References Cited

BOIKE, A. H., JR., and RATHBURN, C. B., JR. 1968. Tests of the susceptibility of Florida mosquitoes to insecticides, 1967. Mosq. News 28(3): 313–316.

RATHBURN, C. B., JR., and BOIKE, A. H., JR. 1967. Studies of insecticide resistance in Florida mosquitoes. Mosq. News 27(3):377–382.

RATHBURN, C. B., JR., and BOIKE, A. H., JR. 1969. A study of factors affecting the susceptibility of mosquito larvae to insecticides in laboratory resistance tests. Mosq. News 29(3):395–401.

ROGERS, A. J., and RATHBURN, C. B., JR. 1964. Present status of insecticides for mosquito control in Florida. Mosq. News 24(3):286–291.

WORLD HEALTH ORGANIZATION. 1960. Instructions for determining the susceptibility or resistance of mosquito larvae to insecticides. Reprinted from WHO: Technical Report Series, No. 191.

A STUDY OF FACTORS AFFECTING THE SUSCEPTIBILITY OF MOSQUITO LARVAE TO INSECTICIDES IN LABORATORY RESISTANCE TESTS

CARLISLE B. RATHBURN, JR. AND ARTHUR H. BOIKE, JR.

West Florida Arthropod Research Laboratory, Florida State Board of Health, Panama City, Florida

The delineation of variables is of great importance to any research and often is itself the object of extensive investigation. The variables encountered in testing the susceptibility of insecticides to mosquito larvae are many but in general are concerned with either the test organism or its environment. Those concerned with the test insects themselves are weight or size, sex, instar, age within instar and the time of year the larvae are tested. These, except for instar, are usually considered random variables and for the most part are uncontrolled. Variables of the other group are environmental in nature and are concerned with the type and quantity of water in which the larvae are tested, the presence or absence of food, the number of larvae per test vessel, the temperature of the test solution, the type of material and dimensions of the testing vessel, the number of replications and the amount and type of insecticide dilution. The importance of many of these variables is generally ignored even though there is considerable evidence to indicate that they may be responsible for large differences in published results.

Standardized tests (WHO, 1960) eliminate many of the above variables and others, such as sex differences and differences in age within instar, are beyond practical consideration. Although WHO procedures stipulate glass testing vessels, many research workers have substituted disposable paper or plastic testing vessels for them without conducting suitable research to first ascertain any difference. Kruse *et al.* (1952) demonstrated a considerably greater loss of DDT in paper than in glass or enameled containers. Curtis (1961), however, obtained little variation in the mortality of mosquito larvae with dilute solutions of DDT between test vessels of aluminum, glass, new polyethylene, paper or enamel vessels although, as he states, there may have been some reduction in toxicity of DDT in used polyethylene test vessels. Bransby-Williams (1965) demonstrated a seven-fold decrease in the effectiveness of fenthion in polyethylene lined containers when compared to unlined enamel pans. Thus it appears that the type of testing vessel may cause significant differences in larval mortality and it is likely that these differences will vary with the type of insecticide used.

World Health Organization procedures also specify that the average temperature of the water should be approximately 25° C. and that it must not be below 20° C. nor above 30° C. This is a wide range of average temperature and does not take into consideration maximum and minimum temperatures. Thomas (1965) found that larvae tested at temperatures of 24°–29° C. gave higher LC_{50} values for DDT than those tested at lower and more constant temperatures of 24°±1° C. and 25°±1° C. The diurnal temperature range experienced in many laboratories might even be considerably wider than those reported by Thomas.

From the foregoing discussion it is apparent that there are many variables which may be responsible for significant differences in the reaction of mosquito larvae to insecticides in laboratory tests. It was the purpose of this research to investigate further the effects of two of the more important of these, namely, the temperature of the test solutions and the type of testing vessel, in regard to differences between insecticides and the reaction of different mosquito species.

