

## EVALUATION OF INSECTICIDES AGAINST *Aedes aegypti* (L.) AND *Culex pipiens quinquefasciatus* Say (DIPTERA: CULICIDAE) IN BANGKOK, THAILAND<sup>1</sup>

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The two species of mosquitoes of primary concern in Bangkok, Thailand are *Aedes aegypti* (L.) and *Culex pipiens quinquefasciatus* Say (= *fatigans* Wiedemann). *Aedes aegypti* is the vector of dengue and the related viral disease, Thai hemorrhagic fever. It breeds profusely in the concrete jugs (ong nam or "Shanghai jars") used for water storage throughout the area. *Culex p. quinquefasciatus* is not known to transmit filariasis or any other diseases in Bangkok, but the species is the most abundant in the area and is a problem. It breeds in great numbers in many of the canals and ditches (klongs) which occur in all sections of Bangkok. Homes are built over and adjacent to these ditches, and the occupants throw most of their garbage into the water. Since this water is stagnant during the dry and premonsoon season, it becomes

foul and makes an ideal situation for breeding.

No organized effort is made to control mosquitoes in the city; however, during outbreaks of Thai hemorrhagic fever, perifocal treatments with DDT are used—that is, the inside of houses is fogged, and a residual spray is applied to all breeding areas. DDT and dieldrin are readily available for anyone who wishes to purchase them for mosquito control or other purposes. On several occasions, we saw DDT being applied as a fuel oil fog with a Swingfog Pulse Jet Fogger® in movie theaters and schools.

During the summer of 1965, we had an opportunity to test the effectiveness of several insecticides as thermal aerosols or as larvicides for control of *A. aegypti* and *C. p. quinquefasciatus* in or near Bangkok. The compounds tested and their mammalian toxicity are shown in Table 1.

**FOGGING TESTS WITH CAGED MOSQUITOES.** Larvae of both species of mosquitoes were collected in the field, brought to the laboratory, and reared to the adult stage. Before a test, twenty adult 1- to 10-day-old females were placed in a screen wire test cage (1½ x 6 inches). These cages were hung 5 feet above ground on stakes in the test area (a new golf course) at distances of 100 and 200 feet downwind and in two lines perpendicular to the path traversed by the fogger. The insecticides were applied with a Swingfog fogger carried by hand past the cages at a speed of 2.5 m.p.h. and operated at an output rate of 4.8 gallons/hour. Immediately after the fog had drifted past the cages, they were removed and replaced with cages containing unexposed mosquitoes. The exposed mosquitoes were transferred to

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TABLE 1.—Mammalian toxicity of compounds tested.

Name	Mammalian toxicity (oral LD <sub>50</sub> in mg./kg.)
Abate® [O,O-dimethyl phosphorothioate O,O-diester with 4,4'-thiodiphenol]	1,766 or higher in rats
Bay 39007 [o-isopropoxyphenyl methylcarbamate]	95 (male) and 104 (female) in rats
Bromophos [O-(4-bromo-2,5-dichlorophenyl) O,O-dimethyl phosphorothioate]	3,750 in rats
Bromophos-ethyl [O-(4-bromo-2,5-dichlorophenyl) O,O-diethyl phosphorothioate]	200 in rats
Dursban® [O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate]	135 (female) and 163 (male) in rats
Fenthion	215 (male) and 245 (female) in rats
Malathion	1,375 (male) and 1,000 (female) in rats
Naled	430 in rats
Schering 34615 [m-cym-5-yl methylcarbamate]	35 in rats
Shell SD-7438 [S-benzylidene O,O-dimethyl phosphorothioate]	280 in rats
Shell SD-8211 [2-chloro-1-(2,5-dichlorophenyl)vinyl dimethyl phosphate]	3,680 in rats; >5,000 in mice
Shell SD-8447 [2-chloro-1-(2,4,5-trichlorophenyl)vinyl dimethyl phosphate]	>2,500 in rats
Sumithion [O,O-dimethyl O-4-nitro-m-tolyl phosphorothioate]	250 in rats

clean cages within 2 hours, and mortality counts were made 24 hours later. Wind velocity during the tests ranged from 5-14 m.p.h. The most favorable test conditions usually occurred from 7 to 10 a.m.

