renewed every three days. Throughout the study period, the range of temperature and salinity of the seawater was 22-27°C and 30-34°/₀₀, respectively. Shrimp were fed daily with fresh oysters at a ration of about 30% of the body weight. The shrimp were maintained in the water table for at least two weeks before experiments were initiated.

Food was withheld for three days before shrimp were taken for experiments, as preliminary studies showed that starvation enhanced behavioral responses to chemical stimuli. The shrimp were transferred to individual glass aquaria (22 cm x 14 cm x 14 cm high) with 2.5 L of 20-μm filtered seawater containing pesticide at a level of 0.1 or 1.0 μg active ingredient (i.e., diazinon, malathion, or paraquat ion) per liter. Shrimp were exposed to the pesticide at 25 ± 1°C for 24 hr before being tested for reponses to an equimolar mixture of glycine and aspartic acid at a total concentration of 0.1 *M* (see below). Control shrimp were kept in aquaria containing filtered seawater alone. The walls of the aquaria were darkened to minimize disturbances to the animals. Shrimp which molted during the exposure period were not used for the test, as they might temporarily suspend feeding behavior during postmoult (Harpaz *et al.* 1987b). Water in the aquaria was renewed after exposure. Aeration was stopped 30 min prior to the test to allow shrimp to achieve a quiescent state. Filtered seawater was then added to the aquaria via a syringe pump through a micropipette at a rate of 2 mL min^{-1}. The point of introduction was no less than 15 cm from the shrimp and 3-4 cm above the bottom of

Table 1. Percentage of *Metapenaeus ensis* which exhibited various behavioral responses to 0.1 *M* mixture of glycine and aspartic acid. n = no. of test animals. Values for shrimp exposed to diazinon are significantly different from the corresponding values for the control ($P<0.05$, 2x2 Contingency Table).

	Perception	Displacement	Approach	Grasping	n
Control	97.1	85.7	80.0	65.7	35
Diazinon (0.1 $\mu g\ L^{-1}$)	60	50	50	40	20

the aquaria. After 15 min, the mixture of glycine and aspartic acid was introduced for another 15 min. The behavior of the shrimp was monitored throughout the test period and the response time for different behaviors (see below) was recorded with a stopwatch.

Metapenaeus ensis reacted to the amino acid mixture by exhibiting a sequential series of responses which were designated as perception, displacement, approach, and grasping the source of stimuli (Table 1). Perception was characterized by an increase in antennular flicking and appendage movement. Displacement occurred when the shrimp moved from its original position by less than one body length. Approach involved direct movement of the shrimp toward the source. Grasping of the source referred to the actual touching of the pipette tip by the shrimp with its feeding appendages. Similar behavioral responses to chemoattractants have been reported in other crustaceans (Carr and Derby 1986; Harpaz *et al.* 1987b; Costero *et al.* 1991).

Pesticides used were commercial grade. Diazinon was a product of Nippon Kayaku Company, Japan. Malathion and paraquat were products of Imperial Chemicals, Inc., U.K. The diazinon and malathion products contained 60 and 81% of active ingredients, respectively. The paraquat herbicide (brand name: Gramoxone), in the form of dichloride, was at a level of 200 g paraquat ion.L^{-1}. Preliminary studies showed that exposure of shrimp to a level of 0.1 or 1.0 μg diazinon, malathion, or paraquat ion per liter caused no mortality after 24-72 hr (K.H. Chu, unpublished data). A series of experiments testing for effects of solvent carriers in the insecticides were also conducted by exposing the animals to benzene, petroleum ether or xylene at a level of 1.0 $\mu g\ L^{-1}$ for 24 hr before behavioral experiments. No solvent control was needed for paraquat as the commercial product was aqueous based.

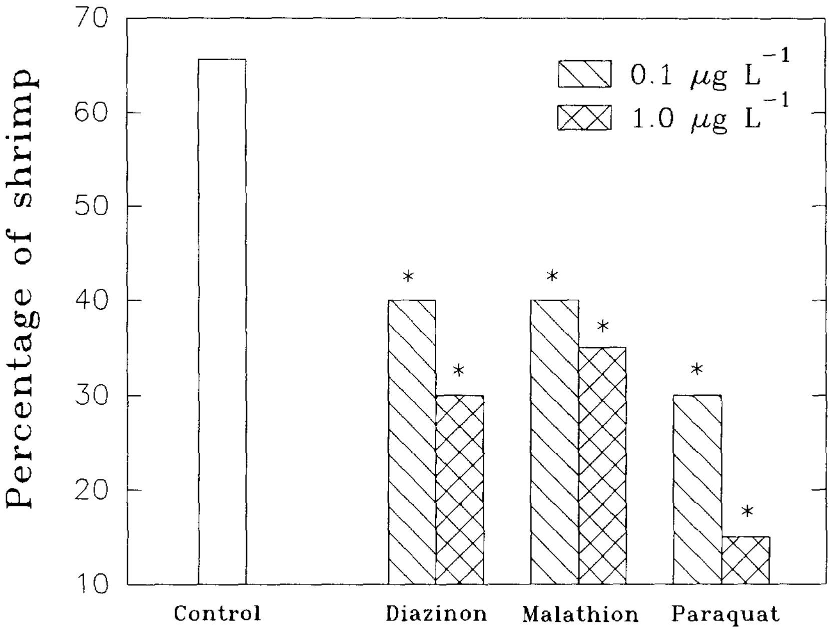

Figure 1. Effect of diazinon, malathion and paraquat on the percentage of *Metapenaeus ensis* that could grasp the source of chemoattractants. The number of test animals in control group is 35. The number in pesticide-exposed groups is 20. Asterisks indicate significant differences from the control group ($P<0.05$).

