EVALUATION OF SOME NEW INSECTICIDES AS MOSQUITO LARVICIDES [1, 2]

FREDERICK W. KUTZ AND PAUL P. BURBUTIS [3]
Newark, Delaware

Insecticides were tested in the laboratory and field to determine their efficiency as mosquito larvicides, particularly against the salt-marsh mosquito *Aedes sollicitans* (Walker). Chemical control of this species is complicated by the presence of high levels of resistance to DDT and BHC (Doll, 1962). This mosquito is an important nuisance pest in eastern tidal marsh areas and has also been cited as a possible vector of eastern encephalitis (Hayes *et al.*, 1962).

The objectives of this study were twofold: (1) to evaluate recently-developed insecticides in the laboratory and (2) to test in the field the more promising of these larvicides using a newly-developed small-plot technique.

LABORATORY TESTS

Ten insecticides (Tables 1 and 2) were tested in the laboratory against *Aedes aegypti* (L.) larvae. Eight of these were recently developed chemicals with approximate acute oral LD-50 toxicities to rats of 125 mg/kg or more, and two were older, reference insecticides used to compare the susceptibility of the *A. aegypti* colony with other colonies of this species.

METHODS AND EQUIPMENT. The laboratory screening procedure used in this study was a slightly modified consolidation of methods previously described by Doll (1962), Brown (1958) and a method of Burbutis and Davis (1955). Technical-grade chemicals were used and subsequent intermediate dilutions were made with ACS-grade acetone.

In this procedure, two 400-ml glass beakers were used for each replicate; one contained the mosquito larvae and the other, the toxicant. In one beaker, 20 four-day-old *A. aegypti* larvae were placed in 100 ml of distilled water by means of a wide-mouth glass pipette; the other beaker contained twice the desired concentration of test chemical in 100 ml of distilled water. To begin the 24-hour exposure period, the larvae in the water were poured into the beaker containing toxicant. Beakers were held in a constant temperature chamber ($79 \pm 2°$ F.) during this period. At the close of the test, larvae were classified and recorded as live, moribund or dead. Larvae exhibiting abnormal behavior, i.e., unable to surface or submerge or to move actively in 10 minutes, were considered moribund and were included with the dead counts.

Each test contained eight treatments made up of seven concentrations of the test material and one control to which 0.5 ml of ACS-grade acetone was added. Each treatment was replicated five times.

Data from these tests were punched on IBM cards and analyzed by an IBM 1620 computer programmed for linear regression analysis. Dosage-mortality regression

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[2] Editor's Note: this article was submitted for publication in the spring of 1964. Publication was delayed by the editor, pending release of information on the composition of several of the test insecticides. In the meantime, all but one have been released. Rather than delay longer, the article is printed herewith. References to the compound in question have been retained, since at this late date it would require the revision of both text and figure, with further delay, to omit the compound.

[3] Research Fellow and Assistant Professor, respectively, Department of Entomology, University of Delaware. The authors wish to acknowledge the assistance of Dr. E. P. Catts and Mr. R. W. Lake, Department of Entomology, University of Delaware, and of the various chemical companies for supplying test materials.

TABLE 1.—Designation, supplier, formulation and control numbers of insecticides tested.

Designation	Supplier	Formulation	Control number
AC-43913	American Cyanamid Co.	Technical	AC 856-94
AC-43913	American Cyanamid Co.	2 lbs./gal. E.C.	AC 826-122
AC-52160	American Cyanamid Co.	Technical	AC 850-141-B
AC-52160	American Cyanamid Co.	2 lbs./gal. E.C.	AC 899-7-1
Bayer 37344	Chemagro Corp.	Technical	885
Bayer 39007	Chemagro Corp.	Technical	808
Bayer 41831	Chemagro Corp.	Technical	8185
DDT	Geigy Ag. Chemicals	Technical	FL-650
NIA 9203	Niagara Chemical Div.	Technical	MR-C-399
Pyramat	Geigy Ag. Chemicals	Technical	FL-472
SD 7438	Shell Development Co.	Technical	4-1-0-0
SD 7438	Shell Development Co.	1.6 lbs./gal. E.C.	4-4-8-1
Zectran	Dow Chemical Co.	Technical	117

lines were drawn from estimated data supplied by the computer and LC-50 and LC-90 values were calculated from linear equations supplied by the computer. The computer output contained the correlation coefficient as well as a test of significance ("t" value) for the analysis.

RESULTS AND DISCUSSION. Results of laboratory evaluation of insecticides against *A. aegypti* larvae are presented in Table 3. Dosage-mortality regression lines of the four most effective compounds and DDT are presented in Figure 1.

The LC-50 and LC-90 of the reference insecticide, DDT, were 0.0048 p.p.m. and 0.0077 p.p.m., respectively. Fay (1959) reported similar values of 0.004 p.p.m., and 0.009 p.p.m., respectively, using third instar *A. aegypti* and DDT. Sutherland and Bast (1961) reported an LC-50 of 0.0064 p.p.m. with third instar *A. aegypti* larvae, and Parker (1957) found an LC-50 of 0.0034 p.p.m. for early fourth instar larvae of the same species.

