ARTICLES

SOME NEW AND HIGHLY EFFECTIVE MOSQUITO LARVICIDES [1]

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INTRODUCTION. Mosquito larvicidal treat-ents comprise a major segment of the osquito control programs in California well as in other states. Although DDT, HC, dieldrin, malathion, parathion and ner synthetic insecticides have been suc-ssfully used for mosquito larval control, e development of resistance in many ecies has rendered some of these larvi-les ineffective in certain areas. The netic plasticity of many mosquito species eatly enhances the development of resist-ce to insecticides. Resistance to DDT d other organochlorine insecticides de-loped rapidly in mosquitoes in Califor-a; therefore, these materials are used ly in localized situations. Direct and oss tolerance phenomena, characteristic the organochlorine insesticides, have rned attention to the organophosphate, rbamate and other groups of insecticides. The organophosphate group of insecti-les includes some highly effective and verse types of mosquito larvicides. Mala-ion, with low mammalian toxicity, was ed in California for a few years only, cause many mosquito species became mune to this material in a short time. rathion, a highly effective larvicide, has en employed for over 6 years in the ntral valley of California without evi-nce of widespread resistance to it. The gh mammalian toxicity of parathion, owever, constitutes an undesirable feature this compound. Although many mosquito abatement districts have used parathion without any serious incidents for many years, its high mammalian toxicity still emphasizes the need for developing safer materials.

In order to find or develop highly effective mosquito larvicides which can be used safely in the presence of mammals, including wildlife and game species, numerous materials were evaluated earlier (Mulla *et al.*, 1960, 1961). The present studies are a continuation of these evaluations for the purpose of enlarging the list of highly effective yet safe materials.

METHODS AND MATERIALS. The materials in the laboratory and field were screened and evaluated in the same manner as described in earlier studies (Mulla *et al.*, 1960, 1961). Laboratory tests were conducted against 4th-instar larvae of a susceptible colony of *Culex p. quinquefasciatus*. Technical grade toxicants were dissolved in acetone and proper dilutions made. Aliquots of the toxicant solutions in acetone were added to 100 ml. of tap water in 6-ounce waxed paper cups. Twenty-five 4th-instar larvae were added to each cup and the per cent mortality read 24 hours after exposure.

The field tests were conducted against 4th instar larvae of *Culex tarsalis*, mostly in breeding ponds located in an agricultural area south of Bakersfield. The sprays were applied at the rate of 8 gallons per acre or 1/2 gallon per each 1/16 acre plot. Fourth instar larvae were counted by taking 15 dips per plot prior to application of the treatments and 24 hours after application of the treatments.

In the field experiments, observations were also made on the effects of test materials against chironomid midge larvae and aquatic predaceous insects such as

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dragonflies, mayflies, dytiscid beetles, etc. However, lack of information relative to these insects is no indication of the ineffectiveness of the materials at the given dosages. It very well may be an indication of the absence of such insects in the plots treated. Information pertaining to this relationship is given in footnotes to the tables and in more detail in Table 8. Many of the materials reported here were also studied for their effects on *Gambusia affinis* and 3 anuran species (Mulla and Isaak 1961 and Mulla *et al.*, 1962).

RESULTS AND DISCUSSION: LABORATORY. In the laboratory 24 new compounds were evaluated (Table 1.) The dosage response lines of these, as well as parathion, DDT, and malathion (for comparison) are presented in Figure 1. At the LC_{90} level, three materials, Bayer 37343, Bayer 29952, and SD-7438 manifested higher biological activity than parathion against the larvae. The activity of SD-7587, Bayer 41831 (Sumithion), and SD-7554 was slightly lower than that of parathion. Sumithion, Bayer 37343, SD-7438 and SD-7587 have much lower mammalian toxicity valu than parathion (Table 2). Sumithion a SD-7438 are especially safe, since th acute oral LD_{50} of 673 mg./kg. and 2 mg./kg. body weight of rats compare ve favorably with an LD_{50} of 113 mg./kg. f DDT and 13 mg./kg. for parathion. terms of their low mammalian toxicity a very high mosquito killing power, sun thion (Bayer 41831) and SD-7438 appe to offer outstanding possibilities as safe a effective mosquito larvicides.

