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Aquatic Bioassay of 11 Pesticides Using Larvae of the Mosquito, *Wyeomyia smithii* (Diptera: Culicidae)

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One of the uses of aquatic bioassays is to determine the presence or absence of toxic contaminants in water from the field. Our Laboratory has developed a bioassay system for this purpose using larvae of the pitcher-plant mosquito, Wyeomyia smithii (Coquillett) (Strickman 1984). The major application of this system is the investigation of minor fish kills which may occur at any U.S. Air Force base. The system was developed around a number of requirements, including: (1) Simplicity so that it may be taught to technicians who rotate from their jobs frequently; (2) Economy of space and time; (3) Tolerance to uncontaminated water from a wide variety of sources; (4) Capability of testing small samples of water in order to reduce shipping costs; and (5) Capability of detecting toxicants at levels at or below the LC_{50} for common species of fish. The Wyeomyia bioassay proved to be a practical tool in the investigation of over 20 fish kills during 1983 and 1984. Toxicants detected by the bioassay were later identified as engine lubricating oil, copper, paint stripper, and high levels of free ammonia. Water from the sites of many of the fish kills produced no effect on larvae, indicating that the fish kills had not involved toxic contaminants or that contaminants had dissipated by the time of sampling or bioassay. Such negative results were important because they indicated that chemical analysis would probably not have been productive. Elimination of unnecessary analyses saved over $15,000 during a single year.

We have tested the effect of known chemicals on larval Wyeomyia smithii in order to establish the limits of sensitivity of the bioassay system. This paper reports results of larval mosquito bioassays of 11 pesticides

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in 4 chemical groups. The pesticides were selected because of their common occurrence in drainage water (U.S. Air Force Occupational and Environmental Health Laboratory, unpublished data) or because of their common use in or near water (Anonymous 1983).

MATERIALS AND METHODS

Samples of pesticides were obtained from manufacturers or the U.S. Environmental Protection Agency. The samples varied in purity from 93% to 100%. The chemical categories and common names of the compounds were as follow: Organochlorines: DDT, heptachlor epoxide; Organophosphates: chlorpyrifos, malathion, temephos; Carbamates: carbaryl, methomyl, propoxur; Pyrethroids: permethrin, phenothrin, resmethrin.

Colony maintenance, production of larvae for bioassays, and the bioassay procedure are described in detail in previous papers (Lillie et al. 1980, Strickman 1983, Strickman 1984). Briefly, larvae for bioassays were reared under uncrowded conditions from eggs removed from a main colony. Eggs were removed daily so that their age never varied by more than 24 hours. Seven days after the date of deposition of the eggs (i.e. 3 to 4 days after eggs hatched), second-instar larvae were selected for use in a bioassay. Each replicate for a bioassay consisted of 30 ml of laboratory water (aged tap water, see Strickman 1984 for specifications) in a clear, glass jar (50-ml capacity). The desired amount of pesticide was added to each replicate by pipetting 0.1 ml of an acetone solution at an appropriate concentration. The concentrations for the bioassays were selected on the basis of 1 or 2 range-finding tests for each chemical. Laboratory-water control treatments received acetone with no pesticide added. Following addition of the acetone solution, 10 larvae and the appropriate amount of food (Tetramin Baby Fish Food "E," Tetra Werke Mille, West Germany) were placed in each replicate and the jars covered with paraffin film to prevent evaporation. Bioassays were maintained in an environmental chamber at 27°C with a 16-hour daily photophase. Larvae were observed daily for survival, stage of development, and behavioral signs of intoxication. Food was added at each observation in proportion to number of survivors and their stages of development. Bioassays lasted 7 days, during which time most larvae in laboratory water completed development to the fourth instar. For each pesticide, larvae from eggs deposited on the same day were used in 9 replicates of each of 3 concentrations and the laboratory-water control.

