Aldrin and Dieldrin Uptake in Insecticide-resistant and Susceptible Mosquitofish (*Gambusia affinis*)

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Abstract

Insecticide-resistant and susceptible mosquitofish (Gambusia affinis) were exposed to ^{14}C-aldrin and ^{14}C-dieldrin for timed intervals. Whole body and tissue levels of the two organochlorine insecticides were compared between the two populations. At the 10 ppb aldrin and dieldrin treatment levels there were no significant insecticide level differences between resistant and susceptible fish. However, at 80 ppb aldrin and 30 ppb dieldrin there were significant differences. There was 8.5 times as much aldrin in whole body samples of susceptible fish as in whole body samples from resistant fish. There was only 1.24 times as much dieldrin in susceptible as in resistant fish, but it was significant ($P < 0.05$). There were significantly higher amounts of both insecticides in tissues (muscle, liver, and brain) examined from susceptible fish, with the exception of the liver in which dieldrin levels were essentially the same for both populations.

Insecticide level comparisons were also made on the basis of whether the fish were exhibiting symptoms of poisoning or not exhibiting symptoms of poisoning. Within the susceptible population there was no difference in the aldrin whole body levels between those fish not exhibiting symptoms and those exhibiting symptoms of poisoning. However, there was a higher level of dieldrin in fish exhibiting symptoms as compared to those not exhibiting symptoms. There were no differences in the insecticide levels in tissues. In comparisons between populations there were significantly higher levels in both whole body and tissue samples of susceptible fish as compared to resistant fish. This was true regardless of whether susceptible fish were exhibiting symptoms or not exhibiting symptoms of poisoning.

Introduction

Vertebrate insecticide resistance in mosquitofish (Gambusia affinis) involves a number of factors. These include a membrane barrier to some insecticides (YARBROUGH and WELLS 1971; MOFFETT and YARBROUGH 1972) and a more effective brain barrier to insecticides in resistant fish than in the susceptible fish (WELLS and YARBROUGH 1972a). To investigate this barrier, resistant and susceptible mosquitofish were exposed to two cyclodienes, aldrin and dieldrin, and whole body and tissue levels of the two insec-

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ticides were compared between the two populations. Comparisons of insecticide levels between the two populations were based on either timed insecticide exposures or the presence or absence of signs of insecticide poisoning at 6 hrs of insecticide exposure.

Materials and Methods

Insecticide-resistant mosquitofish (R) were collected from drainage ditches in Humphreys County, Miss., and susceptible fish (S) were collected from ponds in Oktibbeha County, Miss. All fish were held in the laboratory for at least 48 hrs before use and were adult females.

Fish were placed in all glass aquaria 24 hrs before exposure to the insecticide and food was withheld during this period. Each test aquarium contained 2 fish per liter of water. Ring-labelled ^{14}C-aldrin and ^{14}C-dieldrin were used. The radiochemical purity of aldrin and dieldrin was 98% as determined by TLC. Labelled compounds were dissolved in acetone to yield a 0.1% solution. ^{14}C-aldrin was mixed with non-labelled aldrin and an amount of this mixture added to each test aquarium to yield a final concentration of either 10 ppb or 80 ppb. The final concentration of the ^{14}C-aldrin in each test aquarium was 10 ppb. ^{14}C-dieldrin was mixed with non-labelled dieldrin and added to the test aquaria to yield a final concentration of either 10 ppb or 30 ppb. The final concentration of ^{14}C-dieldrin in each test aquarium was 10 ppb.

The 10 ppb dieldrin and aldrin concentrations were chosen because neither R nor S fish exhibited symptoms of insecticide poisoning during the test period. The 30 ppb dieldrin and 80 ppb aldrin concentrations were chosen because S fish exhibited symptoms of insecticide poisoning during a 6 hr test period. Because of the insolubility of aldrin and dieldrin in water, it was not possible to obtain an insecticide concentration which would elicit symptoms of poisoning in R fish.

Immediately after addition of the insecticide to each test aquarium, the water was stirred with a glass rod to insure uniform insecticide disbursal. The level of insecticide in all test aquaria was monitored at each sampling period. The insecticide level remained relatively constant during the exposure periods.

Two types of fish samples were taken: those fish not exhibiting symptoms of insecticide poisoning (asymptomatic) and those fish exhibiting symptoms of insecticide poisoning (symptomatic). Symptoms were defined as hypersensitivity, disorientation, and gilling (increased movement of the operculum).

