LABORATORY EVALUATION OF SOME ORGANOPHOSPHORUS COMPOUNDS AGAINST THE LARVAE OF *AEDES* (*O.*) *DETRITUS* (HALIDAY), *AEDES* (*O.*) *CASPIUS* (PALLAS) AND *CULEX PIPIENS PIPIENS* L.

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Since 1959, mosquito control operations have been carried out on the Mediterranean shore of Languedoc-Roussillon in southern France. From 1963, the French State has granted financial aid to this program in relation to the tourist facilities of the seacoast.

Following the example of California, larvicidal programs form a major contribution to the suppression of mosquitoes in Languedoc-Roussillon. These mainly include two species of salt marsh mosquitoes, *viz. Aedes* (*O.*) *detritus* and *Aedes* (*O.*) *caspius*.

Excellent results have been achieved using an organophosphorus insecticide, fenitrothion (Folithion®, Bayer 41831 or Sumithion®) at a rate of 0.25 lb. to 0.5 lb. per acre, depending upon the depth of the water (Rioux *et al.*, 1964). The reasons why this larvicide was chosen in 1963 were explained by Gras (1964, 1966) and by Gras and Rioux (1967).

Inasmuch as the development of resistance is often, sooner or later, one of the unfortunate consequences of chemical treatment, it is essential that new insecticides be found in any sustained program of mosquito control.

We report here the first results with some organophosphorus insecticides (Table 1) on three species of local mosquitoes. DDT and carbaryl are included for comparison, as well as tributylgermanium, tributyl tin and tributyl lead acetate which belong to groups of organometallic compounds which have been dealt with in special studies at Montpellier (Gras and Rioux, 1965).

MATERIALS AND METHODS. As was done previously (Gras and Rioux, 1966) the 4th instar *Aedes* (*O.*) *detritus* larvae were collected in the Lower Camargue, Bouches-du-Rhone, France, in a tidal marsh having *Salicornia fruticosa, Obione portulacoides and Aeluropus littoralis.*[3] The larvae of *Aedes* (*O.*) *caspius* came from a habitat in the Smaller Camargue, Gard, France, with *Salicornia fruticosa* and *Juncus maritimus.*

In order to determine the comparative effectiveness of experimental compounds, a procedure used by Brown (1958) was followed. According to their solubility, 0.1 to 1 percent stock solution (w/v) of the technical grade of each compound in acetone was prepared immediately before use. Serial dilutions were made according to need.

Culicidal activity was determined by adding the same volume of acetone (0.5 ml.) to 250 ml. of distilled water (pH $\neq$ 6.5) in which 20 to 25 fourth instar larvae of mosquitoes were placed. Each concentration was run in triplicate. Mortality counts were made 24 hours after treatment, the criterion for mortality being the inability of the larvae to rise to the surface. The temperature during the test was 25° C. The values of the LC_{50} and LC_{90} were then determined by the method used by Finney (1952).

RESULTS AND DISCUSSION. The results giving the values of the LC_{50} and LC_{90} for the three species of mosquitoes are shown in Tables 2, 3 and 4, where the

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[3] The larvae of both species were collected outside the areas now undergoing insecticide treatment.

Table 1.—Chemical description of compounds studied.

Compounds	Chemical description
Abate	O,O,O′,O′-tetramethyl O,O′-thiodi-p-phenylene phosphorothioate.
Bayer 64995	4, 4′-bis (O,O-dimethylthiophosphoryl-oxy) diphenylsulfide.
Bayer SRA-4259	3,3′-dimethyl 4,4′-bis (O,O-dimethylthiophosphoryl-oxy) diphenylsulfide.
Bayer 37342	O,O-dimethyl O-(3,5-dimethyl-4-methylmercaptophenyl)-phosphorothioate.
Bayer 37343	O,O-diethyl O-(3,5-dichloro-4-methylmercaptophenyl phosphorothioate.
Bayer 46676	O-ethyl O-2-ethylthio-4-methyl-6-pyrimidyl éthyl-phosphonate.
Bromophos. (Cela S-1942)	O,O-dimethyl O-(2,5-dichloro-4-bromophenyl) phosphorothioate.
Ethyl bromophos (Cela-2225)	O,O-diethyl O-(2,5-dichloro-4-bromophenyl) phosphorothioate.
Chlorthion (Bayer 22/190)	O,O-dimethyl O-(3-chloro-4-nitrophenyl) phosphorothioate.
Ethyl chlorthion	O,O-diethyl O-(3-chloro-4-nitrophenyl) phosphorothioate.
Dursban	O,O-diethyl O-(3,5,6-trichloro-2-pyridyl) phosphorothioate.
Fenitrothion (Sumithion, Folithion, Bayer 41831)	O,O-dimethyl O-(3-methyl-4 nitrophenyl) phosphorothioate.
Ethyl fenitrothion	O,O-diethyl O-(3-methyl-4-nitrophenyl) phosphorothioate.
Fenthion (Baytex, Bayer 29493)	O,O-dimethyl O-(3-methyl-4-methylmercaptophenyl) phosphorothioate.
Ethyl fenthion (Lucijet, Bayer 29492)	O,O-diethyl O-(3-methyl-4-methylmercaptophenyl) phosphorothioate.
Naled (Dibrom)	O,O-dimethyl O-(1,2 dibromo-2, 2-dichloroethyl) phosphate.
Ronnel (Nankor, Trolene, Korlan)	O,O-dimethyl O-(2,4,5-trichlorophenyl) phosphorothioate.
Shell SD-8211	O,O-dimethyl O-(α-chloromethylene-2,5-dichlorobenzyl) phosphate.
Shell SD-8447	O,O-dimethyl O-(α-chloromethylene-2,4,5-trichlorobenzyl) phosphate.
Shell SD-9020	O,O-dimethyl O-(α-chloromethylene-2,4-dichlorobenzyl) phosphorothioate.
Shell SD-8803	O,O-diethyl O-(α-chloromethylene-2,4-dichlorobenzyl) phosphorothioate.
Sumitomo S-4084	O,O-dimethyl O-(4-cyanophenyl) phosphorothioate
Trichlorfon (Dipterex; Dylox, Bayer L 13/59)	O,O-dimethyl-(1-hydroxy-2,2,2-trichloroethyl) phosphonate.

