LABORATORY STUDIES OF NEW INSECTICIDES AGAINST MOSQUITO LARVAE AND ADULTS[1]

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INTRODUCTION. The search for new and better insecticides for the control of mosquitoes has been intensified as a result of insecticide resistance problems and the initiation of world-wide vector control efforts. A continuing program of laboratory and field evaluation of pesticides has been maintained by the Biology/Chemistry Section of the Communicable Disease Center at Savannah, Georgia. The interest of international agencies in such research is evident from the financial support given by the World Health Organization (WHO) and the Agency for International Development (AID). This paper describes the laboratory techniques employed and presents test data obtained against mosquitoes in 1961 and 1962 in work partially supported by these agencies.

METHODS. The technique and equipment described by Fay *et al.* (1947), and Simmons and staff (1945) were used in these tests except for minor modifications. The exposure chamber consisted of a wooden framework into which 4 plywood panels (3" x 12" x ¼") were fitted to form a chamber of which the total treated surface was 1 square foot and the untreated surface ⅛ square foot. A circular opening (2¾" diameter) was provided in each end of the framework to allow for the introduction and removal of test specimens. Each opening was fitted with a removable metal collar, one of which was closed by a metal screen. Both end openings were closed by sliding panels, wood at the screened end and metal at the other. Chamber construction thus minimized light attraction. All untreated surfaces were replaced or cleaned to prevent cumulative contamination. With the chamber in a vertical position "knocked-down" mosquitoes fell onto an untreated surface.

Three-day-old insectary-reared adults of dieldrin-resistant *Anopheles quadrimaculatus* Say and *Anopheles albimanus* Wiedemann, dieldrin-DDT-resistant *Culex pipiens quinquefasciatus* Say, and DDT-resistant *Aedes aegypti* Linnaeus were the test species. Specimens were anaesthetized with CO_2 for transfer from emergence containers to a screen-wire stock cage. Following a recovery period of 1 hour, 100 to 150 adults were allowed to fly into a glass cylindrical transfer tube and then blown by breath into an exposure chamber. After exposure for 1 hour, the mosquitoes were transferred (Fig. 1) by an air current into a screen-wire holding cage. The specimens then were furnished cotton pads saturated with 10 percent honey solution, and held at 80° F. and 70 percent RH for 24-hour mortality count. Only the female mortality was considered in the results.

Water-wettable formulations of the candidate compounds were used, with the application rates based on available toxicological data. Compounds with an acute oral LD_{50} to rats or mice of less than 100 mg./kg. were applied at dosages up to 100 mg./sq. ft., those with an LD_{50} above 100 mg./kg. at rates up to 200 mg./sq. ft. Each formulation was sprayed upon four plywood panels at the rate of 8 ml./sq. ft., the concentration being varied to give the desired amount of deposit. In treatment the panels were sprayed as a single flat surface as they passed beneath an 8002 Teejet nozzle with 40 lbs./sq. in. pressure on a moving chain-type apparatus. After overnight drying, panels were stored (Fig. 2) in cubicles, each of which was ventilated by a gentle

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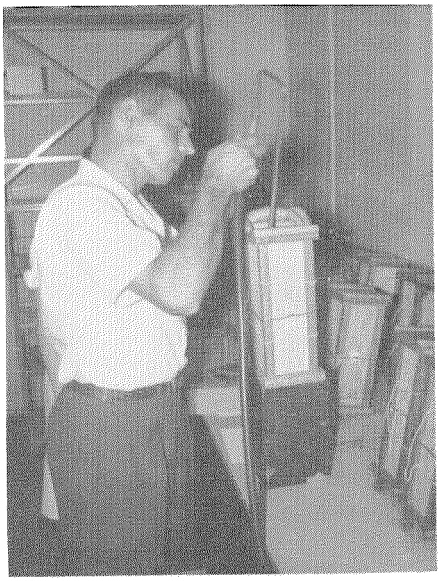

FIG. 1.—Transferring mosquitoes by air current from test chamber into holding cage below.

stream of air diffusing between and around the panels. The residues were tested at periodic intervals, usually 4 weeks. The same panels were evaluated until the deposits no longer gave effective results.

For larvicide determinations the standard WHO technique, WHO (1960), was employed, with mortality counts being made at 24 hours. Mortality counts included larvae found dead or moribund. Each test represented 3 replicates of 25 larvae each. Late 3rd and early 4th instar larvae of the same species and strains used in adult studies were tested except that larvicide tests were not made with *A. albimanus*.

Technical grade material was formulated in ethanol (95 percent) solutions so that the addition of a constant volume (1 ml.) of insecticidal solution to a final volume of 250 ml. of tap water in glass beakers gave the desired concentration (p.p.m.) of the toxicant.