Bayer 41831, SD-7554 and Bayer 372 also indicated a high degree of activ against the larvae. The dosage respon lines of these materials were fairly stee

TABLE 1.—Chemical composition of compounds studied.

Designation	Chemical composition
Bayer 29850	*O,O*-diethyl *S*-1-ethoxy 2,2,2-trichloroethyl dithiophosphate
Bayer 29952	*O*-ethyl *O*-*p*-methylthiophenyl methylphosphonothionate
Bayer 33904	N,N-dimethyl-*O*-*p*-methylthiophenyl diamidophosphate
Bayer 34042	*O*-ethyl *O*-(3-methyl-4-methylthiophenyl) N-methyl phosphonamidothion
Bayer 37289	*O*-ethyl 2,4,5,-trichlorophenyl ethylphosphonothionate
Bayer 37341	*O,O*-diethyl *O*-(3,5-dimethyl-4-methylthiophenyl) phosphorothionate
Bayer 37342	*O,O*-dimethyl *O*-(3,5-dimethyl-4-methylthiophenyl) phosphorothioate
Bayer 37343	*O,O*-diethyl *O*-(3,5-dichloro-4-methylthiophenyl) phosphorothionate
Bayer 37344	3,5-dimethyl-4-methylthiophenyl N-methylcarbamate
Bayer 41831	*O,O*-dimethyl *O*-3methyl-4-nitrophenyl phosphorothionate
Cygon ® (dimethoate)	*O,O*-dimethyl *S*(N-methylcarbamoylmethyl) phosphorodithioate
Diazinon ®	*O,O*-diethyl *O*-(2-isopropyl-6-methyl-4-pyrimidyl) phosphorothioate
G-27365	*O,O*-diethyl *S*-(3,4-dichlorophenylthio)-methyl phosphorodithioate
G-30493	*O,O*-dimethyl *S*-(3,4-dichlorophenylthio)-methyl phosphorodithioate
G-30494	*O,O*-dimethyl *S*-(2,5-dichlorophenyl mercaptomethyl) phosphorodithioate
Hercules 7522 H	2-chloro-5-isopropylphenyl N-methylcarbamate
Ortho 5353	3-*sec*-amylphenyl N-methylcarbamate
Ortho 5655	3-*sec*-butyl 6-chlorophenyl-N-methylcarbamate
SD-3562	3-(dimethyloxyphosphinyloxy)-N,N-dimethyl-cis-crotonamide
SD-4294	α-methylbenzyl 3-(dimethyloxyphosphinyloxy)-cis-crotonate
SD-6460	1,2-dibromo-2,2-dichloroethyl methyl phenylphosphate
SD-7554	diethyl 1-(phenylthio) vinyl phospate
SD-7565	1-(benzylthio) vinyl diethyl phosphate
SD-7587	1-(*p*-chlorophenylthio) vinyl dimethyl phosphate
Stauffer R-1504	phthalimidomethyl-*O*, O-dimethyl phosphorodithioate.

For better visualization of the activi of the materials evaluated, the LC_{50} ar LC_{90} are presented in Table 3. As state earlier (Mulla *et al.*, 1961), materials wi biological activity lower than that of mal thion do not hold much promise for mo quito control in California. However, on compound, SD-4294, may attract attentio in the future due to its low mammalia toxicity (acute oral LD_{50} to rats is 1

