EVALUATION OF INSECTICIDES AGAINST TWO SPECIES OF *CULEX* MOSQUITOES ON OKINAWA[1]

C. S. LOFGREN,[2] NEIL PENNINGTON,[3] AND WILLIAM YOUNG[4]

During August and September 1964 on the island of Okinawa we evaluated twelve insecticides as adulticides and/or larvicides against *Culex tritaeniorhynchus* Giles and *Culex quinquefasciatus* Say. These experiments were part of a continuing cooperative program between military entomologists and personnel of the Insects Affecting Man and Animals Research Branch, Entomology Research Division, U. S. Department of Agriculture.

The purpose of the program is to evaluate new insecticides and equipment against insects of medical importance on military installations around the world. Studies were conducted in Panama in 1962 and in Okinawa in 1963 (Gahan *et al.*, 1965). Our current tests were similar to those conducted in Okinawa except that we used a nonthermal aerosol generator rather than a thermal generator in the tests with adult mosquitoes and conducted laboratory as well as field tests with the larvicides.

The insecticides used and their mammalian toxicity are shown below.

Insecticide	Mammalian toxicity (Oral LD_{50}; mg./kg.)
Abate® (*O,O*-dimethylphosphorothioate, *O,O*-diester with 4,4′-thiodiphenyl)	1,766 (rats)
Bayer 41831 (*O,O*-dimethyl *O*-4-nitro-*m*-tolyl phosphorothioate)	250 (rats)
Baygon (Bayer 39007; *o*-isopropoxyphenyl methylcarbamate)	95 (male rats); 104 (female rats)
Bromophos (CELA S-1942; *O*-(4-bromo-2,5-dichlorophenyl) *O,O*-dimethyl phosphorothioate)	3,750 (rats)
Bromophos-ethyl (CELA S-2225; *O*-(4-bromo-2,5-dichlorophenyl) *O,O*-diethyl phosphorothioate)	200 (rats)
Carbanolate (Upjohn U-12927; 6-chloro-3,4-xylyl methylcarbamate)	30–44 (rats); 300 (mice)
Fenthion	215 (male rats); 245 (female rats)
Malathion	1,375 (male rats); 1,000 (female rats)
Mobil MC-A-600 (benzo[*b*]thien-4-yl methyl carbamate)	500–700 (rats)
Naled	430 (rats)
Shell SD-7438 (*O,O*-dimethyl phosphorodithioate *S,S*-diester with toluene-*alpha, alpha*-dithiol)	280 (rats)
Shell SD-8211 (2-chloro-1-(2,5-dichlorophenyl)vinyl dimethyl phosphate	3,680 (rats); >5,000 (mice)

AEROSOL TESTS

METHODS. Formulations of water emulsions were used except in the test comparing the effectiveness of aerosols of oil and water. Commercial formulations of emulsifiable concentrates of four insecticides (Shell SD-8211, Mobil MC-A-600, carbanolate, and Bromophos-ethyl) were not available. Emulsifiable concentrates of the first three compounds were therefore prepared by dissolving the insecticide in a mixture of equal parts of acetone and xylene and adding 10 percent by volume of

[1] Mention of a proprietary product does not necessarily imply endorsement of this product by the USDA.

[2] Entomology Research Division, Agricultural Research Service, U. S. Department of Agriculture, Gainesville, Florida.

[3] Entomology Branch, Preventive Medicine Division, U. S. Army Medical Center, Ryukyu Islands.

[4] Chief, Entomology Division, 5th U. S. Army Medical Laboratory, St. Louis, Missouri.

emulsifier (Triton X-100). (A 1⅓ pound per gallon concentrate was prepared with Shell SD-8211 and 1 pound per gallon concentrates with Mobil MC-A-600 and carbanolate.) For Bromophos-ethyl 1 pound per gallon emulsifiable concentrate was prepared with xylene as the solvent and 10 percent of emulsifier. The concentrations of the commercial preparations in pounds per gallon were as follows: Baygon, 1.5; Shell SD-7438, 1.6; bromophos, 2; fenthion, Abate® and Bayer 41831, 4; malathion, 5; and naled, 8.

