J. mar. biol. Ass. India, **42** (1&2) 2000 : 173 - 177

Acute toxicity bioassay of endosulfan, HCH, copper and zinc on larva of mangrove crab *Macrophthalmus erato* (de Man)

T. Kannupandi, K. Pasupathi and P. Soundarapandian

Centre of Advanced study in Marine Biology
Annamalai University, Parangipettai - 608 502, Tamil Nadu

Abstract

Acute toxicity test(96hr.) was carried out to determine the toxic effects of the organochlorine pesticides and heavy metals on larvae of crab *Macrophthalmus erato*. The 96hr. LC 50 values were 0.48, 3.2, 90.0 and 152.0 µg/L of endosulfan, HCH, copper and zinc respectively for the first zoeal stage. The order of toxicity to the larvae was Endosulfan > HCH > Copper > Zinc. The calculated safe concentrations were 0.0048, 0.032, 0.900 and 1.52 µg/L for endosulfan, HCH, copper and zinc respectively.

Acute toxicity tests constitute a major tool for evaluating the impact of toxic chemical on marine organisms. In and around Pichavaram mangroves, among the organochlorine pesticides, endosulfan and HCH are widely used (an average of about 871 liters of endosulfan per month) to control the pests (Rajendran, 1984). Copper and zinc are used in the form of copper sulfate and zinc sulfate respectively. Zinc and copper concentrations in the waters of the Pichavaram mangrove area have been reported to range from 10.38 - 62.8 µg/L and 0.28 - 4.6 µg/L respectively (Sundaramoorthy, 1987). Hence endosulfan, HCH, copper and zinc were selected and used in the present study to demonstrate their acute toxicity on the larval (first zoea) stage of the crab *Macrophthalmus erato* which is available throughout the year in Pichavaram mangroves.

Thanks are due to the Director and authorities for providing facilities and CSIR for funding.

Material and methods

The berried females of *M. erato* were collected at low tide from the Pichavaram mangroves (Lat. 11°29'N; Long. 79°49'E) and kept in the laboratory in isolation till the eggs hatched. The filtered, well aerated estuarine water whose salinity, temperature, dissolved oxygen and pH were kept constant as 25 ± 1 ppt, 27 ± 1°C, 4.80 ± 0.25 ml/l and 7.2 to 7.5 respectively throughout the study period.

The required quantity of Endosulfan and HCH (Table 1) were dissolved in 100 ml of technical grade acetone and used as a stock solution. The required test solutions were prepared by adding appropriate volumes of the stock solution to required quantities of water prior to the start of the experiments.

Analytical grade copper sulfate ($C_uSo_4.5H_2O$) and zinc sulfate ($ZnSo_4.7H_2O$)

Table 1. *Pesticide characteristics*

Pesticide	Supplied by	Tech. Grade	Chemical name	Alternate name
Endosulfan	EID Parry (India) Ltd., Ranipet.	99.9%	6, 7, 8, 9, 10 hexachlo 1, 5, 5a, 6, 9, 9a hexahydro 6, 9 methano 2, 4, 3, benzodioxathiepin 3 - oxide	Thiodon Thimul Thifor Thionox
HCH	EID Parry (India) Ltd., Ranipet	97.5%	1, 2, 3, 4, 5 6- hexachlorocyclohexane	Hexachlor Soprocide Hexafor BHC

were used as the source of copper and zinc. Estuarine water was used as the control medium.

Acute toxicity tests were carried out using the continuous flow method as recommended by Parrish (1985). The acute toxicity tests were basic 96 hr. tests using the larvae of *M. erato* reared in the laboratory (Pasupathi and Kannupandi, 1988). Fifty numbers of first zoeal stage larvae of *M. erato* were exposed to each test concentrations viz. 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 µg/l endosulfan; 2.0, 4.0, 6.0, 8.0 and 10µg/l HCH; 20, 40, 80, 100 and 120 µg/l of copper; and 100, 120, 140, 160 and 180 µg/l of zinc. The bioassay was repeated in triplicate. Larvae, obtained from the single females, were used within 10 hr. after hatching. All the larvae were very active and seemed to have sufficient food reserves, hence were not fed during the acute toxicity test (CMTTAO, 1975). Two controls were maintained simultaneously for each bioassay one with acetone and other without acetone. Recording of survival was done at an interval of 24 hours. The criteria for identifying death was the absence of any movement when the larvae were kept for 1-2 minutes. Dead larvae were removed when observations and recordings were made.

