Uptake and Storage of ^{14}C-Labeled Endrin by the Livers and Brains of Pesticide-Susceptible and Resistant Mosquitofish

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In 1963, a DDT-resistant population of mosquitofish (Gambusia affinis) was discovered near Belzoni, Mississippi (VINSON et al. 1963). These pesticide-resistant 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 (FINLEY et al. 1970). CULLEY and FERGUSON (1969) showed these fish to be resistant to most organochlorine pesticides. They reported that mosquitofish from the Belzoni area possessed about a 500-fold resistance to endrin when compared to a susceptible strain.

FERGUSON et al. (1966) rejected mechanical exclusion, rate of uptake, and metabolism as mechanisms of endrin resistance in mosquitofish in favor of increased physiological tolerance. YARBROUGH and WELLS (1971) suggested that endrin resistance in the mosquitofish involved a cellular membrane barrier. They found that endrin inhibited succinic dehydrogenase activity in resistant tissue only after the mitochondrial membrane was ruptured. Enzymatic activity was inhibited in both intact and ruptured mitochondria from susceptible fish. Subsequently, WELLS and YARBROUGH (1973) suggested a greater uptake of endrin by susceptible brain and liver fractions; retention of endrin by resistant cell membranes; a more efficient blood-brain barrier in resistant fish; and increased binding of endrin to myelin in nervous tissue of susceptible fish as additional mechanisms of endrin resistance.

The objectives of this study were to measure the rate of uptake and storage patterns of endrin in the livers and brains of pesticide-susceptible and resistant mosquitofish as possible mechanisms of endrin resistance.

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MATERIALS AND METHODS

Resistant mosquitofish were collected near Belzoni, Mississippi. Susceptible fish were collected from ponds in non-agricultural areas near Mississippi State, Mississippi. The ^{14}C-labeled endrin used in this study had a specific activity of 2.5 mci/mmole. Ten each of susceptible and resistant mosquitofish were treated with 20 or 1000 ppb endrin. All treatments were conducted in 6 liters of dechlorinated tap water.

When a susceptible fish died at the 20 ppb concentration, it was immediately removed from the aquarium along with a live resistant fish. No resistant fish died at this concentration. At 1000 ppb all of susceptible and resistant fish were killed within two hours. When a fish died it was immediately removed from the aquaria for analysis. Cessation of opercular movements was designated as death.

The livers and brains from each fish were assayed to determine any differences in distribution, uptake, and nerve binding patterns between endrin-treated susceptible and resistant fish. The samples were weighed (wet weight) and then homogenized in a glass homogenizer which contained extraction solvent. The livers were extracted with chloroform. The brains were first extracted with n-hexane to remove the endrin not tightly bound to the nerve components. They were then extracted with a chloroform/methanol mixture to remove the tightly bound endrin. The fractions were evaporated to near dryness in a rotary evaporator then diluted in 15.0 ml of scintillation fluid (4.2 gm 2.5-diphenyloxayole, 52.5 mg 2,2'-paraphenylene bis 5-phenyloxayole and 500 ml each of toluene and Triton X-100) for counting. Radioactivity was determined in a Packard Model 3320 Tri-Carb Scintillation Spectrometer. Livers and brains were individually assayed for ^{14}C content, the data pooled and expressed as mean values in ppm.

RESULTS AND DISCUSSION

The brains and livers from susceptible fish killed with 20 ppb endrin contained 1.9x endrin as those from live resistant fish treated for the same time period (Table 1). The brain/liver (B/L) ratios at 20 ppb were the same, indicating no difference in the distribution of endrin between the brains and livers of susceptible and resistant fish. When fish from both strains were killed with 1000 ppb, the brains from susceptible fish again contained more endrin than those from resistant fish. The livers of resistant fish, however, contained

Table 1

Mean Quantities of Endrin (ppm) in the Brains and Livers of Susceptible (S) and Resistant (R) Mosquitofish Treated With 20 and 1000 ppb