The SAS Statistical Analysis System (SAS Institute, Inc. 1979) was used to perform statistical analyses of survival and development. Survival was analyzed by calculating means of total survivors on each day of a bioassay for each concentration. Differences among daily means for each concentration were determined at the 95% level using Duncan's multiple range test. Development was analyzed by examining the number of third-instar larvae on each day of the bioassay. Previous studies (Strickman 1984) had indicated that the third instar is representative of developmental effects throughout the juvenile life of Wyeomyia smithii. Adjustments of number of third-instar larvae for total survival within the replicate (the number of third instars multiplied by 10, then the product divided by the total number of survivors in that replicate) allowed comparison of development independent of survival. This adjustment was possible only in concentrations where at least 1 larva remained alive in each replicate. Results of the entire concentration treatment were eliminated from analysis when any of its replicates contained no living larvae. Means of adjusted number of third instars were analyzed similarly to means of number of survivors.

RESULTS AND DISCUSSION

The bioassay system detected concentrations of pesticides within the range of levels toxic to fish. A comparison of the concentrations detectable by the bioassay to LC_{50} values presented in the literature (Table 1) indicated that the larval mosquito bioassay was within the same range of sensitivity as fish to all of the pesticides tested except DDT and resmethrin. Delayed development was a more sensitive indicator than mortality for detection of 6 of the pesticides (heptachlor epoxide, methomyl, propoxur, permethrin, phenothrin, and resmethrin).

Patterns of the survival curves (Figs. 1-4) suggested a relationship between mortality late in the bioassays and persistence of the chemicals. The slope of the survival curve during the last 3 days of the bioassay was nearly horizontal (indicating cessation of further mortality) for those larvae affected by malathion, carbaryl, permethrin, phenothrin, and resmethrin. The slope was negative (indicating continuing mortality) for DDT, heptachlor epoxide, chlorpyrifos, temephos, methomyl, and propoxur. Literature (Eichelberger and Lichtenburg 1971, Sharom et al. 1980, Miyamoto 1976, Kottkamp el al. 1981, Chapman and Cole 1982) documents

Table 1. Comparison of sensitivity of larval mosquito (Wyeomyia smithii) bioassay and toxic levels to fish[a].

Chemical	Lowest conc. (ppb) detected by bioassay		Lowest LC_{50} (ppb) to fish and species[c]		Highest LC_{50} (ppb) to fish and species[c]	
	Survival	Development				
DDT	50[b]	50[b]	2	LB	21	CC
Heptachlor epoxide	10	5	5	BG	20	RT
Chlorpyrifos	1	1	2	BG	280	CC
Malathion	100	100	62	RS	12900	BB
Temephos	5	5	1	CT	34	FM
Carbaryl	1000[b]	1000[b]	690	LT	20000	BB
Methomyl	5000	1000[b]	530	CC	6800	CT
Propoxur	1000	500[b]	4800	BG	25000	FM
Permethrin	5	1[b]	3	BT	Only 1 tested	sp.
Phenothrin	20	10[b]	41	KF	Only 1 tested	sp.
Resmethrin	200	50[b]	2	LT, BG	17	CC

[a]All data for fish from Johnson and Finley (1980) except for toxicity of phenothrin, which was from Miyamoto (1976).

[b]Lowest concentration bioassayed.

[c]LB = largemouth bass (Micropterus salmoides), CC = channel catfish (Ictalurus punctatus), BG = bluegill (Lepomis macrochirus), RT = rainbow trout (Salmo gairdneri), RS = redear sunfish (Lepomis microplus), BB = black bullhead (Ictalurus melas), CT = cutthroat trout (Salmo clarki), FM = fathead minnow (Pimephales promelas), LT = lake trout (Salvelinus namaycush), BT = brook trout (Salvelinus fontinalis), KF = killifish (Oryzias latipes).

