APPARENT RESISTANCE TO PYRETHROIDS IN ORGANOCHLORINE-RESISTANT MOSQUITOFISH

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ABSTRACT

Organochlorine-resistant mosquitofish from an insecticide contaminated environment in the Mississippi Delta showed a 3.4-fold tolerance to pyrethrum when compared to a susceptible strain. The 24-hr LC50 values for pyrethrum in susceptible and resistant mosquitofish were 27 and 93 ppb, respectively. Results of studies with sesamex, an inhibitor of mixed-function oxidase (mfo) enzymes, indicated that the tolerance to pyrethrum is partially, but not solely the result of increased levels of mfo enzymes. Additional studies with allethrin show that increased carboxylesterase enzyme levels cannot explain the pyrethrum tolerance which was not accounted for by mfo enzymes; thus yet another mechanism must be involved. Resistant mosquitofish that had been maintained in an insecticide-free environment for several years also possessed a tolerance to pyrethrum but to a lesser degree than the original parents from the insecticide-contaminated area. Data suggest that the observed tolerance to pyrethrum is apparently in part a genetically based resistance involving two or more resistance mechanisms.

INTRODUCTION

Mosquitofish (*Gambusia affinis*) from an insecticide-contaminated drainage ditch in the Mississippi Delta are highly resistant to most organochlorine insecticides (Endrin, 523x, Toxaphene, 376x) and show low levels of resistance to organophosphorus insecticides (parathion, 4.1x, Guthion, 1.2x) (Culley and Ferguson, 1969). These authors found mosquitofish to be resistant to some organochlorine and organophosphorus insecticides that had not been applied in the study area (Strobane, 568x; Ethion, 4.8x). Resistant mosquitofish also possess a tolerance to rotenone, a botanical fish poison that has not been used to any extent in the Delta area (Fabacher and Chambers, 1971).

The purpose of the present study was to determine the toxicity of pyrethroids to organochlorine susceptible and resistant mosquitofish, and to investigate the causes of any subsequent differences in toxicity between the two strains.

MATERIALS AND METHODS

"Resistant" mosquitofish were those collected from an insecticide-contaminated drainage ditch near Belzoni, Mississippi. Resistant fish were transported from Belzoni to State College in August 1969 and placed in a pond which contained no susceptible mosquitofish. These fish and their progeny were designated "removed-resistant." Susceptible fish were collected from ponds in non-agricultural areas near State College, Mississippi.

The fish were collected with a fine-meshed siene and held in the laboratory 24 hr prior to testing.

Pyrethrum (20%) was dissolved in acetone at 10 mg actual toxicant/ml, serially diluted at 1 ml of solution/liter of water to yield the desired final concentrations (20-120 ppb). Control groups received an equal volume of solvent alone. LC50 determinations were conducted in glass aquaria with 50 fish in 20 liters of dechlorinated tap water. A minimum of seven dosage concentrations were used.

In addition, the toxicity of 70 ppb pyrethrum for removed-resistant fish was compared to that for susceptible and resistant fish. A single replicate consisting of 50 fish per treatment was run.

The effects of sesamex on the toxicity of pyrethrum were then investigated. Susceptible and resistant mosquitofish were exposed to 2 ppm sesamex for 24 hr. After the sesamex pretreatment, the fish were then exposed to 40 ppb pyrethrum. This test consisted of three replicates of 12 fish per treatment.

Toxicity of allethrin at 120 ppb to susceptible and resistant fish, and at 30 ppb to sesamex (2 ppm, 24 hr) pretreated fish was determined. These tests consisted of one replicate of 50 non-pretreated fish and three replicates of 12 pretreated fish of each of the two populations.

RESULTS AND DISCUSSION

Organochlorine-resistant mosquitofish showed a 3.4-fold tolerance to pyrethrum over the susceptible strain. The LC50 values for pyrethrum treated susceptible and resistant mosquitofish were 27 and 93 ppb, respectively.

Pyrethrum is detoxified in insects and mammals via oxidation by microsomal mixed-function oxidase (mfo) enzymes (Yamamoto *et al.*, 1969; Casida *et al.*, 1971). Sesamex, a known inhibitor of mfo enzymes, was used to determine if any differences in toxicity between the two strains of fish were a result of different levels of mfo enzymes. Data in Table 1 showed that pretreatment of susceptible and resistant mosquitofish with sesamex increased the toxicity of pyrethrum to both strains. The mortality of sesamex-pretreated resistant fish to pyrethrum is 27%, while the expected mortality from bioassay studies on non-pretreated fish is less than 1%. Increased levels of mfo enzymes contribute to, but cannot completely explain the tolerance to pyrethrum in mosquitofish, as sesamex lessens, but does not entirely eliminate the observed tolerance. Thus, tolerance to pyrethrum involves mechanisms other than increased mfo enzymes.

Table 1. Mortality of susceptible and resistant mosquitofish treated with 40 ppb pyrethrum (pretreated 24 hr with 2 ppm sesamex).