compounds are listed in order of decreasing toxicity for the rat.

Table 5 gives the larvicides in order of decreasing activity on *Culex pipiens pipiens,* and our results are compared with those of Mulla *et al.* (1962, 1964, 1966) on *Culex pipiens quinquefasciatus* and of Keppler *et al.* (1965) on *Culex pipiens.* In Figures 1 to 5 we have shown the dosage lines, which allow a visual comparison of the respective activity of each compound.

Compounds showing a high activity on *Aedes detritus* and *Culex pipiens* were Bayer 37343, Bayer 64995, Bayer SRA–4259, Shell SD–9020, Shell SD–8803, fenthion, fenitrothion, ethyl fenthion, ethyl fenitrothion and ethyl chlorthion. On the other hand the activity of trichlorfon, carbaryl, tributyl tin and tributylgermanium acetates is poor. It is worth noticing that Bayer SRA–4259 has practically the same toxicity as Bayer 64995. Bayer SRA–4259, differing from Bayer 64995 only because its molecule has methyl groups in 3 and 3′, is far less toxic for the rat than the latter.

It is also of interest that the methyl analogues of a number of insecticides proved slightly more active than ethyl analogues (Table 6), a fact which confirms what has already been shown by Mulla *et al.* (1961). There are, however, a few exceptions to this, particularly with chlorthion and fenitrothion.

Abate®, Dursban® and Shell SD–9020

TABLE 2.—Toxicity (in p.p.m.) of several insecticides against 4th instar larvae of *Culex pipiens pipiens* L (autogenous), recorded in order of decreasing toxicity for the rat.

Compounds	LC_{50}	LC_{50} 95% Confidence limits	LC_{90}	Oral LD_{50} for the rat ♀ mg/kg
Parathion	0,0047	0,0043–0,0050	0,0064	6, 5
Bayer 64995	0,0016	0,0015–0,0018	0,0023	12
Methyl parathion	0,0042	0,0039–0,0046	0,0067	14
Shell SD-8803	0,0023	0,0021–0,0025	0,0032	17
Ethyl fenitrothion	0,0060	0,0054–0,0066	0,010	17, 5
Bayer 37343	0,00085	0,00081–0,00088	0,0011	25
Ethyl fenthion	0,0058	0,0048–0,0065	0,011	25
Ethyl chlorthion	0,0060	0,0054–0,0066	0,0085	50
Bayer 46676	0,022	0,020–0,023	0,030	50
Shell SD-9020	0,0013	0,0012–0,0014	0,0020	126
Dursban	0,0013	0,0012–0,0014	0,0017	135
DDT	0,024	0,022–0,026	0,039	113
Ethyl bromophos	0,035	0,032–0,038	0,059	200
Fenthion	0,0032	0,0031–0,0034	0,0044	245
Bayer SRA-4259	0,0015	0,0014–0,0017	0,0024	250
Sumitomo S-4084	0,021	0,020–0,024	0,031	400
Naled (Dibrom)	0,038	0,036–0,040	0,070	430
Carbaryl (Sevin)	0,67	0,58–0,75	1,20	561
Trichlorfon (Dipterex)	0,90	0,85–0,96	1,30	560
Fenitrothion	0,0084	0,0081–0,0087	0,011	673
Chlorthion	0,015	0,014–0,016	0,026	980
Malathion	0,042	0,036–0,056	0,065	1200
Ronnel	0,024	0,022–0,025	0,032	2630
Abate	0,00084	0,00078–0,00090	0,0012	2330
Bayer 37342	0,027	0,025–0,028	0,036	>3000
Shell SD-8447	0,192	0,176–0,209	0,342	5000
Bromophos	0,0083	0,0080–0,0086	0,010	5050
Tributylgermanium acetate	2,2	1,07–2,44	3,2	900
Tributyltin acetate	0,38	0,32–0,45	0,69	380
Tributyllead acetate	>2	....	.....	32