For each sampling period, fish were removed from the test aquarium at timed intervals and rinsed with acetone to remove any insecticide which had adhered to the body surface. Samples were homogenized using TenBroeck glass homogenizers in cold, glass distilled, deionized water and an aliquot sample removed for protein determination by the method of LOWRY et al. (1951).

Whole body and tissue (brain, liver, and muscle) samples consisted of three fish pooled per sample. Fish grouped on the basis of the presence or absence of symptoms of insecticide poisoning were sampled at the end of 6 hrs of insecticide exposure.

The sample was homogenized and 1 ml of the homogenate added to scintillation fluid and counted. Undigested tissue samples gave comparable counting results to digested sample preparations; therefore, most samples were undigested tissue homogenates.

The scintillation fluid consisted of 50 mg 2,2'-paraphenylene -bis-5-phenyloxazole, 4.0 gm 2,4-diphenyloxazole, 40 gm Cabosil®, 500 ml scintanalyzed toluene, and 500 ml Triton® X-100. All samples were counted in a Packard Model 3320 Tri-Carb® Liquid Scintillation Spectrophotometer.

Results

There were no statistically significant differences between the levels of either aldrin or dieldrin in susceptible and resistant fish during the 12 hrs of exposure to 10 ppb (Table 1). There were greater dieldrin than aldrin levels in both fish populations.

Table 1

Insecticide concentrations in whole body insecticide-resistant (R) and -susceptible (S) mosquitofish exposed to 10 ppb ^{14}C-aldrin (A) or ^{14}C-dieldrin (D)

Hours	Aldrin S	Aldrin R	S/R	S D/A	R D/A
3	0.72 ± 0.1	0.89 ± 0.35	0.81		
6	1.64 ± 0.18	1.53 ± 0.08	1.07		
9	2.82 ± 0.59	2.50 ± 0.21	1.13		
12	2.90 ± 0.19	3.18 ± 0.45	0.91		
	Dieldrin				
6	12.78 ± 1.54	11.80 ± 1.33	1.08	7.79	7.71
12	24.72 ± 1.53	22.88 ± 1.10	1.08	8.52	7.19

[a] Values are expressed as the mean of 3 determinations in ng/mg protein ± S.E.

[b] Values are expressed as the mean of 4 determinations in ng/mg protein ± S.E.

After exposure to 80 ppb aldrin for 6 hrs, there were no significant differences in the aldrin levels between asymptomatic and symptomatic S fish. The R fish exhibited a significantly lower level of aldrin than either the symptomatic or asymptomatic S fish ($P < 0.01$) (Table 2). When the aldrin levels in asymptomatic fish were compared, S fish exhibited an 8.5 fold higher level than R fish ($P < 0.01$); symptomatic S fish demonstrated an 8.8 fold greater level than asymptomatic R fish ($P < 0.01$). There was no difference between aldrin levels in symptomatic and asymptomatic S fish.

Table 2

Whole body concentrations of aldrin or dieldrin in insecticide-resistant and -susceptible mosquitofish. Exposure to each insecticide was for 6 hrs[a]

Susceptible Asymptomatic Fish (S_a)	Susceptible Symptomatic Fish (S_s)	Resistant Asymptomatic Fish (R_a)	$(S_a/R_a)^b$	$(S_a/S_s)^b$	$(S_s/R_a)^b$
<u>^{14}C-Aldrin</u> (10 ppb)					
1.64 ± 0.2		1.53 ± 0.08	1.07		
<u>^{14}C-Dieldrin</u> (10 ppb)					
12.78 ± 1.5		11.80 ± 1.3	1.08		
<u>^{14}C-Aldrin</u> (80 ppb)					
96.53 ± 12.3	100.03 ± 8.0	11.35 ± 1.0	8.50***	0.97	8.81***
<u>^{14}C-Dieldrin</u> (30 ppb)					
48.41 _ 2.9	78.60 ± 4.3	46.97 ± 3.0	1.24**	0.74*	1.67****

[a] Values are expressed as the mean of 5 determinations in ng/mg protein ± S.E.

[b] Concentrations differ significantly at $\underline{P} < 0.02$ (*), $\underline{P} < 0.05$ (**), and $\underline{P} < 0.001$ (****).

