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Toxicity of the Organophosphorous Insecticide Metamidophos (O,S-Dimethyl Phosphoramidothioate) to Larvae of the Freshwater Prawn *Macrobrachium rosenbergii* (De Man) and the Blue Shrimp *Penaeus stylirostris* Stimpson

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Insecticides, while desirable for insect control, may become a matter of ecological concern where they affect nontarget organisms. Crustaceans are especially sensitive to insecticides, due to their close philogenetic relationship with insects (Williams and Duke 1979). Early larval stages of crustaceans may be even more sensitive to insecticide toxicity than adults (McKenny 1986).

The organophosphorous insecticide O,S-dimethyl phosphoramidothioate (Metamidophos, Tamaron, Monitor, Hamidop) is widely used for pest control in tropical crops. If washed down to streams and estuaries its residues could adversely affect populations of commercially important crustaceans, like those of the palaemonid prawn Macrobrachium rosenbergii and the penaeid shrimp Penaeus stylirostris. This paper presents information on the toxicity of O,S-dimethyl phosphoramidothioate to larvae of M. rosenbergii and P. stylirostris.

MATERIALS AND METHODS

Larvae of M. rosenbergii and P. stylirostris used in this study were hatched from laboratory reared broodstock at the School of Marine Science of the Monterrey Institute of Technology at Guaymas, Sonora, Mexico. Dilutions of the pesticide were prepared using Tamaron 600 (Bayer), an aquous solution containing 600 g/L of the active ingredient, and either seawater (salinity = 35 g/L, pH = 8.4) for tests with P. stylirostris, or a mixture of non-chlorinated tap water (salinity = 0 g/L, pH = 7.2) and seawater, to produce salinities of 15 and 2 g/L for M. rosenbergii larvae and postlarvae, respectively.

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All acute toxicity tests were conducted at a temperature of 28 ± 1 °C in 1-L beakers, filled with 500 mL of solution. Throughout the experiment 450 mL of solution were daily discarded from each beaker, and replaced promptly by a freshly prepared dilution of the pesticide (iterative testing). Gentle aereation was provided with 1-mL glass pippetes connected to an aquarium air pump. This was sufficient to keep dissolved oxigen above 85% saturation in all test containers.

Tests with P. stylirostris were conducted at a density of 20 organisms per beaker. Protozoea larvae were fed unicellular algae, Chaetoceros and Tetraselmis spp at a total concentration of 300,000 cells/mL. P. stylirostris mysis larvae were fed newly hatched Artemia at a density of 4 nauplii per mL of test media. In preliminary tests larval P. stylirostris were exposed to concentrations of 0.0, 0.1, 1.0, 10.0, 30.0, and 50.0 µg/L of the insecticide for a period of 48 hr. Final tests were conducted as outlined above, but at concentrations of 0.0, 0.001, 0.010, 0.10, 1.0, 2.0, and 5.0 µg/L for a period of 96 hr.

M. rosenbergii were tested at a density of 15 individuals per beaker and fed Artemia at a concentration of 5 nauplii per mL of test media. Preliminary tests were conducted at concentrations of 0.0, 0.001, 0.01, 1.0, 2.0, and 5.0 µg/L. Final tests were conducted at concentrations of 0.0, 0.0001, 0.001, 0.01, and 0.1 µg/L for a period of 96 hr.

All acute toxicity data were evaluated by Probit analysis, and the LC50's and 95 % confidence intervals were calculated using the graphical method described by Lichtfield and Wilcoxon (1949) and Matsumura (1975).

Sub-acute toxicity tests were conducted with M. rosenbergii in aereated 18-L glass carboys, using 15 L of test solution and 200 individuals per carboy. Organisms were exposed to concentrations of 0.00, 0.01, and 0.10 ng/L of the pesticide for a period of 33 d. Experimental animals were fed Artemia at a concentration of 5 nauplii per mL of test media. Experiments were conducted at salinities of 15 g/L for larvae and 2 g/L for early postlarvae, in algae-free "clearwater" as well as in phytoplankton-rich "greenwater", consisting of Chaetoceros sp at a density of 350,000 cells/mL. Test water was changed daily in all containers. Concentrations of ammonia and nitrites were determined daily in controls following the indophenol and sulphanilamide methods described by Boyd (1981). pH was measured daily with a Corning 7 (Chicago, Illinois) potentiometer. Survival and percent

of the population in each larval stage were monitored weekly, following the larval stage descriptions of Uno and Soo (1969).

RESULTS AND DISCUSSION

The 12, 24, and 36-hr LC5Ø's of Metamidophos to larval P. stylirostris and their corresponding 95% confidence intervals are presented in Table 1. Nauplii showed the greatest sensitivity to the insecticide, with a 24-hr LC5Ø of 1Ø ng/L, followed by protozoea and mysis larvae, with 24-hr LC5Ø's of 85 and 16Ø ng/L, respectively. The 12, 24, and 36-hr LC5Ø's and their corresponding 95 % confidence intervals generally differed by one order of magnitude.