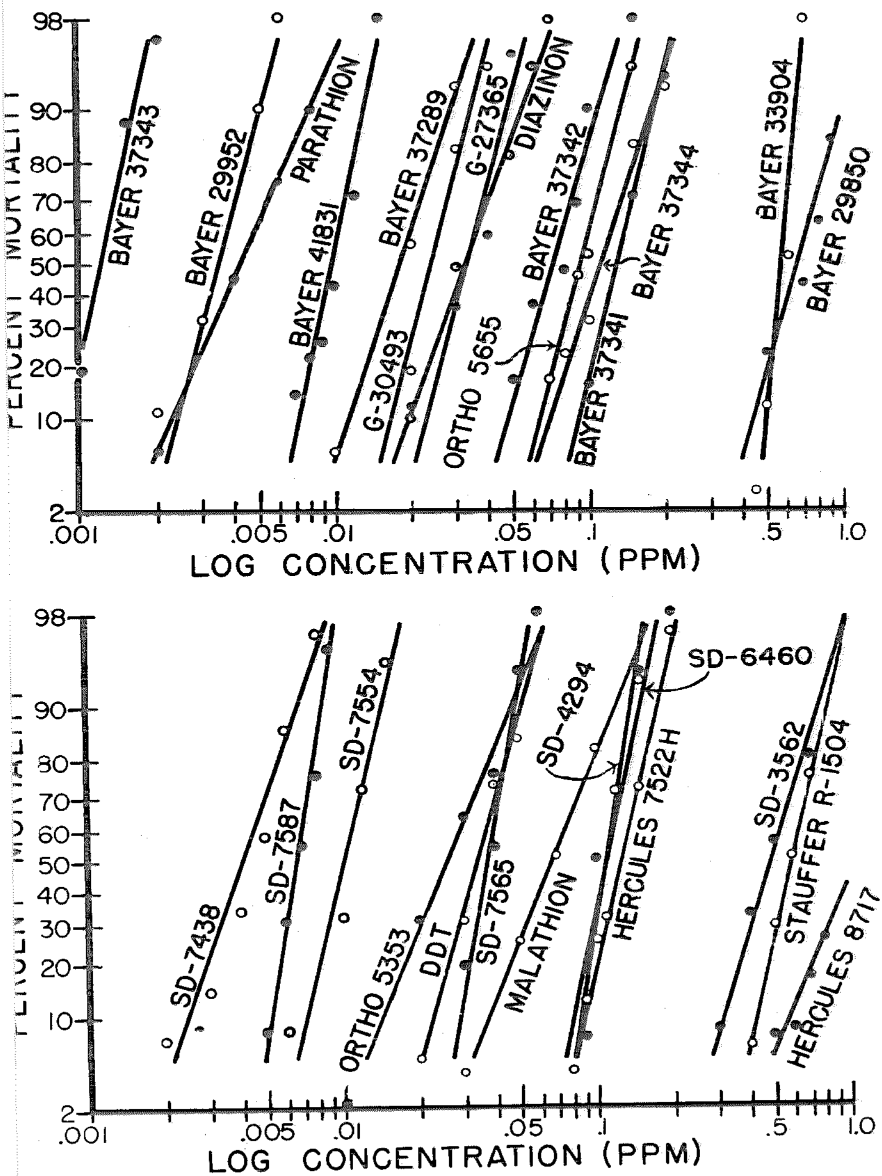

FIG. 1.—Dosage response lines of 24 new insecticides against 4th-instar larvae of *Culex p. quinquefasciatus* in the laboratory. DDT, malathion and parathion lines are included for comparison.

mg./kg.), and also because it belongs to the crotonate group of insecticides. This material may be useful in situations where a high degree of resistance to organophosphates is common. SD-3562 also belongs to the crotonate group, but its low larvicidal activity and high mammalian toxicity (acute oral LD_{50} for rats is 27 mg./kg.) might preclude its widespread use.

TABLE 2.—Mammalian toxicity and larvicidal activity of several new mosquito larvicides

	Mammalian acute oral LD_{50} mg./kg.	Mosquito larvae LC_{50} (p.p.m.)
Bayer 34042	2.0[a]	0.0155
Bayer 29952	5.0[a]	0.0035
Parathion	3.6–13.0[b]	0.0045
Guthion	13.0[a]	0.019
Methyl parathion	17.5[a]	0.0033
SD-7587	58.0[a]	0.0067
Bayer 37343	100.0[a]	0.0012
DDT	113.0[c]	0.035
SD-7438	280.0[a]	0.0041
Baytex (Bayer 29493)	310.0[a]	0.011
Bayer 41831 (Sumithion)	673[d]	0.0098
Bayer 37342	>3000[d]	0.072
Malathion	480–5800[c]	0.068

[a] Data obtained from manufacturers.
[b] From World Health Organization, Tech. Rept. Series No. 114, 1956.
[c] From Guide to the Chemicals used for Crop Protection by Dr. Hubert Martin, 1957, 3rd edition.
[d] World Health Organization data from Dr. J. M. Barnes, Carshalton, England.

FIELD. Most of the field evaluation studies were carried on in artificial breeding ponds (Mulla and Isaak 1961) in the same manner as described earlier (Mulla *et al.*, 1961).