After exposure to 30 ppb dieldrin for 6 hrs, both symptomatic and asymptomatic S fish had significantly higher levels of dieldrin than asymptomatic R fish, 1.24 fold ($\underline{P} < 0.05$) and 1.67 fold ($\underline{P} < 0.001$), respectively (Table 2). Asymptomatic S fish had 0.74 fold lower levels than symptomatic S fish ($\underline{P} < 0.02$).

After 6 hrs of exposure, there was a greater amount of aldrin in fish exposed to 80 ppb than in fish exposed to 10 ppb ($\underline{P} < 0.001$) (Table 2). There was also an increased level of dieldrin in fish exposed to 30 ppb as compared to the level in fish exposed to 10 ppb dieldrin after 6 hrs ($\underline{P} < 0.001$). These differences occurred in both S and R fish.

There were significantly higher aldrin levels of all tissues of both asymptomatic and symptomatic S fish as compared to tissues of R fish, ($\underline{P} < 0.05$ or less) (Table 3). However, there were no statistical differences in tissue aldrin levels between symptomatic and asymptomatic S fish.

There were no differences in the dieldrin liver levels of either S or R fish exposed to 30 ppb dieldrin for 6 hrs (Table 3). However, there was a 1.3 fold greater dieldrin level in muscle in asymptomatic S fish than in R fish ($\underline{P} < 0.05$), and a 1.4 fold greater level in symptomatic S fish than in R fish ($\underline{P} < 0.05$).

Table 3

Tissue concentrations[a] of ^{14}C-aldrin or ^{14}C-dieldrin in insecticide-resistant and susceptible mosquitofish at 6 hrs of exposure

Tissue	Resistant Asymptomatic Fish (R_a)	Susceptible Asymptomatic Fish (S_a)	Susceptible Symptomatic Fish (S_s)	$(S_a/R_a)^b$	$(S_s/R_a)^b$	(S_s/S_a)
<u>^{14}C-Aldrin</u> (80 ppb)						
Muscle	6.33 ± 0.52	46.93 ± 1.81	49.72 ± 6.90	7.4***	7.9**	1.1
Liver	61.72 ± 6.67	221.05 ± 19.84	247.20 ± 31.80	3.6***	4.0*	1.1
Brain	20.30 ± 2.70	104.61 ± 7.32	117.12 ± 14.42	5.2**	5.8***	1.1
<u>^{14}C-Dieldrin</u> (30 ppb)						
Muscle	16.99 ± 1.31	21.29 ± 0.62	23.68 ± 3.97	1.3**	1.4*	1.1
Liver	131.61 ± 2.98	113.00 ± 21.29	126.99 ± 13.23	0.9	1.0	1.1
Brain	45.41 ± 1.99	95.94 ± 7.30	118.00 ± 14.70	2.1**	2.6**	1.2

[a] Values are expressed as the mean of 5 determinations in ng/mg protein ± S.E.

[b] Tissue concentrations differ significantly at $P < 0.05$ (*); $P < 0.01$ (**); and $P < 0.001$ (***) by the *t* test.

There was a 2.1 fold difference in brain dieldrin levels between asymptomatic S and R fish ($P < 0.01$), and a 2.6 fold difference between symptomatic S and asymptomatic R fish ($P < 0.01$). There was no difference in any dieldrin tissue levels between symptomatic and asymptomatic S fish.

Discussion

The whole body levels of aldrin and dieldrin in fish exposed to 80 ppb and 30 ppb respectively, indicate that the differences between the two populations are related to a membrane barrier in R fish. These data agree with earlier reports of an effective membrane barrier to insecticides in R fish (WELLS and YARBROUGH 1972a,b). The lack of differences in aldrin and dieldrin levels between whole body samples of both fish populations exposed to 10 ppb may indicate that at low insecticide levels the membrane barrier is either non-functional or equally effective in both populations. This is similar to reports for R and S cockroaches exposed to dieldrin (RAY 1963) and R and S houseflies exposed to dieldrin and aldrin (SELLERS et al. 1973).

In comparing the levels of the two insecticides, dieldrin was at least 4 times higher than aldrin at all sampling periods in both fish populations. The membrane barrier appears to be more effective to aldrin penetration than to dieldrin. Dieldrin penetrates faster than aldrin in the adult housefly (BROOKS 1960), and perhaps is related to the greater polarity of dieldrin.

