EFFECTIVENESS OF NEW INSECTICIDES AGAINST MOSQUITO LARVAE [1]

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Introduction. In California, the current mosquito control technology is predominantly directed toward the abatement of pest and vector species in their aquatic habitats. Proper management of the larval sources in limited areas has yielded good results. This end has usually been achieved by drainage and other effective engineering schemes. Utilization of biotic control agents such as mosquito fish and other predators under optimum and suitable environmental conditions is also aiding mosquito control in California. Although source reduction and biotic control measures are employed whenever possible, the use of chemical larvicides constitutes a major segment of current mosquito control technology in California.

The physical and biological components of the environment in which mosquito larvae are controlled with larvicides are complex. They vary from one location to another even in a limited area and do not remain constant in time. The types of breeding sources harboring mosquito larvae are diverse too, and no single remedy can be found or recommended for effective control under all circumstances. Insecticidal treatment of larval sources located in agricultural fields poses certain problems pertaining to the residues of the larvicides on food and forage crops. Many effective and choice larvicides cannot be used routinely on crops without obtaining information on the residue levels and the proper establishment of tolerances for the larvicides on the crops to be treated for mosquito control.

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There is a need for the development of a variety of larvicides to be employed under diverse environmental conditions. The need for short-lived materials in agricultural areas is obvious. On the other hand, residual larvicides are preferred in situations where residues on crops do not pose a serious problem. Safety of larvicides to predators of mosquito larvae is another consideration which merits attention. Materials having low mammalian toxicity are also sought for and their use in urban larviciding programs is greatly desired.

The occurrence in mosquitoes of resistance to malathion and parathion further emphasizes the need for substitute materials. Materials having desirable attributes are very few and systematic search for such larvicides should pay off in the long run. To gather information on the behavior and activity of various new materials, studies were conducted on the biological activity of candidate materials against mosquito larvae. As a result of studies conducted in both laboratory and field during the 1959 season, several highly effective and promising mosquito larvicides were found (Mulla *et al.* 1960). The present studies are a continuation of the earlier studies and deal with the effectiveness of several other materials. The toxicity of all the larvicides evaluated in the field during 1959 (Mulla *et al.* 1960) and during the present studies was also determined against the mosquito fish *Gambusia affinis* (Girard and Baird) and will be published elsewhere (Mulla and Isaak 1961). Additional observations on the toxicity to mosquito larval predators of the larvicides which were evaluated in the field earlier (Mulla *et al.* 1960) were also recorded. Such observations, although not definitive in nature and scope, will materially aid in the selection of a proper larvicide.

The effectiveness of most of the insecticides was determined in the laboratory prior to extensive field evaluation. The laboratory information facilitated the determination of approximate dosages of the materials for field evaluation.

MATERIALS AND METHODS. The materials were evaluated in the laboratory against 4th instar larvae of *Culex pipiens quinquefasciatus* Say in the same manner as described earlier (Mulla *et al.* 1960). Twenty-five 4th-instar larvae were placed in 100 ml. of tap water in 6-ounce paper cups. Acetone solutions of the insecticides were added to the cups and larval mortality was recorded 24 hours after exposure. The percent mortality was plotted on probit log scale and the points fitted visually with a straight line. Each treatment was replicated three times and each material was evaluated on two or three different occasions.

