Effect of endosulfan on the physiological activities of the estuarine bivalve *Meretrix casta*

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Nagarajan, D. 2001. Effect of endosulfan on the physiological activities of the estuarine bivalve *Meretrix casta* - Phuket Marine Biological Center Special Publication 25(1): 247-250.

The effect of the pesticide endosulfan on the physiological activities of *Meretrix casta* was assessed. Acute lethal toxicity (LC_{50}) was found to be 0.512 ppm. No mortality was recorded at the higher concentration of 1 ppm, as the clams remained closed throughout the experiment. A 50 % decline of the rate of water filtration was recorded at 0.012 ppm. The non-linear relationship between concentration and mortality was analysed. The shell closing behaviour and water filtration rates were also discussed.

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INTRODUCTION

Pesticides are generally used in agriculture, horticulture, mariculture and also in public pest control. Although these chemicals are designed and used to kill pest organisms, they also affect in some way or other the life of non target beneficial organisms also (Brown 1978). Pesticide residues have been reported in *Crassostrea madrasensis*, *Meretrix casta*, and *Katelysia opima* (Rajendran 1984) in the coastal environment. These residues give some indications that once the pesticides enter the food web, they are concentrated to high levels in the fatty tissues of the organisms. In areas exposed to persistent pesticide pollution, accumulation by aquatic organisms may result in mortality, reduced reproductive capabilities, altered growth rates and reduced ability to withstand natural changes in environmental conditions. Considering the wide use of endosulfan, the present study is carried out to investigate the effect of endosulfan on the clam, *Meretrix casta*.

MATERIALS AND METHODS

M. casta was collected during low tide from clam beds, in the Vellar estuary at Portonovo. The clams were brought to the laboratory, cleaned and acclimated in well-aerated estuarine water for 7 days at 28 °C before experiments. The test organisms were fed with fresh phytoplankton. The experiments were performed using endosulfan (Technical), the product of Bharat Pulversing Mills Private LTD, Bombay. Acetone was used as a carrier solvent, as this solvent is reported to be quite harmless to the test organism (FAO 1978 & 1979). The pesticide was dissolved in appropriate volume of acetone to obtain the stock solution. The different test concentrations were prepared by adding the stock solution to the test aquaria. The filtered estuarine water with temperature (28 $\pm$ 1 °C) pH (7.5) and salinity (20 %) was used throughout the experiments.

The continuous flow through system was followed and toxicity tests were conducted in accordance with the method recommended by the committee on the methods for toxicity test with aquatic organism (Sprague, 1969). Ten randomly selected organisms were tested at each concentration for 7 days. The values of LC_{50} were determined by using probit analysis (Mather and Fisher, 1949).

The rate of filtration of the test animals was studied using the technique followed by Cole

& Hepper (1954) and Nagabushanam (1956), using a neutral red solution. The rate of water filtration at LC_{50} concentration (0.512 ppm) and 1 ppm were measured and a control was also run simultaneously. The concentration, which reduces 50 % filtration rate of control animal, was also measured.

In each concentration of endosulfan 10 clams were examined. Ten glass beakers (500 ml) with 400 ml filtered estuarine water were arranged on a table with white background. One clam was placed in each beaker. At this stage neutral red (4 ml) was added to each test beaker and gently mixed by stirring. Samples of 10 ml were immediately taken with a pipette from each beaker. At 15 minutes intervals 10 ml of solution was removed from each beaker during the period of 1 hour. Dye concentrations in the samples were determined spectrophotometrically at 425 nm in a Hitachi 220 S Spectrophotometer after the addition of a few drops of hydrochloric acid. The readings were calculated into percent concentration.

RESULTS & DISCUSSION

The clams exposed to 0.1 to 1 ppm concentrations during 7 days bioassay showed different behavioural pattern. The shell closure periods gradually increased with increasing concentration and number of days. In 0.1 ppm there was no mortality within 7 days. In this concentration all the clams opened their shell valves slightly, extended their siphons, and foot was rarely protruded out. In 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1 ppm, there was 10, 30, 40, 50, 50 and 60, 30, 10 and 0 % mortality within 7 days respectively. In 1 ppm all the clams remained closed throughout the experimental period. The LC_{50} concentration was 512 ppb (Table 1).