Bayer 34042 proved highly effective against the larvae at the rate of 0.025 lb./acre or higher (Table 4). At 0.025 lb./acre this material did not adversely effect dragonfly naiads. However, at 0.50 lb./acre and 0.10 lb./acre this material resulted in high mortality of the naiads.

Bayer 29952 also proved highly effective against the larvae. But at mosquito larvi-

TABLE 3.—Effectiveness of various insecticid against 4th instar larvae of *Culex pipiens quinquefasciatus*[a] in the laboratory

Materials	LC_{50} (p.p.m.)	LC_{90} (p.p.m
Bayer 37343	0.0012	0.0016
Bayer 29952	0.0035	0.0050
SD-7438	0.0041	0.0068
Parathion	0.0045	0.0082
SD-7587	0.0067	0.0084
Bayer 41831	0.0098	0.013
SD-7554	0.010	0.015
Bayer 37289	0.018	0.029
G-30493	0.024	0.035
Ortho 5353	0.027	0.049
G-27365	0.033	0.048
Diazinon	0.033	0.055
DDT	0.035	0.053
SD-7565	0.037	0.049
Malathion	0.068	0.12
Bayer 37342	0.072	0.11
Ortho 5655	0.094	0.13
SD-4294	0.11	0.14
SD-6460	0.11	0.15
Bayer 37344	0.11	0.18
Bayer 37341	0.13	0.18
Hercules 7522 H	0.13	0.18
SD-3562	0.50	0.78
Bayer 33904	0.58	0.66
Stauffer R-1504	0.60	0.84
Bayer 29850	0.66	1.0
Hercules 8717	1.1	2.0

[a] A susceptible strain, colonized from a colon which was maintained at Kern Mosquito Abat ment District, Bakersfield, California. Parathio DDT and malathion are included for compariso

cidal dosages this material produced hig mortality of dragonfly naiads. Its man malian toxicity is also very high (Table 2 Bayer 41831 indicated high biologic activity against mosquito larvae but w slightly less effective than Bayer 3404 and Bayer 29952. The first material, hov ever, did not adversely affect predaceou beetles at the applied rates, and it has favorable mammalian toxicity value (Tab 2.)

One of the most highly effective mo quito larvicides ever tested was Bayer 3734 (Table 5). This material produced con plete kill of the larvae at the rate of 0.0 lb./acre. This material did not adverse affect mayfly and dragonfly naiads, n did it cause any mortality in *Gambus affinis* at larvicidal rates. (Mulla *et a* 1962).

TABLE 4.—Field evaluation of three Bayer compounds against 4th instar larvae of *Culex tarsalis* in breeding ponds

Material and formulation	Toxicant lbs./acre	Avg. no. larvae/dip Pre-treatment	Avg. no. larvae/dip 24-hour Post-treatment	Percent control
er 34042 EC 4	0.005	11.5	7.1	38
	0.01	18.0	3.8	79
	0.025	2.8[a]	0.0[a]	100
	0.05	3.8[a]	0.0[b]	100
	0.10	2.1[a]	0.0[c]	100
er 29952 EC 4	0.005	3.5	1.1	68
	0.01	14.3	0.5[d]	97
	0.025	5.3	0.0[e]	100
	0.05	4.2	0.0[c]	100
er 41831 EC 4 Sumithion)	0.01	8.0	4.6	42
	0.025	13.5	1.4	89
	0.05	10.8	0.0[e]	100
	0.1	4.1	0.0[f]	100
	0.2	18.8	0.0[e]	100

High dragonfly nymph population.
About 75 percent to 80 percent of dragonfly nymphs dead.
All dragonfly nymphs dead.
Dragonfly nymphs dead, dytiscid adults alive.
Dytiscid larvae and adults alive.
Adult diving beetles and small *Gambusia* alive.

