ARTICLES

LABORATORY AND FIELD EVALUATION OF NEW INSECTICIDES AGAINST MOSQUITO LARVAE [1]

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INTRODUCTION

Since the advent of chlorinated hydrocarbon insecticides, DDT has been one of the major synthetic insecticides used for mosquito larviciding programs in California. Had it not been for the development in mosquitoes of resistance to chlorinated hydrocarbon insecticides and for the passage of federal laws prohibiting the use of DDT on forage crops, the total amount of DDT employed in mosquito control would have increased due to expanding mosquito control activities. Notwithstanding these factors, the amount of DDT employed for mosquito control in California in 1956 and 1958 was essentially the same for the two years (Anonymous, 1957, 1959) and led other synthetic materials in its total quantity used.

From 1956 to 1958 there was a marked increase in the use of toxaphene. Almost 80 percent of this increase was credited to southern California where DDT usually does not give satisfactory control in the summer months. A few mosquito abatement districts in California started using malathion in 1953 and parathion in 1954. There has been a gradual increase in the use of these materials in succeeding years. By 1958, 90 to 95 percent of the total amount used of these two insecticides was used in the central valley for mosquito control, and, based on total area, parathion was the material most widely used (Anonymous, 1959).

It was in the intensively farmed Sacramento and San Joaquin Valleys that mosquito resistance to insecticides was first confirmed. Here the resistance picture is complicated by intensive use of insecticides for the control of agricultural pests. It was in these areas that resistance of mosquito larvae to DDT and other chlorinated hydrocarbon insecticides was reported earlier (Gjullin and Peters, 1952; Gjullin, 1956; Isaak, 1956*a*). Malathion and parathion became the common substitutes for chlorinated hydrocarbon insecticides. However, in due time studies in the San Joaquin Valley confirmed a 30–35-fold resistance in *Culex tarsalis* to malathion at the LC 90 level (Gjullin and Isaak, 1957).

Within the past two years many control failures have been reported for malathion. Mosquito resistance to this material appears to be becoming widespread, but has not been thoroughly substantiated with published field or laboratory studies. A few mosquito abatement districts have undertaken limited studies of this trend and have confirmed the presence in mosquitoes of a high level of resistance to malathion. The Kern Mosquito Abatement District, for example, has been using malathion since 1953—mostly for urban larviciding. During the 1959 season, however, control of *Culex tarsalis* and *C. quinquefasciatus* by means of malathion was poor.

Parathion is used for mosquito control in agricultural areas where the pests are breeding, but resistance to this material in mosquitoes has also appeared recently. Resistance in *Aedes nigromaculis* larvae to parathion was reported toward the latter

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part of 1958 in one locality in the San Joaquin Valley (Lewallen and Nicholson, 1959). This resistance, so far as is known, has not spread to other parts of the valley.

There is a current tendency (with some hesitation) toward a more widespread use of parathion as a mosquito larvicide in California. The greatest drawback of this material is its high mammalian toxicity, which limits its use in urban or suburban areas where there is concern regarding its toxic hazards. Primarily because of the resistance problem, the current studies were initiated to find substitute materials with insecticidal activity against mosquito larvae superior to that of malathion and approximating that of parathion, but having lower mammalian toxicities than the latter material.

Laboratory Screening. Ten new insecticides were screened against susceptible 4th instar larvae of *Culex quinquefasciatus* Say in the laboratory. Acetone solutions of the various compounds were added to 100 ml. of tap water (pH 8 to 8.5) in one-pint cups to which 25 fourth instar mosquito larvae had been added. Each dosage was replicated 3 times and each material was screened 2 to 3 times on separate days. The larvae were set at 75° to 78° F. and the mortality was recorded 24 hours later.