METHODS. Larval tests were conducted with minor modifications according to procedures outlined by the World Health Organization (WHO, 1960). One milliliter of the appropriate insecticide dilution was pipetted under the surface of 200 ml. of water contained in each test vessel and stirred. Twenty-five third instar larvae previously placed in 50 ml. of water in 50 ml. beakers were then added to each test vessel. Alcohol (95 percent ethanol) was used as the diluent for malathion and acetone (99.74 percent ACS) was used as the diluent for the naled, fenthion and Abate.[1]

Four replications of each of five dosages plus a check (alcohol or acetone) were run in each test. Tap water was used in rearing and testing *Culex nigripalpus, Culex salinarius* and *Aedes aegypti* and 25 percent sea water (the same salinity as the rearing water) was used in testing the *Aedes taeniorhynchus*. Larval mortality was determined at 24 hours. Larvae incapable of rising to or submerging from the surface upon probing were considered dead. Test vessels were either 600 ml. glass beakers, 400 ml. polypropylene beakers,[2] or 10 oz. wax treated cold drink cups[3] (See Fig. 1). Test vessels, except for the cups which were discarded after each use, were washed in detergent and water, rinsed in water and then twice in acetone after each use.

Tests at a constant 80° F. were conducted in a water bath 2 ft. by 5 ft. by 6 in. deep (Fig. 2). Temperature control and circulation were by means of a Haake Model E–12[4] constant temperature circulator which allowed water temperature to be controlled at 80°±0.5° F. Testing

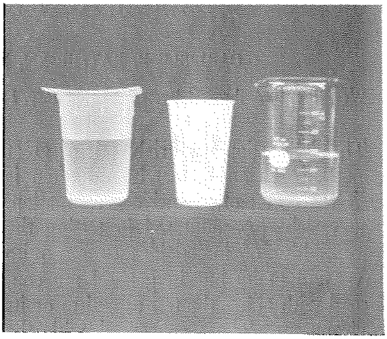

FIG. 1.—Three types of testing vessels. (left to right: 400 ml. polypropylene, 10 oz. waxed paper, 600 ml. glass)

vessels were suspended by their top rims in the 3-inch holes in the cover of the bath. When in position the water level outside the testing vessel equalled that within. The 48 holes in the cover allowed two tests of 24 treatments each to be conducted simultaneously. Cool temperatures reported were the average of the maximum and minimum water temperatures for each test and reflect the temperatures in the room in which the tests were conducted. The average room temperatures for the five tests each of *Ae. taeniorhynchus*-malathion, *Ae. taeniorhynchus*-naled, *C. nigripalpus*-malathion, *C. nigripalpus*-naled were 65°, 63°, 67°, 65° F. respectively. The maxi-

[1] American Cyanamid Co., Princeton, New Jersey.

[2] Biological Research, Inc., Tri-Pour.

[3] Nyman Manufacturing Co., 10431.

[4] Polyscience Corp.

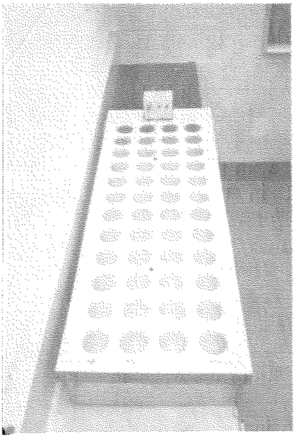

FIG. 2.—The constant temperature water bath.

mum and minimum for the same five tests were 71°–58°, 69°–56°, 72°–62°, and 72°–57° F., respectively. The average of the maximum and minimum water temperatures in all tests of vessel differences was 72° F. The maximum temperature for all tests averaged 74° F. with a range between tests of 70°–77° F. and a minimum temperature for all tests averaged 71° F. with a range between tests of 66°–75° F.

All larvae used in the test were reared in the laboratory from laboratory colonies. All tests either of temperature differences or vessel differences were conducted as comparative tests, thus eliminating day-to-day variations caused by slight differences in larvae and other test conditions. Tests were replicated in time to obtain data that would be more representative of the population of uncontrolled variables.

