TOXICITIES OF FOUR INSECTICIDES TO RESISTANT AND SUSCEPTIBLE MOSQUITOFISH IN STATIC AND FLOWING SOLUTIONS[1]

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INTRODUCTION

Static tests have been extensively used as a bioassey technique. Doudoroff *et al.* (1951), representing the Sewage and Industrial Waste Subcommittee on Toxicity, asserted that constant flow apparatus would be of great utility to research laboratories; but it was concluded that from a practical standpoint static tests should be employed.

Static tests have received considerable opposition from others, however. In static tests the concentration of radioisotope-labelled DDT declined, presumably because of loss to action of detritus and test fishes (Holden, 1962). A similar loss of dieldrin was reported by Weiss (1965). The purpose of our study was to investigate the toxicity of parathion, toxaphene, DDT, and endrin in static and flowing test solutions to susceptible and resistant strains of mosquitofish.

MATERIALS AND METHODS

Resistant mosquitofish (*Gambusia affinis,* Baird and Girard) were seined from a ditch draining several large cotton fields near Belzoni, Humphreys County, Mississippi. The pesticide resistance of these particular fish has been previously described by Ferguson, Ludke, and Murphy (1966) and Ferguson and Bingham (1966).

Susceptible mosquitofish were seined from insecticide-free ponds near State College, Oktibbeha County, Mississippi. All fishes used in the tests described here were collected in June 1968. Fishes for experimentation were kept out-of-doors in two 90-gallon fiberglass tanks, fed BiOrell fish food, and were given such routine care as would insure their health.

The flow-through apparatus developed for this work has been described elsewhere (Burke and Ferguson, 1968). Samples of fish were exposed 36 hr. to a constant insecticide concentration or plain water (controls) at the rate of 300 ml/min. Test chambers were one-gallon soft glass jars with a 2 liter operational capacity. The three dilutions used in all tests were based on a 1:1 serial dilution, e.g., 100, 50, and 25 p.p.b.

In addition to the solution used in the glass jars, 475 ml/min of toxicant solution were diverted to a tank remote from the serial dilutions. This remote plexiglass tank was partitioned with insect screen so that resistant and susceptible fishes could be run simultaneously, assuring identical exposures.

Insecticide solutions for static tests were contained in gallon jars or plexiglass tanks identical to those used in the dynamic tests. The solutions for the static tests were taken from the terminal outfalls of the corresponding test vessels. All static solutions were collected 12 hr. after the flowing tests were initiated.

All experiments were run with 5 adult mosquitofish (mean wt. ca. .28 g) per liter of solution. Data were recorded and dead fish removed at 6-hr. intervals.

Technical grade samples of toxaphene, DDT, parathion, and endrin were used. Stock solutions were prepared from 1 or 10 percent (weight/volume) acetone solutions of technical insecticides, e.g., 1 ml of 1 percent insecticide brought to 1 liter in acetone produced 10,000 p.p.b. For more concentrated solutions the 10 percent solution was used. Test concentrations were varied according to the formula:

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$$\text{Concentration of stock solution} = \frac{\text{volume of diluting water/min}}{\text{volume of stock solution/min}} \times \text{desired test concentration.}$$

This formula gave a very close approximation of desired test concentrations, where the actual value would have required the formula:

$$\text{Concentration of stock solution} = \frac{\text{volume of diluting water/min} + \text{volume of stock solution/min}}{\text{volume of stock solution/min}} \times \text{desired test concentration.}$$

The first formula was used because it represents little error and simplifies calculations.

The test fish were never subjected to more than 3 ml acetone per 1000 ml of water (pH 7.8, hardness 28 p.p.m., temp. 24 C±1). Preliminary tests had shown this concentration of acetone to be nontoxic to *Gambusia* in 48-hr. exposures.

The mortalities for 6-hr. intervals were plotted graphically. Control mortality was rarely encountered; when it did occur the entire series of experiments was rejected and repeated.

MORTALITY-CONCENTRATION CURVES. Tests to determine mortality-concentration curves were conducted in glass jars containing 2 liters of test solution introduced at the rate of 300 ml/min. Ten fish were tested at each concentration.

