Epoxidation and Fate of [^{14}C]Aldrin in Insecticide-Resistant and Susceptible Populations of Mosquitofish (*Gambusia affinis*)

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One susceptible and two resistant populations of mosquitofish (*Gambusia affinis*) were treated with [^{14}C]aldrin to determine differences in aldrin epoxidation between susceptible and resistant mosquitofish brains and livers. Resistant mosquitofish converted aldrin to dieldrin and/or water-soluble material at a greater rate than susceptible mosquitofish. These data would tend to support rate of detoxification as a possible mechanism of resistance to aldrin in the mosquitofish.

Vertebrate resistance to DDT was first demonstrated in populations of mosquitofish (*Gambusia affinis*) found in drainage ditches in the Mississippi Delta (Boyd and Ferguson, 1964). This initial report was expanded to include endrin, aldrin, and dieldrin (Culley and Ferguson, 1969). They demonstrated a 71-fold difference in aldrin resistance (susceptible LC_{50} = 36.17 ppb, resistant LC_{50} = 2558.12 ppb) and a 54-fold difference in dieldrin resistance (susceptible LC_{50} = 8.02 ppb, resistant LC_{50} = 433.60 ppb). The resistant mosquitofish used in this study showed the same resistance as those assayed by Cully and Ferguson.

When aldrin is applied to living organisms, it is converted to the epoxide dieldrin (Earle, 1963; Lichtenstein and Schulz, 1965; Ludwig *et al.*, 1964). Ludke *et al.* (1972) found that freshwater fishes convert aldrin to dieldrin and suggested the presence of enzymes necessary for metabolism of lipid-soluble compounds. They also found aldrin to be toxic to mosquitofish. Wells and Yarbrough (1973) studied aldrin and dieldrin retention patterns in subcellular fractions of livers from the mosquitofish and found that the heavy microsome fraction from resistant fish retained more dieldrin than that from susceptible fish. They suggested that this retention might be related to the metabolism of aldrin or dieldrin by microsomal enzymes.

The purpose of this study was to determine the difference in aldrin epoxidation between susceptible and resistant mosquitofish brains and livers, as it might relate to resistance.

MATERIALS AND METHODS

Resistant mosquitofish were collected from drainage ditches in cotton fields in the Mississippi Delta and susceptible fish were collected from ponds at State College, Mississippi. A second population of mosquitofish was collected from the Mississippi Delta in 1968 and maintained in ponds near State College, Mississippi. The fish from the removed population (denoted as "removed resistant" in this paper) represent the fourth generation. All fish were held in the laboratory for 1 week prior to assay. About 95% of all fish assayed were sexually mature females.

Three groups of nine fish from each population were treated in 5 ppb of [^{14}C]aldrin solutions in 8 l. all-glass aquaria. The [^{14}C]aldrin in acetone was transferred to the glass aquaria and water was added slowly to prevent complete recrystallization of the aldrin. The stock [^{14}C]aldrin was uniformly labeled with a specific activity of 50 mCi/m*M*. Assay showed this material to contain 2.5% dieldrin. Three fish were removed from each aquaria following exposure for 4 and 8 hr, and each was washed carefully with acetone. All tissue samples were extracted in the same manner. The tissues were weighed and homogenized in distilled water (20 mg of tissue/ml of water) using 5 ml Ten Broeck tissue grinders. An aliquot was removed for protein determination by the method of Lowry *et al.* (1951). The residue values (parts per million = ppm) reported in this paper were determined using protein values, although wet weight determinations gave relatively similar values. Samples were then extracted three times with hexane (5 ml each) and twice for recovery of lipid-bound material in the brain with 3:1 chloroform–methanol (5 ml each). On a standard sample, 95% of the aldrin applied was recovered. The remaining aqueous portion of the sample was taken for counting and the hexane and chloroform extracts were concentrated to near dryness before tlc cleanup.

Tlc samples were quantitatively streaked on 20 × 20 cm glass tlc plates coated with a 0.300-mm thick silica gel HF and developed using hexane–acetone (8.5 + 1.5) as the mobile solvent. The solvent front was allowed to migrate for 15 cm and there was complete separation of aldrin and dieldrin. The aldrin and dieldrin zones were scraped into counting vials and taken up to 15 ml with scintillation cocktail. Samples were counted using a Packard Model 3320 Tri-Carb scintillation spectrometer.

RESULTS AND DISCUSSION

There was no difference in the uptake of [^{14}C]aldrin among susceptible, resistant, or removed resistant mosquitofish brains at 4 or 8 hr exposure to 5 ppb of [^{14}C]aldrin (Table I). All populations exhibited increased aldrin and dieldrin residues in the brain during exposure and the ratios of aldrin:dieldrin in the brains of each population were similar (Table I). There was significantly more (p = 0.05) dieldrin bound in the brains of susceptible fish at 4 and 8 hr, as indicated by the aldrin:dieldrin ratios (Table II). There was a highly significant (p = 0.01) difference between susceptible and resistant mosquitofish livers in the unextractable material remaining in the aqueous phase following hexane and chloroform–methanol extraction (Table II). All three populations had significantly (p = 0.05) different amounts of unextractable material in the liver remaining following 8 hr of treatment (Table II).