The compounds tested are listed below. Compounds marked with ** were tested both as larvicides and as residual agents; those marked with an * were tested as larvicides only. All others were tested as residuals only.

Compounds [2] Tested Against Mosquitoes

AR/DDT 10/50	**Dow K 27,820
**Bayer 22684	Dow K 7590
Bayer 29491	Dow K 22,911
*Bayer 29493 (Baytex)	**Dow K 7229
Bayer 30237	General Chemical 4072
*Bayer 30911	*Guthion
Bayer 37341	**Hercules 5727
Bayer 37342	Hercules 6286B
*Bayer 37343	Hercules 7522C
Bayer 37344	Hercules 7845C
Bayer 39007	Hercules 9699
Bayer 39731	Hooker 1422
**Bayer 41831 (Sumithion)	*Shell OS 1741
Bayer 44646	*Shell SD 7587
Bayer 45515	*Stauffer N-2404
Bayer 46650	Stauffer N 3051
Butonate	Stauffer N 2860
Butonate+ DDT (1:2)	Stauffer R 3413
**Cal. Chem. RE 5305-3	Stauffer R 1448
*Dicapthon	**Stauffer R 1504
**Dimethrin	**Stauffer R 1505
**Dimethrin+ piperonyl butoxide (1:1)	**Stauffer R 1571
	Stauffer R 4253
	Union Carbide 10854
	Zectran

[2] Use of trade names is for identification purposes only and does constitute endorsement by the U. S. Public Health Service.

Malathion at 200 mg./sq. ft. and Bayer 29493 served as comparison standards in residual and larvicide evaluations, respectively.

RESULTS. Data for the residual activity of the 9 most effective compounds of the 40 tested are given in Table 1. Each compound was effective for at least 8 weeks against one or more species of mosquito. Effectiveness was measured as the number of weeks during which the compounds gave at least 70 percent female mortality.

Against *A. quadrimaculatus*, Bayer 37344 was the most effective compound tested at 200 mg./sq. ft. where it gave 48 weeks of effective kills as compared to

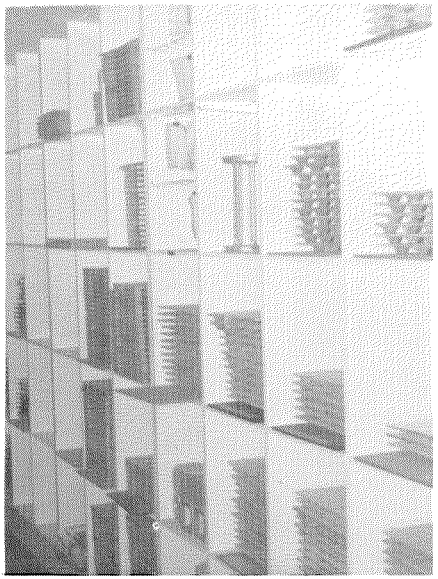

FIG. 2.—Treated panels are stored in ventilated cubicles between tests.

4 weeks for Hooker 1422, 10 weeks for Bayer 39007, and 16 weeks for Bayer 41831, and the malathion standard. At 100 mg./sq. ft., Bayer 29491 and Bayer 30237 gave 24 weeks of satisfactory mortalities against 48 weeks for Bayer 37344. Hercules 9699 gave 28+ weeks of effectiveness at which time the test was terminated.

Against *A. aegypti*, Bayer 37344 again gave 48 weeks of satisfactory kill at both 100 and 200 mg./sq. ft. Bayer compounds 29491, 30237, and 41831, and Hercules 9699 showed the same degree of efficacy against *A. aegypti* as against *A. quadrimaculatus*. Union Carbide 10854, California Chemical RE 5305–3, and Hooker 1422 displayed a marked increase in residual activitiy against *A. aegypti* as compared to *A. quadrimaculatus*.

Of the four compounds tested against *A. albimanus*, Bayer 37344 gave superior results, 48 weeks of effective kills as against 10, 12, and 16+ for Bayer 39007, Union Carbide 10854, and Hooker 1422, respectively. Except for Bayer 37344, all toxicants were inferior to malathion.

Against *C. p. quinquefasciatus*, Hercules 9699 gave 28+ weeks of satisfactory kills at 100 mg./sq. ft. It was superior in effectiveness to the malathion standard and to Bayer Compounds 29491, 30237 and 41831, and Hooker 1422 at 100 mg./sq. ft. Bayer 41831, malathion and Hooker 1422 gave 20 or more weeks of effective mortalities at a dosage of 200 mg./sq. ft.