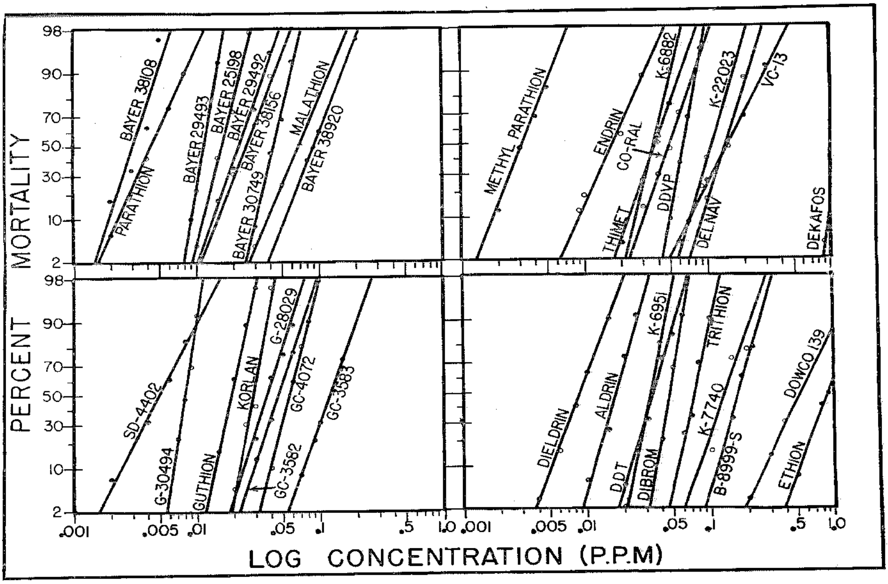

FIG. 1.—Dosage response lines of several insecticides evaluated in the laboratory against susceptible 4th-instar larvae of *Culex p. quinquefasciatus*. Parathion SD-4402, Bayer 29493, DDT, and malathion are included for comparison.

Figure 1 shows dosage response lines of 33 insecticides not previously reported on; for comparison, the dosage response lines of parathion, Bayer 29493[4] (Baytex), DDT, SD-4402 and malathion are also included.

Dosage response lines of a few compounds manifesting high biological activity against mosquito larvae are at the extreme left of the figure, near the activity level of parathion. In this category of highly promising materials are Bayer 38108, Bayer 29493, Bayer 25198, methyl parathion, G-30494, SD-4402 and dieldrin. Bayer 34042, also in this category, is not shown on the figure.

Three materials, Dowco 139 (Zectran), ethion, and Dekafos, with low biological activity against mosquito larvae have dosage response lines located to the extreme right. These materials have such low activity against larvae that their use can be justified only under special conditions.

Most of the remaining materials showed a moderate to high degree of activity. The dosage response lines of these materials are clustered along the dosage response lines established for DDT and malathion. Many promising and versatile materials belong to this group of larvicides.

The effectiveness of all those materials presented in figure 1 and of some of those evaluated in the laboratory during the earlier studies (Mulla *et al.* 1960) but not shown in figure 1 can be more fully visualized in terms of their LC50 and LC90 activity levels against mosquito larvae. This information for materials evaluated thus far is presented in Table 1.

As a general rule, materials manifesting biological activity lower than that of malathion against mosquito larvae do not hold much promise for general use in California larviciding programs. Higher dosages (0.5 lb. or more/acre) of these materials would usually be required, which would not fit into a program aimed at controlling mosquito larvae in agricultural areas; probably such high rates for some of the materials would result in undesirable residues on food and forage crops. Also, the cost of such high rates would preclude their use over wide areas. A few of the materials in this category, however, by virtue of their stability in water, may be applied at economically feasible rates that will yield desired results. These compounds with lower activity may also have a place in the control of resistant strains where other conventional materials do not yield adequate control.

The evaluation of several organophosphate insecticides in the laboratory showed that methyl analogues of a number of insecticides manifested slightly more activity than the ethyl analogues (Table 2). But field evaluation of the activity of methyl and ethyl analogues, although showing the same tendency, has not progressed for many of the analogues to establish this trend definitely. However, ethyl analogues are considered more stable in the field and, therefore, may be used in situations requiring long-lasting materials. Bayer 25141, Ethyl Guthion, and Trithion had considerable residual activity against *Hippelates collusor* in soil (Mulla *et al.* 1961). Possibly these materials will show the same trend when they are used in mosquito larviciding programs.

The results in Figure 1 and Table 1 are presented to provide background information. Laboratory evaluation of candidate larvicides yields useful information on the general trend of activities of insecticides against mosquito larvae. The present procedure of exposing the larvae for 24 hours to the toxicants has been chosen for convenience. Such a short exposure period, however, may fail to discover the potential toxic effects of slow-acting and stable materials. Such materials may even be overlooked in field evaluation where 24-hour mortality counts are taken. The addition of acetone solutions of water-insoluble toxicants to water for laboratory evaluation may result in precipitation or separation of the toxicants and thus reveal some toxicants to be in-

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[4] Chemical designations of proprietary or experimental compounds, are reported before "References Cited."