RESULTS. The effects of temperature on the mortality of mosquito larvae are shown in Figures 3 and 4, and Table 1. Although there was little or no difference in the mortality of *C. nigripalpus* with malathion and *Ae. taeniorhynchus* with naled at the different test temperatures, there was a considerable difference in mortality of the *C. nigripalpus* with naled and the *Ae. taeniorhynchus* with malathion. The *C. nigripalpus*-naled tests at a constant 80° F. resulted in considerably lower mortality than when tested at an average temperature of 65° F. Conversely, the *Ae. taeniorhynchus*-malathion tests at a constant 80° F. gave considerably higher mortality than when tested at an average temperature of 65° F. The reasons for these differences are not known but are obviously dependent upon the reaction to a particular insecticide by a particular mosquito species.

The effects of the type of testing vessel are shown in Figure 5 and Table 2. The dosage-mortality curves in Figure 5 show that there was little or no difference in the toxicity of malathion to *C. nigripalpus* in the glass, plastic or paper vessels. There was no difference in toxicity of fenthion

TABLE 1.—The effect of temperature on the toxicity of malathion and naled to *Aedes taeniorhynchus* (Wied.) and *Culex nigripalpus* Theob.

Insecticide	Species	Temp. °F.	No. of reps.	Lethal concentrations in μg/ml. LC_{50}	LC_{90}
malathion	*Aedes taeniorhynchus*	80°	20	.015	.027
		65°	20	.022	.060
	Culex nigripalpus	80°	20	.031	.042
		67°	20	.030	.041
naled	*Aedes taeniorhynchus*	80°	20	.065	.130
		63°	20	.079	.145
	Culex nigripalpus	80°	20	.065	.083
		65°	20	.040	.058

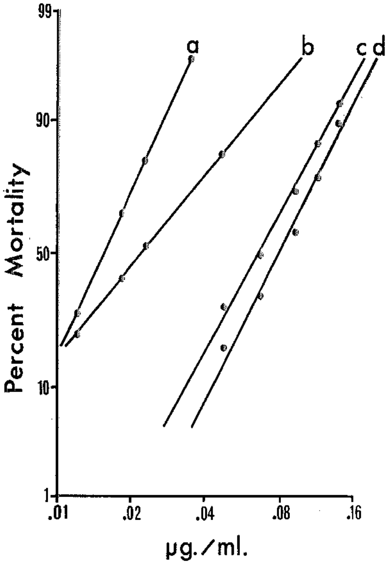

FIG. 3.—Dosage-mortality curves of malathion and naled at warm and cool temperatures for larvae of *Aedes taeniorhynchus* (Wied.) (a—malathion at 80° F., b—malathion at 65° F., c—naled at 80° F., d—naled at 63° F.).

and naled to this species in the glass or plastic vessels; however, there was a slight increase in effectiveness of naled and a slight decrease in effectiveness with fenthion when *C. nigripalpus* was tested in paper vessels. These differences are slight and therefore of questionable significance. In tests with Abate there was a considerable reduction in the mortality of *C. nigripalpus* when tested in plastic or paper as compared to glass vessels. The magnitude of this difference may be emphasized by the fact that a dosage of 0.00073 μg. per ml. resulted in a 91 percent mortality in glass vessels compared to only 1 and 2 percent mortality in plastic and paper vessels, respectively.

Tests of the four insecticides with the three types of testing vessels were also conducted with several other mosquito species. Results of these tests are shown in Table 2. Essentially the same results were obtained with *C. salinarius, Ae. taeniorhynchus* and *Ae. aegypti* as with *C. nigripalpus,* although susceptibility levels were shown to vary considerably between species.