TIME-MORTALITY CURVES. Tests to determine time-mortality curves were conducted in 10 liter plexiglass containers that received 475 ml/min. of solution. Twenty-five resistant and susceptible fishes were separated by a partitioning screen in a common tank. In very high or very low concentrations only one of the populations was exposed, i.e., 25 fish were put in one end of the tank and the other end was left empty, because susceptible fish died immediately in high concentrations and resistant fish did not die in low concentrations. Whenever data were to be compared, fish/volume ratios were identical.

All tests were terminated at 36 hr. if considerable mortality had occurred in one or both samples of fish. If mortalities were low at the end of 36 hr., data were recorded at 42 and 48 hr.

RESULTS

MORTALITY-CONCENTRATION CURVE TESTS. (Fig. 1) In static tests where mortality occurred, increased concentration produced a corresponding increase in mortality. This generalization was true for all insecticides tested against the susceptible population and for all insecticides, except parathion, tested against resistant fish (Fig. 1-D).

In the tests of all four insecticides in flowing solutions, mortality increased with an increase in concentration. This was true of both resistant and susceptible fishes. Flowing tests produced greater and more rapid mortality than did static tests at the same concentration, except when resistant fish were tested in parathion, the pattern was reversed.

In both static and flowing tests mortality was greater in susceptible fishes than in resistant fishes. When the two populations were not compared in a common concentration, a greater concentration was required to produce equivalent mortality in resistant fishes compared with that of susceptible fishes.

TIME-MORTALITY CURVE TESTS. (Fig. 2) DDT, toxaphene, and endrin produced greater mortality in flowing solutions than in static solutions. Resistant fish failed to follow this pattern when tested on high concentrations of parathion, (e.g., 160 p.p.b., Fig. 2-I and 400 p.p.b., Fig. 2-J) in which case static tests produced greater