Data from the acute toxicity tests were processed following the method as described by Ajmalkhan *et al.* (1986), to calculate the 96 hr. median lethal concentration (Lc_{50}) with 95% confidence limits and the slope function(s). The estimation of safe level was based on the recommendation made by the Committee on Water Quality Criteria (1972).

Results

Degree of toxicity

The 96 hr. LC_{50} value of different contamina nts for the first larval stages with confiden-ce limits and slope are shows in Table 2. Based on the 96 hr. LC_{50} values, the degree of toxicity of the pesticides and heavy metals to the larvae were as follows:

Endosulfan > HCH > Copper > Zinc

Behavioral changes

In the acute toxicity tests, when the larvae were first exposed to different concentrations of the contaminants, they often stayed motionless for a few seconds on the bottom of the tanks, then they moved

Table 2. *Median lethal concentration (LC_{50}) in µg/l for different contaminants for 96 hr intervals and other statistical parameters.*

Contaminants tested	LC_{50} and 95% limit	Slope and 95% confidence limit
Endosulfan	0.48 (0.33 - 0.69)	2.32 (1.45 - 3.72)
HCH	3.2 (2.13 - 4.8)	2.48 (1.18 - 5.21)
Copper	90.0 (64.28 - 126)	2.26 (1.43 - 3.57)
Zinc	152.0 (144.06 - 161.36)	1.69 (1.16 - 2.45)

erratically with tail lashing for several seconds before adopting a characteristic vertical swimming pattern. The effects became more pronounced with time and the zoeae manifested abnormal swimming behaviour, followed by disoriented twitching movements. Though similar behavioural patterns were observed in all treated larvae, there were variations in the duration of onset of behavioural abnormalities.

Safe concentration

The safe concentrations for the four different contaminants used in the present study were 0.0048 µg/l, 0.032µg/l, 0.900 µg/l and 1.520 µg/l for endosulfan, HCH, copper and zinc respectively.

Discussion

The 96 hr. LC_{50} reported for endosulfan in the present investigation using *M. erato* larvae was closely similar to 96 hr LC_{50} of methoxychlor, lindane and hepatachlor on crab larvae, *Cancer magister, Eurypanopeus depressus* and *Neoepisesarma (M) teragonium* (Armstrong *et al.,* 1976; Caldwell, 1977; Shirley and McKenney, 1987; Sundaramoorthy, 1987). However, from the LC_{50} values observed in the present study, HCH appeared to be less toxic to the larvae of *M. erato* than other pesticides.

Larvae of *M. erato* used in the present study appeared to be more sensitive to zinc than the larvae of *Paragrapsus quadridentatus* (Ahsanulla and Arnott, 1978), the hermit crab *Claibanarius olivaceus* (Ajmalkhan et al., 1986) and *N (M). tetragonium* (Sundaramoorthy, 1987). Ahsanullah and Arnott (1978) using larvae of *P. quadridentatus* reported that 96 hr. LC_{50} of 170 µg/l copper which is almost twice the value that was obtained in the present study using the larvae of *M. erato* and thus more sensitive to copper also. However, the 96hr. LC_{50} of copper was in close agreement with that observed for *N (M). tetragonum* (Sundaramoorthy, 1987).

The LC_{50} values obtained for copper on the larvae of *M. erato* were closely similar to those observed for *C. olivaceus* and *N(M) tetragonium.* However, the LC_{50} value for copper differed from that of *P. quadridentatus.* LC_{50} values obtained for zinc on the larvae of *M. erato, C. olivaceus* and *N(M). tetragonum* were closely similar but differed from that of *P. quadridentatus.*

Thurberg *et al.* (1973) opined that the interpretation of the effects of contaminants based on LC_{50} values differs among contaminants, among species and among experimental conditions. However, LC_{50} values would certainly reflect the impact of specific contaminants under experimental conditions and the results could be applied to particular species and contaminants in a given environment.

Behavioural abnormalities induced by organochlorine toxicants in crab larvae are characterised by hyperactivity, erratic swimming and loss of balance (Klein and Lincer, 1974). These types of behavioral characters were also observed in the present study with the larvae of *M. erato*. These observations also agree with that of Capaldo (1987), who exposed the larvae of *Uca minax* to carbaryl (sevin).

Organoclorine pesticides were found to affect the central nervous system (Venugopalan and Sasibhushana Rao, 1979) resulting in the impairment of the neuromuscular system and hyperactivity followed by exhaustion and death. Similar behavioral abnormalities were also observed in the present investigation.

