

## **Influence of Salinity on the Toxicity of Phosphamidon to the Estuarine Crab, *Scylla serrata* (Forsk.)**

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Among the various types of pesticides, organophosphates are now being increasingly used in place of organochlorines, due to their biodegradable nature and lesser degree of persistence in the environment (Bookhout & Monroe 1977). Comparatively little work has been done on the toxicity of insecticide to crustaceans (Omkar & Shukla 1985; Omkar & Rammurthi 1985). But, no work seems to have been done on the synergistic effects on interaction between the toxicity of phosphamidon and salinity to crustaceans. The scarcity of literature on the toxicity of insecticides to crabs has initiated the present study. Phosphamidon, an organophosphorous insecticide selected for the present investigation, is being commonly used in various parts of India. In the present study an attempt has been made to investigate the acute toxicity of phosphamidon to estuarine crab, *Scylla serrata* with special emphasis on the synergistic effects of salinity and toxicity.

### **MATERIALS AND METHODS**

Specimens of *S. serrata* were collected from the Buckingham Canal, near Kavali seacoast, Andhra Pradesh, India (14° 50'E and 80° 5'N) brought immediately to the laboratory, and acclimated for 1 wk to the laboratory conditions in aerated seawater of 15 and 25 ppt. During this period crabs were fed on frog muscle pieces. Feeding was stopped 24 hr before the beginning of the experiments and no food was given during experiments, to avoid contamination by excrements in the test solution. All the troughs were continuously aerated to prevent hypoxia in the medium (Khorram & Knight 1977). The pH of the media were found to be 7.6 and 7.3 in 15 and 25 ppt media, respectively. The temperature of the media was  $23 \pm 2^{\circ}\text{C}$ .

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Technical grade phosphamidon (92% w/v; 0,0-dimethyl-0-(1-methyl-2-chloro-2-diethyl-carbomoyl-vinyl) phosphate) was used as the test chemical (obtained from M/S Ciba-Geigy, Bombay). A stock solution of 1000 ppm (1 mg/ 1 ml) and appropriate working concentrations were prepared by dilution with seawater (Reddy 1986). The bioassay methods were similar to standard methods (APHA et al. 1971). Toxicity evaluation studies were conducted in static bioassay system (Doudoroff et al. 1951). Crabs of  $(28 \pm 2$  g in weight) were transferred carefully from the acclimatization troughs to the test solutions and bioassays were carried out. Crabs were exposed to concentrations ranging from 1.0 to 4.5 mg/L with a difference of 0.5 mg/L. For each concentration 25 crabs were exposed. Mortality was recorded after 96 hr of exposure and dead animals were removed regularly from the test solutions. Each experiment was replicated 6 times to get concurrent values. The test solutions were replaced by fresh solutions of respective concentrations after every 24 hr to maintain constant concentration of insecticides in test solution. In order to be more accurate in the determination of  $LC_{50}$  values toxicity evaluation was done through probit analysis, graphical method and from cumulative mortality. To determine the  $LC_{50}$  through graphical method, the following graphs were drawn (i) a graph between log concentration of pesticide vs percent mortality (ii) another graph between log concentration of pesticide vs probit mortality.  $LC_{50}$  values were also determined by adopting the following formula of Dradgstedt - Behren's method as described by Carpenter (1975).

$$\text{Log } LC_{50} = \text{Log } A + \frac{50-a}{b-a} \times \log 2$$

where

- A : Concentration of pesticide, whose concentration is below 50% mortality
- a : Percent mortality observed immediately below 50% mortality
- b : Percent mortality observed immediately above 50% mortality

The data obtained were statistically analysed by the method of probit analysis (Finney 1964) to calculate the  $LC_{50}$  values and 95% confidence limits of  $LC_{50}$ .

## RESULTS AND DISCUSSION

The control crabs behaved normally in the sense that they are very active and the movements of the animals

Table 1. Consolidated table showing values of LC<sub>50</sub> (by different methods) of phosphamidon to Scylla serrata

Exposure salinity ppt	<u>Graphical</u>		Probit analysis	Calculated	Average mg/L
	<u>Sigmoid curve</u>	<u>Straight line</u>			
15	3.4280	3.5080	3.4009	2.8280	3.2912
25	2.7860	2.4830	2.3351	2.4378	2.5105

Table 2. Results showing equation of regression lines, LC<sub>50</sub> values and 95% confidence limits of phosphamidon to Scylla serrata

Exposure salinity ppt	LC <sub>50</sub> mg/L	Regression equation	Slope	95% confidence limits	
				Lower	Upper
15	3.2912	0.2939 +	0.77	2.9451	3.6373
25	2.5105	2.1397 +	0.95	2.2957	2.7253

were well coordinated. They are very alert and at the slightest possible disturbance they squittle fast indicating an escape reaction. The crabs exhibit a tendency to retreat when the experimenter's finger is brought close to them. If the pointed finger was brought too close (1-2") they react aggressively by extending or lifting the chelate legs. The experimental animals appear to be sluggish, running was slow and they stayed where they are in response to threat. The movements are uncoordinated.

