LABORATORY AND FIELD EVALUATION OF NEW MOSQUITO LARVICIDES [1]

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Mosquito larvicidal programs constitute a major portion of the total effort directed toward the suppression of vector and pest mosquitoes in California. Newer synthetic insecticides play an important role in the overall mosquito suppression programs. However, development of mosquito resistance to the various larvicides presently in use indicates that continuing research on their optimum utilization and on the discovery and development of new materials is essential with regard to the future usage of synthetic materials.

A variety of new compounds, however, have become recently available for evaluation which exhibit considerable activity against parathion resistant strains of *Aedes nigromaculis* (Ludlow). The current studies are a continuation of earlier ones (Mulla *et al.* 1960, 1961, 1962, 1964), which are aimed at the evaluation of biological efficacy of new compounds against larvae of several species of mosquitoes. It is expected that the list of safe and more specific compounds can thus be increased for future mosquito control programs in California and elsewhere. Materials having negative correlation or manifesting higher biological activity against resistant strains than against susceptible populations were specifically sought. At least one material of this type was singled out during these studies for further work. The chemical descriptions of compounds evaluated for biological activity in the laboratory are presented in table 1.

METHOD AND MATERIALS

Laboratory. For determining the relative biological effectiveness of experimental compounds, procedures employed in earlier studies (Mulla *et al.* 1960, 1961, 1962, 1964) were closely followed. One percent stock solution (w/v) of the technical grade of each compound in acetone was prepared. Serial dilutions were made as needed. All solutions were kept in a refrigerator when not in use.

Biological activity was determined by adding aliquots (1 ml. or less) of the proper strength solution to 100 ml. of tap water (pH 8–8.5) in which 20–25 4th instar larvae of mosquitoes were placed. Larvae of two species were used. The laboratory colony of *Culex pipiens quinquefasciatus* Say from Bakersfield, California (established in 1946), is a strain which has had no known exposure to organochlorine, organophosphate and other synthetic insecticides. This strain is considered to be susceptible to these materials. The *Anopheles albimanus* Wiedemann strain from Panama and obtained from Shell Development Company, Modesto, California, is one which is resistant to dieldrin and other organochlorine insecticides (Georghiou and Metcalf 1963, Metcalf and Georghiou 1962).

The larvae were exposed for 24 hours; moribund larvae not able to rise to the surface on touch were counted as dead. The final concentrations were expressed in p.p.m. (parts per million). Each concentration was run in duplicates

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or triplicates and each material was run on 2 or 3 different days. The average percent mortality was plotted against log concentration (in p.p.m.) on a probit log paper and the LC_{50} and LC_{90} values in p.p.m. were determined by inspection.

Tests against adult mosquitoes were made in the same manner as in other studies (Georghiou and Metcalf 1961). Briefly, this consisted of evaporating 1 ml. of proper strength w/v acetone solutions on 9-cm Whatman filter paper and exposing the adult mosquitoes for one hour to the treated paper, rolled inside a shell vial. After an hour the mosquitoes were removed from the shell vial and held in uncontaminated containers for 24 hours when the mortality was read. Average values of replicated treatments are expressed as μg./cm.^{2} of deposit. Percent mortality is plotted against log dosage on a probit log paper and the LD_{50} and LD_{90} are read off the established dosage response line.

Field. Evaluation studies in the field were conducted against *Culex tarsalis* Coquillett in experimental breeding ponds (Mulla *et al.* 1964). Ponds utilized were either 1/16 or 1/32 acre, and filled with canal water to a depth of 8–10 inches. Emulsifiable concentrate or wettable powder formulations were used in preparing dilute aqueous sprays. The sprays were applied at the rate of 8 gallons per acre by means of small hand sprayers, using 15–20 lb./in.^{2} pressure.

Pre-treatment and 24-hour post-treatment larval counts were taken. Fourth instar larval population was estimated by taking 10–20 dips per pond. The average number of larvae per dip before and 24 hours after treatment was determined. Percent control was assessed from the average post-treatment and pre-treatment counts.

Observations were also made on non-target organisms. The presence of each one of these organisms in each dip before

TABLE 1.—Chemical description of compounds studied.