The concentrations of aldrin in the livers of resistant and susceptible fish did not differ after 4 hr exposure. There was a significant (p = 0.05) difference following the 8-hr treatment. However, the susceptible fish livers contained less dieldrin than the resistant populations after 4 hr, and the resistant population had more dieldrin in their livers than the susceptible and removed resistant population after 8 hr. The aldrin:dieldrin ratio was the same in all three populations, however (Table I). The unextractable material in the aqueous phase of susceptible liver extracts was lower than in the resistant ones after 4 hr, but all three groups differed after 8 hr exposure (Table II).

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Table I. Aldrin and Dieldrin Residues (ppm Based on Protein) and Aldrin:Dieldrin (A:D) Ratios with Standard Errors (±) in Brains and Livers of Susceptible (S), Removed Resistant (RR), and Resistant (R) Mosquitofish Exposed for 4 and 8 hr to 5 ppb of [14C]Aldrin. Values Represent the Mean of Three Separate Treatments of Three Fish Each

Tissue	Fish	4-hr exposure			8-hr exposure		
		Aldrin	Dieldrin	A:D	Aldrin	Dieldrin	A:D
Liver (hexane)	S	2.39 ± 0.69	1.38 ± 0.14	1.67 ± 0.32	2.47 ± 0.19	1.38 ± 0.11	1.80 ± 0.01
	RR	3.58 ± 0.53	2.34 ± 0.45	1.42 ± 0.37	3.59 ● 1.01	1.98 ± 0.39	1.79 ± 0.25
	R	3.42 ± 0.57	2.36 ± 0.38	1.47 ± 0.09	4.78 ± 0.37	3.14 ± 0.23	1.62 ± 0.09
t-test[a]			S-RR* S-R*		S-R*	S-R** RR-R*	
Brain (hexane)	S	2.16 ± 0.41	1.15 ± 0.15	1.87 ± 0.19	3.96 ± 0.60	1.85 ± 0.31	2.16 ± 0.06
	RR	1.83 ± 0.20	0.92 ± 0.17	2.00 ± 0.43	3.05 ± 0.44	1.48 ± 0.36	2.25 ± 0.56
	R	1.58 ± 0.20	0.88 ± 0.07	1.85 ± 0.34	3.47 ± 0.03	1.46 ± 0.16	2.42 ± 0.24
t-test[a]							
Brain bound (chloroform–methanol)	S	1.00 ± 0.22	0.25 ± 0.17	3.89 ± 0.81	0.55 ± 0.01	0.36 ± 0.27	1.56 ± 1.19
	RR	0.57 ± 0.02	0.05 ± 0.01	12.38 ± 1.97	0.47 ± 0.08	0.05 ± 0.01	9.23 ± 2.14
	R	0.52 ± 0.09	0.06 ± 0.02	11.68 ± 3.33	0.61 ± 0.01	0.10 ± 0.03	7.58 ± 2.79
t-test				S-R** S-RR**			S-R** S-RR**

[a] Significant difference between means at the 0.05 (*) or 0.01 (**) level of confidence as determined by *t*-test.

Table II. Concentrations (ppm ± Standard Error) of Materials Remaining in the Homogenate Aqueous Phase after Hexane and Chloroform–Methanol Extraction of Brains and Livers of Susceptible (S), Removed Resistant (RR), and Resistant (R) Mosquitofish Exposed for 4 and 8 hr to 5 ppb of [14C]Aldrin. Values Represent the Mean of Three Separate Treatments of Three Fish Each

Tissue	Fish	4-hr exposure	8-hr exposure
		Liver	
	S	1.78 ± 0.21	1.73 ± 0.24
	RR	4.45 ± 0.98	2.43 ± 0.10
	R	6.87 ± 0.88	4.50 ± 0.87
t-test[a]		S-RR** S-R**	S-RR* S-R* RR-R*
		Brain	
	S	0.06 ± 0.02	0.03 ± 0.01
	RR	0.04 ± 0.01	0.03 ± 0.01
	R	0.02 ± 0.01	0.05 ± 0.01
t-test[a]			

[a] Significant difference between means at the 0.05 (*) or 0.01 (**) level of confidence as determined by *t*-test.

These results indicate that resistant and susceptible mosquitofish convert aldrin to dieldrin and that a considerable quantity of unextractable material remains in the aqueous phase of the liver following hexane and chloroform extraction. Following 4 and 8 hr treatments, there was greater conversion to water-soluble material in resistant mosquitofish collected directly from the Mississippi Delta as opposed to removed resistant mosquitofish. Resistant fish livers may convert aldrin and/or dieldrin to water-soluble materials at a greater rate, and this could play a part in the resistance mechanism. The resistant population from a pesticide-contaminated area may have been in an induced state. Mayer *et al.* (1970) demonstrated enzyme induction in fish treated with organochlorine pesticides. These data would tend to support rate of detoxification as a possible mechanism of resistance to aldrin in the mosquitofish.

In previous studies, Wells and Yarbrough (1972a,b, 1973) demonstrated a difference in penetration of endrin, DDT, aldrin, and dieldrin in the brain based on comparisons of brain and liver uptake within a mosquitofish population. They found that susceptible fish brains contained 56% of the aldrin taken up by the brains and livers. The resistant fish brains contained only 7% of the total uptake of the brains and livers. Since livers of removed resistant fish are approximately the same size as those of susceptible fish, comparisons of brain:liver ratios within a population are of value as an indirect measurement of the blood–brain barrier. These data support their findings for aldrin and dieldrin.

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