

RESISTANCE OF SALT-MARSH MOSQUITOES TO DDT AND OTHER INSECTICIDES¹

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Studies by members of the Orlando, Fla., laboratory in the Cocoa Beach area of Brevard County, Fla., in the summer of 1949 indicated that aerial applications of DDT at the rate of 0.2 pound per acre and higher were not giving such good control of salt-marsh mosquitoes (*Aedes taeniorhynchus* (Wied.) and *A. sollicitans* (Walk.)) as in previous years. Apparently the mosquitoes in this area, which had been under intensive treatment since 1945, had developed some degree of resistance to DDT (Deonier *et al.* 1950). Other workers have reported on the development of resistance in house flies (King and Gahan 1949) and mosquitoes (Mosna 1947) after several years' exposure to residues of DDT, but so far as is known, there have been no previous reports of mosquitoes developing a resistance to DDT over large areas as a result of aerial spraying. Laboratory tests were made with larvae and adults from treated and untreated areas in Brevard County to compare their susceptibility to DDT, and to evaluate the effectiveness of other insecticides as larvicides and adulticides. The results of these tests are given in this paper.

LARVICIDE TESTS

Tests were made on larvae taken from marshes that had been sprayed intensively with DDT during the last 5 years, and from other marshes that had been sprayed infrequently or not at all. Third- and fourth-instar larvae were brought to the laboratory, where comparative tests were made with acetone-water suspensions of

DDT and several other larvicides. Some difficulty was experienced in getting the larvae into the laboratory at the proper stage for the tests. Fourth-instar larvae were desired, but frequently some pupation occurred before the readings were completed. From past experience it was known that the pupae and prepupal larvae are less readily affected by larvicides than younger larvae. Several attempts to obtain larvae from locations in other counties away from control areas were unsuccessful.

Tests with DDT were made against three lots of *Aedes taeniorhynchus* larvae from the intensively treated area, four lots from an untreated area, and two lots from areas that had been sprayed only occasionally. One lot of *A. sollicitans* from the treated area was also tested. The results with DDT, summarized in table 1, indicated that the larvae from the intensively treated area were much less susceptible to this compound than the larvae from elsewhere. Considerable pupation occurred in some tests, but the pupae and old larvae appeared to be more resistant than similar stages from the untreated area.

The mortalities of *Aedes sollicitans* were distinctly lower than those of *taeniorhynchus* from the treated area, in the one test in which no pupation occurred. No larvae of *sollicitans* from an untreated area were available for comparison.

The results of comparative tests with DDT and other insecticides against seven lots of larvae from five different locations are given in table 2. With *Aedes taeniorhynchus* larvae from Cocoa Beach marsh (in the heavily treated area), DDT and TDE gave much lower kills than any of the other five materials. This was also true for DDT with *A. sollicitans* larvae from the treated area, but TDE was not

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tested against this species. These two compounds gave about the same results as the other materials against larvae from the other marshes. The other materials also gave about as good results against larvae from the treated areas as they did against

those from other locations. Parathion was by far the most toxic of any of the materials, as it gave 100 percent mortality at a dosage of 0.001 p.p.m. (one-fifth of the lowest dosage shown in the table) in three of the four tests against *taeniorhynchus*.

TABLE 1.—Tests against fourth-instar *Aedes taeniorhynchus* and *A. sollicitans*, from different locations in Brevard County, in acetone suspensions of DDT. (Average of 2 beakers of 25 larvae each; number of larvae that pupated before end of test shown in parentheses.)

Date	Location	Percent mortality 48 hours after applications of DDT at—			
		0.05 p.p.m.	0.025 p.p.m.	0.01 p.p.m.	0.005 p.p.m.
<i>Treated areas (Aedes taeniorhynchus)</i>					
Aug. 26	Cocoa Beach	100	76	44	16
Sept. 1	South Causeway	0 (50)	0 (50)	0 (50)	0 (50)
15	Do.	16 (11)	12 (15)	4 (19)	0 (21)
<i>Untreated areas (Aedes taeniorhynchus)</i>					
Aug. 5	Titusville Beach	96 (2)	92 (4)	80 (10)	34 (33)
26	Do.	100	100	100	100
Sept. 1	Do.	100	98 (5)	96 (43)	38 (50)
15	Do.	100	100	58 (21)	22 (39)
<i>Areas occasionally treated (Aedes taeniorhynchus)</i>					
Aug. 19	Shiloh	100	100	96	90
	Haulover	98	98	86	90
<i>Treated area (Aedes sollicitans)</i>					
Aug. 5	Yoder marsh	48	26	18	0

TABLE 2.—Toxicity of several insecticides in acetone suspensions to fourth-instar larvae of salt-marsh mosquitoes taken from different locations in Brevard County, Fla. (Average of 2 beakers of 25 larvae each; number of larvae that pupated before end of test shown in parentheses.)

