Parathion and Methyl Parathion Toxicity to Insecticide-Resistant and Susceptible Mosquitofish (*Gambusia affinis*)

by

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INTRODUCTION

One population of mosquitofish (Gambusia affinis) occurring in the highly agricultural delta region of Mississippi demonstrates resistance to a wide range of organochlorine compounds when compared to a susceptible population. The former population will be designated as R (organochlorine compound-resistant) and the latter as S (organochlorine compound-susceptible). There is reported to be a 4.2 fold difference in the organophosphorus insecticide, parathion, 48-h LC_{50} values (the concentration of toxicant which will cause 50% mortality in 48 h) between the R and S population (CULLEY and FERGUSON, 1969). Because of the low toxicity of methyl parathion, LC_{50} values were not determined for it.

The objectives of this study were to determine the levels of tolerance of mosquitofish to parathion and methyl parathion and to determine whether resistance to these two compounds appears to exist.

MATERIALS AND METHODS

Susceptible mosquitofish (Gambusia affinis) were collected from ponds having no known insecticide exposure near Starkville, Oktibbeha County, Mississippi. Resistant fish were collected from drainage ditches adjacent to cotton fields near Belzoni, Humphreys County, Mississippi. Fish were held in the laboratory in dechlorinated tap water under constant conditions of temperature and nutrition. Over 80% of the fish used were adult females. Insecticides used were 99% pure.

Dosage Mortality Studies

Dosage mortality studies for parathion in the spring and fall and methyl parathion in the fall only were conducted in 8-liter all-glass aquaria in dechlorinated tap water. A fish

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*This study was supported by NIH grant 5 ROI 00412 and NSF Graduate Traineeship grants GZ-1629-1970-71 and GZ-2036-1971-72.

density of 2 fish per liter was used with either 10 or 16 individuals per aquarium. The fish were allowed to acclimate to the aquaria for one day prior to the introduction of the insecticide.

The insecticides were dissolved in methoxyethanol to form stock solutions which were added to the aquaria at 0.1 ml/l of water to yield the appropriate final concentration (0.1 to 1 ppm parathion and 12 to 17 ppm methyl parathion). (Methoxyethanol alone was non-toxic in the quantity used.) LC_{50} values for 48 h were determined from a computerized log probit analysis. Between 70 and 460 individuals were employed for each LC_{50} determination.

RESULTS

The parathion 48-h LC_{50} values in the spring for S and R fish were 0.61 ppm and 0.95 ppm, respectively, while in the fall the values were 0.35 ppm and 0.39 ppm for S and R fish, respectively. Thus, R fish tolerated about 1.5 times more parathion than S fish in the spring, and 1.1 times in the fall. The methyl parathion 48-h LC_{50} for S fish was 13.48 ppm and for R fish 17.48 ppm, yielding a fold difference of about 1.3. The 95% confidence intervals did not overlap for any of the LC_{50}'s determined except parathion values for S and R populations in the fall (Table 1).

TABLE 1

Parathion (P) and methyl parathion (MP) 48-h LC_{50} values and 95% confidence intervals (ppm) for insecticide-susceptible (S) and -resistant (R) populations of mosquitofish.

Insecticide	Population	Season	Lower limit	LC_{50}	Upper limit	Slope[a]
P	S	fall	0.29	0.35	0.43	1.88
P	R	fall	0.12	0.39	0.50	2.00
P	S	spring	0.51	0.61	0.69	10.95
P	R	spring	0.89	0.95	1.01	7.00
MP	S	fall	13.24	13.48	13.72	27.69
MP	R	fall	15.67	17.48	27.38	4.65

[a]slope of the log probit regression line

There was an apparent increase in toxicity of parathion to R fish with the length of time the fish were held in the laboratory. Tolerance was highest when the fish were first collected. Thereafter tolerance gradually declined and by the end of three weeks the tolerance of the R fish approximated that of the S fish. LC_{50} values to demonstrate this decline could not be determined because of small sample size. This trend was not noticed with methyl parathion.

The S mosquitofish tolerated 39 times more methyl parathion than parathion, and R fish tolerated 45 times more (Table 2). The slopes (regression coefficients) of the log probit regression lines for parathion toxicity to S and R populations were statistically parallel in the fall. The slope of parathion toxicity to S fish in the spring was greater than that to R fish. The slope of methyl parathion toxicity to S fish was about six times greater than that to R fish (Figure 1). These latter two differences in slopes indicate a greater homogeneity of response in the S population.