In this series, the six insecticides tested at various concentrations were: fenthion, malathion, naled, Sumithion, Bay 39007, and Schering 34615. Diesel fuel was the solvent for all 6 chemicals, but 10 percent methylene chloride was added as a co-solvent to obtain satisfactory formulations of Sumithion, Bay 39007, and Schering 34615.

Table 2 shows that on the basis of the average mortality of all mosquitos exposed at each rate of application, fenthion gave the best kill of both species. Sumithion and malathion required application rates about double that for fenthion to give similar results. It caused over 98 percent mortality at concentrations of 0.5 percent and 1.0 percent. Concentrations 4 to 8 times that of fenthion were necessary before naled, Bay 39007, and Schering 34615 gave equivalent kill.

**FOGGING TEST WITH A NATURAL POPULATION.** Another test was conducted in a

residential area of Bangkok to evaluate the effectiveness of a 4 percent malathion fog dispensed by the Swingfog in controlling mosquitoes, principally *C. p. quinquefasciatus*. The effectiveness of the fog was determined by making collections of mosquitoes from the bare legs of two men at ½-hour intervals between 2000 and 2130 hours the night before the test and the night of the test. The fogging was done between 1900 and 1930 hours.

It proved difficult to do a thorough job of fogging because the test location was an area where the houses were close together with many built over water. The total area treated was an area about 500 feet long and 300 feet wide, with a street through the middle of it. Since there were no cross streets, treatments to either side could only be made along boardwalks when they were present. The actual quantity of malathion applied was 216 grams, representing an application rate of about 0.14 pound per acre.

The results obtained were good. The pretreatment counts (mosquitoes collected per man hour) were 37, 75, and 137 for

TABLE 2.—Susceptibility of adult females of *Aedes aegypti* and *Culex pipiens quinquefasciatus* to six insecticides applied as thermal aerosols with a Swingfog. (Mosquitoes exposed in small cylindrical screen cages at a height of 5 feet above the ground. Wind velocity ranged from 5 to 14 m.p.h. Three replicates were made at all concentrations except where otherwise indicated.)

Insecticide	Concentration (%)	Percentage mortality at indicated feet from fogger of—					
		<i>Aedes aegypti</i>			<i>Culex quinquefasciatus</i>		
		100	200	Avg.	100	200	Avg.
Fenthion	0.1 <sup>a</sup>	24	1	13	29	10	20
	.25	73	38	56	83	61	72
	.5	100	95	98	100	100	100
	1.0	100	100	100	100	100	100
Sumithion	.25	47	21	34	46	18	32
	.5	86	92	89	91	72	82
	1.0	98	97	98	100	84	92
	2.0	93	100	97	97	99	98
Malathion	.5 <sup>a</sup>	48	16	32	9	15	12
	1.0	98	84	91	74	55	65
	2.0	100	100	100	99	96	98
Naled	.5	7	1	4	9	3	6
	1.0	3	13	8	8	9	9
	2.0	29	23	26	53	19	36
	4.0	64	64	64	100	96	98
Baygon	1.0	81	48	65	50	48	49
	2.0	100	88	94	79	76	78
	4.0	99	90	95	100	97	99
Schering 34615	0.5 <sup>a</sup>	37	29	33	22	18	20
	1.0 <sup>a</sup>	53	39	46	56	46	51
	2.0	76	91	84	84	87	86
	4.0	97	95	96	100	100	100
Check (diesel fuel)	...	6	3	5	4	8	6

<sup>a</sup> Average results from two replicates.

the three successive ½-hour collections. After treatment, the counts were 30, 26, and 11. Also the 24-hour mortality counts showed that all 30 females collected the first ½-hour died, 23 of the 26 collected the second ½-hour died, and 7 of the 11 collected the last ½-hour died. In the check area, the number of mosquitoes caught the second night (night of treatment) increased considerably, perhaps because of windy conditions there the first night. (This area was more open than the test area.) The species and numbers of mosquitoes collected were as follows:

<i>Culex p. quinquefasciatus</i>	542
<i>Mansonia uniformis</i> (Theobald)	14
<i>Mansonia annulifera</i> (Theobald)	13
<i>Culex bitaeniorhynchus</i> Giles	2
<i>Anopheles vagus</i> Dönitz	1

LABORATORY LARVICIDE TESTS. Eleven insecticides were tested against field-collected late third instar or early fourth instar larvae of *A. aegypti* and *C. p. quinquefasciatus*. (Ice cubes added to the water to slow the movement by the larvae aided size determination.) Twenty larvae and 250 milliliters of demineralized water were used per test jar. Then insecticides, either the technical form or emulsifiable concentrates, were diluted in acetone and added to the jar. The desired concentrations in p.p.m. were obtained by varying the insecticide concentrations and using volumes between 0.1 and 1.0 milliliters. A temperature of 80° F. ± 2 was maintained in the test room. Mortalities were recorded after 24 hours.

TABLE 3.—Toxicity of eleven insecticides to *Aedes aegypti* and *Culex pipiens quinquefasciatus* larvae.<sup>a</sup>

Insecticide	Toxicity to species indicated				Ratio of toxicity to Dursban	
	<i>C. p. quinquefasciatus</i>		<i>A. aegypti</i>		<i>C. p. quinquefasciatus</i>	<i>A. aegypti</i>
	LC <sub>50</sub>	LC <sub>90</sub>	LC <sub>50</sub>	LC <sub>90</sub>		
Abate	0.00076	0.0010	0.0026	0.0040	0.8	2.6
Dursban	.00096	.0015	.0010	.0018	1.0	1.0
Fenthion	.0042	.0068	.0035	.0055	4.4	3.5
Shell SD-7438	.0060	.010	.0068	.0125	6.3	6.8
Bromophos	.0072	.010	.022	.035	7.5	22.0
Sumithion	.012	.017	.0056	.011	12.5	5.6
Bromophos-ethyl	.012	.025	.029	.051	12.5	29.0
Malathion	.043	.076	.062	.14	44.8	62.0
Naled	.058	.082	.071	.13	60.4	71.0
Shell SD-8211	.10	.19	.044	.086	104.2	44.0
Shell SD-8447	.24	.40	.064	.12	250.0	64.0

<sup>a</sup> Average of two tests with duplicate pint jars; 20 late third or fourth instar larvae per jar; 250 milliliters of demineralized water per jar.

Table 3 records the LC<sub>50</sub>'s and LC<sub>90</sub>'s for each insecticide and for each mosquito species. In addition, the toxicity of the insecticides relative to that for Dursban is presented. Abate and Dursban were very toxic to both species (LC<sub>50</sub> ranged from 0.00076 to 0.0026 p.p.m.). Fenthion, Shell SD-7438, bromophos, Sumithion and bromophos-ethyl also showed good toxicity, however the LC<sub>50</sub> dosages were from 3.5 to 29.0 times that of Dursban. Malathion, naled, Shell SD-8211 and Shell SD-8447 were the least toxic compounds tested. There were some differences in susceptibility of the two species to the same compound. The most notable differences occurred with Abate and bromophos where *C. p. quinquefasciatus* was about 3 times as susceptible as *A. aegypti*, and Shell SD-8447 where *A. aegypti* was 4 times as susceptible as *C. p. quinquefasciatus*.

LARVAL RESISTANCE TESTS WITH *Aedes aegypti*. Because the Thai Ministry of Health reported that the effectiveness of DDT and dieldrin against *A. aegypti* differed in various sections of Bangkok, we made a series of tests to determine the range in susceptibility of the larvae to DDT, dieldrin, Abate, and malathion.