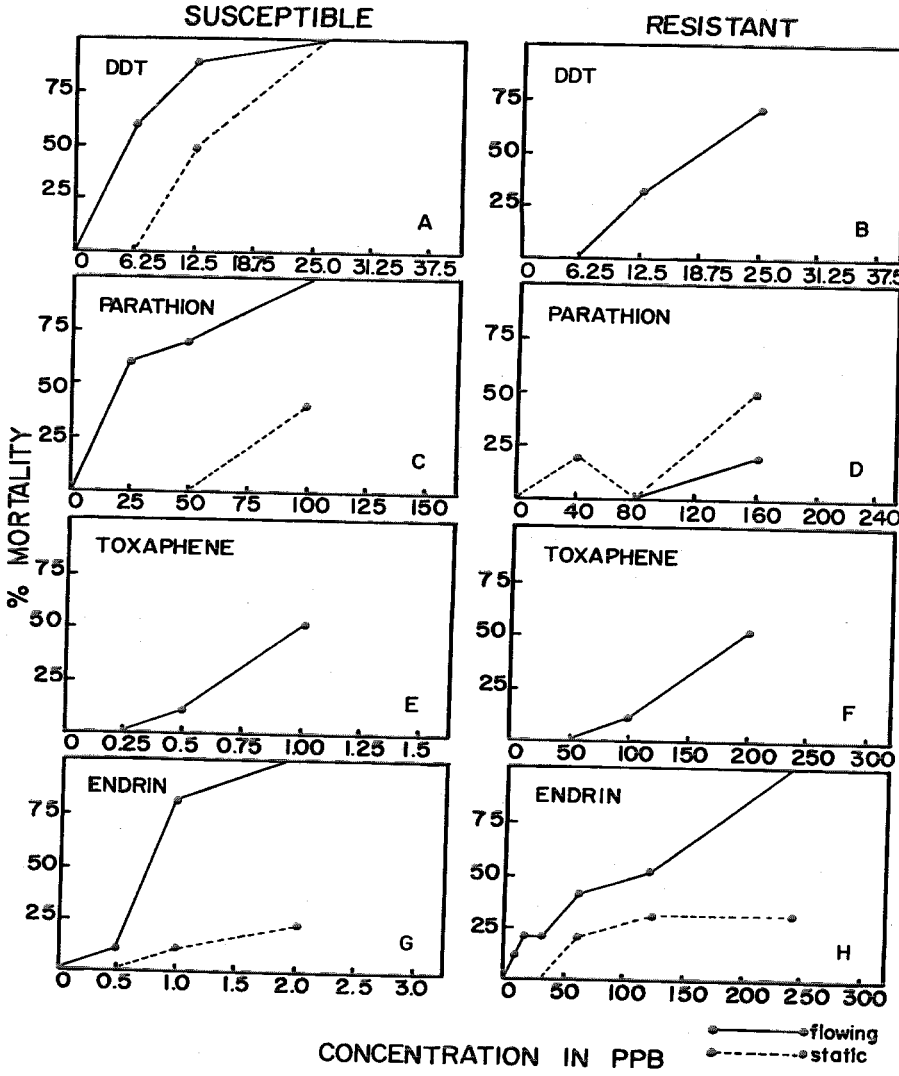

FIG. 1.—Concentration-mortality curves giving comparisons of 36-hr. mortality of resistant and susceptible mosquitofish in various concentrations of four insecticides under static and flowing test conditions. (Note: in B, E, and F the concentrations indicated in the flowing tests proved to be non-lethal in 36-hr. static tests).

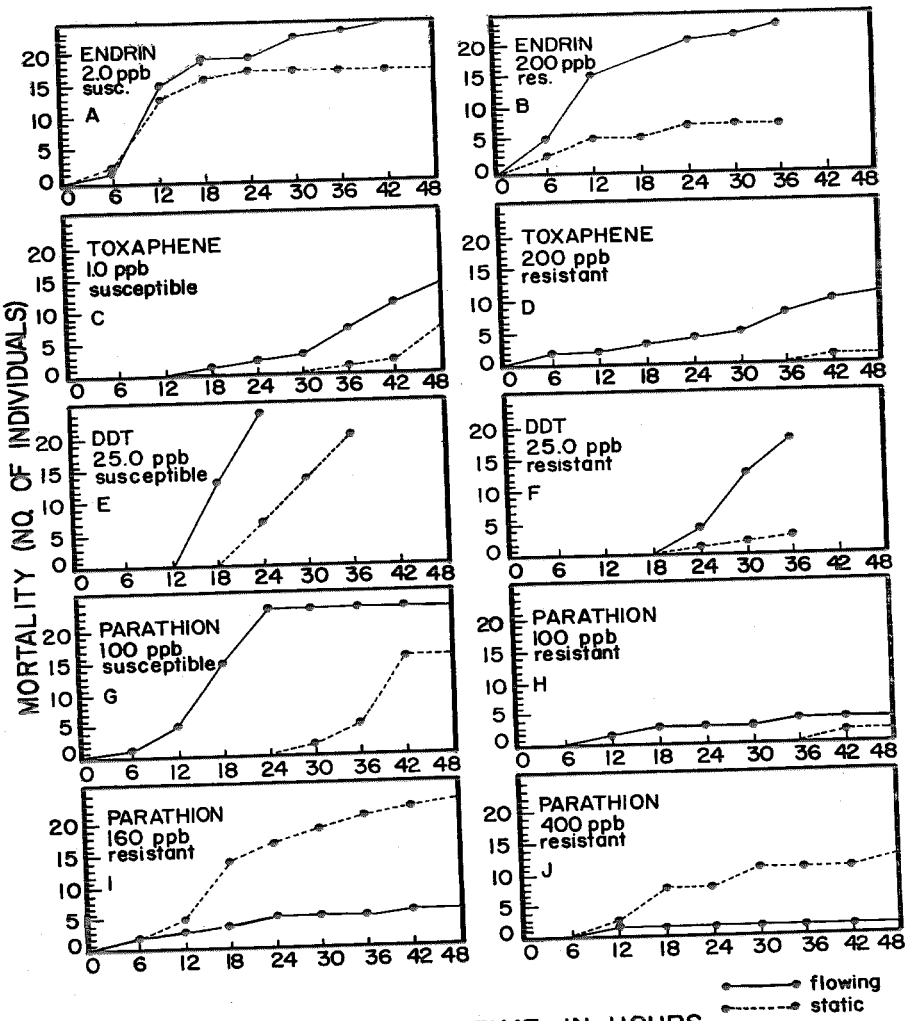

Fig. 2.—Time-mortality curves of resistant and susceptible mosquitofish in various concentrations of four insecticides under static and flowing test conditions.

kills than flowing tests. Resistant fish in relatively low concentrations of parathion produced a more typical mortality curve (e.g., 100 p.p.b., Fig. 2-H). Increase in concentration of parathion failed to produce a corresponding increase in mortality in resistant fishes. A similar phenomenon was demonstrable in susceptible fish (Ferguson and Boyd, 1964), but it was not encountered in our work.