The tests were conducted on one side of a small airfield adjacent to the China Sea. The grass on the field was cut very short, and the entire area was open to provide an unobstructed working area. The wind velocity during the tests usually ranged from 4 to 10 m.p.h.; however, in a few tests it was as low as 2 m.p.h. and in others as high as 12 m.p.h. The wind direction varied from night to night, but usually the wind blew toward the sea (west to northwest).

The mosquitoes for the tests were collected in the field as larvae or pupae. They were placed in water in shallow pans which were inserted in screen cages about 18 inches square. The adults were allowed to emerge in the cages, and a record was maintained of their ages. Three- to eleven-day-old females were used in all tests except one. In this test a small number of one-day-old females was used to provide a full complement of cages. The afternoon before a test, the mosquitoes were aspirated from the emergence cages and placed in cylindrical exposure cages (3 inches diameter x 8 inches long) made of 16-mesh screen wire and open top jar caps. Twenty-five females were placed in each cage.

Two rows of 6-foot stakes with 2-foot crossarms at the top were set out in each test area. There were three stakes per row, 100 feet apart, and 100 feet between rows. The rows were aligned in the direction of the wind. The crossarms had a wire hook at each end. Just before a test two cages, one of each species of mosquito being tested, were hung on these hooks. In some tests with fenthion, the cages were also exposed 3 feet above the ground and at ground level. The aerosol generator was driven past the rows in a direction perpendicular to them and 100 feet upwind from the first stakes. After the aerosol fog had drifted past all the cages, they were taken off the crossarms and replaced with fresh cages. The treated mosquitoes were returned to the laboratory within 30 to 45 minutes of exposure, knocked down with carbon dioxide, and placed in clean holding cages. Posttreatment mortality counts were made the next morning, 14 to 15 hours after the exposure.

The equipment used to apply the insecticides was a truck-mounted nonthermal aerosol generator designed and developed by the U. S. Army Engineer Research and Development Laboratories. It consisted of a gasoline engine, a blower, an insecticide reservoir and pump, a simple flow control system, and a nozzle and discharge assembly. (A commercial model of this aerosol generator is manufactured and sold by the Curtis Dyna-Products Corporation, Westfield, Indiana.) During the tests the aerosol generator was operated at a speed of 5 m.p.h. and at a discharge rate of 40 gallons per hour. For each test the machine was operated over a distance of 300 feet. Under actual control operations the machine is usually driven up and down each street in a residential area; if we assume a distance of 300 feet between streets and the operating conditions listed above, the application rate on an acre basis for a 1 percent formulation would be approximately 0.037 pound (17 g.).

Three series of tests were conducted: (1) a comparison of the effectiveness of eight insecticides at various distances from the path of the aerosol generator, (2) a comparison of oil and water aerosols of fenthion, and, (3) a comparison of the kill of mosquitoes in cages placed at ground level and 3 and 6 feet above ground.

Results. The data for the eight insecticides are presented in Table 1. Against *C. tritaeniorhynchus*, Baygon and Bayer

TABLE 1.—Toxicity of eight insecticides applied with a nonthermal aerosol generator to caged mosquitoes of two species. (Avg. of 3 to 8 tests at each concentration each with 2 cages of 25 females each.)