Of the materials tested as mosquito larvicides, only Bayer 37343 was superior to the Bayer 29493 standard (Table 2) against *A. quadrimaculatus*, *C. p. quinquefasciatus*, and *A. aegypti*. Stauffer N 2404, Dow K 7229, and Guthion followed in decreasing order of effectiveness but all were slightly inferior to the standard.

On the basis of mortality at 0.1 p.p.m., Bayer 30911 and synergized dimethrin were effective against *A. quadrimaculatus* and *C. p. quinquefasciatus* whereas Bayer 41831 was effective against *C. p quinquefasciatus* and *A. aegypti*. Hercules 5727, Bayer 22684, dimethrin, California Chemical RE 5305–3 and Shell SD 7587 gave at least 95 percent mortality of *C. p. quinquefasciatus*.

DISCUSSION. These laboratory data on biological activity indicate that Bayer compounds 29491, 30237, 37344, 39007, and 41831, California Chemical RE 5305–3, Hercules 9699, Hooker 1422, and Union Carbide 10854 warrant further study as residual agents. In later tests using *A. quadrimaculatus*, Bayer 37344 and Bayer 39007 at 100 mg./sq. ft. were shown to be superior to malathion on various surface materials (Schoof *et al.*, 1962; Mathis and Schoof, 1963). Hercules 9699 gave results approximately equal to malathion, whereas Bayer 41831 and Union Carbide 10854 were inferior.

While both Bayer 30237 and Bayer 29491 have given promising results in the laboratory, the available toxicological data

TABLE 1.—Number of weeks during which the indicated compounds on plywood gave at least 70 percent female mortality of resistant strains.

Compound	Mg./sq. ft.	*A. quad.*	*A. albimanus*	*C. p. quinq.*	*A. aegypti*
Bayer 37344	50	4	36	..	15[1]
	100	48[2,3]	48[3]	..	48[1,3]
	200	48[2,3]	48[3]	..	48[1,3]
Bayer 39007	50	4	4	..	0
	100	4	10	..	2
	200	10	10	..	6
UC 10854	50	0	12	..	10
	100	0	12	..	10
Bayer 29491	50	4	..	16	12[1]
	100	24	..	24	24
Bayer 30237	50	4	..	4	0
	100	24	..	24	24
Bayer 41831	50	8	..	12	12
	100	8	..	12	12
	200	16	..	20	16
Hercules 9699	50	24	..	24	24
	100	28[3]	..	28[3]	28[3]
Cal. Chemical RE 5305–3	50	4	..	4	4
	100	4	..	4[4]	12[4]
Hooker 1422	50	0	..[5]	12	0
	100	0	16[3]	12	8
	200	4	16[6]	24[3]	20
Malathion (standard)	200	16	24	24	20

[1] Mortality fell below 70 percent on week 8.
[2] Mortality fell below 70 percent on week 16.
[3] No further tests, material still effective at indicated week.
[4] Mortalities fluctuated between 40 and 100 percent through week 24.
[5] Tested only at week 16.
[6] Not tested again until week 24, at which time mortality was less than 70 percent.

dim the prospects of using these materials in the field. Although results with California Chemical RE 5305–3 showed this material to be ineffective against *A. quadrimaculatus*, the inconsistent mortalities recorded against *A. aegypti* and *C. p. quinquefasciatus* suggest that further tests are needed to determine its effect on these species. Hooker 1422 was ineffective against *A. quadrimaculatus* in comparison with malathion, but against the remaining species the two compounds were essentially equal in biological effectiveness.

The use of chlorinated-hydrocarbon-resistant strains in these tests anticipates possible development of resistance problems which might be encountered with the use of new insecticides in certain areas. Of the test species used, the dieldrin-resistant *A. quadrimaculatus* and the DDT-resistant *A. aegypti* were least susceptible to the candidate compounds.

Species specificity was more notable with the carbamate than the organophosphorus compounds (e.g. Table 1—Bayer 37344, 39007, California Chemical RE 5305–3 versus malathion, Bayer 29491 and 30237). The different response of these species to the carbamate insecticides is of significance because laboratory test evaluations are frequently restricted to one species. Obviously, this limitation can result in misleading information on the potential of a compound if the test species happens

to be one that shows little response to that particular toxicant. Thus, the potential of Hooker Compound 1422 against anopheline mosquitoes would be overlooked if *A. quadrimaculatus* were the only test species used. This aspect of species response is of particular significance since generalized conclusions are often made from tests with a single species.

and 41831) and 6 carbamate materials (Bayer Compounds 37344 and 39007, California Chemical RE 5305–3, Hercules 9699, Hooker 1422 and Union Carbide 10854) were effective for 8 weeks or more. At 200 mg./sq. ft., Bayer 37344 displayed the greatest potential; it gave 48 weeks of kills above 70 percent for *A. quadrimaculatus, A. albimanus* and *A. aegypti*. Her-

TABLE 2.—Concentration (ppm) of indicated compounds required to produce at least 95 percent mortality of mosquito larvae in 24 hours.

