COMPARATIVE TESTS WITH DDT AND PHENOTHIAZINE AGAINST TWO AMERICAN[1] AND THREE NEW GUINEA SPECIES[2] OF MOSQUITO LARVAE

R. C. BUSHLAND
U. S. D. A., Agr. Res. Adm., Bureau of Entomology and Plant Quarantine

DDT has been extensively tested since 1942 against various species of mosquito larvae at the Orlando, Fla., laboratory of the Bureau of Entomology and Plant Quarantine. Deonier *et al.* (1945) reported that DDT applied as acetone-water suspensions at 0.01 p.p.m. caused 98.2 per cent mortality of fourth instar larvae of *Anopheles quadrimaculatus* Say, and was more than 100 times as toxic as phenothiazine to this species. Eide *et al.* (1945) found that DDT at 0.05 p.p.m. was lethal to larvae of *Aedes taeniorhynchus* (Wied.), *A. sollicitans* (Walk.), *A. aegypti* (L.), *Psorophora confinnis* (L.-Arr.), *P. ciliata* (F.), and *Culex quinquefasciatus* Say.

Early in 1943 the writer made tests at Orlando with DDT and phenothiazine against larvae of *Aedes aegypti* and *Culex quinquefasciatus*. During 1944 and 1945, while on military duty in New Guinea, he also made laboratory tests with these insecticides against larvae of *Anopheles punctulatus* Donitz, *Armigeres milnensis* Lee, and *C. annulirostris* Skuse. The results of all these tests are reported herein.

Materials and Methods.—The DDT used in these studies was the technical grade purified by recrystallization from ethyl alcohol. The phenothiazine was a pure compound made by chemists of the Division of Insecticide Investigations of this Bureau.

The test procedure was similar to that described by Bushland and King (1943). Freshly made acetone solutions of the insecticides were employed in every test. DDT was dissolved in acetone to make a 1 to 160,000 stock solution; phenothiazine was used at 1 to 4,000. For each test the desired amount of acetone solution was added to 225 ml. of distilled water in a 400 ml. beaker, and thoroughly mixed. Fourth-instar larvae held in 25 ml. of water were then poured into the test beaker. Thus, each test consisted of a number of larvae in 250 ml. of distilled water containing the desired concentration of insecticide. The amount of insecticidal solution added to the water never exceeded 2.5 ml.

Visible precipitates are formed when concentrated acetone solutions of DDT or phenothiazine are added to distilled water. However, at the dilutions used in these studies the water remained clear.

At least one control beaker was used in every series of tests. Earlier work (Fink *et al.* 1938, Bushland and King 1943) had established that 1 per cent of acetone in distilled water was harmless to *Culex quinquefasciatus* and *Aedes aegypti* larvae. Therefore, in tests against these species the control consisted only of larvae in distilled water. The effect of acetone on the New Guinea species of larvae was unknown, however; so there was included in those tests one jar containing acetone in an amount equal to the maximum applied with any insecticide. There was no mortality in any of the acetone-treated controls during a 48-hour observation period.

The number of larvae used per test varied with the abundance of the test species. The maximum number per beaker was 50 in tests with *Culex quinquefasciatus*, and the minimum was 10, with *Anopheles punctulatus*.

---

[1] Work on American mosquitoes was conducted under a transfer of funds, recommended by the Committee on Medical Research, from the Office of Scientific Research and Development to the Bureau of Entomology and Plant Quarantine.

[2] The New Guinea mosquitoes were identified by W. V. King.

Culex quinquefasciatus and *Aedes aegypti* were reared in 1-gallon battery jars on a diet of ground dog food. *Anopheles punctulatus, Armigeres milnensis,* and *C. annulirostris* were freshly collected in their natural breeding places. For all tests the larvae were held in a wire strainer and gently washed with distilled water. Larvae of uniform size were then counted out into holding beakers containing 25 ml. of distilled water. These beakers were selected at random for testing.

All tests were made at room temperature, which averaged about 26° C. in Florida and about 31° in New Guinea. In Florida the first mortality readings were made after 16 hours and the final readings after 40 hours, as was done in earlier larvicide work (Bushland and King 1943). However, in New Guinea observations were made at intervals of 24 and 48 hours, the intervals used by Deonier *et al.* (1945) in their tests against *Anopheles quadrimaculatus.* All moribund larvae were counted as dead.

Results.—The results of tests against 6 lots of *Culex quinquefasciatus* larvae and 3 lots of *Aedes aegypti* larvae are presented in Table 1. The median lethal concentration of DDT for *Culex quinquefasciatus* was between 1 part in 75 million parts of water and 1 part in 100 million. In the tests against *Aedes aegypti* the lowest concentration of DDT, 1 to 100 million, killed 81 per cent of the insects.