Generally there should be a linear relationship between the mortality of any animal and the concentration of the toxicants. However, in the present work the result was not so. Here, the LC_{50} value obtained was at 0.512 ppm and no mortality occurred in higher concentration (1 ppm). Unlike the fishes and crabs the molluscs have well protective calcareous shells to escape from contaminated environments. In the present work, the ability of *M. casta* to respire anaerobically for long periods (Newell 1964) by closing their shell valves may account for the non-linear relationship between mortality rate and concentration of endosulfan. In 0.1 ppm the frequency of shell opening (aerobic respiration) is more than that observed in 1 ppm concentration. The amount of endosulfan accumulated in the body of the clams during the bioassay test in 0.1 ppm was found to be insufficient to cause mortality, but, in 1 ppm the contact between the animal and the endosulfan-contaminated water was believed to be cut off by closure of shell valves and the animal might have survived by anaerobic respiration. Even though the duration of shell opening was low in the LC_{50} concentration (0.512 ppm), the amount of endosulfan taken up in the body was found to be sufficient to bring mortality up to 50 %.

Table 1. *Meretrix casta*. Assay of Endosulfan. Probit analysis*: Mather, 1949.

Dose (ppm)	Tested (n)	Survived (S)	LC_{50}*
0.1	10	10	
0.2	10	9	
0.3	10	7	
0.4	10	6	
0.5	10	5	0.512 ppm
0.6	10	5	
0.7	10	4	
0.8	10	7	
0.9	10	9	
1	10	10	

The rate of filtration at different concentrations and a control is shown in Figure 1. In the control 59 % of the neutral red was filtered off by the clams while, in 0.512 ppm the filtration was 9% and in 1 ppm it was 3 %. On the other hand at 0.012 ppm the filtration rate was reduced to half to that of the control. During the water filtration experiments, the behaviour of the *M. casta* was also studied. In the control the animal actively

filtered the neutral red and ejected the pseudofaeces and no shell closure was observed. At 0.512 ppm low pumping rate was observed and the majority of the clams opened their shells with the siphon extended during the initial 20 minutes of the experimental period. The rate of pseudofaeces production was also very low when compared to the control. When the clams were introduced into 1 ppm, all the clams opened their shell valves and filtered the suspended material actively for 2 to 5 minutes. Afterwards, all the clams retracted their siphon and remained closed throughout the experimental period. At 0.012 ppm no shell closure was observed. The pumping rate and the amount of pseudofaeces produced was lower than that of the control animals.

In the water filtration experiments a general trend in reduction of filtration rate with increasing concentration was observed (Fig 1). The reason for this reduction was the shell closing behaviour of the bivalves. Generally bivalves can avoid polluted environment by closing their shell valves (Newell 1964) and choose anaerobic respiration. Below 1 ppm concentration the main reason for the reduction in filtration rate might be due to inhibition of important enzymes like succinic dehydrogenase, cytochrome oxidase and carbonic anhydrase as shown by O'Brein (1970). Miller & Kinter (1977) found that the organochlorines, such as DDT, disrupt the membrane transport function in sensitive species. Impairment of aerobic oxidation in *Lamellidens marginalis* due to toxicity of pesticides is also reported (Kinter & Pritchard 1976). Engel *et al.* (1972) demonstrated the sublethal doses of DDT and Lindane interfere with glycolytic and glucogenic enzymes in *Mercenaria mercenaria*.

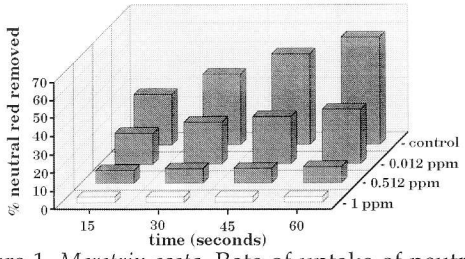

Figure 1. *Meretrix casta*. Rate of uptake of neutral red at different concentrations of endosulfan. Data show a linear relationship.

In the present investigation the concentration of endosulfan required to reduce filtration rate by 50% from that of the control was 0.012 ppm. At this concentration the shell closure was not recorded, and it seems that reduced water filtration might be due to the effect of endosulfan.

In the bioassay study a contradictory result was obtained. According to Sprague (1969, 1970 and 1971) there should normally be a linear relationship between the concentration of toxicant and mortality when LC_{50} is applied. Brown & Newell (1972) also found that higher concentration of copper exerted a direct inhibitory effect on ciliary action in some molluscs. The non-existence of linear relationship in the present study could be due to the shell closure of *M. casta*. The bioassay might therefore not give a clear picture about LC_{50} in the case of *M. casta*. On the other hand, the water filtration experiment could be used as a tool to determine the toxic effect of the toxicant on *M. casta*. This technique can be adopted everywhere because of its simplicity, low time consumption and low cost.

ACKNOWLEDGEMENTS

The author thanks Dr. Sivakumar, V.O.C. College and Dr. A. Murugan, Lecturer, Suganthi Devadason Marine Research Institute, Tuticorin for their valuable suggestions. Dr. Ake Granmo, Sweden, is thanked for comments to the first draft.

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