Insecticide and concentration (oz./gal.)	Percent kill at indicated distance (ft.) from fogger							
	Culex tritaeniorhynchus				*Culex quinquefasciatus*			
	100	200	300	Avg.	100	200	300	Avg.
Baygon								
2	100	100	100	100	99.3	99.3	87	95
1	100	100	100	100	96	75	94	88
.5	100	99	96	98	79	62	57	66
.25	89	83	55	76	..	..	..	..
Bayer 41831								
4	100	100	99	99.7	100	100	100	100
2	100	93	98	97	100	100	100	100
1	98	95	89	94	99	96	95	97
.5	100	88	96	95	99	82	46	76
.25	69	49	25	48	..	..	..	..
Fenthion								
4	100	100	95	98	100	99	99.6	99.5
2	100	100	99	99.7	100	100	100	100
1	97	92	96	95	100	99.4	100	99.8
.5	45	51	59	52	99.3	100	99.2	99.5
.25	11	5	4	7	43	33	32	36
.125	..	..	..	..	6	7	6	6
Shell SD-8211								
4	100	99	95	98	100	92	76	89
2	100	99	100	99.7	81	73	65	73
1	97	96	93	95	53	65	57	58
.5	71	65	55	64	..	..	..	..
Naled								
6	100	100	99	99.7	100	100	93	98
4	100	100	97	99	100	94	79	91
2	90	99.7	98	96	94	88	85	89
1	75	45	50	57	69	45	58	57
Carbanolate								
4	100	100	98	99.3	93	84	59	79
2	90	94	97	94	86	83	53	74
1	43	50	59	51	..	..	..	..
Mobil MC-A-600								
4	97	100	97	98	88	98	65	84
2	52	71	52	58	50	75	62	62
Malathion								
12	72	46	59	59	..	..	..	..
10	33	27	12	24	..	..	..	..
8	32	18	19	23	..	..	..	..
6	28	32	12	24	..	..	..	..
4	44	38	29	37	63	58	60	60
2	..	..	..	..	65	63	64	64
1	..	..	..	..	13	19	10	14

41831 were the most effective compounds. On the basis of the average percent kill in all cages, Baygon gave 100-percent mortality at concentrations of 1 and 2 ounces per gallon and 98-percent mortality at 0.5 ounce per gallon; Bayer 41831 gave kills ranging from 94 to 99.7 percent at concentrations ranging from 0.5 to 4 ounces per gallon.

A kill of 94 percent or more was obtained at concentrations of 1 ounce per gallon with fenthion and Shell SD-8211, of 2 ounces per gallon with naled and carbanolate, and of 4 ounces per gallon with Mobil

MC-A-600. Malathion was practically ineffective; a concentration of 12 ounces per gallon was needed to produce 59 percent kill.

In the tests with *C. quinquefasciatus,* fenthion gave the best results; the average kill for all cages was above 99 percent at concentrations of 0.5 to 4 ounces per gallon; however, at 0.25 ounce per gallon the kill dropped to 36 percent. Bayer 41831 was the next most toxic: it produced over 97 percent mortality at concentrations of 1 to 4 ounces per gallon. Baygon (95 percent kill at 2 ounces per gallon) and naled (91 percent kill at 4 ounces per gallon) were the next most effective. Shell SD-8211, carbanolate, and Mobil MC-A-600 gave kills of 79 to 89 percent at the highest concentration evaluated (4 ounces per gallon). At this same concentration, malathion gave 60 percent kill.

A comparison of our results with those obtained by Gahan *et al.* (1965) shows that the relative toxicity of the three compounds evaluated in both tests was about the same for the two species. However, better kill of both species was obtained with the water emulsions of fenthion and Baygon that we used in our tests. For example, with fenthion in a thermal aerosol Gahan *et al.* obtained average kills of 58 and 80 percent with a concentration of one ounce per gallon with *C. tritaeniorhynchus* and *C. quinquefasciatus,* respectively. In our tests, the results at the comparable concentrations were 95 and 99.8 percent. The comparable results for each species with Baygon at a concentration of 2 ounces per gallon were 92 percent compared with 100 percent in *C. tritaeniorhynchus* and 58 percent compared with 95 percent in *C. quinquefasciatus.* With naled, the third insecticide tested both years, the test results were equivalent. An attempt to compare the thermal and nonthermal aerosol generators directly had to be abandoned because of difficulties in calibrating the two applicators to the same volume output.

The results of the comparison between fuel oil solutions and water emulsions of fenthion are given in Table 2. In this as in the third test, only *C. tritaeniorhynchus* were used. At both concentrations tested the aerosols with fuel oil gave slightly higher average kills than the aerosols with water; the difference was not considered significant. Additional tests will be needed to verify the point.