Marsh location	Treatment	Percent mortality in 48 hours						
		DDT	TDE	Toxaphene	Dieldrin	Lindane	Chlordane	Parathion
0.005 p.p.m.								
Yoder ¹	Heavy	0	...	64	46	58	40	100
Cocoa Beach	do.	16	0	72	56	80	100	100
Haulover	Occasional	90	72	46	52	64	74	100
Shiloh	do.	90	96	64	70	54	90	100
Titusville	None	100	100	100	100	100	100	100
		34 (33)	...	0 (0)	74 (13)	34 (33)	84 (8)	100
0.01 p.p.m.								
Yoder ¹	Heavy	18	...	86	82	100	92	100
Cocoa Beach	do.	44	4	88	100	100	100	100
Haulover	Occasional	86	92	98	100	96	100	100
Shiloh	do.	96	92	98	100	80	100	100
Titusville	None	100	100	100	100	100	100	100
		80 (10)	...	22 (39)	90 (5)	88 (6)	100	100
0.025 p.p.m.								
Yoder ¹	Heavy	26	...	98	100	100	100	...
Cocoa Beach	do.	76	8	100	100	100	100	...
Haulover	Occasional	98	98	100	100	94	100	...
Shiloh	do.	100	100	100	100	96	100	...
Titusville	None
		92 (4)	...	88 (6)	100	78 (11)	94 (3)	98 (1)

¹ *Aedes sollicitans* larvae. All others were *A. taeniorhynchus*.

The test in which it failed at this dosage was the one in which prepupal larvae were used. Against *sollicitans* it gave a kill of 80 percent at this dosage and 100 percent at 0.0025 p.p.m.

SPACE-SPRAY TESTS

Tests were made to compare the susceptibility to DDT and several other insecticides of adult mosquitoes obtained from different salt-marsh areas. The adults were reared in the laboratory from fourth-instar larvae or pupae collected from one untreated and two intensively treated marshes in Brevard County, from one treated marsh near Sarasota, and from one untreated marsh in north Volusia County. One of the treated marshes in Brevard County (Yoder marsh) yielded about equal numbers of *Aedes sollicitans* and *A. taeniorhynchus*, but the others showed over 95 percent of *A. taeniorhynchus*.

The space-spray tests against salt-marsh adults were conducted in 100-cubic-foot cabinets, and the procedure normally used in tests against *Aedes aegypti* (L.) was followed closely. Each insecticide was dissolved in cyclohexanone, and 2 ml. of

the solution was sprayed into each cabinet. After a settling period of 2 minutes, two cages of mosquitoes were exposed for 10 seconds. Tests against one collection of adults were also made at a 60-second exposure. After completion of the exposures the mosquitoes were transferred to clean cages for 24-hour mortality readings.

In the tests with DDT, table 3, the adults of *Aedes taeniorhynchus* from treated areas were markedly less susceptible than those from untreated areas. The mortalities of one lot of *A. sollicitans* from a marsh in the treated area were still lower than those for *taeniorhynchus* from the same location, but no *sollicitans* from an untreated area were available for comparison.

Five insecticides in addition to DDT were tested at two concentrations against four lots of adult mosquitoes, one lot from an intensively treated area and three from untreated areas. The mortalities are shown in table 4. Like DDT, TDE was much less effective against the adults from the treated area than it was against those from untreated areas, an indication that the adults were about equally resistant to

TABLE 3.—Comparative mortalities obtained in space-spray tests with DDT against adult salt-marsh mosquitoes from different sources. (Exposure of 10 seconds unless otherwise shown.)

Date	Species and location	Average number of mosquitoes per test	Concentration (percent) and sex							
			0.25		0.5		1.0		2.0	
			Male	Female	Male	Female	Male	Female	Male	Female
<i>Marshes in treated area</i>										
<i>Aedes taeniorhynchus</i>										
July 18	Yoder marsh (both sexes)	22	91
	Yoder marsh (both sexes, 60-second exposures)	33	54	62	94					92
Aug. 5	Yoder marsh	75	15	35	39	6	31	20	64	52
Sept. 1	South Causeway	180	8	2	51	7	67	11
Aug. 20	Sarasota	115	28	4	38	10	85	27	84	51
<i>A. sollicitans</i>										
July 18	Yoder marsh (both sexes)	55	42
	Yoder marsh (both sexes, 60-second exposures)	32	30	38	46					73
Aug. 5	Yoder marsh	115	32	13	21	13	63	19	89	11
<i>Untreated marshes</i>										
<i>A. taeniorhynchus</i>										
Aug. 5	Titusville Beach	195	93	37	96	62	93	65	97	
	North Volusia County	140	96	71	91	58	100	89	100	51
Sept. 1	Titusville Beach	365	98	84	100	96	98	93
<i>Laboratory colony</i>										
	<i>A. aegypti</i> (both sexes)	81	51		94		100		100	