TABLE 2

Ratios of parathion (P) and methyl parathion (MP) 48-h LC_{50} values.

P	spring	R/S	1.6
P	fall	R/S	1.1
MP	fall	R/S	1.3
fall	S	MP/P	38.5
fall	R	MP/P	44.9
P	S	spring/fall	1.7
P	R	spring/fall	2.5

DISCUSSION

The organochlorine compound-resistant population of mosquitofish can tolerate more parathion in the spring and methyl parathion in the fall than the susceptible population as shown by their respective LC_{50} values. Whether these differences represent a genetically-based resistance to organophosphorus insecticides or an environmentally-induced tolerance to these compounds is not clear.

Three facts indicate that the R fish may be developing a resistance to methyl parathion: 1) they did not lose their methyl parathion tolerance during the time they were held in the laboratory; 2) the methyl parathion LC_{50} value of R fish was greater than that of S fish; and 3) the slope of the log probit regression line for methyl parathion toxicity to R fish was much less than that to S fish. The R population was more heterogeneous in its response to methyl parathion, suggesting a selection for factors which might contribute to increased tolerance. Since the R fish are exposed to methyl parathion,

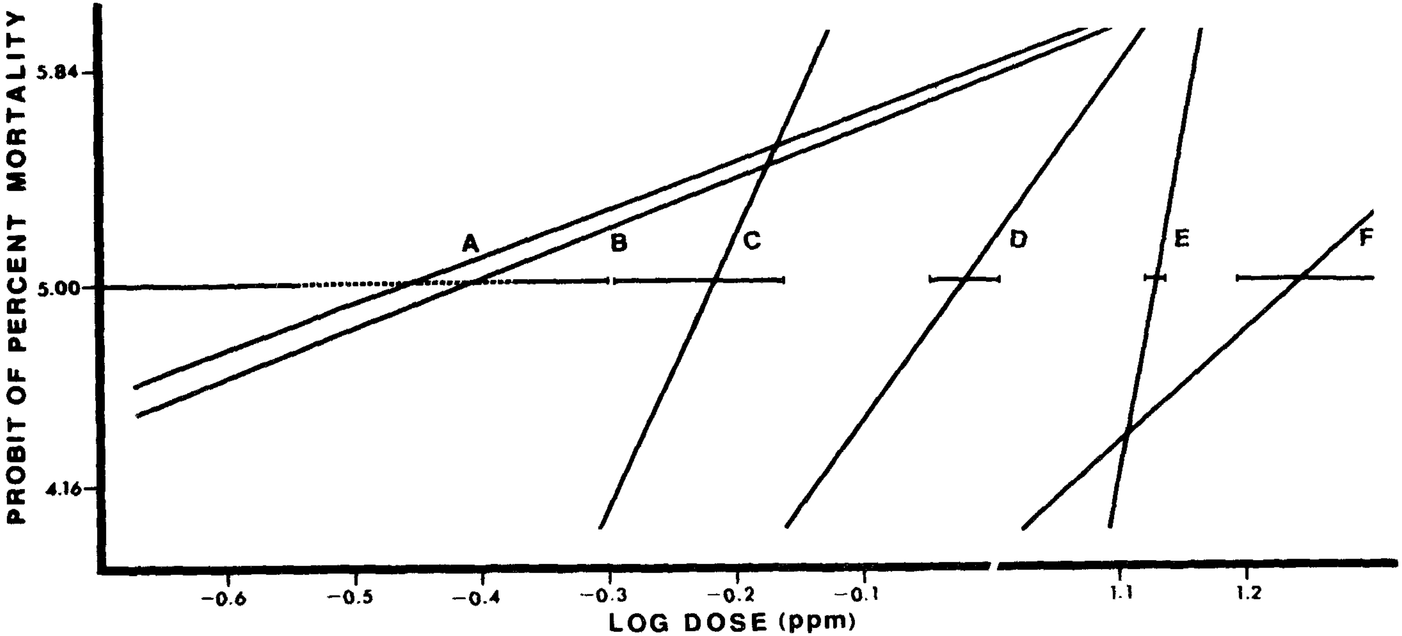

Figure 1. Log probit lines of parathion (P) and methyl parathion (MP) for insecticide-susceptible (S) and -resistant (R) populations of mosquitofish. Probit 4.16 = 20%; probit 5.00 = 50%; probit 5.84 = 80%. A = S, P, fall; B = R, P, fall; C = S, P, spring; D = R, P, spring; E = S, MP, fall; F = R, MP, fall. 95% confidence intervals around LC_{50} values are indicated. Dashed line is confidence interval for LC_{50} for A occurring within confidence interval for B. For extremes of confidence intervals for B and F, see Table 1.

the insecticide could be exerting a selective pressure on this population.