After preliminary evaluation, materials with activity lower than malathion were eliminated. Only those materials that manifested activity between that of malathion and parathion were screened critically in the laboratory. A chlorinated hydrocarbon compound SD-4402 (1,3,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanophthalan) indicated the highest activity against the larvae (Figure 1). Bayer 29493 (*O,O*-dimethyl *O*-3-methyl-4-methylthiophenyl phosphorothioate), Bayer 34042, and Dicapthon (American Cyanamid 4124) followed in order of decreasing activity. Experimental Nematocide (EN) 18133 (*O,O*-diethyl *O*-2-pyrazinyl phosphorothioate) also indicated appreciable activity. The remaining materials—Bayer 22408 (*O,O*-diethyl *O*-naphthaloximido phosphorothioate), Ethyl Guthion, AC-5727 (m-isopropylphenyl *N*-methylcarbamate), Bayer 25141 (*O,O*-diethyl *O-p*-methylsulfinylphenyl phosphorothioate), and Methyl Trithion (*O,O*-dimethyl *S-p*-chlorophenylthiomethyl phosphorodithioate)—were superior in activity to malathion but were not as efficient as the other four organophosphates (figure 1). The regression lines of Bayer 29493 and EN 18133 are rather steep and these materials hold promise for mosquito control. Dicapthon, although indicating greater activity than that of malathion in laboratory screening, did not prove as effective as malathion against *Aedes nigromaculis* in the field (Isaak, 1956*b*).

It is generally accepted among workers in California that *Culex* mosquitoes are relatively harder to kill than the other mosquito species. A toxicant that proves effective against *C. tarsalis* or *C. quinquefasciatus* will generally prove more effective against *Aedes* and *Anopheles* mosquitoes. For this reason the laboratory and field evaluation tests have been directed against the two *Culex* species.

Field Trials

Of the ten new materials showing activity greater than that of malathion in the laboratory, seven were evaluated in the field. Dicapthon was not evaluated because of its poor field performance (Isaak, 1956*b*). Compound GC-3583 [diethyl-1-(2,5-dichlorophenyl)-2-chlorovinyl phosphate] was evaluated in the field but not in the laboratory.

The materials under investigation were evaluated in different types of niches and habitats where mosquitoes breed. These consisted of irrigated pastures, a duck club, a duck club slough, and a river bottom. The water depth in the pastures and the duck club varied anywhere from 2 to 6 inches. Greater water depths were encountered in the plots established in the duck club slough and the river bottom.

Plot size was either 1/16 acre or 1/32 acre. The sprays were applied at the rate of 8 gallons per acre with hand sprayers yielding maximum pressure of 30 psi. Mosquito larval count was obtained by

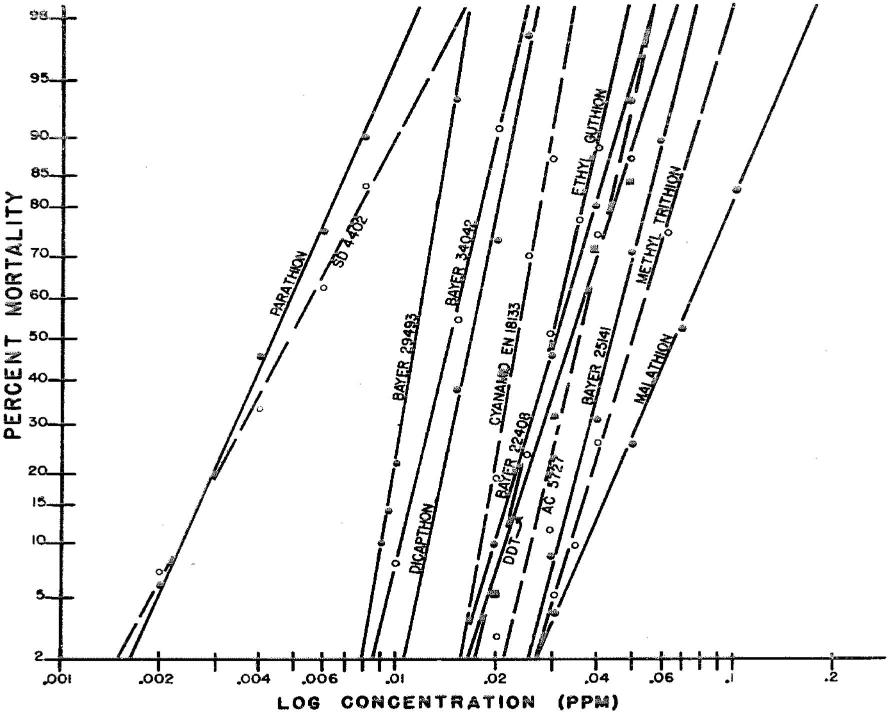

FIG. 1.—Dosage mortality lines of ten new insecticides in laboratory screenings against susceptible 4th instar larvae of *Culex quinquefasciatus* Say. Parathion, DDT, and malathion are included for the purpose of comparison.

dippings. Ten to twelve dips were taken per plot, both before and 24 hours after treatment. Only 4th instar larvae of *Culex tarsalis* were recorded.

