TOXICITY OF DURSBAN TO THREE SPECIES OF FISH[1]

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"Dursban" is the trademark of a new organic phosphorus insecticide (0,0-diethyl 0-3,5,6-trichloro-2-pyridyl phosphorothioate) discovered by the Dow Chemical Company. In experimental tests, the compound resisted rapid leaching in the soil, exhibited desirable residual characteristics, and controlled a variety of arthropod pests when applied at rates of 1-16 oz/acre (Dow Chemical Company, 1965). Because of Dursban's promise as a mosquito larvicide (e.g., the LD_{95}-value for larvae of *Culex fatigans* is 2 ppb; 4.5 ppb for *Aedes aegypti*) and potential value as a broad spectrum economic insecticide, its effects upon various types of fish and wildlife need to be determined. This report concerns the toxicity of Dursban to several populations of golden shiners *(Notemigonus crysoleucas)*, mosquito fish *(Gambusia affinis)*, and green sunfish *(Lepomis cyanellus)*. Certain of these fish populations are known to be resistant to several chlorinated hydrocarbon insecticides (Ferguson *et al.*, 1964) and to have been exposed to the organic phosphorus insecticide, methyl parathion. Since Dursban is an experimental compound and not used in the collecting areas, the possibility that these fish populations might be resistant or cross-resistant to it was of interest. Finally, the symptoms of fish subjected to Dursban were noted in an attempt to identify any that might be specific for the compound.

METHODS AND MATERIALS. Fish from two populations of each species were collected from farm ponds near State College, Oktibbeha County, Miss. All of these ponds are far removed from known potential sources of insecticides and believed to be free from insecticide contamination. Specimens of the same species were obtained from areas in the Mississippi Delta that were known to be contaminated by cotton insecticides. Shiners and green sunfish were collected from a bayou bordering several large cottonfields near Indianola, Sunflower Co., Miss. Mosquito fish were obtained from a ditch that bisects and drains several large cottonfields near Belzoni, Miss. (Humphreys Co.). All fish were collected in a small-mesh seine and held in the laboratory 24 hours prior to testing.

A sample of technical Dursban (99 ± 1 percent purity) provided by the Dow Chemical Company was used to prepare a 1 percent acetone stock solution. This was diluted with tap water (pH 7.4, hardness 24 ppm) to obtain desired test concentrations. Test fish were exposed, three each (5 in some mosquito fish tests), in 2 liters of Dursban solution contained in gallon jars. Test specimens were selected for size, the shiners measured 2.5-4 inches in total length, the mosquito fish 1.0-2.5 inches, and the green sunfish 1.0-3.5 inches. After exploratory tests were performed over a wide range, concentrations were progressively narrowed until the median tolerated limit (TL_m) had been ascertained. Initially fish were tested for 72 hours, however, the exposure period was reduced to 36 hours when it became apparent that little change occurred thereafter. Controls were run in acetone blanks. Dead fish were removed after 6, 12, 24, and 36 hours. Individuals failing to exhibit opercular movements were counted as dead.

In another series of tests, samples of 25 fish (50 in the case of mosquito fish) were placed in 10 liters of 1000 ppb Dursban solution in 10 gallon aquaria. A like number of controls were tested similarly but in tap water. Mortality was recorded at 15-minute intervals for the first

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TABLE 1.—Approximate 36-hour median tolerated limits in ppb of Dursban for three species of fish from an insecticide contaminated area (Delta) and for two populations of the same three species from uncontaminated sources near State College, Miss. Numbers of test fish and controls are indicated in parentheses.

Population	36-hour Median Tolerated Limit (ppb)		
	Golden Shiners	Mosquito Fish	Green Sunfish
Delta	125	595	125
N:# Controls	(211:30)	(426:74)	(160:23)
State College #1	45	230	37.5
N:# Controls	(66:21)	(33:12)	(33:12)
State College #2	35	215	22.5
N:# Controls	(75:24)	(75:21)	(75:21)

hour, after which 30-minute and/or hourly checks were made for 6 to 8 hours. Thereafter, irregular checks were continued until all fish died or until mortality ceased. These tests were intended to reveal the shape and slope of mortality curves and provide LT_{50}-values (exposure period that killed 50 percent of the test sample).