*Aedes aegypti* larvae were collected from the following five points in the Bangkok-Dhonburi area: (1) Suriwong—in the heart of the commercial part of Bangkok near several large hotels catering to tourists; (2) Dhonburi—a semirural area of orchards; (3) Rama VI Road—a rather open area of Thai housing that includes some Chinese shop houses; (4) Din Daeng—a government housing area surrounded by lower class housing; and (5) Klong Toey—a series of squatters' homes along the railroad tracks in the area of the Port of Thailand. We assumed that insecticides had been used frequently in the commercial area (Suriwong) but not in the other areas unless they had been used in Dhonburi for crop protection. (DDT has been used in Bangkok recently and is commercially available from a number of sources; however, dieldrin is used far more commonly.)

All larvae were collected from the large concrete water storage jugs. Standard WHO test kit solutions and procedures were used with the DDT and dieldrin; with Abate and malathion, we followed the procedures described just previously. The estimated LC<sub>50</sub>'s and LC<sub>90</sub>'s we obtained were as follows:

Insecticide	Area	LC <sub>50</sub>	LC <sub>10</sub>
DDT	Suriwong	0.4	3.0
	Dhonburi	.074	.54
	Rama VI	.053	.25
	Din Daeng	.12	.63
	Klong Toey	.07	.33
Dieldrin	Suriwong	.14	.70
	Dhonburi	.007	.072
	Rama VI	.0046	.021
	Din Daeng	.0046	.025
	Klong Toey	.0027	.0062
Malathion	Suriwong	.125	.40
	Dhonburi	.082	.22
	Rama VI	.059	.15
	Din Daeng	.039	.094
	Klong Toey	.062	.14
Abate	Suriwong	.0031	.0048
	Dhonburi	.0018	.0035
	Rama VI	.0015	.0030
	Din Daeng	.0018	.0030
	Klong Toey	.0026	.0040

Resistance to DDT varied in the five localities (highest in Suriwong and lowest in Rama VI) but was definitely present in all five larval collections. A high degree of resistance to dieldrin was present also in larvae from Suriwong and was incipient in three other collections; the fifth collection from Klong Toey was completely susceptible. Susceptibility to Abate and malathion also varied considerably but was in the range obtained with the Gainesville laboratory strain.

The areas where resistance to DDT and dieldrin occurred were those where we believed the most insecticide had been used.

FIELD TESTS WITH LARVICIDES FOR THE CONTROL OF *C. p. quinquefasciatus*. Even though good areas for tests against larvae of *C. quinquefasciatus* were unavailable, a preliminary evaluation of four chemicals was made against larvae by using areas of heavy larval concentration in ditches and canals under and between houses as plots (all were quite small). Since these areas between the houses were difficult to measure accurately, we trained men carrying standard 2-gallon compression-type sprayers containing emulsifiable concentrates diluted in water to spray at a uniform rate of 1 liter per 1,000 square feet. The water

in this area did not flow, so cross-contamination of plots did not occur, even though the water was more or less continuous under the houses.

The effectiveness of the treatments was determined by counting the number of third and fourth instar larvae in 10 dips made with a 150-milliliter dipper before and 24 hours after the treatment. However, so many larvae were present in the pretreatment samples and in some post-treatment samples that complete counts were not made. Instead, one-eighth subsamples of the total collections were counted. By this estimate, the total pretreatment counts ranged from 728 to 4,272.

The larvicides, rates of application, and results are shown below. Results are the average of three plots, except the untreated check which was two plots.

Insecticide	Application rate (lb./acre)	Average percentage reduction
Abate	0.005	99.1
Dursban	.025	99.3
	.005	99.7
Malathion	.025	99.7
	.05	70.1
None	.25	100.0
	...	33.5

LARVICIDE TESTS AGAINST *A. aegypti* IN CONCRETE WATER JUGS. The effectiveness of Abate, Dursban, Shell SD-8211, and bromophos against *A. aegypti* breeding in the concrete water storage jugs was determined. Twenty-seven of the jugs were placed on a cement floor in an open area under a military school building where they were exposed to general atmospheric conditions but protected from wind-blown debris. Each jug was filled with 45 gallons of water, and the larvicides, either emulsifiable concentrates or acetone solutions, depending on the quantity of insecticide required, were added at various rates. All tests were made in triplicate, and three jugs were also left untreated as checks. Initially, and thereafter, at weekly intervals 100 third or fourth instar larvae

were added; then 72 hours later the water was examined for surviving larvae. The water was not changed, and additional water was not added during the 6 weeks of the test.