PASTURE TRIALS. Six new materials and malathion were evaluated in two pastures where emergent vegetation covered 10 to 30 percent of the total area. Because of this variable some differences were observed in the results. However, the results with the various dosages of a material indicated the range of activity of the materials tested.

In the first pasture, Methyl Trithion, Bayer 29493, EN 18133, and malathion were evaluated (Table 1). Bayer 29493 proved to be the most effective material, followed by EN 18133. The latter material did not yield as good results at the highest dosage (0.1 pound per acre) as at the two lower dosages. Methyl Trithion proved effective at dosages of 0.2 and 0.5 pound per acre. This material probably has the same range of activity as malathion against *C. tarsalis*.

In the second pasture, AC-5727, Bayer 25141, Bayer 22408 and malathion were evaluated (Table 2). The population in this pasture was reported in 1955 to have a 75-fold resistance to DDT at the LC 50 level (Isaak, 1955). In this test Bayer 25141 was the only material that man-

TABLE 1.—Field trials with organophosphorous insecticides against *Culex tarsalis* larvae (Baby Pony Pasture, Wasco, California) [a]

Insecticide and formulation	Dosage, pounds of toxicant/acre	Avg. no. larvae/dip		Percent of control
		Pre-treatment	Post-treatment	
Methyl Trithion EC 4	0.100	12	2.0	83
	0.200	8	0.0	100
	0.500	13	0.9	93
Bayer 29493 EC 2	0.025	19	4.0	79
	0.050	12	0.0	100
	0.100	2	0.0	100
EN 18133 EC 4	0.025	27	1.4	95
	0.050	10	0.4	96
	0.100	10	2.3	77
Malathion EC 5	0.200	16	0.7	96
	0.400	10	0.2	98

[a] Plot size 1/16 acre. Water temperature 75°–78° F. during the day.

TABLE 2.—Field evaluation of 4 insecticides against *Culex tarsalis* larvae (Bloomfield pasture, Bakersfield, California) [a]

Insecticide and formulation	Dosage, pounds of toxicant/acre	Avg. no. larvae/dip		Percent of control
		Pre-treatment	Post-treatment	
AC–5727 EC 1.1	0.02	9.4	19.4	0.0
	0.04	11.8	11.8	0.0
	0.10	9.0	7.5	16.7
Bayer 25141 EC 2	0.02	7.8	4.2	46.7
	0.04	14.8	4.8	67.6
	0.10	9.4	0.0	100.0
Bayer 22408 EC 2	0.02	6.2	2.4	61.3
	0.04	9.4	17.3	0.0
	0.10	7.1	12.2	0.0
Malathion EC 4	0.02	12.6	20.5	0.0
	0.05	6.3	10.8	0.0

[a] Plot size 1/32 acre.

ifested a high degree of activity against the larvae at the 0.1 pound per acre dosage. This material, although showing less activity in the laboratory than AC-5727 and Bayer 22408, proved superior to these two in the field. None of the other materials manifested any high degree of activity at the administered dosages.

DUCK CLUB TRIALS. Compounds SD-4402, GC-3583 and Bayer 22408 were evaluated in a duck club covered with a considerable amount of emergent vegetation (Table 3). Bayer 22408 was administered at higher dosages than in the previous test in the pasture and proved ineffective again at the applied dosages. SD-4402 proved to be highly effective and GC-3583 showed greater activity only at the 0.2 and 0.3 pound per acre rates. Gross observations on the activity of these materials against predaceous insects were also made. SD-4402 resulted in greatest mortality, and at the highest dosage proved toxic to frogs. On the other hand, slight damage to predaceous and scavenger insects was observed at the two highest and most effective dosages of GC-3583. Similarly, Bayer 22408 proved harmless to aquatic predators and scavengers, but proved highly lethal to adult Ephydrid-like (probably *Hydrellia* sp.) flies hovering over the treated water.