Impairment of swimming activity was observed in the larvae treated on the copper and zinc contaminants. The larvae displayed rapid twitching movements of the body accompanied by rapid movements of the maxilliped. Furthermore, spinning or circular swimming patterns were observed in the treated larvae. These swimming irregularities were very similar to the behavioural changes described for zoeae of *U. minax* exposed to mercury (DeCoursey and Vernberg, 1972).

Long term bioassays are important in determining the safe level of a pesticide. However, in the absence of such studies, the Committee on Water Quality Criteria (CWQC, 1972) suggested an arbitrary factor, 0.01 as the 'application factor' to provide some margin of safety for sensitive species and to obtained the maximum permissible concentration for the receiving water, so as to safeguard the various uses of aquatic environments. Thus, the safe concentrations for endosulfan, HCH, copper and zinc are 0.0048 µg/l, 0.032 µg/l, 0.900 µg/l and 1.520 µg/l respectively. The safe levels reported by CWQC (1972) and Ahsanullah and Arnott (1978) for the contaminants used in the present investigation *viz*., endosulfan, copper and sinc are 0.003 µg/l, 1.7 µg/l and 12.3 µg/l respectively and are therefore within the range of safe levels.

References

Ahsanullah, M. and G. H. Arnott. 1978. *Aust. J. Mar. Freshw. Res.* **29** : 1-8.

Ajmalkhan, S., K. Rajendran and R. Natarajan. 1986. *Proc. Indian Acad. Sci. (Animal Sci.)* **95** : 515-524.

Armstrong, D. A., D. V. Buchanan, M. H. Mallon, R. S. Caldwell and R. E. Millemann., 1976. *Mar. Biol.*, **38** : 239-252.

Caldwell, R. S. 1977. Biological effects of pesticide on the Dungeness crab. EPA - 600/3-77-131, pp 125.

Capaldo, P. S. 1987. *Estuaries*, **10** : 132-135.

CMTTAO, 1975. The Committee on method for Toxicity Test with Aquatic Organisms, Methods for acute toxicity test with fish, macroinvertebrate and amphibians. USEPA Ecological Series, EPA - 660/3-75-099 p. 61.

CWQC., 1972. A report of the Committee on the Water Quality Criteria. Ecological Research Series. EPA-R3-73-003.

DeCoursey, P. J. and W. B. Vernberg 1972. *Oikos*, **23** : 241-247.

Klein, M. L. and J. L. Lincer 1974. Behavioural effects of dieldrin upon the fiddler crab, *Uca pugilator*. In : Pollution and Physiology of Marine Organisms. (eds. F. J. Vernberg and W. B. Vernberg). Academic Press, New York, pp. 181 - 196.

Parrish, P. R. 1985. Acute toxicity tests. In : Fundamental of Aquatic Toxicology. (eds. G. M. Rand and S. R. Petrocelli), Hemispher Publishing Corporation. Washington. New York, pp 659.

Pasupathi, K. and T. Kannupandi 1988. *Hidrobiologia*, 169 : 327-338.

Rajendran, N., 1984. Organochlorine pesticide residues in Vellar estuary. Ph. D. Thesis, Annamalai University, India, p 148.

Shirley, M. A. and C. L. Mckenney, Jr. 1987. Influence of lindane on survival and osmoregulatory, metabolic responses of the larvae and adults of the estuarine crab, *Eurypanopeus depressus*. In : Pollution Physiology of Estuarine Organisms. (eds. W. B. Vernberg, A. Callbrese, F. P. Thurberg and F. J. Vernberg). Univ. of South Carolina Press, Columbia, SC., pp 275-297.

Sundaramoorthy, S. 1987. Taxonomical developmental and larval bioassay studies in the mangrove crab *Neoepisesarma* (*Muradium*) *tetragonum* Fabricius, 1798 (Grapsidae, Brachyura, Decapoda). Ph. D. Thesis, Annamali University, India, p 210.

Thurberg, F. P., M. A. Dawson and R. S. Collier. 1973. *Mar. Biol.*, 23 : 171-175.

Venugopalan, V. K. and P. Sasibhushana Rao 1979. Pesticide induced impairment in incubation and post embryonic development and planktonic eggs of estuarine fish of Porto Novo (South India) water. In : Environmental Biology. (eds. S. R. Verma, S. K. Bansal and A. K. Tyagi). The Academy of Environmental Biology, Muzffar Nagar, India.