A Possible Mechanism of Insecticide Resistance in Mosquitofish

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Mosquitofish (Gambusia affinis) from a heavily insecticide contaminated site in the Mississippi Delta are resistant to most organochlorine pesticides (1). Insecticide-resistant populations of mosquitofish inhabit drainage canals adjacent to cotton fields, where direct uptake of insecticide residues from the water is a major selective factor in the development of resistance (2). Insecticide resistance in mosquitofish is an excellent example of the effects of chronic environmental pollution, and the mechanisms of this resistance should be completely understood.

Dieldrin-resistant strains of the mosquito, Aedes aegypti (L.), contained 20-50% more larval fat than susceptible strains (3). Holden (4) reported that lipid content is an important factor in DDT tolerance in brown trout.

This study compares the percent lipid content in insecticide-resistant and susceptible mosquitofish, and compares lipid content to survival of susceptible mosquitofish treated with endrin.

Materials and Methods

Insecticide-resistant and susceptible mosquitofish were collected for lipid analysis in February, 1970. The resistant mosquitofish were collected from an insecticide contaminated drainage ditch near Belzoni, Mississippi. "Removed-resistant" mosquitofish were collected from an uncontaminated pond near State College, Mississippi. These fish were transported from Belzoni

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to State College in August, 1969. The susceptible fish were collected from an uncontaminated pond near State College.

The fish were frozen and periodically thawed for analysis. The percent lipid content was individually determined in the whole bodies of 19 resistant, 20 removed-resistant, and 19 susceptible fish. In addition, livers and brains were also analyzed from pooled samples of 25 fish from each of the 3 groups. These analyses were replicated twice.

Susceptible mosquitofish were collected in October, 1970 to compare lipid content in dead and living fish after treatment with endrin. Three endrin concentrations (20, 30 and 40 ppb) were selected, and 50 fish were treated at each concentration according to the static conditions described by Finley (5). At 6, 12, 24, and 30 hours post exposure, both dead and living fish were analyzed for lipid content. A minimum of 3 samples with 3 fish per sample were used for each analysis.

Lipids were extracted with anhydrous ethyl ether in a microsoxhlet apparatus, weighed to the nearest 0.1 mg and expressed as percent lipid on a dry weight basis (6).

Results and Discussion

The lipid content of resistant mosquitofish was 1.8 times that of susceptible fish (Table 1). Lipid content of removed-resistant fish was only 1.2 times that of susceptible fish. Similarly, the lipid content of livers from resistant fish was 1.7 times that of livers from susceptible fish. Lipid content of livers from removed-resistant fish was only 1.1 times that of livers from susceptible fish. The lipid content of the brain was essentially the same for all 3 populations of fish.

Susceptible mosquitofish that survived exposure to endrin consistently contained more lipid than dead fish at the time intervals and concentrations tested (Table 2). This difference was most pronounced at the highest endrin concentration. Lipid content of surviving susceptible mosquitofish approached that found in resistant fish. It should be noted that susceptible fish collected in October (Table 2) contained considerably more lipid than those collected in February (Table 1).

TABLE 1

Lipid Content of Whole Bodies, Livers and Brains of Resistant, Removed-Resistant, and Susceptible Mosquitofish. (Standard Deviations in Parenthesis)

Population	Lipid Content (%)			
	Whole Bodies		Livers	Brains
Resistant	16.9	(3.2)	25.7	26.8
Removed-Resistant	11.7	(2.9)	16.9	25.0
Susceptible	9.4	(2.2)	15.1	25.3

TABLE 2

Lipid Content of Both Dead and Living Susceptible Mosquitofish Exposed to Endrin.

Time (hrs)	Conc. (ppb)	Lipid Content (%)	
		Dead	Alive
6	40	10.4	15.7
12	20	12.2	15.3
24	20	13.3	16.2
30	30	12.2	15.8

Ludke (7) found that the liver size of insecticide-resistant mosquitofish was about twice that of susceptible mosquitofish. Our studies show that the increased liver size of resistant mosquitofish results, at least partially, from high lipid content.

The mechanism of endrin resistance in mosquitofish has been attributed by Ferguson et al. (8) to toleration of the active toxicant within the body. However, these authors did not suggest how the toxic effects of endrin are avoided. We have shown a direct correlation between high lipid content and endrin tolerance in susceptible fish. Yamasaki and Narahaski (9) found that the nervous tissue of dieldrin-resistant houseflies was less sensitive to direct application of dieldrin than was that of susceptible flies. Although no direct evidence is available, we feel that the nervous system of resistant fish may also be less sensitive to low concentrations of endrin. We are presently investigating this possibility. Organochlorine insecticides are highly lipophilic compounds and lipids could serve as a reservoir to protect the nervous system from high concentrations of toxicant. Finley (5) reported that high winter residues of organochlorine insecticides in resistant mosquitofish were accompanied by increased susceptibility. He also reported that resistant populations were more susceptible during the winter and early spring than in the summer and fall. These changes in susceptibility could be the result of seasonal changes in lipid content.

There are several possible explanations for the presence of high lipid concentrations in resistant mosquitofish. The drainage ditches inhabited by resistant fish are extremely fertile (5), thus high lipid levels may result from high fertility. Insecticide residues are usually present in these waters. Our studies indicate that high lipid levels may be selected for if this phenomenon is genetically controlled. Preliminary studies in our laboratory suggest that organochlorine insecticide residues induce lipid deposition in susceptible mosquitofish.

Mosquitofish are highly resistant to some lipid-soluble organochlorine insecticides (endrin 523X, toxaphene 376X) and only slightly tolerant to others

(DDT 5X) (10). This suggests that resistance to organochlorine insecticides in mosquitofish probably results from other factors in addition to increased lipid content. These may include decreased uptake, "resistant" nervous tissue, stress-tolerance, or others. The total mechanism of resistance is probably a complex interaction of many factors.

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