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Effects of Temephos on the Respiratory Rate of the Salt Marsh Amphipod, *Gammarus mucronatus*

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Many years ago, the senior author reported on a number of studies in which the effects of various pesticides on copepods were assessed in the laboratory and an attempt was made to extrapolate to field results (RUBER & JOBBINS 1961; RUBER & FERRIGNO 1964). It was apparent that standard 24-h mortality tests had their limitations. When the tests were extended to 48 and 72-h and beyond, sublethal concentrations of insecticides became lethal in some cases, while in other cases, mortality rates did not increase even after six days (Fig. 1).

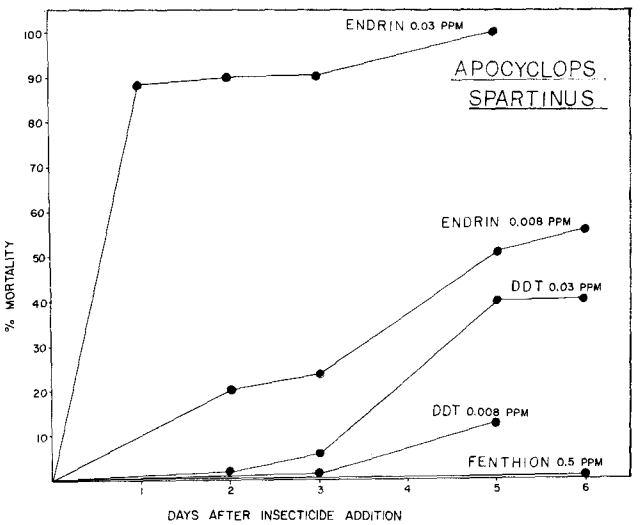

Fig. 1. Time and dose dependence of mortalities of the salt marsh copepod, Apocyclops spartinus (Cyclopoida, Crustacea). Fenthion is not toxic at high doses even after 6 days, but even low doses of DDT and endrin result in increasing mortalities with time.

Some years ago we began to study amphipod crustaceans, some of which live in streams and must maintain themselves and migrate upstream into a current (HUGHES 1970; WILLIAMS & HYNES 1976). We asked whether sublethal doses of insecticides might affect the behavior of the amphipod, Gammarus fasciatus, in such a way that their upstream migration might be inhibited and found that they can (RUBER & KOCOR 1976). This led us recently to consider

whether overall changes in metabolic rate of amphipods can be detected at sublethal concentrations of insecticides and whether this could lead to predictions of mortality after 48-h or longer. To study this question, we chose for an organism, the amphipod crustacean, Gammarus mucronatus, which is found extensively in salt marsh pools in mats of the alga, Cladophora, and chose for an insecticide, the organophosphate, temephos.

METHODS

Preliminary tests were run to determine sub-lethal doses of temephos. Technical grade material was diluted in acetone to a 1% concentration and then serially diluted in water. Amphipods were treated and held in beakers of marsh water; mortality was recorded at 24 and 48-h. As a matter of routine, 3rd and 4th instar Aedes sollicitans larvae (the major salt marsh mosquito on the east coast) were similarly treated and checked. Based on the results of these studies (Table 1), further experiments were done with temephos at 0.1 and 0.01 ppm and with acetone controls at 100 ppm.

Table 1. Mortality rates of Gammarus mucronatus and Aedes sollicitans larvae treated with temephos.

	Acetone	Temephos, ppm			
	100 ppm	0.01	0.10	0.50	1.0
Gammarus					
24-h	2.5	5.0	5.0	40.0	95.0
48-h	12.5[a]	35.0	21.0	90.0	100
Aedes					
24-h	0	100	100	100	95.0

a. Mortality rates increase at 48-h even in controls; the tendency is erratic. The differences between 0.01 and 0.10 are not statistically significant.

Gammarus were tested in the following sequence. A reserve of insecticide-treated test water was prepared (Millipore filtered and treated with 65 mg streptomycin and 65 mg penicillin/L to inhibit bacterial respiration) and held for later use. The gammarids were held in temephos-treated but antibiotic-free test water for 16-h. Natural food materials were available during this interval. After this, the food was rinsed away and the gammarids were placed in temephos (for controls, acetone) and antibiotic treated water which was replaced a last time after 4-h. For the last 4-h of the 24-h period 4 to 6 gammarids were placed in each 20 ml vial and oxygen concentrations determined. Blanks were also established which contained only the antibiotics and acetone. At the end of the test period concentrations of dissolved oxygen were determined, times recorded, and the gammarids from each vial separately preserved. The changes in dissolved oxygen values in test vials were corrected by the changes observed in the blanks.

Oxygen concentrations were measured with an Orbisphere model 2607 meter. This instrument is stable, can be used in a small vial, and usually reaches a stable reading in less than a minute. This made possible a number of replications in small vials with final readings over a short period of time which would have been impossible with Winkler titrations. Gammarids were preserved in 95% ethanol and then measured. Based on previous work (LAFRANCE 1982), lengths were converted to weights by the equation: log Weight (mg) = log 0.0049 + 2.71 log Length (mm). Values were expressed as mg O_2/300 min/mg dry weight of gammarid. The means of the 3 sets of tests were compared for statistical significance by t-tests.

RESULTS

Oxygen concentrations in the acetone controls was compared with that in the temephos-dosed vials (Table 2). The reduction at a concentration of 0.01 ppm temephos is not statistically significant; however, the reduction at 0.1 ppm temephos is. We have, therefore, been able to show significant respiratory inhibition at 24-h at concentrations of insecticide which do not cause significant 24-h mortality rates.

Table 2. Effects of temephos on respiratory rate of Gammarus mucronatus.

	Acetone	Temephos, ppm	
	100 ppm	0.01	0.10
N	8	8	7
O_2	0.0150[a]	0.0146	0.004**

a (EXPERIMENTALS - BLANKS) /N given as mg O_2/300 min/mg dry weight of organism

** t-Test Significant at p=0.99.

DISCUSSION

Two major points occur to us. First, that we might have had an effect at 0.01 ppm, but trouble with two blanks led us to make the most conservative analysis regarding controls. With further refinement of our technique, we should be able to pick up effects at lower concentrations if they exist. It should also be possible to test the practicality of this approach with smaller test animals such as copepods.

The objective continues to be to secure an early indicator of later mortality without going through the difficulty of holding test animals for several days to a week.

The second point is that this was not a realistic impact study. Temephos gave 100% Aedes mortalities at 0.01 ppm. We did not test lower than this but assumed that lower doses of temephos might still give adequate mosquito control. Therefore, our impact tests

on Gammarus mucronatus were set at a higher concentration than might actually need to be applied in the field. What is presented in this paper is a first approach at a technique for impact analysis, not an impact case study.

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