Table 2 shows the results obtained with *Anopheles punctulatus* larvae collected near Oro Bay, New Guinea, and used in one series of tests made in June and a second series in October 1944. Only 10 larvae were available for each test, but the results after 48 hours checked closely. At the 24-hour observation it was difficult to differentiate between moribund larvae and those not so seriously affected. Those observations are therefore omitted and only the 48-hour records are shown.

TABLE 1. Mortality of *Culex quinquefasciatus* and *Aedes aegypti* Larvae Exposed to Various Concentrations of DDT and Phenothiazine, Florida.

Concentration of Insecticide	*Culex quinquefasciatus* [1]		*Aedes aegypti* [2]	
	After 16 Hours	After 40 Hours	After 16 Hours	After 40 Hours
	Per Cent	*Per Cent*	*Per Cent*	*Per Cent*
DDT (1 part in indicated million parts of water):				
16			100	100
20	99.7	100.0	99	100
30	98.3	99.3	85	99
40	92	94	85	93
50	81.3	87.7	73	95
75	70.8 (5 tests)	79.2 (5 tests)	68	87
100	36.3	40.7	55	81
Phenothiazine (1 p.p.m.)	82.3	88	85	92
Control (no treatment)	0	0	0	4

[1] 6 tests, 50 larvae per test.
[2] 3 tests, 25 larvae per test.

Only one series of tests was made (October 1944) against larvae of *Armigeres milnensis.* For each concentration of insecticide 20 larvae were employed, but in computing the mortality (Table 3) those larvae that pupated during the test were not considered. The number of larvae lost through pupation is also shown. The median lethal concentration of DDT against this species appeared to be between 1 part in 50 million parts of water and 1 part in 75 million.

One series of tests was made in January 1945, at Hollandia, Dutch New Guinea, using 25 larvae of *Culex annulirostris* at each concentration. This species seemed unusually susceptible to DDT, as all larvae were killed within 24 hours at

TABLE 2. Mortality of *Anopheles punctulatus* Larvae Exposed to Various Concentrations of DDT and Phenothiazine (10 Larvae per Test). New Guinea.

Concentration of Insecticide	Number of Tests	Mortality After 48 Hours
		Per Cent
DDT (1 part in indicated million parts of water):		
40	1	90
50	2	90
75	1	80
100	2	45
200	2	30
400	1	10
Phenothiazine (p.p.m.):		
1	2	100
0.75	1	90
0.50	2	35
0.25	2	15
0.10	2	0
Controls:		
1% acetone	1	0
No treatment	2	0

TABLE 3. Mortality of *Armigeres milnensis* Larvae Exposed to Various Concentrations of DDT and Phenothiazine. (20 Larvae per Test.) New Guinea

Concentration of Insecticide	Mortality After 24 Hours	Mortality After 48 Hours	Number Pupated in 48 Hours
DDT (1 part in indicated million parts of water	*Per Cent*	*Per Cent*	
16	100	100	0
30	95	100	0
40	5	79	6
50	25	80	3
75	10	36	6
100	5	8	8
Phenothiazine (p.p.m.):			
2.5	65	72	2
2	35	60	0
1.5	20	62	7
1	15	19	4
0.5	0	6	3
Controls:			
1% acetone	0	0	5
No treatment	0	0	0

concentrations of 1 part in 30, 40, 50, 75, 100, and 150 million parts of water. At 1 to 200 million 23 larvae died during the first 24 hours, and after 48 hours the 2 survivors still appeared to be of normal vigor. The results with phenothiazine are shown in Table 4. The larvae seemed to be no more susceptible to this material than were certain lots of *C. quinquefasciatus* tested in earlier work in Florida.

TABLE 4. Mortality of *Culex annulirostris* Exposed to Various Concentrations of Phenothiazine. New Guinea.

Concentration	After 24 Hours	After 48 Hours
	Per Cent	*Per Cent*
(P.p.m.):		
2.0	100	100
1.5	96	96
1.0	92	100
0.50	44	60
0.25	4	16
Controls:		
1% acetone	0	0
No treatment	0	0

Discussion.—It is difficult to ascribe definite minimum and median lethal concentrations for mosquito larvicides, because of the variation in resistance among different lots of the same species. In tests against 56 lots of laboratory-reared larvae of *Culex quinquefasciatus,* Bushland and King (1943) found that 1 p.p.m. of phenothiazine caused a mean mortality of 69.1 ± 2.08 per cent, with kills for the various lots ranging from 40 to 100 per cent. Therefore, it seemed desirable to compare DDT with phenothiazine. The results reported in this paper show that the lots of *quinquefasciatus* larvae used were somewhat more susceptible to phenothiazine than the average, and that a DDT concentration of 1 part in 20 million parts of water might allow some survivors if further tests were made. Against this species DDT appeared to be about 50 times as effective as phenothiazine.