Table 3 compares the kill of mosquitoes exposed in cages at ground level and at 3 and 6 feet above ground. Based on the averages for all cages at each height, the highest kill was obtained at the 3-foot height (47 percent compared with 38 and 36 percent at ground level and 6 feet, respectively). It is interesting to note that the percentage kills at the different distances from the generator declined progressively for the cages at the 3- and 6-foot heights whereas, in contrast, the highest kill in the ground level cages was obtained

TABLE 2.—Toxicity of fenthion aerosols produced from fuel oil solutions and water emulsions in a nonthermal aerosol generator to caged females of *Culex tritaeniorhynchus*. (Avg. of 3 tests at each concentration, each with 2 cages of 25 females each; 1 test in each check.)

Type of fog and concentration of fenthion (oz./gal.)	Percent mortality at indicated distance (ft.) from fogger			
	100	200	300	Average
Oil				
1	92	83	85	87
.5	85	78	70	78
0 (check)	13	9	12	11
Water				
1	93	77	80	83
.5	86	58	59	68
0 (check)	5	6	8	6

TABLE 3.—Mortality of female *Culex tritaeniorhynchus* exposed in cages at different heights above ground to aerosols of fenthion produced with a nonthermal aerosol generator. (Fenthion concentration 0.5 oz./gal. avg. of 6 tests per height, each with 2 cages of 25 females each.)

Height of cage above ground, ft.	Percent mortality at indicated distance (ft.) from fogger			
	100	200	300	Average
0	39	26	50	38
3	62	44	36	47
6	39	36	33	36

at a distance of 300 feet, apparently because the particles of insecticides were settling to the ground as they drifted from the applicator.

LARVICIDE TESTS

METHODS. Laboratory tests were conducted with both mosquito species to determine the relative toxicity of eight compounds as larvicides. Acetone solutions of the larvicides were prepared from formulations of emulsifiable concentrates except for Shell SD-8211, for which the technical product was used. The standard test procedure for larvicides outlined in the WHO larval resistance test kit was used except that: (1) the insecticides were prepared in acetone solution instead of alcohol, and (2) the volume of solution added to each container was not kept constant at 1 ml. The actual volumes added varied from 0.1 to 2 ml., depending on the concentration of the stock solution. Larvae were collected in the field the same day the test was started. Only late third or early fourth instar larvae were used. Twenty larvae were placed in each 600-ml. test beaker; three replications with duplicate beakers were made with *C. tritaeniorhynchus* and two replicates with duplicate beakers for the *C. quinquefasciatus*.

In the field tests, the larvicides were evaluated against infestations of *C. tritaeniorhynchus* in rice paddies. These natural infestations were much lower than usual during 1964, probably because many farmers on Okinawa were switching from the production of rice to sugar cane and the resulting decrease in breeding area made it easier for the insect control units to treat the entire infested area. Because we lacked sufficient test areas, the procedures of Gahan *et al.* (1965) (entire rice paddies used as single plots) were not followed. Instead, plots 10 feet wide and 25 to 50 feet long were established along the edges of infested paddies. This was done only in paddies where the water was stationary. While this was not a completely satisfactory procedure, general observations of treated and check plots indicated little kill of larvae outside the plots, and the control obtained was in line with anticipated results based on the laboratory tests and the experience of other investigators.

The insecticides were applied as uniformly as possible with 2-gallon compressed air sprayers. About 2 ml. of formulation were applied per square foot. The actual dosages of insecticide applied ranged from 0.001 to 0.25 pound per acre; on a square foot basis this is an application rate of about 0.01 to 2.6 mg.

Three to six plots were treated at each application rate, except that only two plots were treated with the very high dosages of Abate® and Shell SD-7438. The effectiveness of the treatments was determined by the reduction in the number of third and fourth instar larvae picked up in 20 dips before and after application. The average number of larvae per dip for the various plots ranged from 1 to 273, with the majority in the range of 2 to 10.

RESULTS. The results of the laboratory tests are presented in Tables 4 and 5.

