A BIOASSAY METHOD FOR MEASURING MOSQUITO LARVICIDE DEPOSITIONS

P. A. GILLIES, D. J. WOMELDORF, AND J. D. WALSH [1]

Larviciding is the primary method of mosquito control in California, with over one million acres treated annually by aircraft alone. A substantial acreage is also treated by calibrated ground equipment. Adequate dispersal of the chemicals applied is therefore essential to control efficacy.

Mosquito control agencies in California have used various methods (e.g., indicator dyes applied to paper cards or strips) to determine the swath width of aircraft, mistblowers, and other calibrated equipment. The dye technique, while useful for visual pattern analysis, does not lend itself well to quantitative measurements. Sophisticated chemical and physical recovery methods, although indispensable to equipment research and development, are rarely recommended for use by control agencies. Measurement of chemical depositions determined by toxicity to mosquito larvae appeared to be a logical and inexpensive approach to the problem.

Bioassay, which utilizes the response of a living organism to a test environment, is an accepted means of measuring unknown amounts of insecticide. The various uses of bioassay in entomology have been discussed by Brown (1958) and by Hoskins and Craig (1962). A commonly used technique calls for placing mosquito larvae in water treated or to be treated with insecticide. At a given endpoint, usually 24 hours, mortality is compared with that for larvae from the same population treated with a control series of known

[1] California Department of Public Health, Bureau of Vector Control.

concentrations of the chemical. An estimate is then made of the concentration of the unknown sample. The sensitivity range of this method is extremely limited. When larvicides are applied at field dosage rates to typical shallow water sources, the resulting insecticide concentration far exceeds the sensitivity of this test. When materials are applied for the purpose of swath analysis, 100-fold variations in concentrations recovered within the swath are not uncommon. With the endpoint mortality method, preparation of numerous serial dilutions is usually necessary to arrive at detectable concentrations.

Our need was for a bioassay method capable of directly assessing the output of calibrated insecticide application equipment. A modified time-knockdown method was devised to meet this need. The method is based on the observation that members of a homogeneous population, when exposed to a series of concentrations of an insecticide, are affected at time intervals directly related to the insecticide concentration. That is, the higher the concentration, the quicker the knockdown.

Knockdown of the test animals at timed intervals following treatment, rather than endpoint mortality versus a series of concentrations, is a bioassay method which has been employed for various purposes. Time of knockdown has been used to detect insecticide resistance in mosquito larvae (French and Kitzmiller, 1965) and as a technique for studying the genetic constitution of larval populations (French and Kitzmiller, 1963). Kaminski (WHO/EBL/66.63) used the aquatic isopod, *Asellus aquaticus,* to detect and measure traces of insecticide in water.

TEST PROCEDURE. Mosquito larvae are added to water which has been exposed to an application of insecticide. The observed times of knockdown are compared with those of a standard series of concentrations to obtain estimates of the unknown insecticide concentrations.

For measuring swath width of larviciding equipment, it was necessary to find a container possessing a surface/volume relationship that would result in insecticide concentrations corresponding to the limits of the test. Disposable waxed paper cups, 8-ounce capacity, 2½ inches outside height, are satisfactory. When chemicals are applied at the rate of 0.1/acre to the container filled with 200 ml. of water, the resulting deposit/volume is 0.346 mg/l (= p.p.m.).

The cartons are placed in a straight line across the equipment's swath for a distance exceeding the assumed operational swath width. For example, for an assumed swath of 60 feet, test cartons are set out in a line 100 feet long to insure recovery of the entire swath. The intervals between containers depend upon test needs, but are usually from 2 to 5 feet. Two (sometimes three) replicates are used, with the separate rows spaced 5 to 10 feet apart. The cartons are filled with water and the insecticide is applied by the equipment to be tested.

A control series is prepared to serve as a standard for comparison. Cartons identical to those in the test are filled with water from the same source and are placed so that they are exposed to the same environmental conditions as the test containers. The control series is treated with a weight-volume solution of technical insecticide, with a series of concentrations spaced at approximately equal logarithmic intervals. A typical control series includes final concentrations of 0.0015, 0.0020, 0.0030, 0.0045, 0.0070, 0.010, and 0.015 p.p.m., with the cycle being repeated to a maximum of 0.45 p.p.m. when using larvicides ordinarily applied at the rate of 0.1 lb/acre. The series of control cartons is either removed or covered during the actual application to prevent contamination. Following application, lids are placed on the containers of both the control and test samples and they are transported to the laboratory for the remainder of the test.