The other reference insecticide, Zectran, gave LC-50 and LC-90 values of 0.9968 p.p.m. and 1.7134 p.p.m., respectively, indicating that this insecticide is not a very effective mosquito larvicide. Georghiou and Metcalf (1961) also reported results

TABLE 2.—Chemical composition and mammalian toxicity of test materials.

Designation	Chemical Composition	Approx. Acute Oral LD-50 to Rats (mg/kg)	Dermal LD-50 (rats) (mg/kg)
AC-43913	Called "an organophosphate" composition not divulged. See footnote page 000	6,150	>2,500
AC-52160	o,o,o′,o′-tetramethyl o,o′-thiodi-*p*-phenylene phosphorothioate	440	>821
Bayer 37344	4-(Methylthio)-3,5-xylyl methylcarbamate	135 (♀)—130 (♂)	>1,000
Bayer 39007	o-Isopropoxyphenyl methylcarbamate	95 (♂)—104 (♀)	>1,000
Bayer 41831	o-o-Dimethyl o-4-nitro-m-tolyl phosphorothiolate	250 (♀ & ♂)	200—765
DDT	2,2-Bis(*p*-chlorophenyl)-1,1,1-trichloroethane	113	..
NIA 9203	o-o-dimethyl s- (benzoxazolin-2-on-3-yl) methyl phosphorothiolate	131±25	12,500±3,600
Pyramat	2-n-propyl-3-methylpyrimidyl-(6)-dimethylcarbamate	225	..
SD 7438	toluene-*a*,a-dithiol-bis(o,o-dimethyl phosphorodithioate	280	..
Zectran	4-Dimethylamino-3,5-xylyl methylcarbamate	15—63	..

TABLE 3.—Results of laboratory screening of test materials against *Aedes aegypti* larvae.

Material	LC-50 (ppm)	LC-90 (ppm)	Correlation coefficient	No. of observ.	"t" Value
AC-52160	0.0012	0.0017	0.7807	60	9.5163[1]
SD 7438	0.0029	0.0052	0.8745	85	16.4302[1]
AC-43913	0.0030	0.0066	0.7729	80	10.7567[1]
Bayer 41831	0.0044	0.0065	0.9646	37	21.6573[1]
Bayer 39007	0.6162	1.1959	0.8981	35	11.7324[1]
Bayer 37344	Between 0.05–0.5		..	15	..
NIA 9203	Between 0.05–0.5		..	15	..
Pyramat	Greater than 2.0		..	35	..
DDT	0.0048	0.0077	0.9235	35	13.8347[1]
Zectran	0.9968	1.7134	0.9290	70	20.7080[1]

[1] Significance at the 0.01 level.

with Zectran using susceptible *Culex pipiens quinquefasciatus* and dieldrin-resistant *Anopheles albimanus* larvae that would appear to support this conclusion.

The most effective new insecticide tested in the laboratory was AC-52160 ("Abate") giving LC-50 and LC-90 values of 0.0012 p.p.m. and 0.0017 p.p.m., respectively.

The LC-50 values of SD 7438 and AC-43913 (i.e., 0.0029 p.p.m. and 0.0030 p.p.m., respectively) were very similar; however, the LC-90 values exhibited more variation. Recent laboratory tests by Mulla *et al.* (1962) using SD 7438 and fourth instar *C. p. quinquefasciatus* larvae found rather similar, low LC-50 and LC-90 values of 0.0041 p.p.m. and 0.0068 p.p.m., respectively.

Preliminary laboratory tests with Pyramat, Bayer 39007, Bayer 37344 and NIA 9203 indicated that these compounds had high LC-50 values, so further testing was

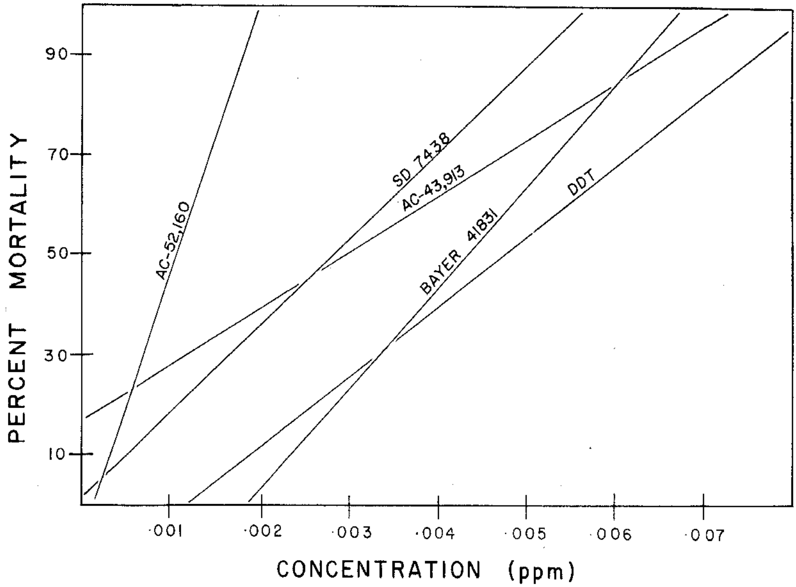

FIG. 1.—Dosage-mortality regression lines of the four most effective larvicides and DDT, for *Aedes aegypti* larvae after 24-hours exposure.