Abate® was outstanding in toxicity to both species. The LC_{50} was 0.00049 p.p.m. for *C. tritaeniorhynchus* and 0.00067 p.p.m. for *C. quinquefasciatus*. Shell SD-7438, fenthion, Bromophos-ethyl, Bayer 41831, and Bromophos were next in ef-

TABLE 4.—Mortality of larvae of *Culex tritaeniorhynchus* exposed to various larvicides in laboratory tests. (Avg. of 3 replicates with duplicate beakers, 20 late 3rd or early 4th instar larvae per beaker.)

Insecticide	Percent mortality in 24 hr. at concentration (p.p.m.) indicated															
	.04	.02	.015	.01	.008	.006	.004	.003	.002	.001	.0008	.0006	.0004	.0002	LC_{50}	LC_{90}
Abate®	..	..	..	..	..	..	..	..	..	98	93	66	35	0	.00049	.00075
Shell SD-7438	..	..	..	..	99	99	91	..	42	4	..	..	..	..	.0023	.0041
Fenthion	..	..	..	..	100	98	77	33	0	..	..	..	..	..	.0032	.0048
Bromophos-ethyl	..	..	..	100	94	87	33	..	2	..	..	..	..	..	.0044	.0066
Bayer 41831	..	..	..	100	99	71	20	..	2	..	..	..	..	..	.0045	.0066
Bromophos	..	99	..	87	57	26	2	..	..	..	..	..	..	..	.0078	.012
Shell SD-8211	..	89	65	11	4	3	..	..	..	..	..	..	..	..	.014	.023
Naled	100	70	..	12	4	..	..	..	..	..	..	..	..	..	.017	.026

TABLE 5.—Mortality of larvae of *Culex quinquefasciatus* exposed to various larvicides in laboratory tests. (Avg. of 2 replicates with duplicate beakers, 20 late 3rd or early 4th instar larvae per beaker.)

Insecticide	Percent mortality in 24 hr. at concentration (p.p.m.) indicated																			
	.2	.1	.08	.06	.05	.04	.03	.02	.01	.008	.006	.004	.003	.002	.001	.0008	.0006	.0004	LC_{50}	LC_{90}
Abate®	..	..	..	..	..	..	..	..	..	..	..	..	..	100	83	62	41	7	.00067	.0012
Fenthion	..	..	..	..	..	..	..	..	99	88	70	29	9	..	..	..	..	..	.0049	.0078
Bayer 41831	..	..	..	..	..	..	..	100	83	56	33	3	..	..	..	..	..	..	.0076	.011
Bromophos	..	..	..	..	..	..	..	99	75	62	32	6	0	..	..	..	..	..	.0076	.013
Shell SD-7438	..	..	..	..	..	100	100	98	67	44	18	..	..	..	..	..	..	..	.0086	.016
Bromophos-ethyl	..	..	..	100	100	77	51	36	..	0	..	..	..	..	..	..	..	..	.024	.040
Naled	..	100	99	72	59	34	6	..	..	..	..	..	..	..	..	..	..	..	.045	.069
Shell SD-8211	98	48	14	4	0	..	0	..	..	..	..	..	..	..	..	..	..	..	.103	.154

fectiveness, with LC_{50}'s of 0.0023 to 0.0078 p.p.m. for *C. tritaeniorhynchus;* Bromophos-ethyl was less effective against *C. quinquefasciatus.* Shell SD–8211 and naled were the least toxic.

Malathion, the insecticide currently used for larviciding on Okinawa, was not included in our tests. Young and Pennington (unpublished data) in a series of resistance tests in 1964 employing the standard WHO procedures showed that the LC_{50} for malathion against *C. tritaeniorhynchus* in 1962 ranged from 0.0066 p.p.m. to 0.011 p.p.m.; against *C. quinquefasciatus,* the range was 0.0077 p.p.m. to 0.0291 p.p.m. More recent tests have and differences in ability of individuals to dip larvae.

The control of the two species obtained in our series of tests with malathion and fenthion was a little better than that obtained by Gahan *et al.* in 1963. For example, with malathion they had to use dosages of 0.5 pound per acre to obtain 99 percent control; we obtained that percent control with 0.25 pound per acre. The reasons for this difference are not known though it may be attributed to the much heavier larval infestations in 1963; thus, in that situation nonuniform application would be more apt to result in survival of larvae.