On the other hand, the R population no longer appears to be resistant to parathion, as was reported earlier (CULLEY and FERGUSON, 1969), because the fish became less tolerant to parathion as they were retained in the laboratory. In addition the slopes of the log probit regression lines within a season were similar for the two populations. Although the overall dosage mortality response in the fall was not statistically different between the two populations, there was a decrease in tolerance of the R population with time held in the laboratory. This is probably the reason for the large confidence interval around the LC_{50}. The LC_{50} value for the R population was significantly greater than that for the S population in the spring, and is probably the result of an environmentally-induced tolerance. However, the slope of the log probit regression line for the R population was less than that for the S population. This more heterogeneous response could indicate a developing resistance. However the R population is not known to have been exposed to parathion, so parathion has probably not exerted a selective pressure. If a genetically-based resistance to parathion exists in the R population, it is at such a low level that it is masked by environmental effects.

The parathion LC_{50} values for S and R populations were originally reported to be 48 ppb and 199 ppb, respectively (CULLEY and FERGUSON, 1969). The disparity between the LC_{50}'s reported in 1969 and those presented here could be attributed to differences in assay techniques or purity of insecticides used. The tolerance of the R fish could have changed since they no longer appeared to be resistant to parathion and seemed to be developing a resistance to methyl parathion. Although the S population was not expected to change, other environmental pressures could have caused a coincidental alteration of their tolerance to insecticides. It seems unlikely, however, that a change of this magnitude could be related to only a non-insecticide selective pressure.

Both populations of fish tolerated more parathion in the spring than in the fall. The greater slopes of the log probit regression lines indicated that the population was more homogeneous in the spring. A heterogeneous population would be expected in the fall because of the gene recombinations occurring during reproduction in the summer. It is possible that only the hardier individuals can survive the stresses of the winter and thus form a more homogeneous, stress-tolerant population in the spring. The difference in toxicity between spring and fall could also be attributed to nutritional factors.

When the toxicity of the insecticides is compared, parathion is much more toxic than methyl parathion. In general this is consistent with the toxicity patterns in most animals (O'BRIEN, 1960). The greater degradation of dimethyl esters as well as a cholinesterase which is less sensitive to methyl paraoxon inhibition probably contribute to the difference in toxicity seen in mosquitofish (CHAMBERS, 1973).

Organophosphorus insecticides have probably not exerted a major selective pressure on the R population of mosquitofish for three reasons: 1) organophosphorus compounds are less toxic than organochlorine insecticides; 2) organophosphorus insecticides are used in smaller quantities than organochlorine insecticides; and 3) because of their lability, organophosphorus insecticides have a shorter persistence in the environment than organochlorine insecticides. Thus, organochlorine compounds have exerted the major selective pressure (FINLEY et al., 1970) and some of the mechanisms functioning in organophosphorus insecticide resistance or tolerance (such as possible microsomal mixed function oxidase induction) may be coincidental to organochlorine compound effects.

SUMMARY

This study determined the toxicity of parathion and methyl parathion to organochlorine compound-resistant and -susceptible populations of mosquitofish (Gambusia affinis). Mosquitofish can tolerate about 40 times more methyl parathion than parathion. The resistant population demonstrates a 1.3 fold greater tolerance of methyl parathion than the susceptible population, which may be a developing resistance. The resistant population also demonstrates an environmentally-induced tolerance to parathion (1.6 fold) in the spring but no overall tolerance to parathion in the fall when compared to the susceptible population.

ACKNOWLEDGEMENT

The authors are grateful to Dr. Howard Chambers, Department of Entomology, Mississippi State University, for providing the parathion used in this investigation and for his advice and assistance.

LITERATURE CITED

CHAMBERS, J. E.: Studies of organophosphorus insecticide toxicity and possible mechanisms of tolerance in insecticide-susceptible and -resistant populations of mosquitofish (Gambusia affinis). Ph.D. Thesis. Mississippi State University, Mississippi State. 40 p.

CULLEY, D. D., and D. E. FERGUSON: J. Fish. Res. Bd. Can. 26, 2395 (1969).

FINLEY, M. T., D. E. FERGUSON and J. L. LUDKE: Pestic. Monit. J. 3, 212 (1970).

O'BRIEN, R. D.: Toxic Phosphorus Esters, New York-London: Academic Press. 1960.