The concentrations of insecticides used and the results are shown below. When the test was discontinued after 6 weeks, most of the larvicides were still effective.

Insecticide	Rate of application (p.p.m.)	No. of consecutive weeks with no live larvae in indicated replication		
		A	B	C
Shell SD-8211	1.0	>6	>6	>6
	10.0	>6	>6	>6
Dursban	0.1	>6	5	6
	1.0	>6	>6	>6
Abate	0.1	>6	>6	>6
	1.0	>6	>6	>6
Bromophos	0.1	4	3	4
	1.0	5	5	>6
None	..	0	0	0

In a separate test of the same type, the effect of a complete change of the water in the jugs after application of the insecticide was investigated. The insecticides and rates of application were the same, but one week after the insecticides were added (larvae not yet introduced), all the water was poured out. Two days later, the jugs were refilled with water, and the larvae were added. After 3 days, surviving larvae were found in all jugs except those treated with the two concentrations of Abate and 1.0 p.p.m. of Dursban. These particular jugs were therefore emptied 4 days later and immediately refilled with water; larvae were again added. After 3 more days, two jugs treated with Dursban contained live larvae, but all larvae in jugs treated with Abate were dead. The procedure was therefore repeated with the Abate containers at weekly intervals. Live larvae appeared in the containers treated at 0.1 p.p.m. Abate after the fourth reflooding; one jug treated with 1.0 p.p.m. contained a few live larvae after the fifth flooding and two after the sixth flooding. The test was then discontinued.

**SUMMARY.** Thirteen insecticides were evaluated as larvicides and/or adulticides for control of *Aedes aegypti* and *Culex pipiens quinquefasciatus* in Bangkok, Thailand. Fenthion was the most toxic of six compounds dispersed as thermal fogs in tests with both species. Over 98 percent kill was obtained with concentrations of 0.5 percent and 1.0 percent. Sumithion and malathion were a little less effective (1 percent to 2 percent necessary for more than 90 percent kill). Bay 39007, Schering 34615, and naled required concentrations of 2 percent to more than 4 percent for greater than 90 percent kill.

Malathion applied as a 4 percent thermal fog gave a good reduction of a natural infestation of mosquitoes (predominately *C. p. quinquefasciatus*) within 2 hours.

In laboratory tests with larvicides, Abate and Dursban were highly effective against both species with LC<sub>50</sub>'s of 0.0026 p.p.m. or less. Fenthion, Shell SD-7438, Sumithion, bromophos, and bromophos-ethyl gave LC<sub>50</sub>'s ranging from 0.0035 to 0.029 p.p.m. Tests with *A. aegypti* collected from five different parts of the Bangkok-Dhonburi area showed definite but variable resistance to DDT in all collections. Definite resistance to dieldrin occurred in one collection, incipient resistance in three, and none in the fifth. There was no resistance to Abate or malathion.

In field tests, Abate, Dursban (0.005 and 0.025 pound/acre), fenthion (0.05 and 0.25 pound/acre), and malathion (0.25 pound/acre) gave 99+ percent control of *C. p. quinquefasciatus* when applied as water emulsion sprays. Malathion at 0.05 pound/acre gave 70 percent control.

In tests conducted in concrete water storage jugs with *A. aegypti* larvae, Abate (0.1 and 1.0 p.p.m.), Dursban (1.0 p.p.m.), and Shell SD-8211 (1.0 and 10.0 p.p.m.) gave complete kill of third or fourth instar larvae placed in the water at weekly intervals for the duration of the test (six weeks) Dursban (0.1 p.p.m.) and bromophos (1.0 p.p.m.) produced complete kills in the various replicates from five to more than six weeks.