	S	R	S/R
		20 ppb	
Brain	16.98	8.83	1.9
Liver	33.28	16.84	1.9
B/L	0.51	0.52	1.0
		1000 ppb	
Brain	149.31	57.52	2.6
Liver	160.27	353.42	0.45
B/L	0.93	0.16	5.8

2.2x more endrin than those of susceptible fish. At this concentration all susceptible fish were killed within 45 minutes; all resistant fish were killed within 2 hours. A time lag of 75 minutes existed between the death time of susceptible and resistant fish at the 1000 ppb concentration. A better understanding of the rate of uptake of endrin can be had if these data are converted to ppm/hr. The data would then be: S-brains, 198.6; R-brains, 28.76 (6.90x); S-livers, 213.6; R-livers, 176.7 (1.2x). These data then show a faster uptake of endrin by the brains and livers of susceptible fish. The B/L ratio at the 1000 ppb concentration is 5.8x lower in resistant fish. Thus, endrin is entering the brain at a slower rate in resistant fish and is accumulating in the liver. There is a faster uptake of endrin by susceptible fish and, at least at high lethal concentrations, endrin is entering the nervous tissue of resistant fish at a slower rate indicating a more efficient blood-brain barrier in resistant fish. Similarly, WELLS and YARBROUGH (1972) reported a greater rate of endrin uptake by the brain and liver fractions of susceptible mosquitofish and a more efficient blood-brain barrier in resistant fish.

The hexane fraction contained the majority of the endrin extracted from the brains of susceptible and resistant mosquitofish treated with 20 ppb (Table 2).

Table 2

Mean Quantities and Percentages of Endrin in n-Hexane and Chloroform-Methanol Extracts of Brains From Susceptible (S) and Resistant (R) Mosquitofish Treated With 20 and 1000 ppb Endrin

	S		R	
	ppm	%	ppm	%
		20 ppb		
C-M	2.12	12.5	1.44	16.3
H	14.86	87.5	7.39	83.5
C-M/H	0.14		0.19	
		1000 ppb		
C-M	39.72	26.6	6.84	11.9
H	109.59	73.4	50.68	88.1
C-M/H	0.36		0.13	

There was no significant difference in the ratios of endrin extracted with hexane or with chloroform-methanol (C-M) in susceptible and resistant fish brains. Previously, POLLES (1970) found that in endrin-treated tobacco budworms, endrin was more readily extractable with C-M from the nerve cords of resistant insects than from the nerve cords of susceptible insects. He suggested that the C-M extractable endrin represented a greater binding of endrin to non-essential protein complexes in resistant nervous tissue, thus decreasing the amount of endrin available to produce a toxic effect. Most of the extractable endrin from the brains of fish treated with 1000 ppb was again extracted in the hexane fraction. Unlike the data of POLLES (1970), of the total endrin in the brain, the C-M/H ratios show that significantly more endrin was extracted with C-M from the brains of susceptible fish than from the brains of resistant fish. Assuming that the C-M fraction represents the endrin

bound complexes in the nerve, there is a greater degree of binding in susceptible fish brains than in resistant fish brains.

It appears that at low concentrations, slower uptake of endrin in resistant fish may represent a major mechanism of resistance. At higher concentrations, other mechanisms seem to become important. A more efficient blood-brain barrier in resistant fish, for example, could prevent large amounts of endrin from accumulating in the brain, thus allowing a more rapid accumulation of endrin in the lipid stores of the liver. FABACHER and CHAMBERS (1971) reported that livers of resistant fish have a higher lipid content than those of susceptible fish. YARBROUGH and COONS (1974) found a marked increase in cell size and lipid inclusions in the hepatocytes of resistant mosquitofish as compared to susceptible mosquitofish, and also suggested that the inclusions could serve as a storage area to compartmentalize highly lipid soluble organochlorine insecticides. Additionally, if the chloroform-methanol/hexane ratio can be interpreted as an index of binding of endrin to tissues (e.g., protein), the brains of resistant fish show a lower degree of binding of endrin than do those of susceptible fish following exposure to high insecticide concentrations.

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