In a slough where water depth averaged about 2 feet, Bayer 22408 was again eval-

TABLE 3.—Investigations with 3 insecticides against *Culex tarsalis* larvae (Auree Duck Club, Gun Club Road, Wasco, California) [a]

Insecticide and formulation	Dosage, pounds of toxicant/acre	Avg. no. larvae/dip		Percent of control
		Pre-treatment	Post-treatment	
SD-4402 EC 1.25 [b]	0.05	4.5	0.4	91
	0.10	5.0	0.1	98
	0.20	4.6	0.0	100
GC-3583 EC 4	0.05	4.9	3.5	28
	0.10	2.3	0.9	61
	0.20 [c]	4.6	0.2	95
	0.30 [c]	1.6	0.0	100
Bayer 22408 EC 2 [d]	0.05	4.5	6.1	0
	0.10	3.4	0.7	79
	0.20	5.5	3.3	40

[a] Plot size 1/32 acre. Water depth varied from 2 to 6 inches with medium plant cover.
[b] May-fly naiads and scavenger beetle larvae found dead. Sick frogs seen in plot treated with the highest dosage. Mosquito pupae alive at all dosages.
[c] Predators such as backswimmers and diving beetles observed to be alive at these dosages.
[d] Diving beetles and second stage mosquito larvae found alive. Large number of Ephydrid-like flies hovering over the water seen dead.

TABLE 4.—Field trials with Bayer 22408 and AC-5727 against *Culex tarsalis* larvae (Tulare Duck Club slough, Garcess Highway, Delano, California) [a]

Insecticide and formulation	Dosage, pounds of toxicant/acre	Avg. no. larvae/dip		Percent of control
		Pre-treatment	Post-treatment	
Bayer 22408 EC 2	0.1	3.1	0.9	71 [b]
	0.2	11.9	2.6	78 [b]
	0.3	7.4	2.0	73 [b]
AC-5727 EC 1.1	0.1	13.6	3.2	77 [c]
	0.2	6.3	0.5	92 [d]
	0.3	9.1	0.4	96 [d]

[a] Plot size 1/32 acre. Water depth ranged from a few inches to 3 feet.
[b] Second stage larvae also alive, indicating material ineffective at these dosages.
[c] Young larvae (2nd and 3rd) dead. Fourth stage instars only ones observed alive. Rat-tail maggots alive.
[d] Rat-tail maggots dead.

uated but at still higher dosages (Table 4). It proved to be ineffective under the test conditions, and even young instar larvae (second) of mosquitoes were not touched by the higher dosages of this material. AC-5727, on the other hand, proved to be effective at the highest two dosages. This material, however, proved lethal to predaceous rat-tail maggots at the effective dosages.

RIVER BOTTOM TRIALS. The plots in the river bottom were diked to avoid water flow and diffusion of toxicant from one plot to another. Compound SD-4402 was evaluated at three dosages and it proved highly effective even at the lowest dosage of 0.05 pound per acre (Table 5). This material at the lowest dosage proved harmless to frogs but killed *Gambusia* fish. At the highest two dosages (0.1 and 0.2 pound per acre) this material resulted in complete mortality of frogs.

It is important to note that certain materials investigated here at the effective dosages against mosquito larvae are harmful to predators and scavengers living together with the pests. Certain other materials, on the other hand, are quite harmless to such organisms at the effective

TABLE 5.—Control of *Culex tarsalis* with SD–4402 sprays in river bottom (Kern River, Oildale, California)[a]

Dosage, pounds of toxicant/acre	Avg. no. larvae/dip		Percent of Control
	Pre-treatment	Post-treatment	
0.05[b]	12	0	100
0.10[c]	6	0	100
0.20[c]	8	0	100

[a] Plot size 1/32 acre arranged in Kern River bottom; water depth 2 feet. No emergent or submerged vegetation except some algal growth on surface.
[b] Frogs alive but *Gambusia* fish all dead. No mortality of mosquito pupae observed.
[c] All frogs dead in these treatments. No fish observed either before or after treatments.

dosages. Emphasis on selective materials in mosquito control has been largely overlooked and future investigations should arrive at an integrated control of mosquito pests. This subject has gained emphasis in the control programs of agricultural pests.

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