EVALUATION OF NEW MOSQUITO LARVICIDES, WITH NOTES ON RESISTANT STRAINS[1]

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INTRODUCTION. Larvicidal treatments with synthetic organic compounds comprise a major portion of current mosquito control programs in California. Recent spread of acquired resistance in *Aedes nigromaculis* to the most effective and commonly used materials, namely, parathion and methyl parathion (Lewallen 1961, Lewallen and Nicholson 1959) has caused concern among the abatement agencies in California. A need for substitue materials or for measures other than chemical agents for the abatement of pest and vector mosquito species is strongly felt through the central and southern San Joaquin Valley where resistance in *Aedes nigromaculis* to organophosphate insecticides has become apparent.

At the present time the most practical and quick answer to the problem of resistance appears to be the screening and development of substitute materials. Compounds with biological activity approximating that of parathion or methyl parathion (as tested against susceptible populations) but with more favorable mammalian, fish and avian toxicity are sought. Greater safety factors to non-target invertebrate species are also considered in the development of newer mosquito larvicides. The current studies were aimed at finding and developing materials which would have these desirable attributes.

METHODS AND MATERIALS. Laboratory evaluation of experimental compounds was accomplished against a susceptible strain of *Culex pipiens quinquefasciatus*. Testing procedure similar to the one described earlier (Mulla *et al.*, 1960, 1961, 1962) was followed. This procedure is discussed briefly below.

Small quantities of the technical materials of the experimental compounds were dissolved in acetone. Proper strength solutions were obtained by means of serial dilutions. For determining the biological activity, 25 fourth instar larvae were placed in 100 ml. tap water (pH 8-8.5) in 6-oz. wax paper cups. Aliquots of the acetone solutions (not more than 1 ml./cup) were added to the cups. Each cup was replicated 3 times and the mortality was read 24 hours after start of the exposure period. The cups were placed in a room with a temperature of 75°–80° F. Controls were concurrently run with each test. The mean percent larval mortality was plotted on probit log paper against concentration, and the LC_{50} and LC_{90} values were read off the dosage response lines plotted.

Fourth instar larvae of *Culex tarsalis* from an area where all the field evaluation was accomplished, were brought into the laboratory. These were exposed to standard and new insecticides in the same manner as explained above. No failures of control with parathion have been reported from this area, but the susceptibility level of the population was ascertained for future reference.

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Similarly, larvae of *Aedes nigromaculis* from two areas in southern San Joaquin Valley (Blaise pasture and Baker alfalfa field) were transported into the laboratory and tested for susceptibility within 3 hours after collection. Several control failures using parathion were reported for both locations during the 1962 and 1963 seasons. The larvae were tested for susceptibility in the same manner as described above.

Only limited tests were made on the susceptibility of field collected *Culex tarsalis* and *Aedes nigromaculis.* But the data, although not extensive, show certain trends in the susceptibility levels and therefore are included here.

In actual field evaluation work against *Culex tarsalis,* 1/16 acre or 1/32 acre field plots were sprayed with the toxicants. Sprays were prepared from the emulsifiable concentrate formulations and were applied at the rate of 8 gallons of spray per acre. Pre-treatment and post-treatment counts were taken by 10–20 dips per plot. Only 4th instar larvae were counted. Post-treatment counts were taken 24 hours after treatment. The percent control was obtained from the two counts.

Also, observations were made on the effects of the test compounds on other organisms. Such observations, although not definitive, are useful and are there-

TABLE 1.—Chemical description of compounds studied.[a]