TABLE 4.—Comparative mortalities obtained in space-spray tests with two concentrations of six insecticides against adults of *Aedes taeniorhynchus* from different locations.¹

Material and concentration	Treated area				Untreated areas			
	South Causeway		Titusville Beach		North Volusia			
	Sept. 1		Aug. 5		Sept. 1		Aug. 5	
	Male	Female	Male	Female	Male	Female	Male	Female
0.5 percent								
DDT	8	2	96	62	98	84	91	58
TDE	4	0	87	30	86	52	93	54
Toxaphene	40	3	87	26	91	8	93	25
Lindane ²	71	76	97	49	90	74	87	49
Technical benzene hexachloride	9	0	76	47	78	16	78	26
Chlordane	16	2	73	29	86	40	75	24
2.0 percent								
DDT	67	11	97	95	99	98	100	99
TDE	13	0	99	74	97	93	97	90
Toxaphene	83	28	91	54	99	79	97	92
Lindane ²	100	100	100	100	100	100	100	100
Technical benzene hexachloride	92	75	98	53	100	90	96	65
Chlordane	95	84	94	57	99	86	99	67

¹ The approximate numbers of specimens (both sexes) in the different series were as follows: South Causeway 190; Titusville Beach, Aug. 5, 200; Titusville Beach, Sept. 1, 365; and North Volusia 150. From 40 to 50 adults per cage were exposed in each test. The figures in the table are percentages killed.

² Lindane used at concentrations of 0.25 and 1.0 percent instead of 0.5 and 2.0 percent.

TDE and DDT. At the lower concentration (0.5 percent), the other materials also showed less toxicity to the mosquitoes from the treated area, but at the higher concentration (2 percent) this difference was not in evidence, except possibly with toxaphene. Lindane, although tested at only half the concentrations of the other materials, was the most effective against the resistant mosquitoes, followed by chlordane and technical benzene hexachloride (12 percent gamma isomer). Against mosquitoes from untreated areas, lindane and DDT were the most effective, with TDE third and the others only slightly less effective. Technical benzene hexachloride at a concentration of 2 percent was somewhat more effective than lindane at 0.25 percent, an indication that the technical material is slightly higher in relative toxicity than the 1 to 8 gamma ratio.

RESIDUE TESTS

Tests were made with adult salt-marsh mosquitoes from two of the lots in the previous space-spray tests—one lot from a treated area and one from an untreated

area—to determine their relative susceptibility to DDT. One of the lots was also tested on residues of lindane, technical benzene hexachloride, toxaphene, chlordane, and TDE. Each chemical was applied as a wettable-powder spray to plywood panels at the rate of 25 mg. per square foot, and the mosquitoes, confined under petri dishes, were exposed on each treatment for different periods of time. In the first series of tests *Aedes aegypti* adults from the laboratory colony were included for comparison.

As shown in table 5, *Aedes taeniorhynchus* from the intensively treated areas (Yoder marsh) required much longer exposure to DDT than those from an untreated area (Titusville Beach). The exposure times required to produce 50 percent mortality (LT-50) as determined from the test data, were 61 minutes for the former and 10 minutes for the latter, or about six times as long. The LT-50 for *Aedes sollicitans* from the treated area was 111 minutes, or about 1.8 times that for *taeniorhynchus* from the same location. The LT-50 for *A. aegypti* was only 2.5 minutes.

TABLE 5.—Residue tests on three species of adult *Aedes* exposed to DDT spray deposits of 25-mg. per square foot on plywood panels. Mosquitoes from Yoder marsh collected on July 18, and those from Titusville Beach on August 7. (Two dishes of insects at each exposure on two consecutive days.)