Because there are varying degrees of insecticide tolerance within both fish populations, insecticide levels were compared on the basis of the presence or absence of symptoms of poisoning. The whole body samples of R fish at 80 ppb aldrin and 30 ppb dieldrin treatments had lower levels of both insecticides than those of S fish. Susceptible fish exposed to 30 ppb dieldrin and not showing symptoms had lower insecticide levels than S fish showing symptoms. However, when a similar comparison was made for aldrin, there was no difference in aldrin levels between asymptomatic and symptomatic S fish. Further, when tissue insecticide level comparisons were made between symptomatic and asymptomatic S fish, there were no differences for either insecticide. A possible explanation for this lack of difference may involve an insensitivity at the target site. If insecticide levels were the only consideration of the toxic effect, the tissue insecticide concentration ratio of symptomatic to asymptomatic fish would be expected to be greater than one at the target site(s). Indicative of the target site is muscle tissue which contains the highest concentration of peripheral nerves of the tissues examined. Although muscle ratios are similar to the other tissues, the actual insecticide concentrations in muscle tissue are much lower than the other tissue levels.

The higher aldrin and dieldrin tissue levels in S fish as compared to R fish tissue levels are an indication that lower insecticide uptake is a factor involved in resistance. A comparison of the insecticide levels in brain tissue to those in liver tissue supports earlier findings that there is a more efficient brain barrier to insecticides in the R population than in the S population (WELLS and YARBROUGH 1972a,b). Dieldrin brain/liver ratio for asymptomatic S fish was 0.85, for symptomatic S fish was 0.93 and for R fish was 0.34. An earlier study reported ratios of 0.08 for R fish and 0.62 for S fish after exposure to 25 ppb dieldrin. Aldrin ratios of 0.23 for S and 0.1 for R fish have been reported (WELLS and YARBROUGH 1973). Aldrin brain/liver ratios of 0.32 in R and 0.45 in the S fish agree with WELLS and YARBROUGH (1973) in which these differences in the effectiveness of a brain barrier were proposed as a factor in resistance. WELLS et al. (1973) reported a greater rate of aldrin epoxidation in R fish than in the S fish, and indicated the presence of a water-soluble metabolite of aldrin in the aqueous fraction of the liver homogenate after extraction with hexane and chloroform:methanol. Therefore, lower levels of aldrin in R fish than in S fish may also reflect a more effective metabolism of aldrin and excretion of the metabolic products by R fish.

In summary, these studies demonstrate that within the S fish population there is a direct correlation between whole body dieldrin concentrations and the appearance of toxic symptoms. However, there is no apparent relationship between tissue insecticide concentrations and toxic symptoms. This suggests that there is an insensitivity at the target site, although the nature of this insensitivity is not clear. Further, the fact that there was always a greater amount of the two insecticides in both whole body and tissues of S fish than R fish is suggestive of an effective insecticide barrier in R fish. However, this barrier can only be effectively demonstrated at high concentrations. The possibility of a greater turnover rate in R fish than in S fish cannot be ignored. However, the equal insecticide levels in R and S fish at the lower insecticide treatments seem to indicate equal turnover rates in both populations. This does not rule out the possibility that at higher insecticide treatments R fish may eliminate the insecticides at a greater rate than S fish.

References

BROOKS, G.T.: Nature 186, 96 (1960).

LOWRY, O.H., N.J. ROSEBROUGH, A.L. FARR, and R.J. RANDALL: J. Biol. Chem. 193:265 (1951).

MOFFETT, G.B. and J.D. YARBROUGH: J. Agr. Food Chem. 20:558 (1972).

RAY, J.W.: Nature 197, 1226 (1963).

SELLERS, L.G., F.E. GUTHRIE, and L.B. COONS: J. Econ. Entomol. 65(2):378 (1973).

WELLS, M.R. and J.D. YARBROUGH: J. Agr. Food Chem. 20:14 (1972a).

WELLS, M.R. and J.D. YARBROUGH: Appl. Pharmacol. 22:409 (1972b).

WELLS, M.R., J.L. LUDKE, and J.D. YARBROUGH: J. Agr. Food Chem. 21:428 (1973).

WELLS, M.R. and J.D. YARBROUGH: Tox. Appl. Pharmacol. 24:190 (1973).

YARBROUGH, J.D. and M.R. WELLS: Bull. Environ. Contam. Tox. 6:171 (1971).

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