Compound	Chemical description
AC-38023 (Famophos®)	*O, o*-dimethyl *o-p*-(dimethylsulfamoyl) phenylphosphorothioate
AC-38906	2-(methylcarbamoyloximino)-1, 3-dithiolane
AC-43064	2-(diethoxyphosphinothioylimino)-1, 3-dithiolane
AC-43356	2-(dimethoxyphosphinothioylimino)-1, 3-dithiolane
AC-47031	2-(diethoxyphosphinylimino)-1, 3-dithiolane
AC-47071	2-(diethoxyphosphinothioylimino)-4-methyl-1, 3-dithiolane
AC-47470	2-(diethoxyphosphinylimino)-4-methyl-1, 3-dithiolane
AC-47548	2-methylcarbamoyloximino)-4-methyl-1, 3-oxathiolane
AC-47772	2-(dimethoxyphosphinylimino)-4-methyl-1, 3-dithiolane
AC-47826	2-(diethoxyphosphinylimino)-4-ethyl-1, 3-dithiolane
AC-47938	2-(diethoxyphosphinylimino)-4, 5-dimethyl-1, 3-dithiolane
Bayer 73	2-aminoethanol salt of 2′, 5-dichloro-4′-nitrosalicylanilide
Bayer 37342	*O*-ethyl *o*-2-ethylthio-4-methyl-6-pyrimidyl ethylphosphonothionate
Bayer 44646	3-methyl-4-dimethylaminophenyl N-methylcarbamate
Bayer 46676	*O*-ethyl *o*-2-ethylthio-4-methyl-6-pyrimidyl ethylphosphonothionate
Bayer 47940	*O, o*-dimethyl-*o*-(3-Chloro-4-Cyanophenyl)-thionophosphate
Bayer 52957	*O, o*-diethyl *o*-(5-chlorobenzisoxazoly(3)-phosphorothioate
Baytex®	*O*, o-dimethyl o-[4-(methylthio)-*m*-tolyl] phosphorothioate
Bomyl® (GC-3707)	Dimethyl 1, 3-(dicarbomethoxy)-1-propen-2-ylphosphate
Cela S-1942 (OMS-658)	*O, o*-dimethyl *o*-(2, 5-dichloro-4-bromophenyl) thionophosphate
Cela S-2225 (OMS-659)	*O, o*-diethyl *o*-(2, 5-dichloro-4-bromophenyl) thionophosphate
Dimetilan®	2-dimethylcarbamyl-3-methylpyrazolyl-(5)-dimethylcarbamate
Endosulfan (Thiodan®)	6, 7, 8, 9, 10, 10-hexachloro-1, 5, 5a, 6, 9, 9a-hexahydro-6, 9-methano-2, 4, 3-benzodioxathiepin 3-oxide
Endothion	*O, o*-dimethyl S-[(5-methoxy-4-oxo-4H-pyran-2-yl) methyl] phosphorothioate
Isolan®	Dimethyl 5-(1-isopropyl-3-methylpyrazolyl) carbamate
Mirex® (GC-1283)	Dodecachlorooctahydro-1, 3, 4-metheno-2*H*-cyclobuta(cd) pentalene
Morocide®	2-*sec*-butyl-4, 6-dinitrophenyl 3-methyl-2-butenoate
N-2788	*O*-ethyl-*S-p*-tolyl-ethylphosphonodithioate
NIA-9205	N-methyl-5-(diethoxyphosphinothioylthio)-3- thiapentanamide
N-2789	*O*-ethyl-*S-o*-tolyl-ethylphosphonodithioate
Ortho 5305	3-*sec*-butylphenyl-N-methylcarbamate
Pyramat®	2-*n*-propyl-4-methylpyrimidyl(6) dimethylcarbamate
Sumithion®	*O, o*-dimethyl *o*-(3-methyl-4-nitrophenyl) phosphorothioate
UC-8305	P-chloro-2, 4-dioxa-5-methyl-P-thiono-3-phosphabicyclo-(4.4.0) decane

[a] American Cyanamid compounds are interchangeably coded as AC-, or CL- compounds.

fore added to the tables in the form of footnotes.

RESULTS AND DISCUSSION

LABORATORY. A large number of experimental compounds (Table 2) were evaluated for their biological activity against 4th instar larvae from a susceptible colony of *Culex p. quinquefasciatus* (Fig. 1 and see Table 2). Eight materials, namely AC-52160, SD-9020, SD-9320, CL-43913, Bayer 47940, SD-8803, AC-47921 and Bayer 52957, manifested an exceptionally high degree of biological activity. These materials approximated or exceeded the activity of parathion and had steep dosage response lines. The dosage response lines of SD-9020, SD-9320, SD-8803 and SD-9321 are not included. The biological activity of these 4 compounds can be noted in Table 2.

Based on the LC_{50} and LC_{90} levels, 14 materials were more active than DDT and 21 materials showed greater activity than that of malathion (see Table 2). The remaining materials showed activity lower than that of malathion. Thirteen materials had very low activity ($LC_{50}>0.5$ p.p.m.) and the dosage response lines of these materials were not determined.