Concentrations resulting in 50% mortality and 95% confidence limits are shown in Table 2 in both 15 and 25 ppt salinity media. The toxicity of phosphamidon to be more at 25 ppt rather than at 15 ppt salinity, thus confirming with an increase in salinity to produce increased toxicity of insecticides at higher salinities (Eisler 1970a). The differences in the LC<sub>50</sub> values at different salinities may be attributed to the uptake of phosphamidon. The rate of uptake may be higher at lower salinity and lower at higher salinity. Similar observations were also reported in mosquito fish with DDT (Murphy 1970). The changes in the pH of the two media should have also contributed to some extent to the differential rates of toxicity reflecting the phenomenon of degradation of a pesticide by alkaline hydrolysis, which generally increases the apparent LC<sub>50</sub> values (Eisler 1970b). Phosphamidon, an organophosphorous insecticide to produce greater mortality of Metapenaeus monoceros at higher salinity (Vijayalakshmi et al. 1986). Similar kind of observations were also reported in the american lobster (MeLeese 1974) and in crab, Uca pugnator (O'Hara 1973) in the toxicity evaluation studies with heavy metals. Such a type of influence of salinity on the toxicity of phosphamidon to the crab, Scylla may also be visualised. It may be concluded from the present investigation that an alteration not only in the physiochemical properties of insecticides but also the exposure media influences the apparent LC<sub>50</sub> values in aquatic bioassays. The differences in the LC<sub>50</sub> values at 15 and 25 ppt salinity media may be attributed to the quality of the water which can act to reduce the toxicant present by way of aiding chemical breakdown or precipitation or hydrolysis. The bioassay experiments in the present study were conducted under laboratory conditions. The laboratory bioassay provides quickly reliable information about the toxicity of chemicals, although the results differ from field results (Sanders 1969).

Bridges (1961) concluded that acute toxicity appears at higher concentrations in the field than in the laboratory. Such laboratory bioassays were also used to study physical, chemical and biological factors influencing the toxicity of different chemicals. The evaluation of toxicity of chemicals to crab has been recommended as one of the methods in water pollution (USEPA 1975). Determination of  $LC_{50}$  values is a starting point in studies of toxicological research, since it provides fundamental data for the design of more complex animal studies.

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#### REFERENCES

- APHA, AWWA, WPCF (1971) Standard methods for the examination of water and waste water ed 13. New York
- Bookhout CC, Monroe RJ (1977) Effects of malathion on the development of crabs. (eds) FJ Vernberg, A Calabrese, FP Thunberg and WB Vernberg. Physiological responses of marine biota to pollutants. Academic Press, New York. p3-19
- Carpenter PL (1975) In: Immunology and serology, 3rd ed. WB Saunders Company, Philadelphia. p254
- Doudoroff P, Anderson BG, Burdick GE, Galtsoff PS, Hart WB, Patrick R, Strong ER, Surber EW, Van Horn WM (1951) Bioassay methods for the evaluation of acute toxicity of industrial wastes to fish. Sewage Industr Wastes 23:1380-1397
- Eisler R (1970a) Factors affecting pesticide induced toxicity in an estuarine fish. US Fish Wildl Serv Tech Pap 45
- Eisler R (1970b) Acute toxicities of organochlorine and organophosphate insecticides to estuarine fishes. US Fish Wildl Serv Tech Pap 46
- Finney DJ (1964) Probit Analysis. Cambridge University Press, London
- Khorram S, Knight AW (1977) The toxicity of kelthane to the grass shrimp, Ceratonereis acronotus. Bull Environ Contam Toxicol 18:674-682
- McLeese DW (1974) Toxicity of copper at two temperatures and three salinities to the american lobster. J Fish Res Bd Can 31:1949-1952
- Murphy PG (1970) Effect of salinity on uptake of DDT, DDE and DDD by fish. Bull Environ Contam Toxicol 5:404-407

- O'Hara J (1973) The influence of temperature and salinity on the toxicity of cadmium to the fiddler crab, Uca pugilator. Fish Bull US 70: 140-153
- Omkar, Rammurthi (1985) Toxicity of some pesticides to the freshwater prawn, Macrobrachium dayanum (Henderson) (Decapoda, Caridea). Crustaceana 49:1-6
- Omkar, Shukla GS (1985) Toxicity of insecticides to Macrobrachium lamarrei (H.Milne Edwards) (Decapoda, Palaemonidae). Crustaceana 48:1-5
- Reddy MS (1986) Subacute toxic impact of phosphamidon on the carbohydrate metabolism of penaeid prawn, Metapenaeus monoceros (Fabricius) - a tissue metabolic profile. Ph D Thesis Sri Venkateswara University, Tirupati, India
- Sanders HO (1969) Toxicity of pesticides to crustacean Gammarus lacustris. Bur Sport Fish W Wildl Tech Pap 25:1-18
- US Environmental Protection Agency (1975) Methods for acute toxicity tests with fish, macroinvertebrates and amphibians. Ecol Res Ser EPA-600/3-75-009:1
- Vijayalakshmi P, Sastry DVS, Rao KVR (1986) Interaction between salinity and toxicity of phosphamidon in Metapenaeus monoceros (Fabricius) Bull Environ Contam Toxicol 37:797-801

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