Prior to the test, 20 fourth instar mosquito larvae are placed in about 50 ml of water in each of as many small dis-

posable paper cups as there are test and control samples. The test cartons are arranged on a work surface and the precounted larvae are then transferred to the treated water samples. To avoid unnecessarily diluting the samples, it is necessary to strain out the larvae in transfer. A 3-inch square of nylon net, about 26 mesh, is finger-shaped to an inverted cone. The cup containing the larvae is emptied into it and the larvae are quickly rinsed into the treated water samples. A fresh nylon square is used for each transfer to avoid contamination.

The time at which the larvae are added is recorded. Cursory observations begin almost immediately. At the first sign of abnormality, usually within one-half hour, the time is recorded and the percent knockdown is noted for each test and control sample. This procedure is repeated throughout the test. The intervals between observations should be short enough so that several counts may be recorded between zero and 100 percent knockdown. At the higher concentrations, the counts may need to be taken less than 5 minutes apart, with longer intervals at the lower dosages. A slightly conservative criterion of knockdown is more realistic than recording initial signs of intoxication, since larvae sometimes appear to recover from the first effects of the chemical. Our criterion is that a larva is knocked down when it is obviously moribund and is unable to surface or exhibit normal escape responses when lightly probed. Counts are continued until about 90 percent of the larvae in each container have been knocked down. The total time during

TABLE 1.—Example of percent knockdown * by elapsed time from addition of the larvae to the treated water for several control and unknown concentrations of insecticide. These figures are representative of chemicals commonly applied at 0.1 lb/acre such as parathion, methyl parathion and fenthion.

Time (min.)	Control concentrations (p.p.m.)				Unknown concentrations		
	0.3	0.1	0.03	0.01	A	B	C
34	0	.....	.....	....	.....	.....	.....
36	20.0	.....	.....	....	.....	.....	.....
38	90.0	.....	.....	....	0	.....	.....
40	100.0	.....	.....	....	2.5	.....	.....
45	.....	0	.....	....	55.0	.....	.....
50	.....	7.5	.....	....	97.5	.....	.....
55	.....	40.0	.....	....	100.0	.....	.....
60	.....	65.0	.....	....	.....	0	.....
70	.....	97.5	.....	....	.....	5.0	.....
80	.....	100.0	.....	....	.....	40.0	.....
90	.....	.....	0	....	.....	67.5	.....
100	.....	.....	5.0	....	.....	90.0	.....
110	.....	.....	20.0	....	.....	100.0	.....
120	.....	.....	42.5	....	.....	.....	.....
135	.....	.....	80.0	....	.....	.....	0
150	.....	.....	95.0	....	.....	.....	10.0
170	.....	.....	100.0	0	.....	.....	20.0
200	.....	.....	.....	2.5	.....	.....	42.5
240	.....	.....	.....	7.5	.....	.....	80.0
280	.....	.....	.....	20.0	.....	.....	97.5
320	.....	.....	.....	45.0	.....	.....	100.0
360	.....	.....	.....	55.0	.....	.....	.....
400	.....	.....	.....	67.5	.....	.....	.....
450	.....	.....	.....	70.0	.....	.....	.....
500	.....	.....	.....	90.0	.....	.....	.....
560	.....	.....	.....	97.5	.....	.....	.....

* Percent knockdown is the average of two replicates for each control concentration and test station. Test station samples are designated by unknown concentrations A, B, and C.

which observations are made varies with temperature, larval condition, and the needs of the test, but usually covers several hours. Examples of percent knockdown at timed intervals for both unknown and control concentrations are shown in Table 1. Containers showing no mortality, or in which only a few larvae are moribund at the end of several hours, are set aside and held for a 24-hour mortality determination.

The total number of minutes between addition of larvae to the treated samples and the time at which each count is recorded is determined. For each sample the percent knockdown is then plotted versus time, in minutes, on probability-log paper. A straight line is fitted to the points by eye and the time necessary to knock down 50 percent of the larvae (KT_{50}) is read from the line. Figure 1 shows lines and KT_{50} values derived from the data given in Table 1. The $KT_{50's}$ determined for the control dosages are then plotted against concentrations on log-log graph paper and a curved line is drawn through the points (Fig. 2). An estimate of the concentrations of insecticide in each unknown sample is obtained by comparing its KT_{50} to the curve for the controls.