Compound	Chemical description
Abate	*O,O,O′,O′*-tetramethyl *O,O′*-thiodi-*p*-phenylene phosphorothioate
Bay 64995	4,4′-Bis (*O,O*-dimethylthiophosphoryl-oxy) diphenyldisulfide
Dursban	*O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate
GC–6506	*O,O,*-dimethyl-*O*-(4-methylmercaptophenyl) phosphate
GC–9160	Δ-(5-hydroxy-1,2,3,4,6,7,8,9,10,10-decachloro-pentacyclo $[5.3.0^{2,6}.0^{3,9}.0^{4,8}]$decyl) ethyl levulinate
GC–9879	α-(diethoxyphosphinothioylthio)-γ-butyrolactone
GS–12968	*O,O*-dimethyl-S-[5-ethoxy-1,3,4-thiadiazol-2-(3H)-on-3yl-methyl]-dithiophosphate
GS–13005	*O,O*-dimethyl-S-[5-methoxy-1,3,4-thiadiazol-2-(3H)-on-3yl-methyl]-dithiophospate
N–3794	*O*-methyl-*S*-phenyl-ethylphosphonodithioate
N–4328	*O*-methyl-*S*-(P-toluyl)-ethylphosphonodithioate
N–4330	*O*-methyl-*S*-(P-tert-butylphenyl)-ethylphosphonodithioate
NIA–10242	2,3-dihydro-2,2-dimethylbenzofuranyl-7 N-methylcarbamate
Ronnel	*O,O*-dimethyl *O*-2,4,5-trichlorophenyl phosphorothioate
RP–11974	*O,O*-diethyldithiophosphorylmethyl-3-chloro-6 benzoxasolone
SD–8211	Phosphoric acid, 2-chloro-1-(2,5-dichlorophenyl) vinyl dimethyl ester
SD–8447	Phosphoric acid, 2-chloro-1-(2,4,5-trichlorophenyl) vinyl dimethyl ester
SD–8530	3,4,5-trimethylphenyl *N*-methylcarbamate
SD–8803	Phosphorothioic acid, *O*-[2-chloro-1-(2,4-dichlorophenyl) vinyl] *O,O*-diethyl ester
SD–9020	Phosphorothioic acid, *O*-[2-chloro-1-(2,4-dichlorophenyl) vinyl] *O,O*-dimethyl ester
SD–9129	3-(dimethoxyphosphinyloxy)-N-methylcrotonamide
SD–9321	Phosphorothioic acid, *O*-1-(2-bromo-4-chlorophenyl)-2-chlorovinyl *O,O*-diethyl ester
UC–20047	3-chloro-6-cyano-2-norbornanone *O* (-methyl-carbamoyl) oxime
UC–21149	2-methyl-2-[(methylthio) propionaldehyde O-methylcarbamoyl] oxime
UC–21427	2,4-dinitro-6-tert-butylphenyl carbonate
UC–22463	3,4-dichlorobenzyl methylcarbamate

and 24 hours after treatment was noted. Actual numbers of these organisms were not recorded. Disappearance or obvious die-off of these organisms when observed 24 hours after treatment was considered to be a good indication of acute toxic effects of a given treatment. Lack of such observations, however, is no indication of the safety of a given treatment. In case of drastic acute toxicity, marked changes in the prevalence of the non-target organisms were recorded. This information is made part of the tables presenting the field evaluation data for mosquitoes.

RESULTS AND DISCUSSION

Laboratory. Dosage response lines of some of the compounds evaluated against *Culex pipiens quinquefasciatus* are presented in Fig. 1. It is apparent from these data that Dursban, SD-9020, Bay 64995, GS-13005 and SD-8803 manifested a high degree of biological activity against this susceptible strain. Several other compounds, namely, SD-9321, ronnel, GS-12968, NIA-10242 and SD-8211, also showed a good degree of biological activity against this species. The remaining materials are considered to have low levels of activity, but one material (GC-9160) proved to be exceptionally active against field populations of *Culex tarsalis* and *Aedes nigromaculis,* both species having acquired resistance to some chlorinated hydrocarbon and phosphate insecticides. This material apparently shows negative correlation with resistance (see below).

The relative biological activity of some of the compounds tested varied a great deal against the larvae of *Anopheles albimanus* and *Culex p. quinquefasciatus* (Table 2). Abate and Bay 64995, closely

