EFFECT OF VARYING DEPTHS OF WATER WITH IDENTICAL SURFACE AREA ON MOSQUITO LARVAL MORTALITY USING COMMERCIAL MALATHION AND PARATHION EMULSION CONCENTRATES

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There are many factors which may affect the results of tests of mosquito larvicides. Some of these which have been studied are age, nutritional state and density of larvae, surface area-volume relationships, type of container and amount and type of solvents (Kruse *et al.*, 1952, and Weidhaas and Gahan, 1958). Strong evidence of joint action of solvent and toxicant was demonstrated by Hawkins (1956). Hawkins and Kearns (1956) published results of ionic environment and zeta potential in relation to suspension stability of DDT. Differences in the susceptibility of larvae of various species of mosquitoes to a particular insecticide, and of the same species to different insecticides, were observed by Schmidt and Weidhaas (1958). These authors (1959) also studied the effect of variations of technique on the mortality of mosquito larvae in laboratory tests. In spite of all these observed differences in mortality due to testing method, it is customary to recommend larvicide application rates on an area basis without regard to the depth of water.

Most larviciding formulations available are emulsion concentrates which are diluted with water, and when applied to water disperse rather uniformly through it. It is therefore to be expected that sprays applied to deep water will be less effective than when shallow water is treated. The objective of this study was to test the effect of water depth on larval mortality when emulsion concentrate solutions of insecticides are applied to the water surface.

MATERIALS AND METHODS. Wide-mouth, glass, gallon jars, 14.6 cm. in diameter, were employed as test containers. The surface area of water inside each jar was 190 cm.^{2} Tap water of pH 7.4 was used in all tests.

Malathion and parathion emulsion concentrates, 57 and 25 percent by weight respectively, were selected for these studies. Both insecticides were formulated by American Cyanamid Company, New York, N. Y.

Fourth instar larvae of the Bakersfield strain of *Culex pipiens quinquefasciatus* Say were utilized in the tests. This strain of mosquitoes was colonized in 1952 by the Kern County Mosquito Abatement District from adults collected in Bakersfield. A sub-colony was started at the Bureau of Vector Control Field Station at Fresno in 1955.

Thirteen one-gallon jars of identical size were used for the tests. Four hundred thirty ml. of water placed in a jar provided a medium of 2.5 cm. deep. Each successive 475 ml. of water increased the depth by an increment of 2.5 cm. Two sets of 6 jars each were prepared for the two dosage rates of each insecticide. Within each set, an appropriate volume of water was added to give depths of 2.5, 5.0, 7.5, 10.0, 12.5, and 15.0 cm. One jar containing water 7.5 cm. deep was employed as an untreated control for the two sets. Each insecticide was tested at two dosage levels. These two dosages were selected to kill approximately 50 and 90 percent of the larvae when applied to water 2.5 cm. deep. These dosages were 0.3241 and 0.4140 mg. malathion/cm.^{2} of water surface and 0.0065 and 0.0084 mg. of parathion.

One hundred larvae were introduced into each jar before the addition of the insecticide. Appropriate amounts of malathion and parathion formulations were weighed on an analytical balance and serial dilutions were made in distilled water. One ml. of the final dilution was added to each jar by pipette, dropwise, care being taken to treat only the water surface. The jars were left undisturbed for the entire exposure period to avoid mechanical mixing of the test medium. After 24 hours each larva was probed with a needle and those which failed to respond were tabulated as dead. All tests were performed at $25 \pm 3^\circ$ C. Each test was replicated three times.

In another experiment, 3 series of jars with 3 jars in each series were employed to determine the distribution pattern of the emulsion concentrate of malathion in varying depths of water. To the first, second, and third series of jars were added, respectively, 430, 860 and 1720 ml. of water to produce depths of 2.5, 5.0, and 10.0 cm. Malathion was applied to each of the three series at the rate of 0.4140, 0.8280, and 1.6560 mg./cm.^{2} of water surface. Although these three dosages differ in terms of weight applied per unit of surface area, the final concentration of malathion in the water of each series would be approximately the same if it were homogeneously distributed.

RESULTS. The effect of depth (or volume) of water on mortality of larvae is shown in Table 1. Each figure in the table is an average of three replicates. It can be seen that the effectiveness of the insecticides was greatly reduced by increasing the depth of the medium. Larval mortality was negligible in tests where the water was 10 cm. or more in depth. Increasing the depth of the water affected the performance of the two insecticides similarly.