TABLE 1.—Effectiveness of various insecticides against 4th instar larvae of *Culex pipiens quinquefasciatus*[1] in the laboratory

MATERIALS	LC50 (P.P.M.)	LC90 (P.P.M.)	MATERIALS	LC50 (P.P.M.)	LC90 (P.P.M.)
BAYER 38108	0.0031	0.0078	BAYER 30749	0.043	0.061
PARATHION	0.0045	0.0082	PHORATE (THIMET)	0.040	0.064
SD-4402	0.0050	0.010	BAYER 25141	0.046	0.064
G-30494	0.0082	0.010	DIBROM	0.048	0.064
BAYER 29493	0.0110	0.014	GC-3582	0.046	0.070
DIELDRIN	0.0088	0.015	CO-RAL	0.051	0.072
BAYER 34042	0.0115	0.018	METHYL TRITHION	0.050	0.081
BAYER 25198	0.016	0.022	GC-4072	0.058	0.081
ALDRIN	0.017	0.025	DDVP	0.066	0.084
GUTHION	0.019	0.026	TRITHION	0.072	0.10
E.N. 18133	0.023	0.031	MALATHION	0.068	0.12
ENDRIN	0.017	0.032	BAYER 38920	0.09	0.16
BAYER 29492	0.023	0.036	DOW K-22023	0.11	0.17
RONNEL (KORLAN)	0.029	0.038	GC-3583	0.12	0.21
ETHYL GUTHION	0.031	0.041	DOW K-7740	0.14	0.21
BAYER 38156	0.027	0.043	DELNAV	0.15	0.23
BAYER 22408	0.031	0.046	VC-13	0.15	0.30
DOW K-6951	0.034	0.046	B-8999-S (ASP-51)	0.18	0.24
AC-5727	0.036	0.052	DOWCO 139	0.58	1.2
DOW K-6882	0.038	0.052	ETHION	0.90	2.0
DDT	0.035	0.053	DEKAFOS	1.30	1.5

[1] A susceptible strain, colonized from a colony which was maintained at Kern Mosquito Abatement District, Bakersfield, California.

effective. Such materials when used in the field as emulsifiable concentrates may yield promising results. Settling of some toxicants in the testing cups, absorption on the waxed surface of the cups, and codistillation with water are some variables which may render the results doubtful.

FIELD EVALUATION. Many of the materials listed in table 1 have already been evaluated in the field. Results of field trials with some of the materials are not conclusive, but a general trend of the activity of the materials against mosquito larvae has been established. Phorate (Thimet), Guthion, Trithion, ronnel, Co-

TABLE 2.—The toxicity of methyl and ethyl analogues of some organophosphorus insecticides to 4th-instar larvae of *Culex p. quinquefasciatus* in the laboratory [a]

Methyl analogues			Ethyl analogues			Toxicity of methyl/ethyl	
Material	LC50 (p.p.m.)	LC90 (p.p.m.)	Material	LC50 (p.p.m.)	LC90 (p.p.m.)	LC50	LC90
Methyl parathion	0.0033	0.0053	Parathion	0.0045	0.0082	1.4	1.6
G-30494	0.0082	0.010	G-28029	0.038	0.060	4.6	6.0
Bayer 29493	0.011	0.014	Bayer 29492	0.023	0.036	2.1	2.6
Bayer 25198	0.016	0.022	Bayer 25141	0.046	0.064	2.9	2.9
Guthion	0.019	0.026	Ethyl Guthion	0.031	0.041	1.6	1.6
Dow K-6951	0.034	0.046	Dow K-6882	0.038	0.054	1.1	1.2
Methyl Trithion	0.050	0.081	Trithion	0.072	0.10	1.4	1.3

[a] Susceptible strain.

Ral, DDVP, and Delnav have been evaluated in the field against *Aedes nigromaculis* and *Culex tarsalis* (Isaak 1956, Gjullin and Lewallen 1958, Lewallen 1958); Guthion was highly effective against *A. nigromaculis* larvae. SD-4402, Bayer 29493, E.N. 18133, Bayer 24408, AC-5727, Bayer 25141, G-30494, and Methyl Trithion were evaluated against *C. tarsalis* and *A. nigromaculis* larvae (Lewallen and Gjullin 1960, Mulla *et al.* 1960). SD-4402, Bayer 29493, E.N. 18133, and Bayer 25141 were highly effective larvicides. A few materials were evaluated in olive vats against *C. p. quinquefasciatus and C. peus* (Mulla 1961). Results of trials with several other materials in the San Joaquin Valley against *C. tarsalis* larvae are presented in this paper. Field investigation of the remaining highly active materials is in progress.

In field evaluation emulsifiable concentrate formulations of the materials were used for preparing the dilute sprays. Size of plots was 1/16 acre and the spray was applied by hand sprayers at the rate of ½ gallon per plot or 8 gallons per acre. Small hand sprayers with TeeJet No. 8002 nozzles were used for applying the sprays; pressure was maintained at 20 to 30 pounds per square inch.