It is interesting to note that in all comparisons between the glass and plastic vessels the LC_{50} values are always slightly higher with the plastic than with the glass except in two instances, those of *C. salinarius* with fenthion and *Ae. aegypti* with malathion. It has been shown by others (Schmidt and Weidhaas, 1959; Kruse *et al.,* 1952) that the surface to volume ratio of the test vessel may also affect larval mortality. The surface diameter and the depth of the water in the glass, plastic and waxed paper testing vessels were 3-5/16 inches by 1-11/16 inches, 2-15/16 inches by 2-7/16 inches, and 2-13/16 inches by 3-5/16 inches, respectively. Whether or not the slight increase in LC_{50} values when the tests were conducted in plastic vessels as compared to glass vessels was due to

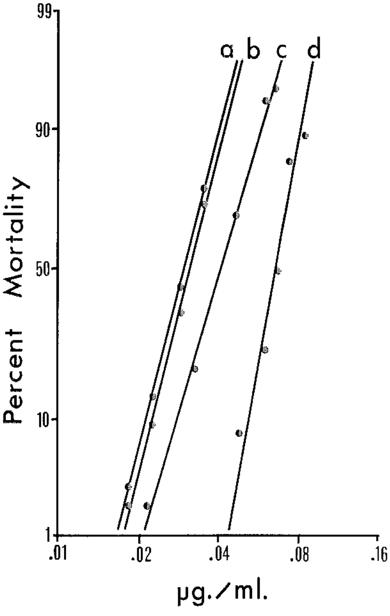

FIG. 4.—Dosage-mortality curves of malathion and naled at warm and cool temperatures for *Culex nigripalpus* Theob. (a—malathion at 67° F., b—malathion at 80° F., c—naled at 65° F., d—naled at 80° F.).

TABLE 2.—Effect of type of testing vessel on the toxicity of four insecticides to four species of mosquitoes.

Insecticide	Species	No. of reps.	LC_{50} in μg./ml.			LC_{90} in μg./ml.		
			Glass	Plastic	Paper	Glass	Plastic	Paper
Abate	*Culex nigripalpus*	12	.00055	.00119	.00112	.00070	.00150	.00145
	Culex salinarius	4	.00056	.00130	......	.00098	.00200	......
	Aedes taeniorhynchus	10	.00100	.00180	.00195	.00140	.00255	.00275
	Aedes aegypti	8	.00160	.00240	......	.00260	.00430	......
fenthion	*Culex nigripalpus*	14	.0031	.0033	.0040	.0041	.0046	.0055
	Culex salinarius	2	.0030	.0029	.....	.0039	.0038	.....
	Aedes taeniorhynchus	6	.0010	.0010	.....	.0019	.0023	.....
	Aedes aegypti	2	.0040	.0045	.....	.0053	.0054	.....
malathion	*Culex nigripalpus*	12	.031	.032	.029	.043	.046	.039
	Culex salinarius	12	....	.045	....	....	.063	....
	Aedes taeniorhynchus	4	.031	.032	....	.054	.058	....
	Aedes aegypti	4	.094	.082	....	.153	.128	....
naled	*Culex nigripalpus*	12	.057	.057	.048	.074	.072	.061
	Culex salinarius	4	.080	.083	....	.095	.098	....
	Aedes taeniorhynchus	4	.094	.098	....	.132	.130	....
	Aedes aegypti	6	.188	.195	....	.272	.285	....

the difference in the surface to volume ratio of the two types of vessels is not known. However, it appears unlikely since there was no similar increase in LC_{50} values between the plastic and the waxed paper vessels.

Overall control mortality averaged 1.1 percent for the 424 replications of test vessels and temperatures with all mosquito species. There was less than one percent difference in control mortality between the type of test vessel, between the warm and cool temperatures or between mosquito species.

DISCUSSION. Although the standardized test of the World Health Organization specifies glass testing vessels, much of the current research on the susceptibility of different species of mosquito larvae to various insecticides is conducted with plastic beakers or paper cups. This research has shown that, depending on the insecticide, larval mortality may vary with the type of testing vessel and in order to be comparable tests must be conducted in vessels of the same material. It therefore seems desirable that all insecticide susceptibility tests with mosquito larvae be conducted in like vessels in order to be comparable. Because of its inertness, impermeability and availability, glass appears to be the vessel of choice.