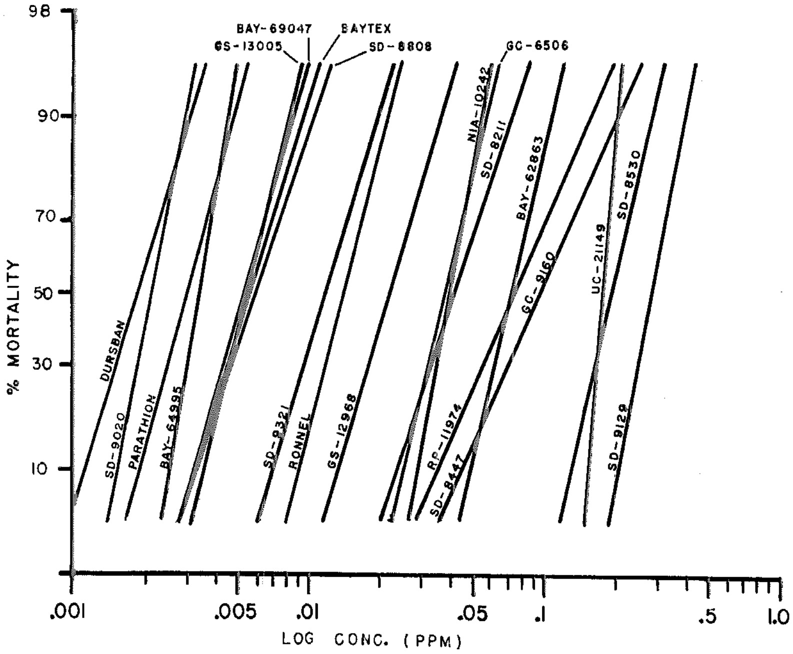

FIG. 1.—Dosage response lines of new insecticides tested against 4th instar larvae of a susceptible laboratory strain of *Culex pipiens quinquefasciatus.* Parathion and Baytex lines are included for comparison.

TABLE 2.—Biological activity of new insecticides against adults and larvae of mosquitoes in the laboratory

		Anopheles albimanus				*Culex quinquefasciatus*			
		Adults (μg./cm.^{2})		Larvae (PPM)		Adults (μg./cm.^{2})		Larvae (PPM)	
Compound	OMS no.	LD_{50}	LD_{90}	LC_{50}	LC_{90}	LD_{50}	LD_{90}	LC_{50}	LC_{90}
Abate	786	>16.0	....	0.015	0.045	>16.0	....	0.001	0.002
Bay 64995	1012	>16.0	....	0.47	>1.0	>16.0	....	0.005	0.007
Bay 69047	1066	>16.0	....	0.066	0.11	>16.0	....	0.02	0.037
Bay 62863	1094	2.3	3.0	0.34	0.48	5.2	12.5	0.19	0.24
Dursban	971	0.18	0.38	0.006	0.016	0.10	0.19	0.003	0.005
GC-9160	1053	>16.0	....	0.033	0.14	>16.0	....	0.046	0.072
GC-6506	1096	>16.0	....	0.21	0.31	>16.0	....	0.039	0.049
GC-9879	1054	>16.0	....	>1.0	....	>16.0	....	>1.0	....
GS-12968	958	>16.0	....	0.112	0.24	>16.0	....	0.02	0.04
GS-13005	844	4.75	12.0	0.068	0.15	>16.0	....	0.006	0.008
Malathion	1	4.3	6.6	0.12	0.20	6.2	9.5	0.08	0.10
N-3794	1005	1.75	2.9	0.62	0.87	5.4	11.0	0.365	0.56
N-4328	1006	1.4	2.1	0.21	0.27	2.7	4.6	0.22	0.33
N-4330	1007	>16.0	....	>1.0	....	>16.0	....	>1.0	....
NIA-10242	864	1.02	1.6	0.16	0.23	2.9	6.3	0.054	0.072
Parathion	17	0.46	0.78	0.005	0.012	1.2	2.0	0.004	0.008
Ronnel	123	2.8	3.7	0.066	0.11	0.61	1.5	0.030	0.044
RP-11974	....	....	....	....	....	....	....	0.07	0.15
SD-8211	711	>16.0	....	0.24	0.42	>16.0	....	0.13	0.21
SD-8447	595	>16.0	....	>1.0	....	>16.0	....	0.28	0.38
SD-8530	597	1.1	2.0	0.50	0.80	12.0	23.0	0.3	0.37
SD-8803	774	4.5	7.0	0.0094	0.013	5.0	7.0	0.004	0.008
SD-9020	775	0.70	2.1	0.0077	0.014	0.16	0.26	0.0021	0.003
SD-9129	834	>16.0	....	0.68	1.0	>16.0	....	0.49	0.60
SD-9321	777	4.05	7.3	0.0024	0.0067	4.8	10.0	0.01	0.02
UC-20047	....	>16.0	....	>1.0	....	>16.0	....	....	>1.0
UC-21149	....	0.3	0.9	0.14	0.18	0.28	0.46	0.18	0.22
UC-21427	1057	8.9	18.1	0.47	>1.0	>16.0	....	>1.0	....
UC-22463	1058	>16	....	>1.0	....	>16.0	....	....	>1.0

related compounds, showed high activity against the larvae of both species, but were not active against the adults. The latter compound was much less effective against the larvae of *A. albimanus* than the larvae of *C. p. quinquefasciatus.*

Abate was 14 times and Bay 64995 was 23 times as active (at the LC_{90} level) against the larvae of the latter species as against *Anopheles freeborni* Aitken (Lewallen—personal communication). Dursban proved to be effective against larvae and adults of both species. GC–9160 showed a moderate level of activity against larvae of both species, but was not active against the adults, while GC–9879 showed little activity against both species. GS–12968 and GS–13005 were more active against the larvae of *C. p. quinquefasciatus* than that of *A. albimanus,* but they were inactive against the adults of both species. N–3794, N–4328 and N–4330 showed little or no activity against larvae of both species.