FIELD. *Culex tarsalis* larvae collected from and in the vicinity of experimental ponds (Mulla and Isaak 1961) were susceptible to parathion and other organophosphate insecticides (Fig. 2). They were tolerant to DDT. The susceptibility level of this field strain to parathion and other organophosphates was comparable to the susceptibility level of the sus-

TABLE 2.—Effectiveness of various insecticides against 4th instar larvae of *Culex p. quinquefasciatus* in the laboratory.

Materials	LC_{50} (p.p.m.)	LC_{90} (p.p.m.)	Materials	LC_{50} (p.p.m.)	LC_{90} (p.p.m.)
AC-52160	0.0014	0.0017	AC-43064	0.12	0.25
SD-9020	0.0026	0.0036	AC-47470	0.17	0.30
SD-9320	0.0032	0.0049	AC-47031	0.19	0.34
CL-43913	0.0027	0.005	AC-47826	0.19	0.34
Bayer 47940	0.0045	0.007	AC-47938	0.20	0.33
SD-8803	0.004	0.0078	AC-38906	0.36	0.60
Parathion	0.0045	0.0082	AC-47548	0.45	0.70
AC-47921	0.005	0.0085	AC-47772	0.70	1.20
Bayer 52957	0.005	0.0085	AC-43356	>0.5	..
SD-9321	0.01	0.019	Bayer 73	>1.0	..
Cela S-1942 (OMS-658)	0.01	0.02	Bayer 44646	>1.0	..
N-2404 (OMS-405)	0.018	0.03	Bomyl (GC-3707)	>0.5	..
Famophos (CL-38023)	0.016	0.04	Dimetilan	>0.5	..
UC-8305	0.025	0.04	Endothion	>0.5	..
Cela S-2225 (OMS-659)	0.03	0.05	Isolan	>0.5	..
DDT	0.035	0.053	Mirex (GC-1283)	>0.5	..
N-2788	0.04	0.07	Morocide	>0.5	..
Bayer 46676	0.04	0.07	Nia-9205	>2.0	..
Ortho 5305	0.04	0.07	Pyramat	>0.5	..
N-2789	0.032	0.075	Imidazole	>1.0	..
AC-47071	0.035	0.076	SD-7727	>1.0	..
N-2790	0.054	0.11			
Bayer 37342 (OMS-362)	0.072	0.11			
Malathion	0.068	0.12			
Endosulfan (Thiodan)	0.066	0.14			