For concentrations less than 0.01 p.p.m., there is generally little mortality during the test observation time. To estimate

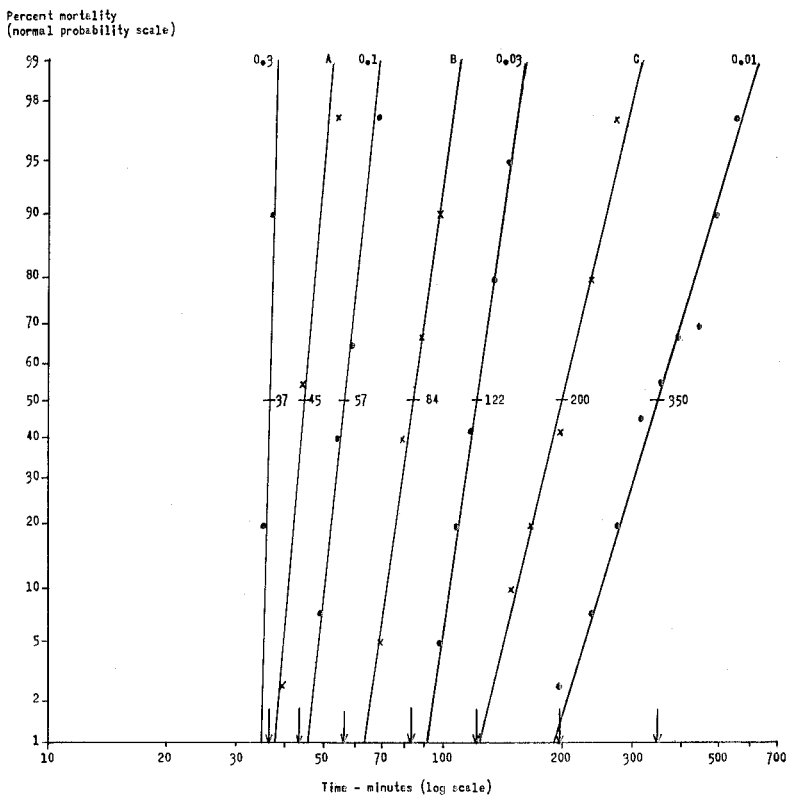

FIG. 1.—Time-mortality lines fitted to the data given in Table 1. The KT_{50} in minutes determined from each line is shown to the right at the 50 percent level.

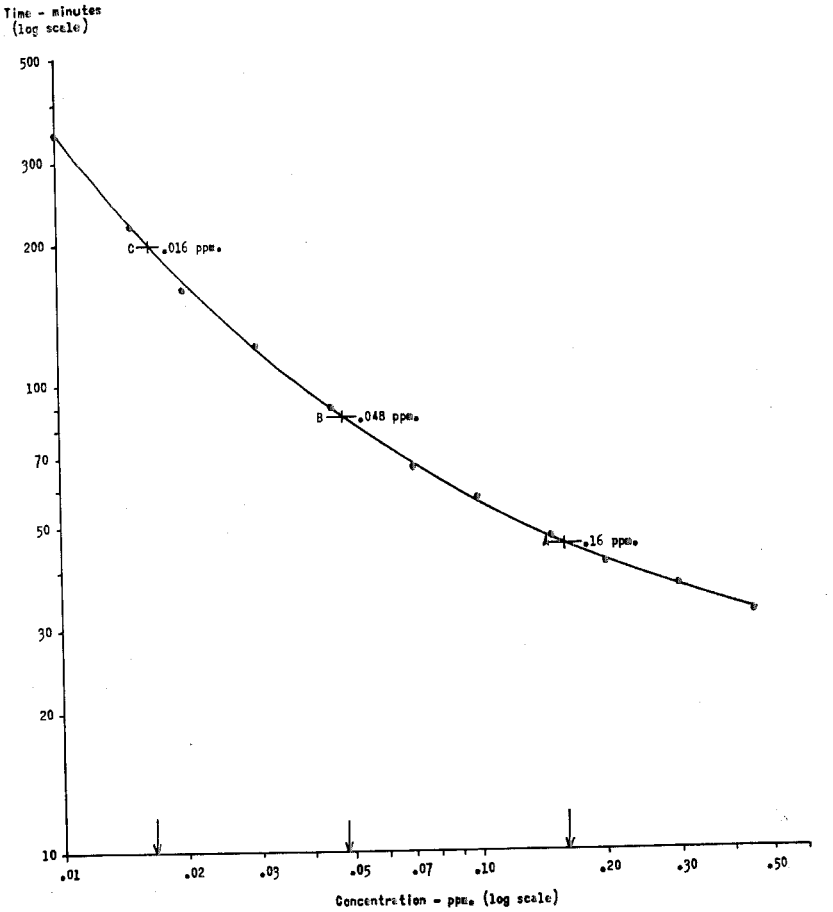

FIG. 2.—Example of a curve of KT_{50} versus concentration for a control series of concentrations of 0.01 to 0.45 p.p.m. including the control KT_{50}s of Figure 1. KT_{50}s of test samples A, B, and C are marked on the curve and the estimates of concentration determined from the curve are shown to the right.

concentrations for test samples in which mortality has occurred at 24 hours, the 24-hour mortality for the controls is plotted on probability-log paper as a standard log-dosage-probit line. From the fitted line, mortality from the unknowns can be compared to that of the controls and the concentrations estimated (Fig. 3). Test samples in which no larvae are dead at 24 hours are assumed to contain a concentration less than that in the lowest control sample showing mortality.