Endrin, DDT, toxaphene, and parathion produced greater mortality in susceptible fishes than in resistant fishes under both static and flowing tests. In both types of tests susceptible fishes proved less tolerant than resistant fishes.

DISCUSSION AND CONCLUSIONS

The toxicity of a given concentration of DDT, toxaphene, and endrin is greater in constantly renewed solutions than in static solutions. The decrease of insecticide concentrations in static water is presumably due to absorption by test fish, adsorption onto test vessel surfaces, and metabolism by the test fish.

Parathion does not produce the same mortality pattern in resistant fish as do endrin, DDT, and toxaphene. The increase in mortality in static parathion tests suggests the generation of a metabolite more toxic than parathion itself. Such a metabolite could be removed from the flowing system at a rate to preclude its buildup to toxic levels. Weiss (1965) described the conversion of parathion to paraoxon *in vivo* in fishes. He showed this metabolite to be highly effective in reducing acetylcholinesterase activity, even after the fishes were removed from the parent toxic material.

Ferguson and Boyd (1964) described irregular patterns in tests of mosquitofish in which parathion (erroneously labelled methyl parathion) failed to produce increased mortality within certain limits of increased concentration. Any toxin which would produce increased mortality with an increase in concentration should prove more toxic in a flowing system than under comparable static tests.

Perhaps related to this phenomenon is the observation of Prévost, Lanouette, and Grenier (1948) in which a given concentration of DDT or rotenone produced greater mortality with increased volume. A flow-through test may simply be comparable with a static test of great volume. For example, in a 36-hr. test having a flow rate of 300 ml/min, test fishes are exposed to 648 liters of solution.

Results of bioassay under flowing conditions are likely to give more conservative estimates of tolerated limits of a given toxin than results obtained under static conditions. Differential abilities of populations to tolerate given concentrations of insecticides are as demonstrable using static tests as using more elaborate continuously flowing tests.

The use of flowing tests is not as well standardized as is the use of static tests. Freeman (1953) has proposed a very elaborate static bioassay scheme in which all controllable factors are considered, including even the formulation of a "standard reference water." The work of Doudoroff *et al.* (1951) has come to be regarded as a standard reference in conducting static bioassay tests.

The objections to static tests have been described elsewhere in this paper, and the advantages of the alternative are obvious. Mount and Warner (1965) assert that it is necessary to use tests in which the concentration in water of the material being tested is known and maintained constant. They maintain that continuous flow tests are the best tool available to evaluate toxic effects under controlled conditions; such tests have been conducted at the Newtown Laboratory of the Robert A. Taft Sanitary Engineering Center at Cincinnati, Ohio, since 1962.

There is a paucity of information on actual comparisons of bioassay results using both static and flowing tests. Jensen and Gaufin (1964) proposed to establish procedures for conducting continuous-flow bioassays and ultimately concluded with regard to stonefly naiads, that parathion and Di-Syston under continuous-flow conditions were only as toxic as the corre-

sponding concentrations under static conditions. Dylox, DDT, and malathion were found to be less toxic under continuous-flow conditions than under static conditions.

Most investigators merely give the conditions under which their research has been performed, e.g., flow rates, drip rates of stock solutions, and test chamber size. These factors are highly diverse and should be standardized to make various workers' findings more correlative.

Ferguson, Ludke, and Murphy (1966) questioned the realistic nature of continuous flow tests. Normally, pesticide concentrations in natural waters decline as do concentrations in static tests (Ferguson, Ludke, Wood, and Prather, 1965).

The present study shows dynamic tests to be more stringent than static tests, perhaps unrealistically so.

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