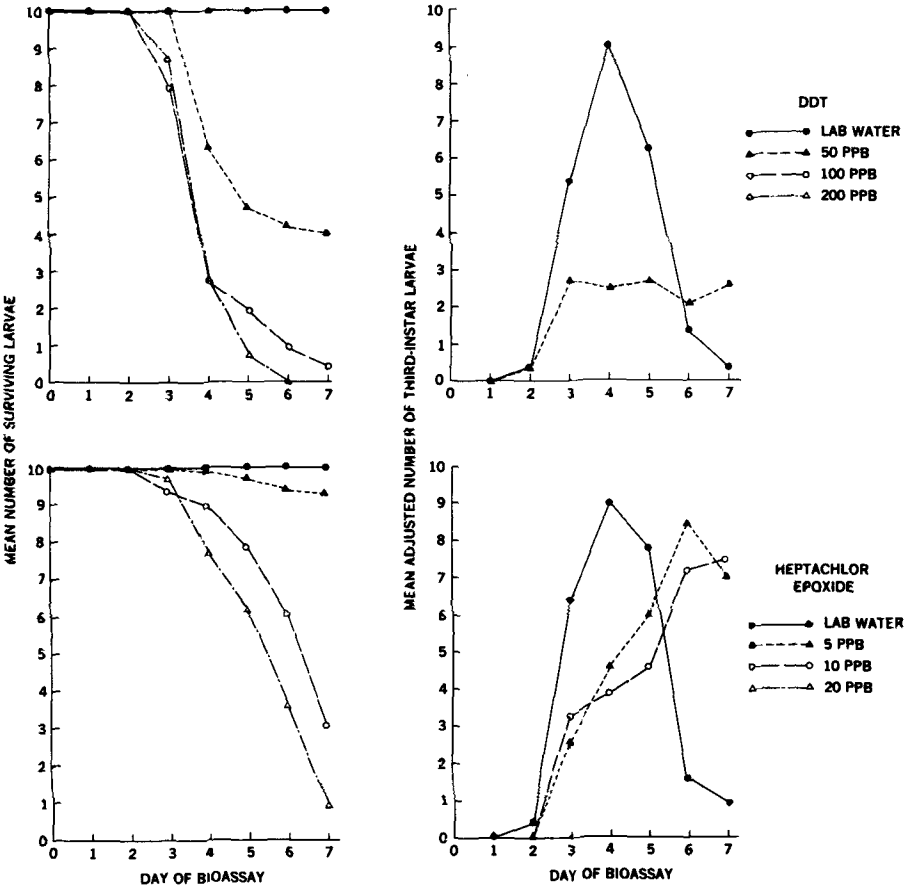

Figure 1. Results of bioassays of organochlorine pesticides using larval mosquitoes (Wyeomyia smithii). Data for heptachlor epoxide previously reported in a different format (Strickman 1984).