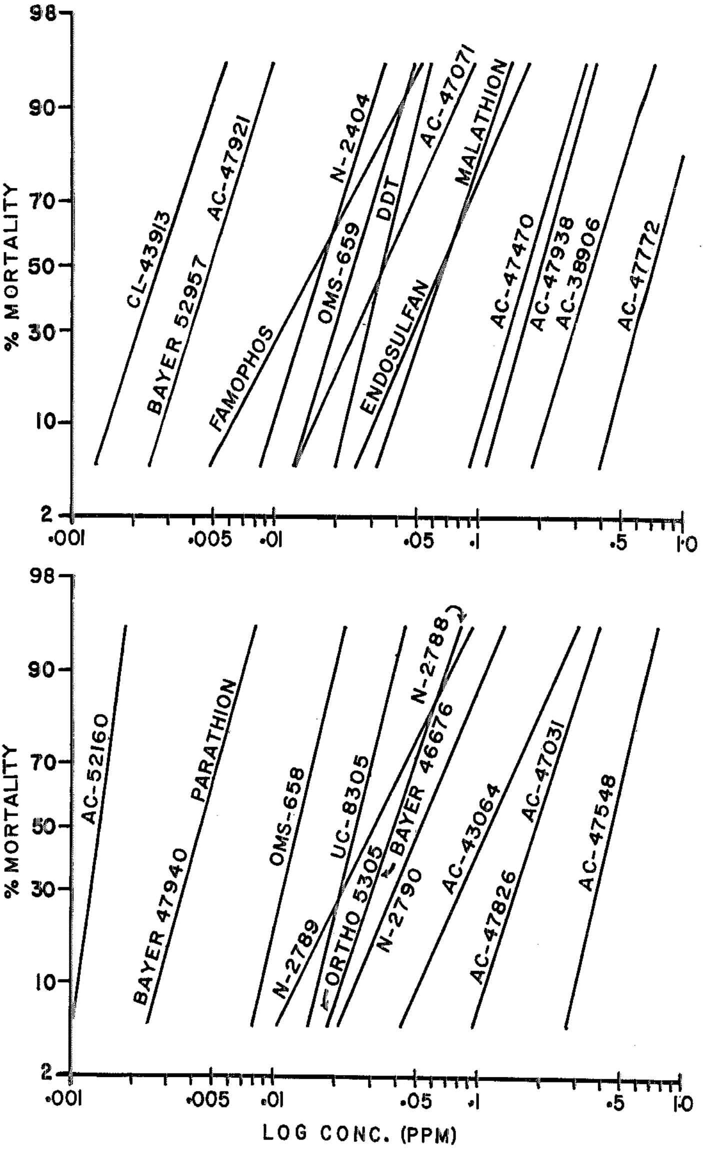

FIG. 1.—Dosage response lines of new insecticides against 4th instar larvae of a susceptible laboratory strain of *Culex p. quinquefasciatus*. DDT, malathion and parathion lines are included for comparison.

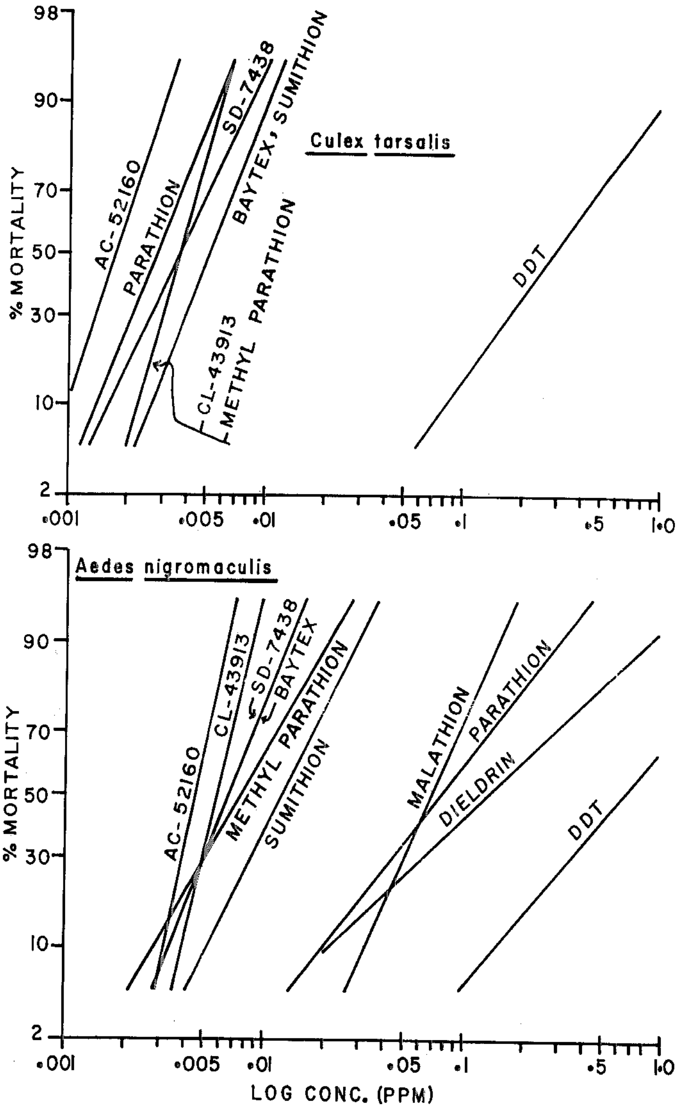

FIG. 2.—Susceptibility levels of field collected 4th instar larvae of *Culex tarsalis* and *Aedes nigromaculis* to parathion and other insecticides. All field plot evaluation tests were conducted against this *Culex tarsalis* strain.