Toxicity data for M. rosenbergii are shown in Table 2. Zoea IV and VII were extremely sensitive to the pesticide, with 24-hr LC5Ø's of Ø.48 and Ø.5Ø ng/L, respectively. Zoea I and early postlarvae were more resistent, with 24-hr LC5Ø's of 24 and 42 ng/L, respectively.

The LC5Ø's of Metamidophos to larvae of P. stylirostris and M. rosenbergii found in this study are well below the corresponding values found for other insecticides with larvae and postlarvae of decapod crustaceans; i.e., Summer and Eversole (1978) reported a 24-hr LC5Ø of 1Ø4 µg/L Mirex to M. rosenbergii postlarvae; and McKenney (1986) reported that the 96-hr LC5Ø value for Lindane to larvae of the mud crab Euripanopeus depresus was Ø.66 µg/L. This shows that Metamidophos is more toxic to crustaceans than other insecticides previously tested.

In tests with M. rosenbergii through its complete larval cycle increasing concentrations of Metamidophos reduced survival and slowed development (Fig. 1). Lengthening of the larval development period has been observed by Brookhout et al. (1972) for the crab Rhithropanopeus harrisi when exposed to sublethal concentrations of the chlorinated insecticide Myrex. Similarly, McKenney (1986) reported that continual exposure to a sublethal concentration of the pyrethroid insecticide Fenvalerate (1.6 µg/L) delayed completion of metamorphosis by 2 d for developing larvae of the grass shrimp Palaemonetes pugio. McKenny (1986) reported reductions in survival of estuarine mysids Mysidopsis bahia through a complete life cycle as responses to subacute exposures to Fenthion and Thiobencarb (organophosphorous and carbamate insecticides, respectively), and suggested that extension of the larval phase in crustaceans, as a response to subacute concentrations of pesticides in

Table 1. Calculated LC5Ø's of Metamidophos to larval shrimp Penaeus stylirostris exposed for 12, 24 or 36 hr.

Larval stage	Time (hr)	LC5Ø (ng/L)	95% Confidence Intervals Lower	Upper
Naupliae	12	16Ø.Ø	1ØØ.Ø	256.Ø
	24	1Ø.Ø	8.3	12.Ø
	36	Ø.6	Ø.3	1.Ø
Protozoea	12	3ØØ.Ø	1Ø4.Ø	866.Ø
	24	85.Ø	26.Ø	274.Ø
	36	2.4	2.Ø	3.Ø
Mysis	12	8ØØ.Ø	392.Ø	1632.Ø
	24	16Ø.Ø	5Ø.Ø	453.Ø
	36	8.Ø	7.Ø	9.Ø

Table 2. LC5Ø's of Metamidophos to larval and postlarval prawn Macrobrachium rosenbergii exposed for 12, 24, 48, or 96 hr.

Stage	Time (hr)	LC5Ø (ng/L)	95% Confidence Intervals Lower	Upper
Zoea I	12	--	--	--
	24	24	11	54
	48	8	2	32
	96	4	1	11
Zoea IV	12	Ø.99	Ø.38	2.5
	24	Ø.48	Ø.24	Ø.97
	48	Ø.28	Ø.15	Ø.5Ø
	96	Ø.22	Ø.13	Ø.37
Zoea VII	12	Ø.75	Ø.47	1.1Ø
	24	Ø.5Ø	Ø.36	Ø.7Ø
	48	Ø.46	Ø.29	Ø.7Ø
	96	Ø.38	Ø.19	Ø.74
Postlarvae	12	--	--	--
	24	42	26	65
	48	3Ø	14	64
	96	2Ø	12	34