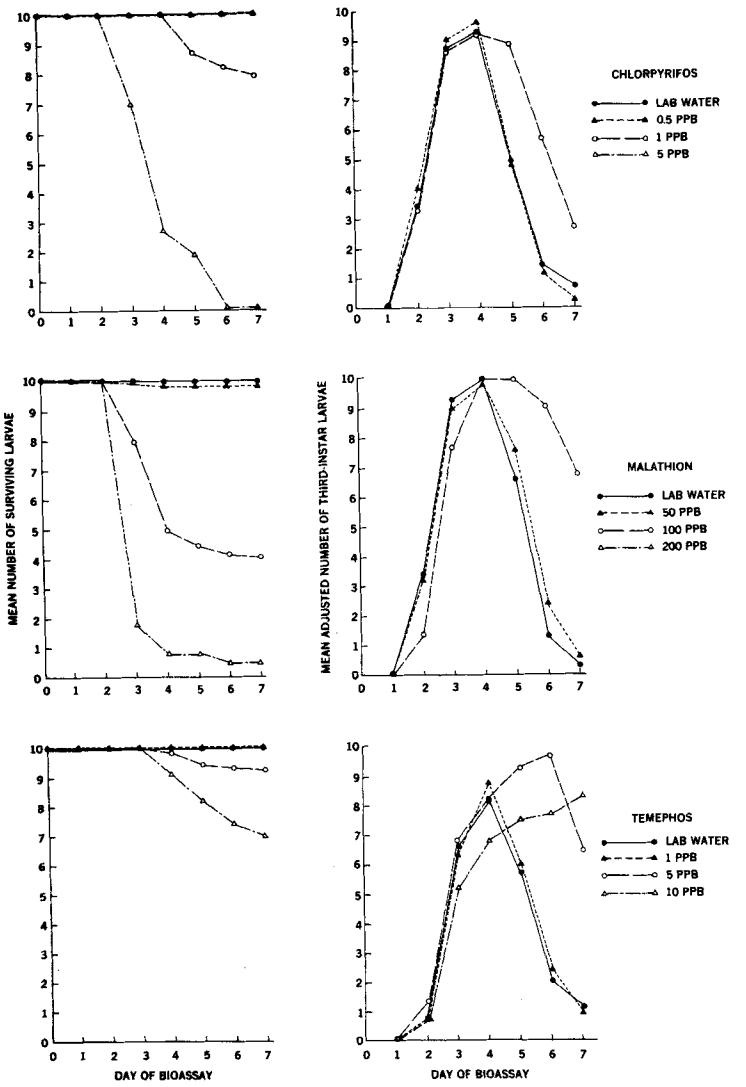

Figure 2. Results of bioassays of organophosphate pesticides using larval mosquitoes (Wyeomyia smithii). Data for malathion previously reported in a different format (Strickman 1984).

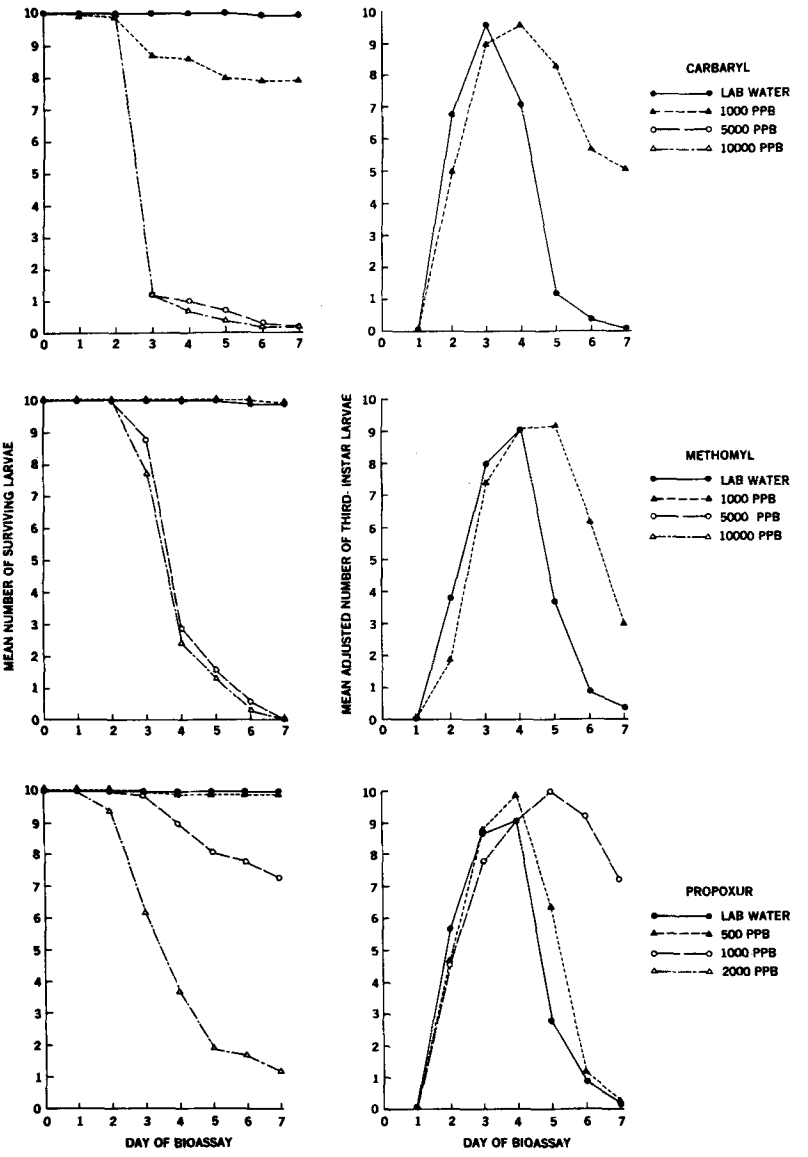

Figure 3. Results of bioassays of carbamate pesticides using larval mosquitoes (Wyeomyia smithii).