TABLE 3.—Susceptibility of field strains of *Culex tarsalis* and *Aedes nigromaculus* from southern San Joaquin Valley to some common and new insecticides.[a]

Culex tarsalis [b]			*Aedes nigromaculis* [c]		
	p.p.m.			p.p.m.	
Materials	LC_{50}	LC_{90}	Materials	LC_{50}	LC_{90}
AC-52160	0.0018	0.003	AC-52160	0.0045	0.0065
Parathion	0.003	0.0056	CL-43913	0.0058	0.009
CL-43913	0.0036	0.007	SD-7438	0.0068	0.013
Methyl parathion	0.0036	0.006	Baytex	0.0068	0.015
SD-7438	0.0037	0.008	Methyl parathion	0.008	0.03
Sumithion	0.005	0.009	Sumithion	0.0125	0.03
Baytex	0.0056	0.011	Malathion	0.07	0.15
DDT	0.30	>1.0	Parathion	0.08	0.30
			Dieldrin	0.13	1.0
			DDT	0.65	3.0

[a] Larvae from the field were transported into the laboratory and 4th instar larvae were utilized for the determination of susceptibility levels. Tests run during August to October 1963.

[b] A strain from experimental ponds in the vicinity of which parathion has been used for 6 or 7 years for mosquito control. Two runs about a month apart were made on these larvae.

[c] Larvae collected from Blaise pasture and Baker alfalfa field. The data from these two areas although slightly different were pooled together and averaged. Difficulty in achieving control with parathion was experienced during the 1963 season.

ceptible laboratory colony of *Culex p. quinquefasciatus* (Table 3).

Aedes nigromaculis larvae collected from Blaise pasture and Baker alfalfa field where standard parathion treatments (0.1 lb. active/acre) had failed to give adequate control, showed considerable tolerance to parathion, dieldrin and DDT (see Fig. 2 and Table 3). In these areas, parathion has been used for over 8 years and during the current season methyl parathion was used to control the larvae. Other organophosphate materials such as AC-52160, CL-43913, SD-7438, Baytex®, methyl parathion and Sumithion®, were not as effective against this strain as they

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

Insecticide and formulation	Dosage lb./acre	Avg. no. larvae/dip Pre-treatment	Avg. no. larvae/dip Post-treatment	% control
AC-47921 EC2	0.01	15.0	2.2[a]	85
	0.025	11.0	1.0[b]	91
	0.05	15.0	0.1	99
N-2790 EC4	0.05	2.5	0.4	84
	0.1	4.0	0.1	98
	0.2	8.2	0.0[b]	100
Bayer 46676 EC2	0.05	10.5	7.3	30
	0.1	10.7	2.0	80
	0.2	13.5	0.1[b]	99
Bayer 44646 EC1.5	0.025	2.0	2.0	0
	0.40	10.0	8.0[c]	20
Ortho 5305 EC2	0.05	2.0	1.5	25
	0.10	5.0	0.1	99
	0.20	4.0	0.0[c]	100

[a] Mayfly naiads alive.

[b] Diving beetle larvae and adults alive.

[c] Diving beetle larvae and adults and tadpoles of *Bufo boreas* were alive in these treatments.

were against the field collected *Culex tarsalis,* and the laboratory colony of *Culex p. quinquefasciatus.* It is apparent that some vigor tolerance existed in this strain to these other organophosphate materials. Nevertheless, it is noted that AC-52160 and CL-43913 still manifested a high degree of activity against this parathion resistant strain of *Aedes nigromaculis.* Baytex and SD-7438 also were still relatively effective against this resistant strain.

Aedes nigromaculis larvae from a pasture located less than one mile from the Blaise pasture (where the parathion resistant strain was collected) showed no sign of tolerance to parathion or the other organophosphates. It seems that the resistance in this area has been confined for the last two seasons and has not spread even to the adjacent locations. It may be postulated that the various environments play an important role in the selection and survival of insecticide tolerant strains.

FIELD PLOTS. In field plots 12 materials either having high biological activity against mosquito larvae or low toxicity to