Larval mortality was assessed by dippings; 10 to 15 dips were taken before and 24 hours after treatment of the plots. Only 4th-instar larvae of *Culex tarsalis* were counted. Observations on the presence of other stages were also made and recorded in the tables. The percent control based on the 4th-instar larval mortality was arrived at by comparing the average posttreatment counts with the average pretreatment counts.

The plots were nonreplicated, but certain dosages of some materials were run two or three different times under varied conditions. In this manner the general trend of activity of the materials was established.

All the field trials were conducted in the southern part of San Joaquin Valley, California, in cooperation with Kern Mosquito Abatement District. Some of the trials were conducted at a duck club 10 miles northwest of Wasco. Here, salt grass and other plants covered from 20 to 50 percent of the water surface. The remaining tests were conducted in artificial ponds constructed and maintained by the Kern Mosquito Abatement District. The ponds are 15 miles south of Bakersfield, near Greenfield, in an intensively farmed area. Each pond is 1/16 acre and the water surface is clear of vegetation. The water depth ranged from 6 to 8 inches; water pH was 7.5. Water temperature during the tests varied from 80° F. to 90° F. during the day.

Duck Club Trials. In the duck club trials Bayer 25198 and Bayer 38156 were highly effective against mosquito larvae (Table 3).

Bayer 38156, although highly effective in the duck club trials, did not prove as effective when evaluated in the ponds

TABLE 3.—Field trials with new insecticides against 4th-instar larvae of *Culex tarsalis* (Donald Duck Duck Club, Wasco, California)

Insecticide and formulation	Dosage, lbs. tox./acre	Avg. no. larvae/dip		% Control
		Pre-treatment	Post-treatment	
Bayer 25198 50% EC[a]	0.01	11.5	4.9	58
	0.025	13.1	0.3	98
	0.050	9.3	0.0	100
Bayer 38156 50% EC[a]	0.01	4.3	0.8	81
	0.025	3.0	0.0	100
	0.050	2.3	0.0	100
GC-4072 50% EC	0.10[b]	10.0	3.6	64
	0.20[b]	8.3	0.0	100
	0.40[c]	11.3	0.0	100

[a] Insect predators such as mayfly naiads, adults and larvae of diving beetles and backswimmers were alive in all treatments of these materials.

[b] Backswimmers both dead and alive. Dytiscid adults alive. Water boatman, mayfly naiads, and mosquito adults dead on water surface. Few mosquito pupae also dead.

[c] All predators dead in plot treated at this rate; mosquito pupae also dead.

(see Table 5) or in olive vats; whereas Bayer 25198 did prove effective in the olive vats (Mulla 1961). Therefore, the status of Bayer 38156 in mosquito control is not yet clearcut. Further trials will be necessary to understand clearly the biological activity of this material against mosquito larvae in the field.

Compound GC-4072 also proved effective but not as effective as Bayer 25198 and Bayer 38156 (see Table 1). GC-4072 proved slightly more effective than its structural isomer GC-3583 in the laboratory (see Table 1); this trend held true in the field as well. Both materials seem to be residual in water and studies on their residual effectiveness are in progress. GC-4072 was harmful to aquatic insects that are assumed to be predators of mosquito larvae. Both materials were toxic to the mosquito fish at larviciding dosages (Mulla and Isaak 1961).

Dow K-6882, an organic amidophosphate, had appreciable activity even at the

TABLE 4.—Field trials with new Dow Chemical Company compounds against 4th-instar larvae of *Culex tarsalis* (Donald Duck Duck Club, Wasco, California)

Insecticide and formulation	Dosage, lbs. tox./acre	Avg. no. larvae/dip		% Control
		Pre-treatment	Post-treatment	
Dow K-6882 25% EC[a]	0.05	8.8	1.0	88
	0.075	11.3	0.1	99
	0.10	7.2	0.5	93
	0.20	16.5	0.0	100
Dowco 139 25% EC[b]	0.05[c]	4.0	4.5	0
	0.10[c]	3.3	3.1	7
	0.20[c]	2.3	1.1	52
	0.40	8.7	1.8	79

[a] Predacious diving beetles were alive in all plots treated with this material.

[b] Insect predators such as mayfly naiads, adult and larval diving beetles and backswimmers were alive in all treatments with this material.