These results indicate that when equal amounts of insecticide are applied to equal surface areas, the mortality rate is inversely

TABLE 1.—Twenty-four hour mortality of *Culex pipiens quinquefasciatus* larvae exposed to malathion and parathion emulsions applied at different dosage rates (mg./cm.^{2}) to varying depths of water

Depth of water (cm.)	Malathion		Parathion	
	0.3214 mg./cm.^{2}	0.4140 mg./cm.^{2}	0.0065 mg./cm.^{2}	0.0084 mg./cm.^{2}
2.5	53.7	88.0	35.3	91.0
5.0	18.7	31.0	5.3	37.7
7.5	6.7	15.7	2.0	6.0
10.0	0.7	4.3	1.0	3.7
12.5	0.0	2.3	2.0	1.3
15.0	0.0	0.0	1.3	1.3

related to the depth of water. If the mortality rate is plotted against the square of the depth of water, an approximately straight line results, suggesting that the mortality rate is inversely related to the volume of medium. If the mortality rate is plotted against the dosage in terms of weight of insecticide per volume of water an approximately straight line results which suggests a uniform dispersion of insecticide throughout the aqueous medium. This is especially true of malathion. With parathion the kill of larvae at high dilutions is greater than expected which suggests a less homogeneous distribution of the insecticide with larger amounts occurring in the zone where larvae are concentrated.

When similar tests were done in which the weight of insecticide per unit of surface area was increased as the depth increased, the mortality rate did not vary with increasing depth of water (Table 2).

DISCUSSION. The data presented in Table 1 indicate that a given dosage of insecticide applied to different volumes of water is homogeneously distributed throughout the water so that the concentration of the insecticide is decreased as the volume of water is increased. These results are confirmed by the data shown in Table 2 which indicate that when the weight of insecticide per volume of medium is held constant, the mortality rate does not change with depth of medium. It is expected that emulsion concentrates of many other insecticides will be found to behave in a similar fashion. It remains to be shown whether these laboratory findings will prove true under field conditions. The present studies strongly suggest, however, that the usual method of calculating dosages, i.e., weight per unit of surface area, has some limitations. If all mosquito breeding places are treated with the same dosage rate of larvicide (based on area), many larvae, especially those in deeper standing water, will undoubtedly survive. From this the impression may be created that the population has become resistant to the insecticide. The depth of water over the area to be treated should therefore be considered when selecting an effective dosage, especially if the formulation used can be expected to attain a state of homogeneous distribution in the water.

TABLE 2.—Twenty-four hour mortality of *Culex pipiens quinquefasciatus* larvae exposed to malathion emulsion applied at similar dosage rates (mg./cm.^{3}) with respect to volume of water [1]

Depth of water (cm.)	Dosage (mg./cm.^{2})	Mortality (percent)			Average
2.5	0.4140	95	90	96	93.7
5.0	0.8280	95	93	94	94.0
10.0	1.6560	96	93	91	93.3

[1] Actually, the dosages were 0.183, 0.174, and 0.170 mg./cm.^{3}, respectively, the variation being due to the non-uniform nature of the bottoms of the jars.

SUMMARY. The effects of similar rates of application (mg./cm.^{2} of water surface) of malathion and parathion emulsion concentrates to varying depths of water with identical surface area were investigated by employing *C. p. quinquefasciatus* larvae. A given dosage (based on mg./cm.^{2}) was less effective as the depth of water was increased. The lower mortality with increasing depth of water was attributed to homogeneous distribution of the insecticide throughout the water.

When malathion was applied at a constant rate of weight of insecticide to volume of water, varying depths did not affect the larval mortality.

This information suggests that the common practice of applying mosquito larvicides on the basis of surface area of water without consideration of water depth may result in ineffective larval control in deep water.

Literature Cited

HAWKINS, W. B. 1956. The joint action of DDT and ethyl alcohol upon anopheline larvae in bioassay suspensions. Jour. Econ. Ent. 49(4): 433–5.

HAWKINS, W. B., and KEARNS, C. W. 1956. The stability of a DDT suspension. Bull. Ent. Res. 47(1):197–203.

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

SCHMIDT, C. H., and WEIDHAAS, D. E. 1958. Absorption and toxicity of three radioactive insecticides in larvae of two species of mosquito. Jour. Econ. Ent. 51(5):640–4.

SCHMIDT, C. H., and WEIDHAAS, D. E. 1959. Effect of varying conditions in a laboratory testing technique on the mortality of mosquito larvae. Jour. Econ. Ent. 52(5):977–9.

WEIDHAAS, D. E., and GAHAN, J. B. 1958. Effect of certain changes in testing technique on mortality of mosquito larvae. Mosquito News 18(4):275–9.