Tests against *Aedes aegypti* were too few to establish lethal concentrations for the species, but DDT again appeared to be about 50 times as toxic as phenothiazine.

The results of tests with *Anopheles punctulatus* are of special interest because of the importance of that species as a vector of malaria in the southwest Pacific. Field-collected larvae brought into the unnatural conditions of laboratory testing should be easier to kill than laboratory-reared *quadrimaculatus* from an established colony. However, *punctulatus* seemed much more resistant to DDT than the *quadrimaculatus* tested by Deonier *et al.* (1945). A DDT concentration of 1 to 100 million, which gave nearly 100 per cent mortality of *quadrimaculatus,* caused only 45 per cent mortality of *punctulatus.* The highest concentration tested (1 to 40 million) failed to kill all the *punctulatus* larvae. The *punctulatus* larvae used in these tests were not extremely resistant to all insecticides, because phenothiazine at 1 p.p.m. was 100 per cent lethal, whereas the same treatment killed only 79 per cent of the *quadrimaculatus* larvae.

Armigeres milnensis larvae were very resistant to phenothiazine. This compound caused only 72 per cent mortality at 2.5 p.p.m., a concentration which in earlier work the author found to be lethal to larvae of *Aedes aegypti, A. taeniorhynchus, A. mitchellae* (Dyar), *Psorophora confinnis, Culex nigripalpus,* Theob., and *C. quinquefasciatus.* However, *Armigeres milnensis* seemed to be about as susceptible to DDT as were *C. quinquefasciatus* and *A. aegypti,* since a treatment of 1 part in 30 million killed all the larvae. DDT appeared to be about 100 times as toxic as phenothiazine to *Armigeres milnensis.*

In the tests against *Culex annulirostris* it was pointed out that this species was very susceptible to DDT, a concentration of 1 to 150 million being lethal. The particular groups of larvae tested were about as susceptible to phenothiazine as the least resistant group of *Culex quinquefasciatus.* The results indicated that DDT was about 150 times as toxic as phenothiazine to this species.

Summary.—DDT and phenothiazine were tested against fourth-instar larvae of two American and three New Guinea species of mosquitoes according to a standard laboratory procedure for evaluating organic insecticides.

DDT caused 100 per cent mortality of *Culex quinquefasciatus* Say and *Aedes aegypti* (L.) larvae at 1 part in 20 million parts of distilled water, and appeared to be about 50 times as toxic as phenothiazine to these species. The median lethal concentration for *C. quinquefasciatus* was between 1 to 75 million and 1 to 100 million. Against *A. aegypti* the lowest concentration tested, 1 to 100 million, killed 81 per cent of the test insects.

DDT caused 90 per cent mortality of *Anopheles punctulatus* Donitz at the two highest concentrations tested, 1 to 40 million and 1 to 50 million. The median lethal concentration appeared to be near 1 to 100 million. DDT was indicated to be about 70 times as toxic as phenothiazine to this species.

DDT was lethal to *Armigeres milnensis* Lee at a concentration of 1 to 30 million and the median lethal concentration appeared to be between 1 to 50 million and 1 to 75 million. DDT appeared to be about 50 times as toxic as phenothiazine to this species.

DDT was lethal to *Culex annulirostris* Skuse at a concentration of 1 to 150 million. The lowest concentration tested, 1 to 200 million, caused 92 per cent mortality. DDT appeared to be about 150 times as toxic as phenothiazine to this species.

Literature Cited

BUSHLAND, R. C., AND W. V. KING. 1943. Laboratory tests with organic compounds as larvicides for *Culex quinquefasciatus* Say. U. S. Bur. Ent. and Plant Quar. E-585, 15 pp. (Processed.)

DEONIER, C. C., J. D. MAPLE, H. A. JONES, E. HINCHEY, AND P. M. EIDE. 1945. DDT as an anopheline larvicide—Laboratory tests. Jour. Econ. Ent. 38(2):241–3.

EIDE, P. M., C. C. DEONIER, AND R. W. BURRELL. 1945. DDT as a culicine larvicide. Jour. Econ. Ent. 38(5):537–41.

FINK, D. E., L. E. SMITH, D. L. VIVIAN, AND H. V. CLABORN. 1938. Toxicity tests with synthetic organic compounds against culicine mosquito larvae. U. S. Bur. Ent. and Plant Quar. E-425, 34 pp. (Processed.)