Species and place collected	Average number of mosquitoes per test	Percent kill of mosquitoes 24 hours after indicated exposure period (in minutes)								Calculated time (in minutes) required to produce mortalities			
		1	5	10	20	30	60	120	240	LT-50	LT-70	Slope coefficient	
<i>A. taeniorhynchus</i>													
Titusville Beach (untreated)	88	26	29	48	73	75	100	100	..	10	16	2.59 ¹	
Yoder marsh (treated)	30	2	18	33	34	34	36	67	96	61	99	2.42 ¹	
<i>A. sollicitans</i>													
Yoder marsh (treated)	53	7	0	4	4	10	19	43	93	111	150	3.93	
<i>A. aegypti</i>													
Laboratory colony	107	22	74	88	95	96	100	100	..	2.5	4.7	1.9	

¹ 1-minute exposures omitted in computing the regression line.

In the tests with six insecticides against *Aedes taeniorhynchus* adults from an untreated marsh (table 6), lindane proved the most effective material, causing 100 percent mortality after a .1-minute exposure. DDT, chlordane, and technical benzene hexachloride each caused complete mortalities with 60-minute exposures. Benzene hexachloride was not tested at a shorter exposure period, so its minimum lethal time was not indicated. Exposures of 60 minutes to toxaphene and TDE produced mortalities of 85 and 78 percent, and the next higher exposure time, 240 minutes, caused 100 percent mortality. It is probable that a 2-hour exposure would have been close to the minimum lethal time for these two materials.

SUMMARY

Studies in the Cocoa Beach area of Brevard County, Fla., in June 1949 showed that the usual aerial treatments with DDT were not giving such effective control of either the larvae or the adults of salt-marsh mosquitoes (*Aedes taeniorhynchus* (Wied.) and *A. sollicitans* (Walk.)) as in previous years. It was suspected that these species had developed a resistance to DDT after 4 or 5 years of intensive spraying operations, and laboratory tests with larvae and adults from several treated and untreated marshes

were made to compare their susceptibility to DDT and other insecticides. DDT and TDE gave much lower kills of larvae from frequently treated marshes than toxaphene, dieldrin, lindane, chlordane, and parathion, but gave about the same results as the other materials against larvae from untreated areas. In one comparative test with larvae from treated areas, all the insecticides except parathion showed distinctly less toxicity to *A. sollicitans* than to *A. taeniorhynchus*. Parathion was by far the most toxic material against both species.

In space-spray tests DDT and TDE were much less effective against adults from treated areas than against those from untreated areas. Lindane was the most effective material against the adults from treated areas, followed by chlordane and technical benzene hexachloride (12 percent gamma isomer). Against adults from untreated areas, lindane and DDT were the most effective materials, with TDE third, and the others only slightly less effective. As with the larvae, the mortalities of *Aedes sollicitans* adults were lower than those of *A. taeniorhynchus*.

In residue tests adult *Aedes taeniorhynchus* from treated areas required much longer exposure than those from an untreated area, the LT-50's being 61 minutes and 10 minutes, respectively. Against

TABLE 6.—Residue tests with different insecticides against adult *Aedes taeniorhynchus* collected from an untreated area (Titusville Beach) August 7. (Chemicals applied to panels at the rate of 25 mg. per square foot.)

Chemical	Exposure period (minutes)	Number of insects	Percent kill in 24 hours		
			Male	Female	Both sexes
DDT	1	68	45	17	26
	5	62	50	19	29
	10	75	74	27	48
	20	109	91	60	73
	30	121	81	70	75
	60	88	100	100	100
	120	96	100	100	100
Chlordane	1	51	57	75	73
	10	73	66	54	60
	60	99	100	100	100
	240	63	100	100	100
Toxaphene	10	89	32	28	29
	60	96	90	80	85
	240	73	100	100	100
TDE	10	88	59	32	39
	60	69	88	74	78
	240	62	100	100	100
Lindane	1	66	100	100	100
	10	45	100	100	100
	60	54	100	100	100
Benzene hexachloride (12% gamma isomer)	60	56	100	100	100
	240	39	100	100	100

adults from an untreated area, lindane was the most effective residue, followed by DDT and technical benzene hexachloride.

Literature Cited

- DEONIER, C. C., CAIN, T. L. JR., AND McDUFFIE, W. C. 1950. Aerial spray tests on DDT-resistant salt-marsh mosquito adults. *Jour. Econ. Ent.* (In press.)
- KING, W. V., AND GAHAN, J. B. 1949. Failure of DDT to control house flies. *Jour. Econ. Ent.*, 42(3):405-409.
- LINDQUIST, A. W., AND MADDEN, A. H. 1946. A special chamber for testing insecticidal sprays. *U. S. Bur. Ent. and Plant Quar. ET-229*, 3 pp. (Processed.)
- MOSNA, E. 1947. Su una caratteristica biologica del *Culex pipiens autogenicus* di Latina. *Riv. di Parassitol.*, 8(2/3):125-126.