RESULTS AND DISCUSSION

Almost all control shrimp exhibited perceptive behavior in response to amino acids, and over 65% of the animals eventually grasped the source of chemoattractants (Table 1). In the control assays (by using filtered seawater alone) included in the test of each animal, less than 2% of the animals reached the source. The difference was significant ($P<0.01$, 2x2 Contingency Table; Zar 1984). To illustrate the effect of pesticides, Table 1 shows that only 60% of the shrimp exposed to 0.1 $\mu g\ L^{-1}$ of diazinon showed perceptive behavior and 40% grasped the source. The percentage of shrimp exhibiting each behavioral response was significantly lower than that in control group ($P<0.05$, 2x2 Contingency Table).

Since grasping the source of chemoattractants by a shrimp could be clearly defined and observed, the percentage of shrimp exhibiting this

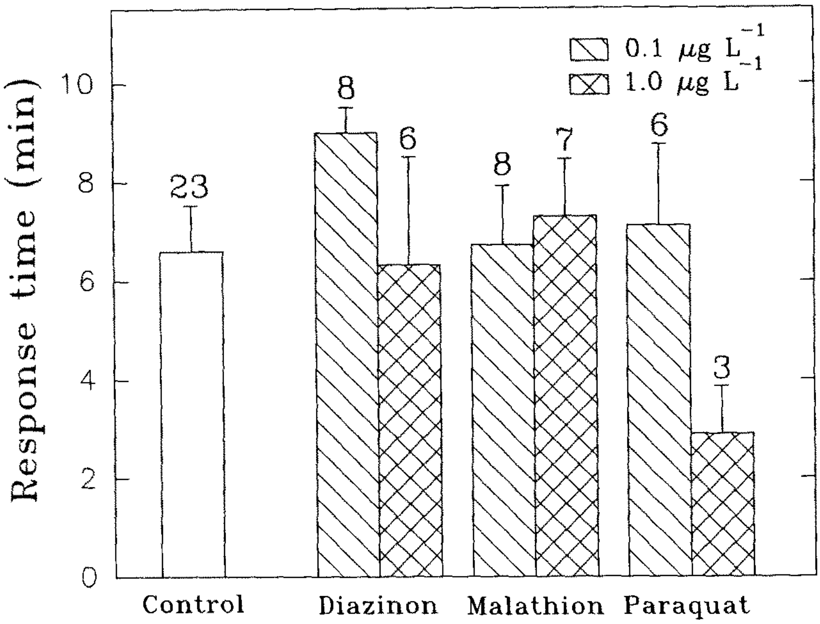

Figure 2. Time required for *Metapenaeus ensis* to reach the source of chemoattractants in different groups of shrimp. Error bars represent standard errors of the means. Number of shrimp that grasped the source is indicated above the error bar.

response and the response time were used as the parameters to compare the effects of pesticides with the control. Fig. 1 shows that 40% of the shrimp could grasp the source after exposure to 0.1 $\mu g\ L^{-1}$ of diazinon or malathion. In comparison, 30% of the animals exposed to the same level of paraquat reached the source. Both values were significantly different from that of the control animals ($P<0.05$, 2x2 Contingency Table). At a pesticide level of 1.0 $\mu g\ L^{-1}$, the percentage of shrimp that could locate the source was further reduced to 30, 35 and 15% for diazinon, malathion and paraquat, respectively. After exposure to benzene, petroleum ether, or xylene, the percentage of animals which could grasp the source was not significantly different from that of the control ($P>0.05$; data not shown).

For those shrimp which could grasp the source of chemoattractants, the time required for them to reach the source did not differ significantly among the different groups ($P>0.05$, 1-factor ANOVA; Zar 1984) (Fig. 2). This result indicates that the pesticides have little effects on this parameter, probably due to its high individual variation.

In acute toxicity tests, the LC_{50} values of paraquat to microcrustaceans such as cladocerans were in the order of mg L^{-1} (Sanders and Cope 1966). The values for diazinon and malathion were generally between 1 and 10 $\mu g\ L^{-1}$ (Sanders and Cope 1966; Goodman *et al.* 1988; Naqvi and Hawkins 1989). Bigger animals such as fish and shrimp are less susceptable (Trim 1987; Arias *et al.* 1991). The pesticide levels tested in the present study were lower than the reported LC_{50} values for microcrustaceans, yet such low levels of pesticides could still inhibit chemical induction of feeding in shrimp. It is likely that long-term survival and growth of the animal would then be affected. Reddy *et al.* (1986) showed that sublethal malathion level affects the carbohydrate metabolism of crustaceans. These studies demonstrate the importance of studies on the sublethal effects of pollutants. The present study has shown that pesticides can affect the behavior of shrimp in response to chemical stimuli. Further studies will aim to establish the use of this behavioral test as a sensitive assay to evaluate the sublethal effects of pesticides on crustaceans.

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