TABLE 5.—Field evaluation of organophosphate larvicides against 4th instar larvae of *Culex tarsalis* in breeding ponds

Material and formulation	Toxicant lbs./acre	Avg. no. larvae/dip Pre-treatment	Avg. no. larvae/dip 24-hour Post-treatment	Percent control
er 37343 EC 2	0.0025	7.33	5.93[a]	19
	0.005	1.53	0.07[b]	95
	0.01	3.60	0.00[b]	100
7587 EC 2	0.005	10.60	6.53[c]	38
	0.01	1.47	0.80[d]	46
	0.025	12.00	1.90	84
	0.05	7.30	0.10	99
7438 EC 2	0.005	11.93	5.00	58
	0.01	1.47	0.20[e]	86
	0.025	11.40	0.10[e]	99
athion EC 2	0.0025	1.4	3.5[f]	0
	0.005	8.90	4.00	55
	0.01	3.80	0.30[g]	92
	0.025	2.70	0.0[h]	100
	0.05	11.40	0.0[h]	100
	0.10	10.10	0.0[h]	100

Average of 20 pupae/dip alive.
Mayfly and dragonfly naiads alive; few live mosquito pupae found.
All stages alive—dragonfly naiads and diving beetles alive.
Some 2nd and 3rd instars alive. Chironomid midge larvae dead on water surface.
Mayfly naiads and tadpoles alive. Dead chironomid midge larvae observed on water surface, 24 rs after treatment.
All stages of mosquito larvae alive.
Pollywogs present and alive.
Chironomid midge larvae present and were found dead on the water surface 24 hours after treat- it.

Two other materials, SD-7587 and SD-7438, were also found to be highly effective (Table 5). In field tests, SD-7438 proved to be twice as effective as SD-7587, a trend similar to the one determined in laboratory studies. Both materials were relatively safe to aquatic predaceous insects present in the ponds.

Parathion was also included in the tests during May, 1962. The use of parathion in the study area in routine control operations for more than 6 years has caused no tolerance in the natural mosquito population. Complete larval mortality was obtained at 0.025 lb./acre of parathion (Table 5).

G-30494 proved more effective against the larvae in the ponds than in a duck club (Table 6). Due to different conditions existing in the two places, this difference in activity has to be expected. It is assumed that the dosage required to give complete kill in the duck club would be around 0.2 lb./acre, a dosage double the amount used in the breeding ponds. Also the material was observed in these tests to yield good control of chironomid midge larvae (Table 6).

Ortho 5353 a carbamate insecticide ga almost complete mortality of 4th inst larvae at 0.2 lb./acre (Table 7). Th carbamate seems to be the most effecti one of those evaluated in the field th far. This compound was also observ to cause considerable mortality in chiro omid midge larvae at the rate of c lb./acre or higher. The material produc some initial mortality in *Gambusia affi* at the two highest rates.

Cygon ® (dimethoate) proved ineff tive against mosquito larvae at 0.6 lb acre (Table 7). At the 0.2 lb./acre r Cygon produced no mortality of *Gambu affinis.* Toxicity of Cygon at higher ra to fish was not determined.

SD-7554 proved very effective agai the larvae (Table 7). It did not advers affect most of the insect predators. Ba 37289 proved more effective than ant pated. Both these materials advers affected some predaceous aquatic inse

Applications of highly effective mater at very low rates have several advanta over other materials which have to be u at higher rates. The problem of resid in crops, toxicity to wildlife and ben

TABLE 6.—Field evaluation of G-30494 against 4th instar larvae of *Culex tarsalis* in a duck club and in breeding ponds

Material and formulation	Toxicant lbs./acre	Avg. no. larvae/dip		Perc cont
		Pre-treatment	24-hour Post-treatment	
		Duck club[a]		
G-30494 EC 2	0.025	7.0	1.3[b]	81
	0.05	2.3	0.9[c]	61
	0.1	9.8	1.7[d]	83
		Ponds		
	0.01	4.7	0.3[e]	94
	0.02	5.0	0.2[e]	9[illegible]
	0.025	2.3	0.0[c]	100
	0.05	13.7	0.4[c]	97
	0.1	2.6	0.0[c]	100
	0.2	2.8	0.0[c]	100
	0.3	3.1	0.0[c]	100

[a] Wind blowing at about 15 mph caused considerable water movement. Plots were separated b 6 ft. buffer zone. Plot size was 1/16-acre.
[b] Second and third instar larvae alive.
[c] Chironomid midge larvae dead, floating on surface 24 hours after treatment.
[d] One dip yielded about 40 first instar larvae alive.
[e] Dytiscid larvae alive.