Compound	*A. quadrimaculatus*	*C. p. quinquefasciatus*	*A. aegypti*
Bayer 37343	0.004	0.004	0.004
Stauffer N 2404	0.02	0.02	0.1
Dow K 7229	0.1[1]	0.02	..
Guthion	0.1	0.1	0.1
Bayer 29493 (standard)	0.01	0.005	0.02
Bayer 30911	0.1	0.1	0.5[2]
Hercules 5727	0.5	0.1	0.5
Bayer 22684	0.5	0.1	0.5
Dimethrin	0.5[2]	0.1	>2.5
Dimethrin+piperonyl butoxide (1:1)	0.1	0.1	<2.5
Cal. Chem. RE 5305–3	0.5	0.1	..
Bayer 41831	0.5[3]	0.1	0.1
SD 7587	0.5	0.1	0.5

[1] 91 percent mortality at 0.02 p.p.m.
[2] 90 percent mortality at 0.1 p.p.m.
[3] 85 percent mortality at 0.1 p.p.m.

Results of larvicide tests showed that Bayer 37343 was the most effective of the 17 larvicides tested against the 3 species. Stauffer N-2404, Dow K 7229 and Guthion were highly effective against *A. quadrimaculatus, C. p. quinquefasciatus,* and *A. aegypti* at 0.1 p.p.m. Mulla *et al.* (1962), in tests with many of these same compounds against *C. p. quinquefasciatus,* also found Bayer 37343 one of the most promising toxicants.

SUMMARY. The technique and equipment used in the laboratory evaluation of insecticides against adult and larval mosquitoes at Savannah, Georgia, are described. Of the 40 compounds tested as residuals against adult *A. quadrimaculatus, C. p. quinquefasciatus, A. aegypti* and/or *A. albimanus,* 3 organophosphorus toxicants (Bayer Compounds 29491, 30237, cules 9699 and Bayer Compounds 29491 and 30237 were superior to the malathion standard in efficacy against *A. quadrimaculatus, C. p. quinquefasciatus* and *A. aegypti*.

Of the 17 materials evaluated as mosquito larvicides, Bayer 37343 was the most effective, followed by Stauffer Compound N-2404, Dow K 7229 and Guthion. A definite difference in susceptibility response to the different toxicants was apparent among the species used as test insects. Their variations suggest the need for using several test species in order to measure the true potentiality of a chemical for mosquito control.

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ESCAPE-PROOF COLONY CAGE (*AEDES AEGYPTI*)

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In laboratory studies on insecticide resistance in mosquitoes, it is frequently necessary to maintain colonies of many strains of the same species in the same room. As each strain represents a potentially different gene pool, a major concern is the possibility that escapees during insertion and removal of food pad, oviposition bowls, and emergence containers may cause a cross-contamination of the different strains. Such escapees cause annoyance to the workers, which is a particular problem during mass rearing for sterile-male-release studies, when numerous colonies must be maintained for egg production (Morlan *et al.*, 1962; Fay *et al.*, 1963). In addition, it is important to avoid the release of laboratory strains in uninfested areas. To minimize these problems, the existing cages (Morlan *et al.*, 1963), which had a port in front closed by a cloth sleeve through which all containers were inserted and removed, were modified.

The modified colony cage (Fig. 1) is 23″ long x 18″ wide x 18″ high, and the top, bottom, front, and back are made of ½″ plywood. The two sides are closed with 20-mesh galvanized screening on a ½″ plywood frame. The sides and the top and bottom are nailed together, and the back and front panels are fastened in place with screws. Openings in the front panel (see figure) accommodate (A) a screened observation port 5″ wide x 8″ high; (B) a stainless steel tray 6″ long x 3½″ wide x ¾″ high, supported inside the cage by a wooden cleat and containing a cellulose sponge to retain liquid food; (C) a stainless steel tray 22½″ long x ¾″ high, for holding pupae; (D) a removable 8½″ wide x 9″ high panel of ½″ plywood which forms a frame attached to the front of a tunnel for the blood-host; (E) a galvanized tray, 21″ long x 4″ wide x 1½″ high, for an oviposition tray; (F) a 22-gauge stainless steel oviposition strip, 22″ long x 3″ high, which is inserted through a ½″ wide x 4″

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