[c] Large numbers of second- and third-instar larvae of *C. tarsalis* were alive in these treatments, indicating the ineffectiveness of this material at the indicated dosages.

lowest dosage used against the larvae (Table 4). It manifested considerable activity against *Culex* larvae breeding in olive vats also (Mulla 1961). It proved harmless to some aquatic predacious insects at the larviciding dosage. Dowco 139 proved much less effective especially at the three lowest dosages against the larvae than the former compound; higher dosages of this material were needed for complete kill of mosquito larvae in the ponds (see Table 5). At all dosages employed in the duck club tests Dowco 139 proved harmless to aquatic predacious insects.

Bayer 25198 proved more effective than its ethyl analogue (Bayer 25141) against 4th-instar larvae of *Culex p. quinquefasciatus* in the laboratory. This trend was also noticed in the field trials conducted last year (Mulla *et al.* 1960) and in the current studies. In field trials, Bayer 25198 was 4 times as toxic as Bayer 25141. The biological activity of Bayer 29493 (Baytex) and Bayer 25198 against mosquito larvae in the laboratory (see Table 1) and field was essentially the same. Both materials were investigated for their toxicity against the mosquito fish and were relatively safe (Mulla and Isaak 1961). These three Bayer compounds were relatively harmless to insect predators of mosquito larvae at effective larviciding dosages.

BREEDING PONDS TRIALS. The ponds were constructed to reduce variation caused by mixing of water and diffusion of toxicants from one plot to the other. Variation due to plant cover was also eliminated in the ponds. The ponds were located in an agricultural area where parathion applications for mosquito larval

TABLE 5.—Control of 4th-instar larvae of *Culex tarsalis* with insecticides applied to breeding ponds (Greenfield, California) [a]

Materials and formulation	Dosage, lbs. tox./acre	Avg. no. larvae/dip: Pre-treatment	Avg. no. larvae/dip: Post-treatment[b]	% Control
Parathion 50% EC	0.0005	157.0	165.0	0
	0.001	122.0	20.0	84
	0.003	115.0	0.0	100
	0.005	27.6	0.0	100
Dekafos 25% EC	0.20	14.5	15.5[c]	0
	0.40	11.5	6.0[c]	48
	0.80	6.1	0.3[c]	95
	1.60	15.8	0.3	98
K-6882 25% EC	0.01	5.6	7.7[c]	0
	0.05	4.1	0.2	95
	0.10	16.1	0.2	99
Dowco 139 25% EC	0.10	16.1	7.7[c]	57
	0.20	13.6	3.0[c]	80
	0.40	12.1	2.1	83
	1.0	17.8	0.0	100
Bayer 38156 50% EC	0.008	2.4	0.7[c]	68
	0.01	10.5	3.0[c]	72
	0.02	25.1	1.6[c]	92
	0.05	6.9	0.5[c]	93

[a] Water depth in the ponds was 6 to 8 inches, and water pH was 7.5. Water temperature measured during the day ranged from 80° to 90° F.

[b] All insect predators such as damselfly larvae, dytiscid larvae and adults (diving beetles) and dragonfly naiads were alive in all the treatments. Dead mosquito pupae were not observed in the treatments.

[c] Young mosquito larvae (1st through 3rd stage) were also alive in these treatments, thus indicating the ineffectiveness of the materials at these dosages.

control have been made for 7 or 8 years.

Parathion, Dekafos, Dow K-6882, Dowco 139, and Bayer 38156 were evaluated in the ponds against 4th-instar larvae of *Culex tarsalis* (Table 5). Parathion proved highly effective beyond expectation. Many mosquito abatement agencies in California have applied and are applying parathion at the rate of 0.1 pound per acre as a mosquito larvicide. No published work indicates that this dosage is the minimum effective rate for mosquito control in California. The dosage yielding kill less than 100 percent had not been established prior to the wide use of parathion as a mosquito larvicide. In the current studies a dosage as low as 0.003 pound per acre of parathion resulted in complete kill of the larvae in the ponds. Whether this susceptibility of the population to parathion is due to a reversal of the tolerance level or was present at the beginning of the larvicidal applications is not known. The dosages of parathion were applied late in the season when water temperature ranged from 55° F. to 60° F. Tests conducted earlier in the season showed complete kill of the larvae at 0.005 to 0.0075 pound per acre of parathion. This information is rather significant in view of the somewhat increased tolerance of *Aedes nigromaculis* to parathion in certain parts of the San Joaquin Valley (Lewallen 1960). Further studies on mosquito population in the ponds as well as on populations in other areas are contemplated for the future.