NIA–10242, a carbamate insecticide, was slightly more active against larvae of *C. p. quinquefasciatus* than *A. albimanus* but the reverse was true for the adults of the two species. Ronnel was slightly more active against the former species.

SD–8211, SD–8447, SD–9129 and SD–9321 were quite ineffective against the adults of both species. Activity of the former three compounds against larvae of both species was mediocre, but SD–9321 proved more active against larvae of *A. albimanus* than that of *C. p. quinquefasciatus.* SD–8530 was more active against adults of the former species and had low activity against the larvae of both species.

SD–8803 and SD–9020 showed high activity against larvae of both species, but the latter compound was more effective against the adults of both species, having higher activity against adults of *C. p. quinquefasciatus.*

Among the newer carbamates, UC–21149 showed good activity against adults of both species and mediocre activity against larvae. The remaining three compounds evaluated manifested little if any activity against the larvae as well as adults of both species.

Field. Some of the compounds evaluated in the laboratory were subjected to field evaluation against *Culex tarsalis.* The carbamate insecticides showing good to mediocre activity in the laboratory were evaluated in the field (Table 3). NIA–10242 gave complete kill of 4th instar larvae at 0.25 lb./acre. At the larvicidal rates this compound was toxic to mayfly naiads. Diving beetle adults and possibly larvae were not seriously reduced at the administered effective larvicidal rates. UC–21149 tested as emulsion concentrate did not show promise but a 10 percent granular (corncob grit) formulation proved effective at 0.25 lb./acre. The emulsifiable concentrate formulation apparently was unstable thus yielding poor results. This carbamate also proved to be toxic to mayfly naiads, although diving beetle larvae and adults were not seriously affected.

Dursban (Dow Chemical Co.), a phosphate insecticide, proved highly effective in field tests (see Table 3). Complete control of larvae was obtained at 0.005 lb./acre. At this rate this compound did not manifest acute toxicity to corixids, and diving beetle larvae and adults. No information on the kill of mayfly naiads at this rate could be obtained. Mayfly naiads, however, were noticeably affected at 0.01 and 0.05 lb./acre rates.

In contrast to laboratory tests against *A. albimanus* and *C. p. quinquefasciatus,* GC–9160 (a chlorinated hydrocarbon) showed exceptionally high level of activity against larvae of *C. tarsalis* under field conditions.

Complete control was obtained at the rate of 0.1 lb./acre. At this rate some reduction in the diving beetle larval and adult populations was noticed. Mortality of these non-target organisms at the higher rate of 0.25 lb./acre was more severe. GC–9160, when tested against parathion resistant field strains of *Aedes nigromaculis,* showed negative correlation (Lewallen 1965 personal communication). This material was eight times as effective against the resistant strain of this species as it was against a susceptible strain.

SD–9020 and SD–8803 both proved to

TABLE 3.—Field evaluation of new mosquito larvicides against *Culex tarsalis* in field ponds.