World Health Organization procedures also specify that the average temperature of the water should not be below 68° F. nor above 86° F. This research has demonstrated that larval mortality may vary greatly between test temperatures of 65° and 80° F. depending upon the type of insecticide and/or the species used. An average temperature of 68° to 86° F. allows for temperatures well below 68° F. and above 86° F. This appears to be too great a range and may lead to highly variable results that are not comparable between species or insecticides. The obvious solution appears to be the use of a constant temperature bath. If this is not

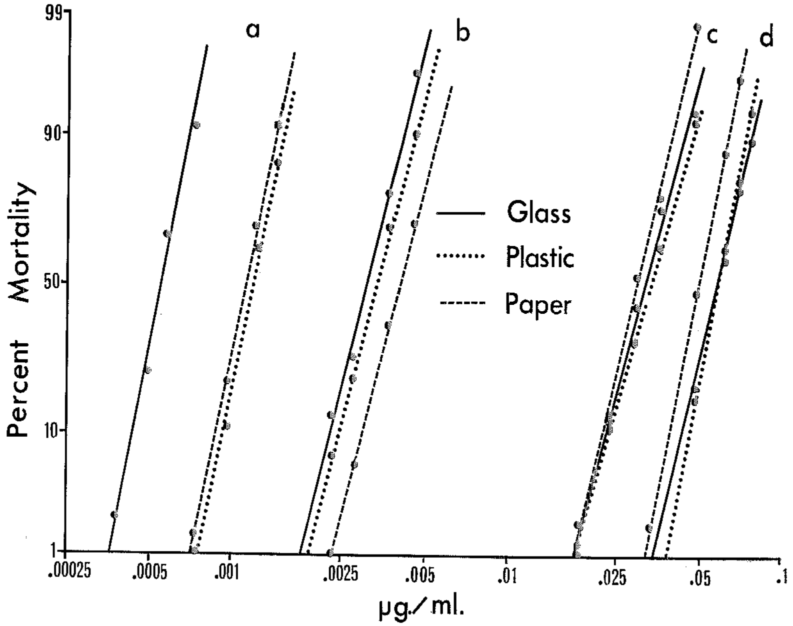

FIG. 5.—Dosage-mortality curves of Abate, fenthion, malathion and naled in glass, plastic and paper testing vessels for larvae of *Culex nigripalpus* Theob. (a—Abate, b—fenthion, c—malathion, d—naled).

available, the temperature of the test solutions should be carefully recorded and reported since in reviewing most methods in published literature, it usually cannot be readily ascertained whether the temperature, if reported, was the room temperature or the water temperature, whether it was recorded at treatment time or count time, or whether it is some type of average temperature.

Although the maximum difference in LC_{50} or LC_{90} values obtained in these tests was only 2- to 3-fold, the effects of temperature and type of testing vessel on larval mortality are important. The effects of these variables, as well as those previously mentioned, may be additive and therefore result in differences of a much greater magnitude. On the other hand the effect of these or other variables may be offsetting, the effect of one variable negating the effect of another. In any event, uncontrolled variables of unknown effect can only result in highly variable data of poor reliability.

Literature Cited

BRANSBY-WILLIAMS, W. R. 1965. Some effects of polyethylene on the efficacy of larvicides tested against *Culex pipiens fatigans*. Bull. Wld. Hlth. Org. 33(5):737–740.

CURTIS, L. C. 1961. The effect of various container materials on dilute DDT solutions. Mosq. News 21(2):139–140.

KRUSE, C. W., LUDVIK, G. F., and HAWKINS, W. B. 1952. Factors affecting evaluation of insecticide against *Anopheles* larvae. Jour. Econ. Ent. 45(4):598–601.

SCHMIDT, C. H., and WEIDHAAS, D. E. 1959. Effects of varying conditions in a laboratory testing technique on the mortality of mosquito larvae. Jour. Econ. Ent. 52(5):977–979.

THOMAS, V. 1965. Effects of certain extrinsic and intrinsic factors on the susceptibility of larvae of *Culex pipiens fatigans* Wied. to DDT. Mosq. News 25(1):38–43.

WORLD HEALTH ORGANIZATION. 1960. Instructions for determining the susceptibility or resistance of mosquito larvae to insecticides. Reprinted from WHO: Tech. Rept. Series, No. 191.