Population	Mortality (%)
Susceptible	91
Resistant	27[1]

[1]Expected mortality with 40 ppb of pyrethrum alone is <1%.

The possibility also exists that pyrethrins are hydrolyzed via carboxylesterases. The synthetic pyrethroid, allethrin, is chemically similar to pyrethrum; however, allethrin is metabolized exclusively by mfo enzymes (Casida 1972). Data indicated that resistant fish also possess a tolerance for allethrin (Table 2). Pretreatment with sesamex did not overcome the tolerance mechanism in the resistant fish since these fish were tolerant even after sesamex treatment. Again, a mechanism of tolerance other than increased mfo enzymes is suggested. These data infer that tolerance to pyrethroids involves factors other than detoxication by mfo and/or esterase enzymes. The tolerance to pyrethrum in resistant fish may result from a slower rate of uptake or desensitization of nervous tissue. Since the time period before the onset of symptoms is similar in both strains, but the severity of poisoning differs, the latter explanation is more probable.

Table 2. Mortality of susceptible and resistant mosquitofish treated with 30* ppb and 120 ppb allethrin.

Population	Mortality (%)	
	30 ppb	120 ppb
Susceptible	80	80
Resistant	11	30

*Pretreated 24 hr with 2 ppm sesamex.

Removed-resistant mosquitofish also possess a tolerance for pyrethrum (Table 3). Since the average life span of a mosquitofish is about a year, the majority of these removed-resistant mosquitofish would be progeny and not the original parents. Tolerance to pyrethrum in removed-resistant mosquitofish suggests that higher mfo activity is, in part, genetically inherited and that the observed tolerance is apparently a resistance phenomenon.

Table 3. Mortality of susceptible, removed-resistant, and resistant mosquitofish treated with 70 ppb pyrethrum.

Population	Mortality (%)
Susceptible	84
Removed-resistant	44
Resistant	24

Since resistant mosquitofish from the Belzoni area have had no known exposure to pyrethrum, high levels of mfo enzymes are apparently not the result of selection by this chemical. Mosquitofish from the Belzoni area are known to contain organochlorine residues and since organochlorine compounds are known inducers of mfo enzymes in mammals (Street 1969), we cannot rule out the possibility that they might also induce high levels of mfo enzymes in resistant mosquitofish. Murphy (1967) reported that when mosquitofish from the Belzoni area were maintained in an insecticide-free environment, none showed reversion toward susceptibility to endrin. In fact, the F1, F3, and F4 generations had a lower percent mortality when exposed to endrin than did the original Belzoni parents (F2 generation fish were not tested). The data in Table 3 showed that when resistant fish were removed from the insecticide-contaminated environment and placed in an uncontaminated environment, successive generations (removed-resistant) tended to revert toward susceptibility to pyrethrum. The same was found to be true for tolerance to rotenone in removed-resistant mosquitofish (Fabacher and Chambers, 1971). This suggests that decreased tolerance is the result of decreased mfo levels in the progeny. Attempts to induce mfo enzymes in the laboratory in both susceptible and removed-resistant fish have been unsuccessful.

These data showed that mosquitofish from a pesticide polluted environment possess altered enzymic activity when compared to fish of the same species from an uncontaminated environment. We feel that any alterations in the biochemistry of pesticide-resistant mosquitofish which allow them to tolerate or become resistant to various toxic materials are environmentally important and deserve further investigation.

ACKNOWLEDGEMENTS

This investigation was supported by funds provided by Grant R200-W-5540 from the Department of Health, Education and Welfare, U.S. Public Health Service.

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EFFECTS OF SUB-LETHAL CONCENTRATIONS OF PHENOL ON EVENTS IN THE PRE-REPRODUCTIVE PERIOD OF THE CLADOCERAN, *DAPHNIA MAGNA*[1]

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ABSTRACT

The purpose of this investigation was to determine the effect of continuous exposure of pre-adult *Daphnia magna* to low, presumably sub-lethal doses of phenol.

The experimental data were obtained through use of a standard 24-hour toxicity bioassay and a modified long-term toxicity bioassay. Control and test animals were cultured in a synthetic pond water and fed with dried yeast. Six concentrations of phenol were tested.

Data were applied to least squares linear regression analysis, multiple linear regression analysis, crossed covariance analysis, and several related tests in order to quantitatively interpret the total effects of the chronic poisoning.

It was found that phenol exhibited strong interaction with temperature of the culture medium and with the age of the individual to retard ecdysis. This results in a prolonged generation time. Mortality was also increased and reproduction was greatly inhibited. Combinations of these effects act to greatly reduce population growth rates.

It is concluded that even minute amounts of phenol introduced into a natural environment may have deleterious effects on the ecological balance of an ecosystem.

[1]The funds for this research were received from the Federal Water Pollution Control Administration, Grant WP-00927-03.

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