Material and formulation (%)	Tox. lb./acre	Av. no. larvae/dip Pre-tr.	Av. no. larvae/dip Post-tr.	Percent cont.	Non-target organisms[a]
NIA-10242 WP-50	0.01	1.6	1.7	0	
	0.05	4.3	2.1	51	DBA, DF, P alive
	0.1	7.6	1.0	87	DBA, DBL, P alive, MF dead
	0.25	9.7	0.0	100	DBA, P alive, MF dead
UC-21149 EC-12	0.05	6.2	7.8	0	DBA, DBL, MF, P, TPS alive
	0.1	6.3	7.8	0	DBA, DBL, P, TPS alive
	0.25	10.6	5.6	47	DBL, FNS, MF, P alive
UC-21149 GR-10	0.05	5.1	6.5	0	DBL, DF, MF alive
	0.1	5.7	1.1	80	DBA, DBL, MF alive, MF dead
	0.25	4.4	0.0	100	DBA, DBL, P alive, MF dead
Dursban EC-40	0.001	16.9	6.8	60	C, DBL, MF, P alive
	0.002	13.8	2.4	83	C, DBA, DBL, P alive
	0.005	7.2	0.0	100	C, DBA, DBL, P alive
	0.01	6.6	0.0	100	DBA, DBL alive, C, DBA, MF dead
	0.05	8.0	0.0	100	P alive, C, MF dead
GC-9160 EC-25	0.01	3.3	2.5	22	DBA, DBL, MF, P alive
	0.05	3.0	0.5	83	DBA, DBL, MF, P, TP alive
	0.1	4.7	0.0	100	DBA, DBL, P alive; some DBA, DBL dead
	0.25	3.0	0.0	100	DBA, DBL dead
SD-9020 EC-25	0.005	6.6	1.0	87	DBA, DBL, P alive; some MF dead
	0.01	4.0	0.0	100	C, Cr, DBA, DBL, P alive; some MF, DBA dead
	0.05	5.0	0.0	100	DBL, TPS, P alive, MF, DBL, FNS dead
SD-8803 EC-25	0.001	5.0	7.0	0	
	0.005	5.7	1.3	77	DBA, DBL, MF, P alive
	0.01	8.0	0.0	100	DBA, DBL, MF, P alive, MF dead
	0.05	9.0	0.0	100	C, DBL, P alive, DBA dead
Ronnel EC-25	0.01	2.3	0.3	86	
	0.05	1.5	0.1	92	DBL, DF alive
	0.1	4.0	0.0	100	DBL, P alive
	0.5	5.0	0.0	100	DBL dead, P alive
GS-13005 EC-40	0.01	10.0	4.0	60	DBL, P alive
	0.02	10.4	0.0	100	
	0.05	10.0	0.0	100	DBA, DBL, TPS, FNS, CL, MF dead
GS-12968 EC-40	0.01	2.9	2.1	26	MF, P, Y alive
	0.05	6.0	0.4	94	DBL, P alive
	0.10	3.0	0.0	100	DBA, TPS, P alive, CL, DBL dead
Bay 64995 EC-40	0.005	6.2	3.0	55	DBA, DBL, DF, MF alive
	0.01	4.3	0.1	69	DBA, DBL, P alive
	0.05	4.0	0.0	100	DBA, DBL alive and dead
SD-8211 EC-25	0.01	20.5	8.7	58	DBA, DBL, MF, P alive
	0.05	15.6	1.8	88	C, DBL, P alive, MF dead
	0.10	8.1	0.0	100	C, DBA, DBL, P alive, MF dead
	0.20	5.5	0.0	100	DBL alive, MF dead
SD-9129 EC-32	0.05	1.7	2.6	0	C, DBL, MF, P alive
	0.10	3.5	3.2	9	

[a] Observations on non-target animals were made prior to and 24 hours after treatment. Survival or death is indicated 24 hours after treatment.

C—Corixids, CL—Chironomid midge larvae, DBA—Diving beetle adults, DBL—Diving beetle larvae, DF—Dragonfly naiads, FNS—Finger nail shrimp, MF—Mayfly naiads, P—Pupae of mosquitoes, TP—Tadpoles of toads, TPS—Tadpole shrimp, Y—Young mosquito larvae.

be effective at 0.01 lb./acre. These two compounds at this and a higher rate of 0.05 lb./acre, adversely affected some non-target organisms (see Table 3). Ronnel proved effective at 0.1 lb./acre and produced mortality of diving beetle larvae at the higher rate of 0.5 lb./acre. At the larvicidal rate of 0.1 lb./acre mortality of these organisms was not significant.

GS–13005 was more effective than GS–12968. Both compounds at slightly higher rates (0.05 lb./acre and 0.1 lb./acre respectively) caused mortality of some non-target organisms (see Table 3). They both proved to be highly effective against larvae of certain chironomid midges.

Bay 64955 proved effective at 0.05 lb./acre rate, and produced some mortality in diving beetle larvae and adults. SD–8211 was effective at 0.1 lb./acre and caused mortality of mayfly naiads, but not other non-target organisms which were observed. SD–9129 at the rates tested (see Table 3) proved ineffective.

During the course of these studies, it was observed that mayfly naiads are highly sensitive to insecticidal treatments. Other non-target organisms observed were not nearly so affected as these ephemeropteran species.

From the field evaluation data, several new materials offer a good deal of promise for mosquito suppression in their breeding habitats. Dursban with its low mammalian toxicity offers excellent possibilities. SD–9020, SD–8803, SD–8211, GS–13005, GS–12968 and Bay 64995 are also very promising. GC–9160 has negative correlation with resistance and its potentialities against resistant field strains should be further explored.

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