# Insecticide Tolerances of Two Crayfish Populations (*Procambarus Acutus*) in South-Central Texas[1]

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Resistance to insecticides has been found in several non-target organisms, primarily in the delta region of Mississippi, which is one of the most intensively treated areas in the world (1). Resistant animals in that region include copepods (2), fishes (3), and frogs (4). In Virginia, resistance to endrin has been found in pine mice in heavily treated orchards (5).

Populations of crayfish in agricultural areas are frequently exposed to insecticide contamination. I performed bioassays with crayfish in south-central Texas to determine levels of tolerance to DDT, toxaphene, and methyl parathion, and to compare the tolerance of specimens from an area of intensive insecticide use with that of specimens from an area where use is minimal. Procambarus acutus (Girard) was chosen for study because it is eurytopic over a large geographic range and inhabits both permanent ponds and streams and temporary bodies of water such as those often found in roadside ditches.

## METHODS

The collection area of low insecticide use is in the Navasota River floodplain, Brazos County, Texas. Crayfish were obtained from four isolated pools, all within 500 m of the river, 13 km E and 29 km NE of College Station. Gas chromatographic studies have shown a variety of aquatic and terrestrial organisms from this region of range land and woods to be relatively free of insecticides (6,7). The population exposed to high insecticide use

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inhabits a bayou in the Brazos River floodplain in Burleson County, 16 km SW of College Station. This bayou is surrounded by cotton fields from which it receives both natural and irrigation runoff. Methyl parathion, DDT, and toxaphene have been applied to these fields for control of insect pests of cotton. Total application rates often have exceeded 11 kg of insecticide per hectare per year.

Five consecutive bioassays were conducted in January and February, 1972. Crayfish were collected two days before the beginning of a bioassay. They were then weighed, sexed, and maintained in individual jars at room temperature. All animals weighed between 0.25 and 0.40 g and measured 11.8 to 14.6 mm in cephalothorax length. Equal numbers of males and females were subjected to each treatment. Crayfish from both areas were tested at three to five concentrations of DDT, methyl parathion, and toxaphene in each bioassay. The range of concentrations was reduced in successive bioassays, as approximate median lethal concentrations were determined. For each insecticide, a total of at least 38 crayfish from each area were tested at concentrations which killed some, but not all. These concentrations were between 1 and 10 ppb for DDT and methyl parathion, and between 50 and 100 ppb for toxaphene.

Technical grade insecticides were dissolved and diluted in acetone so that desired concentrations were obtained when 1 ml was added to 500 ml of aged tap water (pH 8.7, hardness as $CaCO_3$ 10 ppm). Crayfish were tested individually (due to cannibalism) in 500 ml of test solution in a one-gallon jar. Controls received 1 ml of acetone in 500 ml of water. Animals were considered dead when probing elicited no movement. After 48 hr exposure, survivors were transferred to aged tap water and held for two weeks.

Median lethal concentrations ($LC_{50}$) and 95% confidence intervals were determined by probit analysis (8) on an IBM 360 digital computer.

## RESULTS

Table 1 shows $LC_{50}$ values determined from the bioassays. The two crayfish populations were significantly different in their responses to all three insecticides. $LC_{50}$ values for DDT, methyl parathion, and toxaphene were 2.4, 1.4, and 1.5 times greater respectively for animals from the area of high insecticide use than for those from the area of low insecticide use. For crayfish from the clean area, DDT and methyl parathion had similar toxicity, but the $LC_{50}$ for toxaphene was more than 20 times greater.

TABLE 1

Comparative 48 hr $LC_{50}$ concentrations of insecticides (ppb) and 95% confidence intervals for two populations of crayfish (*Procambarus acutus*).

| Insecticide | Clean Area | Cotton Field Area |
|---|---|---|
| DDT | 3.0 (2.5-3.6) | 7.2 (5.8-8.8) |
| Methyl Parathion | 2.4 (1.9-3.0) | 3.4 (3.0-4.0) |
| Toxaphene | 60.7 (55.2-66.8) | 90.2 (79.4-102.5) |

The sequence of symptoms most commonly observed upon exposure to each insecticide was hyperactivity, loss of equilibrium and coordination, paralysis, and death. Death occurred 1-24 hr after symptoms were first observed. When survivors were transferred to tap water after 48 hr exposure, little additional mortality (10 of 195 individuals) occurred, and some crayfish which had shown advanced symptoms of poisoning appeared to recover completely. Since the ten additional deaths (eight of which occurred within 9 hr after transfer to tap water) were presumed to be the result of the 48 hr exposure, they were included in calculating the 48 hr $LC_{50}$. There was no mortality among control animals.

## DISCUSSION

The higher tolerance of crayfish from the cotton field area, compared to that of specimens from the uncontaminated area, presumably results from the selective pressures of exposure to the test chemicals. However, the differences in tolerance to toxaphene and methyl parathion are too small to be termed resistance in view of the larger resistances which have evolved in some fishes and invertebrates exposed to these chemicals (9,10). The 2.4 fold resistance to DDT is nearly as great as the largest DDT resistance reported for any crustacean (2,10).

Toxicity data published by federal agencies show that fish are less susceptible to methyl parathion than to DDT and toxaphene (11,12). For bluegill (Lepomis macrochirus), 96 hr $LC_{50}$ values were: DDT, 8 ppb; methyl parathion, 572 and 800 ppb; and toxaphene, 3.5 and 18 ppb (11). $LC_{50}$ values for 24 hr tests were 7, 8500, and 7.2 ppb, respectively (12). This indicates that fish and crayfish have similar tolerance to DDT, but that fish are more tolerant to methyl parathion than are crayfish, and vice versa for toxaphene.

A comparison of my data with those of Naqvi and Ferguson (10) reveals similar toxicity of the test chemicals to Procambarus acutus and the freshwater shrimp, Palaemonetes kadiakensis, from uncontaminated areas. Estimated 24 hr $LC_{50}$ values for crayfish are roughly the same as those shown for freshwater shrimp. Of the three insecticides tested, toxaphene was least toxic to both crayfish and shrimp. Crayfish were slightly more tolerant to DDT than to methyl parathion--the reverse of Naqvi and Ferguson's findings (10) for freshwater shrimp. In contaminated habitats in the Mississippi delta, freshwater shrimp showed resistance to seven insecticides (including toxaphene and methyl parathion) 6 to 25 times greater than tolerances of shrimp from an uncontaminated area (10). However, the difference in response to DDT was about the same as that between these two Texas populations of crayfish.

My findings, as well as fish bioassays conducted by L. J. Dziuk and F. W. Plapp, Jr. (pers. comm.) of the Texas A&M University Entomology Research Laboratory, suggest that some aquatic organisms in Texas cotton field areas have evolved more resistance to DDT but less resistance to toxaphene and methyl parathion than have similar populations in the Mississippi delta. Presumably, this is a consequence of differences in relative amounts of these insecticides used in the two regions over the past 25 years.

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