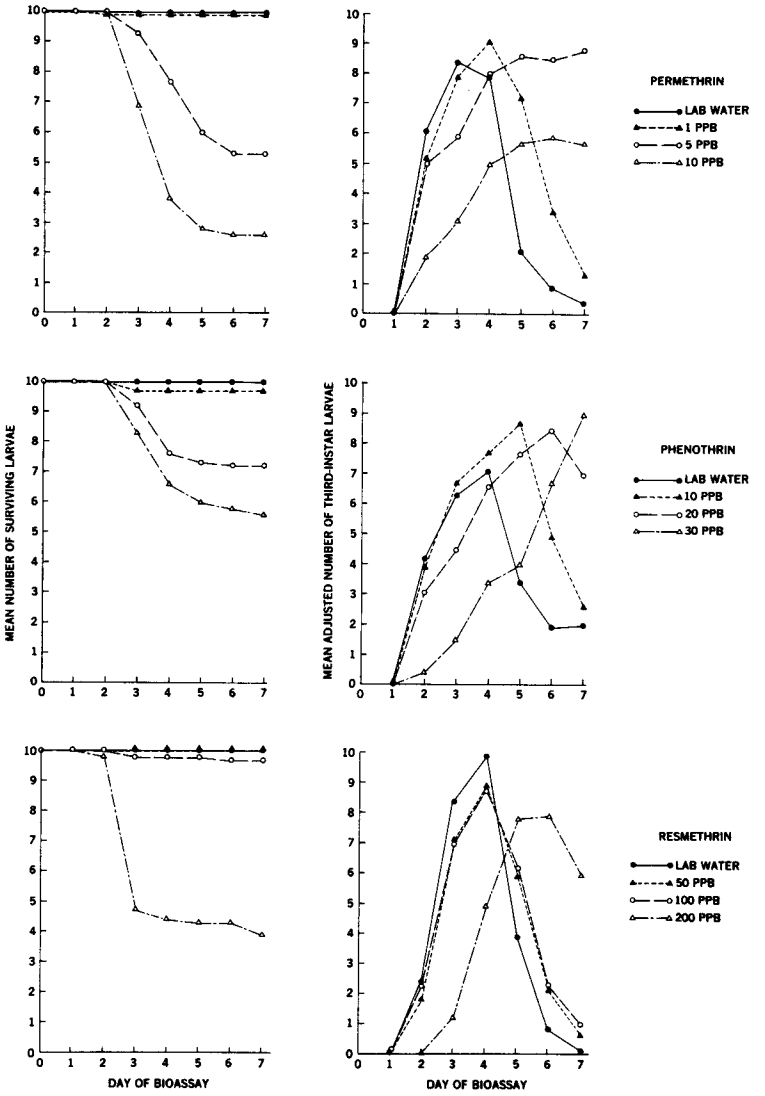

Figure 4. Results of bioassays of pyrethroid pesticides using larval mosquitoes (Wyeomyia smithii).

that the latter group of chemicals is more persistent than the former at neutral pH similar to that of the bioassays.

Behavioral signs of intoxication by pesticides included uncoordinated movement, jerky movement, difficulty unflexing, inactivity, and tonic contraction of longitudinal muscles resulting in shortening and thickening of larvae. Although all of the toxicants tested caused some or all of these signs of intoxication, the following distinctions between the chemicals were possible: heptachlor epoxide caused a quivering paralysis of larvae, but never tonic contraction of longitudinal muscles; pyrethroids caused a more pronounced inability to unflex than the other chemical groups; and, organophosphates caused tonic contraction of longitudinal muscles more uniformly than the other toxicants.

Aquatic bioassay using larval Wyeomyia smithii was capable of detecting a wide variety of pesticides at levels comparable to the LC_{50} of various fish species. Consideration of practical application of the technique suggests several topics for additional research. First, the influence of natural water chemistry on toxicity would contribute to more accurate detection of toxicants. Second, careful testing of heavy metal salts, solvents, and other common contaminants would expand the ability to interpret bioassay results. Finally, systematic evaluation of signs of intoxication could lead to a means of identifying contaminants from responses of the larvae.

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