Dekafos proved effective but only at high dosages. Rates as high as 1.6 pound per acre or higher would be needed for complete kill of the larvae. It showed no appreciable activity against the young instar larvae at 0.8 pound per acre or lower rates. Dow K-6882 manifested appreciable activity in the ponds against the larvae and showed the same trend of activity observed in the duck club and olive vat treatments. Dowco 139 proved effective but at the relatively high dosage of 1.0 pound per acre. At the lowest two dosages this material did not show much activity against the young instar larvae.

Bayer 38156 did not prove as effective in the ponds as it did in the duck club studies. Early instar larvae were alive at all treatment levels of this material. Whether this difference in activity is due to mosquito population characteristics or to environment will be further investigated.

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CHEMICAL DESIGNATIONS OF PROPRIETARY MATERIALS

AC-5727	*m*-isopropylphenyl *N*-methyl carbamate
B-8999-S (NPD)	Tetra-*n*-propyl dithionopyrophosphate
Bayer 22408	naphthaloximido-*O,O*-diethylphsophorothioate
Bayer 25141	*O,O*-diethyl *O-p*(methylsulfinyl)phenyl phosphorothioate
Bayer 25198	*O,O*-dimethyl *O-p*-(methylsulfinyl)phenyl phosphorothioate
Bayer 29492	*O,O*-diethyl *O*-[4(methylthio)-*m*-tolyl)] phosphorothioate
Bayer 29493	*O,O*-dimethyl *O*-[4 (methylthio)-*m*-tolyl)] phosphorothioate
Bayer 30749	*O*-ethyl *O-p*-ethylsulfinylphenyl methylphosphonothioate
Bayer 34042	*O*-ethyl *O*-(3-methyl-4-methylthiophenyl) *N*-methyl phosphoramidothionate
Co-Ral ®	*O,O*-diethyl *O*-(3-chloro-4-methyl-7-coumarinyl) phosphorothioate
DDVP	dimethyl 2,2-dichlorovinyl phosphate
Dekafos ®	3-pentadecylphenol *O,O*-diethylthiono phosphate and related compounds

Delnav ®	2,3-*p*-dioxanedithol *S,S*-bis (*O,O*-dimethyl phosphorodithioate)
Dibrom ®	1,2-dibromo-2,2-dichloroethyl dimethyl phosphate
Dowco 139 (Zectran ®)	4-dimethylamino-3,5-xylol-*N*-methylcarbamate
Dow K-6882	*O*-ethyl *O*-(2,4,5-trichlorophenyl) methyl phosphoramidothioate
Dow K-6951	*O*-methyl *O*-(2,4,5-trichlorophenyl) methyl phosphoramidothioate
Dow K-7740	*O*-methyl *O*-(2,4,5-trichlorophenyl)phosphoramiothioate
Dow K-22023	*O*-methyl *O*-(2,4-dichlorophenyl) isopropylphosphoramidothioate
EN 18133	*O,O*-diethyl *O*-(2-pyrazinyl) phosphorothioate
Ethyl Guthion ®	*O,O*-diethyl *S*-4-oxo-1,2,3-benzotriazin-3(4*H*)-ylmethyl phosphorodithioate
G-30494	*O,O*-dimethyl-*S*-2,5-dichlorophenyl mercaptomethyl phosphorodithioate
GC-3582	Diethyl-1-(2,5-dichlorophenyl)-2,2-dichlorovinyl phosphate
GC-3583	Diethyl-1-(2,5-dichlorophenyl)-2-chlorovinyl phosphate
GC-4072	Diethyl-1-(2,4-dichlorophenyl)-2-chlorovinyl phosphate
Guthion ®	*O,O*-dimethyl *S*-4-oxo-1,2,3-benzotriazin-3(4*H*)-ylmethyl phosphorodithioate
Korlan ® (ronnel)	*O,O*-dimethyl *O*-2,4,5 trichlorophenyl phosphorothioate
Methyl Trithion ®	*O,O*-dimethyl *S*-(*p*-chlorophenylthiomethyl) phosphorodithioate
Thimet ® (phorate)	*O,O*-diethyl (*s*-ethyl mercaptomethyl) dithiophosphate
Trithion ®	*O,O*-diethyl *S*(*p*-chlorophenylthiomethyl) phosphorodithioate
SD-4402	1,3,4,5,6,7,8,8-octachloro 3a,4,7,7a-tetrahydro-4, 7-methanophthalan
VC-13	*O*,2,4-dichlorophenyl *O,O*-diethyl phosphorothioate

trict in the field evaluation work in the Bakersfield area.

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