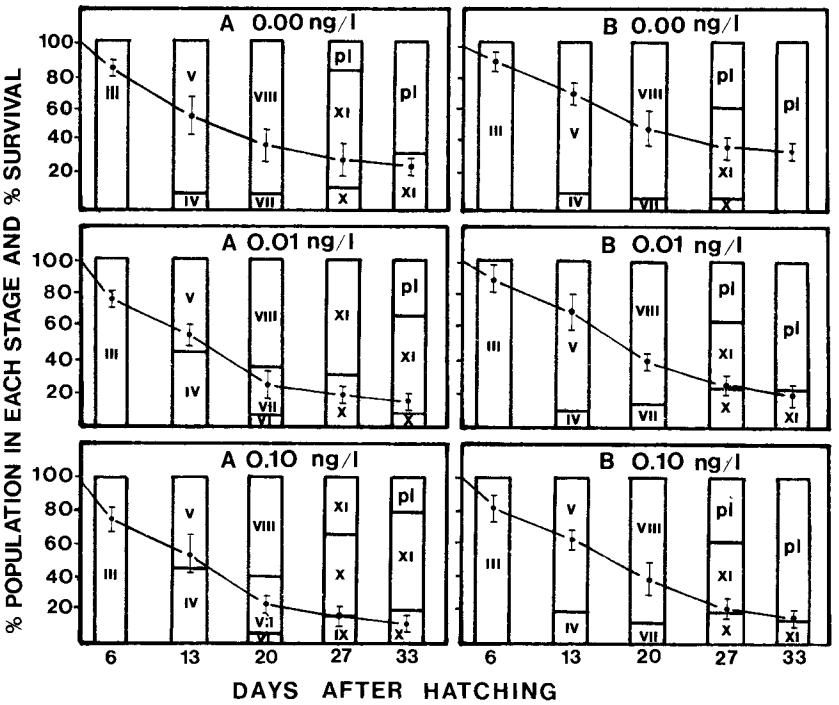

Figure 1. Larval stage composition (roman numerals) and survival (mean and 95% confidence interval) of Macrobrachium rosenbergii exposed to sub-acute concentrations of Metamidophos for 33 d in two test environments: (A) clearwater and (B) phytoplankton-rich "greenwater". Data represent means of 3 replicates (PL=postlarvae).

the environment, may increase predation pressure on the species and reduce the number available for recruitment into adult populations.

In clearwater concentrations of Ø.Ø1 and Ø.1Ø ng/L Metamidophos decreased survival of M. rosenbregii (by 13 and 15%, respectively) and postlarvae production (by 35 and 48%), when compared with clearwater controls. In greenwater concentrations of Ø.Ø1 and Ø.1Ø ng/L of the pesticide also decreased survival (by 24 and 16%), compared to greenwater controls, but this effect was less pronounced than in the corresponding clearwater treatments. After 33 d of testing, greenwater-reared larvae of M. rosenbergii showed an average 5.6% higher survival and 46.3% higher production of postlarvae than their clearwater-reared counterparts, regardless of the pesticide concentration. The greenwater method is used by commercial prawn hatcheries in Hawaii and is belived to increase postlarvae production by 1Ø-2Ø% in comparison to systems not using phytoplankton (Malecha

1982). Several mechanisms have been postulated as possible means of algal enhancement of M. rosenbergii larval cultures, including direct or indirect (via herbivorous nauplii of Artemia) ingestion af algae, production of algal metabolites that could act as growth factors (Maddox and Manzi 1976), and reduction by algae of nitrogenous compounds in the culture media (Cohen et al. 1976). The above mentioned differences in survival and postlarvae production after 33 d of testing were, however, very small, when compared with a ten-fold increase in the pesticide concentration, reflecting the very low concentrations and long exposure periods used in the experiment.

Total ammonia nitrogen concentrations throughout these experiments ranged from Ø.11 to Ø.2Ø mg/L in the clearwater treatments, and from Ø.15 to Ø.22 mg/L in greenwater. pH varied between 7.2 and 8.2 in clearwater, and between 7.3 and 8.7 in greenwater. These ranges are below levels reported as dangerous for M. rosenbergii larvae by Armstrong et al. (1976) and Armstrong et al. (1978).

Survival rates in controls of sub-acute toxicity tests with M. rosenbergii larvae are below those reported by Malecha (1982) as normal in commercial prawn hatcheries. Daily handling stress and the limitations of rearing larvae in small containers offer a probable explanation for these low survival values.

Survival curves in Figure 1 show an increase in their (negative) slopes between days 13 and 2Ø. This is in agreement with the results of acute toxicity tests (Table 2) which show the intermediate larval stages tested (zoea IV and VII) to be more sensitive to the pesticide than zoea I and early postlarvae. The relative low sensitivity of zoea I larvae may be due to their dependance only on internal reserves for nutrition and/or to the absence of molting-associated stress. The relative resistence to the pesticide exhibited by early postlarvae could result from a completed development of structures and functions after the critical period of metamorphosis.

Metamidophos is highly toxic for both P. stylirostris and M. rosenbergii larvae. The low LC5Ø values found in this study and the fact that concentrations as low as Ø.1Ø ng/L can reduce survival and increase time to metamorphosis show that even small quantities of this pesticide could adversely affect crustacean populations. Clearly the presence of pesticides is highly undesirable in waters that support populations of commercially important crustaceans and in the water supplies for crustacean aquaculture facilities, where

screening of the water for pesticides should be an important step in the site-selection process. In order to assess the biotic hazard and to detect the environmental damage of pesticides entering natural bodies of water more information is needed on the long-term, subacute effects of these compounds on crustaceans, especially during their larval phases.

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