RESULTS AND CONCLUSIONS. In 36-hour tests (Table 1), Dursban is less toxic to fish than most chlorinated hydrocarbon insecticides (Ferguson *et al.*, 1964; Boyd and Ferguson, 1964) but generally more toxic than other organic phosphorus insecticides (Henderson *et al.*, 1960). Our tests show mosquito fish to be more tolerant of Dursban than either golden shiners or green sunfish. Test fish from insecticide-contaminated environments in the Mississippi Delta are 3 to 5 times more tolerant than the same species from a nonagricultural area. These findings agree with those of Ferguson and Boyd (1964) in a similar study involving methyl parathion. Control mortality seldom exceeded 5 percent. In two shiner tests all treated and control fish died, apparently due to fungus. These data were not included in determining TL_m-values shown in Table 1.

In general, fish from uncontaminated sources survived increases in concentration to a point, above which mortality increased abruptly within a narrow range of concentrations. Mortality among Delta fish was sporadic and increased slowly over a wide range of concentrations. A similar phenomenon was observed in fish populations resistant to chlorinated hydrocarbon insecticides (Ferguson *et al.*, 1964).

Although it is not uncommon for mortality to sharply decline after the first 24 to 36 hours of a static bioassay test, it was unusually pronounced in these Dursban tests. Rapid hydrolysis of organic phosphorus insecticides is known to cause a reduction in toxicity in short term bioassay tests (Henderson *et al.*, 1960).

The higher tolerances of Delta populations are evident in prolonged exposures to 1000 ppb Dursban (Table 2). When the results are expressed as LT-values, the LT_{50}-, and especially the LT_{75}-values tend to agree with the findings from the bioassay tests in showing mosquito fish to be most tolerant and green sunfish least tolerant. The LT_{25}-values for State College populations fail to show the same relationships.

Many fish lose equilibrium rapidly in Dursban solutions, turn on their backs, come to the surface, but continue to survive in a state of partial paralysis for several days. With most insecticides, par-

TABLE 2.—LT-values (hours) for 1000 ppb Dursban in three species of fish from an insecticide-contaminated locality (Delta) and from an insecticide-free source (State College).

Species	State College			Delta		
	LT_{25}	LT_{50}	LT_{75}	LT_{25}	LT_{50}	LT_{75}
Shiners (N=25)	0.2	3.5	8.5	7.5	13.75	18.0
Mosquito Fish (N=50)	1.45	2.5	8.5	5.75	34.0	60.0*
Green Sunfish (N=25)	3.0	3.75	4.5	4.0	5.0	5.75

* Estimated from 48-hour test.

ticularly the chlorinated hydrocarbon compounds, fish tend to race around the test container and become hypersensitive to disturbances as symptoms of poisoning. In Dursban, green sunfish moved to the bottom of the containers, turned on their sides, and died without becoming highly active. The other test species came to the surface and showed little evidence of spasms or struggling. However, gravid female mosquito fish in the terminal stages of gestation tend to abort in the presence of Dursban. Boyd (1964) reported mosquito fish abortion with several chlorinated hydrocarbon insecticides, and we have noted the same phenomenon in tests with methyl parathion in our laboratory.

Dursban is not likely to be harmful to fish at rates of application that have controlled arthropod pests in experimental tests. Its high toxicity to target organisms, such as mosquito larvae (e.g., LD_{95} less than 5 ppb), provides a wide margin of safety even for green sunfish, the most susceptible species in our tests. In fact, low tolerances of target organisms and high tolerance of mosquito fish suggest that Dursban and mosquito fish might be an effective combination for use in mosquito control.

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