discontinued. Several investigators have formed essentially the same conclusions: Mulla *et al.* (1962) and Georghiou and Metcalf (1961) using Bayer 37344, and Georghiou and Metcalf (1961) using Bayer 39007.

SMALL-PLOT FIELD EVALUATIONS

Of the eight new insecticides tested in the laboratory, four (AC-52160; AC-43 913; SD 7438; and Bayer 41831) exhibited sufficient larvicidal activity to warrant field evaluation.

METHODS AND EQUIPMENT. Basically, the small-plot field testing technique developed for this study consisted of using shallow polyethylene-lined pools (one square yard by one foot deep) containing second and third instar *A. sollicitans* larvae and mixed instars of the northern house mosquito, *Culex pipiens pipiens* L.

Pre- and post-treatment sampling was made with an enameled dipper (4.5 inches in diameter and 450 cc capacity). Insecticides, obtained as emulsifiable concentrates and diluted with #2 fuel oil, were applied with a hand-operated sprayer. Post-treatment sampling was made 24 hours after application. Five dips were taken from each pool.

Each test comprised four treatments composed of three concentrations of the test material and one control; each treatment was replicated three times. The average number of larvae per dip in the pre- and post-treatment sampling was used to calculate the percent reduction in larvae.

RESULTS AND DISCUSSION. Results of the small-plot field evaluations are presented in Table 4. Complete control of both species was obtained using Abate (AC-52160) at a rate of 0.005 pound per acre. Complete control was also obtained with AC-43913 at a rate of 0.01 pound per acre and with SD 7438 at a rate of 0.025 pound per acre. At 0.01 pound per acre, Bayer 41831 almost completely controlled *A. sollicitans* larvae, but failed to control adequately *C. p. pipiens* larvae.

Mulla *et al.* (1962) field-tested both SD 7438 and Bayer 41831 against fourth instar *Culex tarsalis* larvae. The latter gave

TABLE 4.—Results of small-plot field evaluation of the more effective test materials.

Material	Rate (lbs./A)	Percent Reduction	
		Aedes sollicitans [1]	*Culex p. pipiens* [2]
AC-52160	.025	100.	100.
	.01	100.	100.
	.005	99.9	100.
	.001	55.8	1.8
AC-43913	.05	100.	100.
	.025	100.	100.
	.01	100.	100.
	.005	82.4	55.6
	.001	43.6	5.9
SD 7438	.1	100.	..
	.05	99.9	..
	.025	100.	100.
	.01	90.5	96.5
	.005	48.7	47.3
Bayer 41831	.01	99.6	87.7
	.005	81.9	20.4
	.001	42.4	42.1

[1] *Aedes sollicitans:* second and third instar larvae.
[2] *Culex pipiens pipiens:* mixed instars.

complete control at 0.05 pound per acre and 99 percent reduction was obtained with the former at 0.025 pound per acre. Lewallen and Wilder (1963) found that SD 7438 gave complete control of *Aedes dorsalis* larvae at 0.01 pound per acre, and 99 percent reduction of *C. tarsalis* and *Aedes nigromaculis* larvae at 0.025 pound per acre; complete control of the latter species was obtained at a rate of 0.05 pound per acre.

SUMMARY

Ten insecticides were evaluated in laboratory tests against four-day-old *Aedes aegypti* larvae; eight were recently-developed insecticides with low mammalian toxicities, and two were older insecticides used for references to compare the susceptibility of this *A. aegypti* colony with colonies of this species used by other workers. Results of laboratory tests were analyzed by an IBM 1620 computer programmed for linear regression analysis.

Of the eight new insecticides tested in the laboratory, four (AC-52160; AC-43 913; SD 7438; and Bayer 41831) exhibited sufficient larvicidal activity to warrant field evaluation. A small-plot field technique using a small polyethylene-lined pool containing *A. sollicitans* and *C. p. pipiens* larvae was utilized for field evaluations.

In small-plot field tests, AC-52,160 (Abate) gave complete control of both species of larvae at a dosage rate of 0.005 pound per acre. Complete control also was obtained with AC-43,913 at a rate of 0.01 pound per acre and with SD 7438 at a rate of 0.025 pound per acre. At a rate of 0.01 pound per acre, Bayer 41831 almost completely controlled *A. sollicitans* larvae, but failed to effectively control *C. p. pipiens* larvae.

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