SUMMARY AND DISCUSSION. Larvae of laboratory strains of *Culex tarsalis* and *C. pipiens*, as well as field-collected larvae of *Aedes nigromaculis* and *A. melanimon* have been used as test animals. Probably any nonresistant species would be satisfactory. The range of test sensitivity will vary slightly with the different species, but is commonly from about 0.010 to 0.45 p.p.m. when parathion, methyl parathion, fenthion, or any other material applied at 0.1 lb/acre is used. A series of control con-

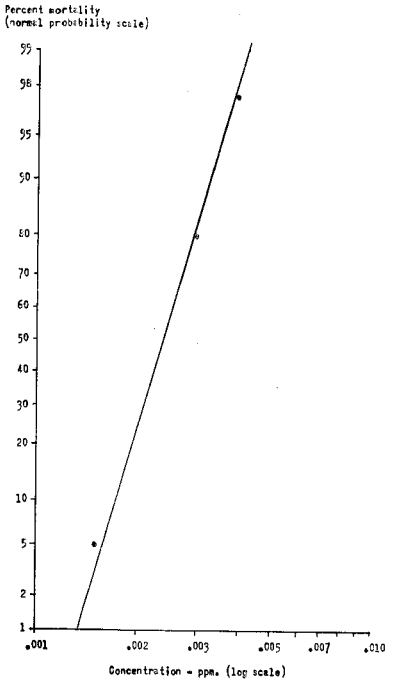

FIG. 3.—Example of a 24-hour mortality concentration line fitted to control data. Estimates of concentration in unknown samples can be made by comparing the percent mortalities at 24 hours to the control line.

centrations in the 24-hour mortality range is included to estimate unknown concentrations lower than those detectable by the time-knockdown test.

This technique has also been used to test applications of malathion, another widely used mosquito larvicide. Field dosages of malathion are considerably higher, usually 0.5 lb/acre, but can be measured satisfactorily by increasing the control concentrations. The inclusive range of the time knockdown and the 24-hour concentrations for this material and dosage rate is from about 0.015 to 4.5 p.p.m.

The test allows a certain amount of flexibility. A higher range of expected dosages can be detected by using containers with a greater volume-to-surface relationship. If concentrations are too high for the test, all larvae in a particular container will be knocked down almost simultaneously. If this occurs, the dead larvae can be removed and the sample diluted as necessary. Fresh larvae are then added, the time and dilution are recorded, and the test is continued. The lower dosages, those which do not affect susceptible populations of mosquito larvae within 24 hours, are of no consequence in operational practice.

This method has been used successfully to determine the swath patterns of aircraft, mistblowers, and other types of liquid larvicide application equipment used in mosquito control. It has also been used to measure concentrations of insecticide in water following treatment.

The graphic methods employed in the analysis of the bioassay data provide only rough estimates of the swath pattern concentrations. The advantages of the graphic method are that it is quick and simple and can be used in the field. More refined statistical methods could be used to fit the time-knockdown lines and the regression curve of mean knockdown time on concentration. In addition, these methods would provide confidence intervals about the point estimates of concentrations. However, the width of such confidence intervals increases with the amount of variability in the data and with the number of swath positions for which estimates are made. A comparison of the two methods of analysis on several sets of data revealed little difference in the point estimates of concentration. Moreover, for the large number of test samples required for the evaluation of the swath pattern, the confidence intervals computed for conventional probability levels were too wide to be meaningful. It was concluded that in general the more time-consuming statistical analysis of the data is not warranted in using the bioassay method of evaluation of insecticide dispersal.

ACKNOWLEDGMENTS. Special thanks are due Dr. Kathleen E. White for reviewing the statistical aspects of the test. Mr. Thomas D. Mulhern and many California

mosquito abatement district personnel have given advice and encouragement throughout the development of the technique.

References Cited

BROWN, A. W. A. 1958. Insecticide resistance in arthropods. World Health Organ. Monogr. Ser. No. 38, 240 p.

FRENCH, W. L., and KITZMILLER, J. B. 1963. Determination of genotypes for dieldrin resistance in anopheline larvae. Proc. N.J. Mosquito Exterm. Assoc. 50:241–250.

FRENCH, W. L., and KITZMILLER, J. B. 1965. Time in concentration: A simple technique for the accurate detection of resistance to insecticides in mosquito larvae. WHO Bul. 32(1):133–142.

HOSKINS, W. M., and CRAIG, R. 1962. Uses of bioassay in entomology. Ann. Rev. Entomol. 7: 437–464.