Table 6.—Control of larvae of *Culex tritaeniorhynchus* in tests in rice field plots. (Avg. of 2 to 6 tests.)

	Percent reduction in larvae in 24 hr. at doses (lb./acre) indicated							
Larvicide	.25	.1	.05	.025	.01	.005	.0025	.001
Abate®	..	99.9	99.5	99.9	96	99	92	73
Shell SD-7438	..	99.6	99	99	95	52	29	..
Bromophos-ethyl	..	98	93	90	78	..	..	..
Fenthion	..	..	99	85	82	26	..	..
Shell SD-8211	..	98	94	87	71	..	..	..
Bayer 41831	..	99.7	94	68	68	..	..	..
Bromophos	..	..	99	49	58	..	..	..
Malathion	99	68	52	..	..	..	..	..

shown an increased tolerance of malathion by both species; during the past year LC_{50} ranges for the species were, respectively, 0.0272 p.p.m. to 0.0768 p.p.m. and 0.0125 p.p.m. to 0.0798 p.p.m.

The data from the field tests (see Table 6) correlate well with the laboratory results. Abate® was extremely effective; it gave 92 percent control at 0.0025 pound per acre. Control of 90 percent or more was obtained with Shell SD-7438 at 0.01 pound per acre, with Bromophos-ethyl at 0.025 pound per acre, with fenthion, Shell SD-8211, Bayer 41831, and Bromophos at 0.05 pound per acre, and with malathion at 0.25 pound per acre. The average percent reduction in the 12 check plots was 23.6 percent, not excessive considering the many variables in the sampling procedures such as pupation of fourth instar larvae, changing water levels,

Summary. In field tests on Okinawa, caged female mosquitos (*Culex* sp.) were exposed to aerosols produced by a nonthermal generator. In tests with *C. tritaeniorhynchus* Giles, kills of 94 percent or more were obtained with concentrations as low as 0.5 ounce per gallon of Baygon (*o*-isopropoxyphenyl methylcarbamate) and Bayer 41831 (*O,O*-dimethyl *O*-4-nitro-*m*-tolyl phosphorothioate) with 1 ounce per gallon of fenthion and Shell SD-8211 (2-chloro-1-(2,5-dichlorophenyl) vinyl dimethyl phospate), with 2 ounces of naled and carbanolate (6-chloro-3,4-xylyl methylcarbamate), and with 4 ounces of Mobil MC-A-600 (benzo[*b*]thien-4-yl methyl carbamate). Against *C. quinquefasciatus* Say, the concentrations required to give at least 91 percent kills were 0.5 ounce per gallon with fenthion, 1 ounce with Bayer 41831, 2 ounces with Baygon,

and 4 ounces with naled. The other insecticides and malathion did not give 90 percent kills at 4 ounces.

In a separate set of tests with fenthion against *C. tritaeniorhynchus* only, fuel oil formulations gave slightly more control than those prepared in water. A better average kill was obtained in cages placed 3 feet above ground than in those at ground level or 6 feet above ground.

Tests were conducted against natural populations of *C. tritaeniorhynchus* larvae in rice paddies. Control of 90 percent or better was obtained with Abate® (*O,O*-dimethylphosphorothioate *O,O*-diester with 4,4′-thiodiphenyl) at 0.0025 pound per acre, Shell SD-7438 (*O,O*-dimethyl phosphorodithioate *S,S*-diester with toluene-*alpha,alpha*-dithiol) at 0.01 pound per acre, fenthion, Bromophos (*O*-(4-bromo-2,5-dichlorophenyl) *O,O*-dimethyl phosphorothioate), Bayer 41831, Bromophos-ethyl (*O*,(4-bromo-2,5-dichlorophenyl) *O,O*-diethyl phosphorothioate), and Shell SD-8211 at 0.05 pound per acre, and malathion at 0.25 pound per acre.

References Cited

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