TABLE 7.—Field evaluation of 4 insecticides against 4th instar larvae of *Culex tarsalis* in breeding ponds

Material and formulation	Toxicant lbs./acre	Avg. no. larvae/dip Pre-treatment	Avg. no. larvae/dip 24-hour Post-treatment	Percent control
tho 5353 EC 2	0.025	7.7	10.8	0
	0.05	10.3	2.3	78
	0.1	13.1	0.9 *	93
	0.2	15.9	0.2 * [a]	99
	0.3	12.1	0.0 * [a]	100
gon EC 4	0.1	5.1	7.2 [b, c]	0
	0.2	5.0	5.5 [b, c]	0
	0.4	10.1	9.9 [c]	2
	0.6	7.6	5.4 [c]	30
-7554 EC 2	0.025	3.3	0.3 [c]	92
	0.05	8.1	0.0 [d]	100
	0.1	7.1	0.0 [d, e]	100
yer 37289 EC 4	0.05	9.7	0.0 [d, f]	100
	0.1	6.1	0.0	100

* Mortality of chironomid midge larvae was observed in these treatments.
[a] Slight mortality of *Gambusia affinis* occurred when exposed for 24 hours after treatment.
[b] No mortality of *G. affinis* when exposed for 24 hours immediately after treatment. The material s observed to be safe to tadpoles at these rates.
[c] All stages of mosquito larvae alive.
[d] Dytiscid larvae observed to be alive.
[e] Water boatmen adults dead. Pollywogs alive, hydrophylids alive, dragonfly nymphs sick and alive.
[f] Mayfly larvae and adult water boatmen dead.

TABLE 8.—Observations on the toxicity of various insecticides to dytiscid beetle larvae (all instars) found in breeding ponds

Material and formulation	Toxicant lbs./acre	Avg. no. larvae/dip Pre-treatment	Avg. no. larvae/dip Post-treatment	Percent reduction
tho 5353 EC 2	0.025	0.9	0.9	0
	0.05	0.4	0.7	0
	0.1	0.7	0.8	0
	0.2	0.8	0.4	50
	0.3	1.3	0.6 [a]	54
-7587 EC 2	0.01	1.1	0.9 [b]	18
	0.025	1.5	1.5	0
	0.05	0.6	0.5	17
-7438 EC 2	0.01	0.5	0.7 [a]	0
	0.025	1.0	0.7	30
30494 EC 2	0.025	1.3	1.3	0
	0.05	1.5	0.4	67
	0.10	0.9	0.0	100
	0.20	1.1	0.0	100
rathion EC 2	0.005	1.2	4.0	0
	0.01	0.7	0.6	14
	0.025	0.6	0.7	0
	0.05	1.6	1.3	19
	0.10	0.8	0.7	12
gon EC 4	0.1	0.5	0.5	0
	0.2	0.6	0.7 [a]	0

[a] Adult diving beetles alive.
[b] Mayflies, Belastomatids alive.

cial arthropods, and persistent pollution of treated water can be greatly diminished by applying larvicides at low rates. In addition, the treatment cost for highly effective larvicides is deemed to be lower than for materials having low or intermediate degrees of biological activity.

Toxicity to Dytiscid Beetle Larvae. All stages of predaceous dytiscid beetle larvae were observed in some of the ponds. Pre-treatment and post-treatment counts were recorded in treatments where the beetle larvae could be detected by the sampling method.

Counts of beetle larvae prior and 24 hours after treatment were made in the plots treated with ortho 5353, SD-7587, SD-7438, G-30494, parathion and Cygon (Table 8). Ortho 5353 at the two higher rates produced some reduction in the beetle larval population. SD-7587 and SD-7438 for all practical purposes were innocuous. G-30494 at 0.1 and 0.2 lb./acre produced complete kill of the predaceous larvae. Due to the low counts of the larvae, it is believed that the other materials at the rates used produced no marked reduction in the larval population.

There was no apparent relationship between the predator population and mosquito larvae. It is not known as to what extent these predaceous larvae play a role in suppressing mosquito larval populations under natural conditions. From gross observations made in these studies it seems that the dytiscid larvae are not suppressi the mosquito larval populations sufficien to eliminate the need for administeri chemical control measures.

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