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

Insecticide and formulation	Dosage lb./acre	Avg. no. larvae/dip		
		Pre-treatment	Post-treatment	% control
CL-43913 EC4	0.005	5.5	5.8	0
	0.01	8.0	3.0[a]	63
	0.02	3.5	0.13	96
	0.05	7.0	0.0[b]	100
Bayer 52957 EC2	0.01	6.2	1.8	71
	0.02	5.5	1.0	80
	0.05	4.7	0.13[b]	97
	0.10	16.0	0.1	100
Cela S-1942 EC4	0.025	15.0	5.0	68
	0.05	13.0	2.5[a]	81
	0.1	4.0	0.0[b]	100
	0.2	8.0	0.0[a]	100
N-2404 EC4	0.01	24.1	5.0	78
	0.025	16.2	2.0	88
	0.05	13.0	0.8	94
	0.1	10.5	0.0[b c]	100
	0.5[d]	..	..	..
Bayer 37342 EC4	0.025	4.0	1.7	60
	0.05	4.5	0.6	87
	0.1	6.0	2.0	97
	0.2	9.0	0.0[b]	100
	0.4	7.0	0.0	100
Cela S-2225 EC4	0.01	8.1	4.4	46
	0.025	11.1	1.0	91
	0.05	7.4	0.1	98
	0.1	22.0	0.0	100
	0.2	6.0	0.0	100
	0.4	11.0	0.0[b]	100
AC-52160 EC2	0.002	13.0	8.3	36
	0.005	9.3	0.3	97
	0.01	25.0	0.4	98
	0.02	18.0	0.0	100
	0.05	27.0	0.0	100
	0.10	30.0	0.0[b]	100

[a] Tadpole shrimp alive.
[b] Beetle larvae and adults and mayfly naiads alive.
[c] Tadpole shrimp dead after treatment.
[d] Diving beetle adults, larvae and chironomid midge larvae dead. Pronounced mortality of dragonflies resulted from this treatment.

warm-blooded animals or both were evaluated. In the first series of tests AC-47921 proved highly effective (Table 4), while N-2790, Bayer 46676 and Ortho 5305 also manifested quite high activity. Bayer 44646 was not effective. Generally, the high dosages of most of these compounds did not cause any noticeable mortality of non-target invertebrates and vertebrates present at the time of the treatments.

In the second series of tests, 7 materials were evaluated. AC-52160 proved to be the most outstanding material followed by CL-43913, Bayer 52957, N-2404, Cela S-2225 (OMS-659), Cela S-1942 (OMS-658) and Bayer 37342 (Table 5).

It is apparent that certain materials such as Bayer 37342, N-2404, Cela S-1942 (OMS-658) and Cela S-2225 (OMS-659) although manifesting moderate biological activity in the laboratory tests, showed a good deal of biological activity in the field. Chemical stability, physical factors of the field environment and biological strains of the test organisms are undoubtedly influencing the biological activity of test materials.

Most of the highly effective materials evaluated in the field have low mammalian toxicities (Table 6). Among these Cela S-2225 (OMS-659), AC-52160, Cela S-1942 (OMS-658), Bayer 37342 and CL-43913 have very favorable mammalian toxicities. These materials seem to be outstanding from the standpoint of larval control as well as low toxicity to warm blooded animals.

Those materials evaluated in field plot studies were also evaluated against the mosquito fish *Gambusia affinis* in the manner discussed elsewhere (Mulla and Isaak 1961, Mulla *et al.*, 1963). Of the highly effective mosquito larvicides, Cela S-1942 (OMS-658) and Cela S-2225 (OMS-659) did not produce any significant fish mortality at the rate of 1.0 lb./acre (Mulla 1963 unpublished data). CL-43913 was safe to the fish at 0.4 lb./acre while AC-52160 and Ortho 5305 produced no fish mortality at the rate of 0.1 lb./acre. At higher rates these two materials were toxic to the fish. N-2404 and AC-47921 proved toxic to the fish at 0.1 lb./acre. The former material also proved effective (at 0.1 lb./acre) against the rice tadpole shrimp which is a pest species.

Table 6.—Mammalian toxicity (against rat or mouse) and larvicidal activity of some new mosquito larvicides.

Larvicide	Mammalian acute oral LD_{50} mg./kg.	Mosquito larvae[a] LD_{50} (p.p.m.)
Parathion	3.6– 13[b]	0.0045
AC-47921	21[c]	0.005
N-2404 (OMS-405)	32[c]	0.018
DDT	113[d]	0.035
Cela S-2225 (OMS-659)	250[c]	0.030
AC-52160	293– 440[c]	0.0014
Cela S-1942 (OMS-658)	3000[c]	0.01
Bayer 37342	>3000[e]	0.072
Malathion	480–5800[d]	0.068
AC-43913	6150[c]	0.0027

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

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