TABLE 3.—TOXICITY (p.p.m.) of several insecticides against 4th instar larvae of *Aedes (O.) detritus.*

Compounds	LC_{50}	LC_{50} 95% Confidence limits	LC_{90}
Parathion	0,0028	0,0021–0,0036	0,0052
Methyl parathion	0,0053	0,0050–0,0056	0,0071
Shell SD-8803	0,0085	0,0082–0,0088	0,010
Ethyl fenitrothion	0,0052	0,0047–0,0055	0,0072
Bayer 37343	0,00062	0,00052–0,00072	0,0018
Ethyl Fenthion	0,002	0,0018–0,0022	0,0028
Shell SD-9020	0,0017	0,0014–0,0019	0,0023
Dursban	0,0014	0,0012–0,0016	0,0026
DDT	0,042	0,028–0,094	0,13
Ethyl bromophos	0,0067	0,0062–0,0073	0,011
Fenthion	0,0015	0,0014–0,0016	0,0024
Bayer SRA 4259	0,0017	0,0016–0,0018	0,0025
Fenitrothion	0,0037	0,0028–0,0048	0,0062
Malathion	0,020	0,017–0,023	0,044
Abate	0,0011	0,0010–0,0012	0,0019
Bayer 37342	0,056	0,027–0,078	0,082
Shell SD-8211	0,027	0,023–0,031	0,069
Shell SD-8447	0,025	0,023–0,027	0,038
Bromophos	0,0076	0,0072–0,0080	0,0105

TABLE 4.—Toxicity (p.p.m.) of several insecticides against 4th instar larvae of *Aedes (O.) caspius*.

Compounds	LC_{50}	LC_{50} 95% Confidence limits	LC_{90}
Dursban	0,00095	0,00084–0,0010	0,0016
DDT	0,010	0,007–0,017	0,020
Shell SD-9020	0,0013	0,0012–0,0014	0,0019
Fenthion	0,0021	0,0019–0,0023	0,0032
Naled (Dibrom)	0,061	0,058–0,065	0,090
Carbaryl (Sevin)	0,532	0,504–0,555	0,681
Fenitrothion	0,0051	0,0048–0,0054	0,0067
Malathion	0,023	0,021–0,026	0,043
Abate	0,00081	0,00075–0,00085	0,00013
Bromophos	0,0105	0,0098–0,011	0,015

TABLE 5.—Toxicity (in p.p.m.) of several insecticides recorded in order of decreasing toxicity against the 4th instar larvae of *Culex pipiens* L. (autogenous). Comparison with the results obtained by Mulla (1962, 1964 and 1966) against *Culex pipiens quinquefasciatus* Say., and by Keppler & al. against *Culex pipiens* L.

Compounds	*Culex pipiens pipiens* L. (autogenous) [Gras & Rioux] LC_{50}	*Culex pipiens quinquefasciatus Say.* [Mulla & al.] LC_{50}	*Culex pipiens* L. [Keppler & al.] LC_{50}
Abate	0,00084	0,0014	0,00070
Bayer 37343	0,00085	0,0012	....
Shell SD–9020	0,0013	0,0021	....
Dursban	0,0014	0,0030	....
Bayer SRA–4259	0,0015	....	....
Bayer 64995	0,0016	0,0050	0,00058
Shell SD–8803	0,0023	0,004	....
Fenthion	0,0032	0,011	0,0038
CL–43913	....	0,0027	....
Methyl parathion	0,0042	0,0033	0,0028
Parathion	0,0047	0,0045	0,0032
Ethyl fenthion	0,0058	0,023	....
Ethyl chlorthion	0,0060	....	....
Ethyl fenitrothion	0,0060	....	....
Bromophos	0,0083	0,010	....
Fenitrothion	0,0084	0,010	0,0041
Chlorthion	0,015	....	....
Sumitomo S–4084	0,021	....	....
Bayer 46676	0,022	0,040	....
Ronnel	0,024	0,029	0,020
DDT	0,024	0,035	0,048
Bayer 37342	0,027	0,072	....
Ethyl bromophos	0,035	0,030	....
Naled (Dibrom)	0,038	0,048	0,019
Malathion	0,042	0,068	0,043
Shell SD–8447	0,192	0,28	....
Trichlorfon (Dipterex)	0,90	....	0,18
Carbaryl (Sevin)	0,67	....	2,2

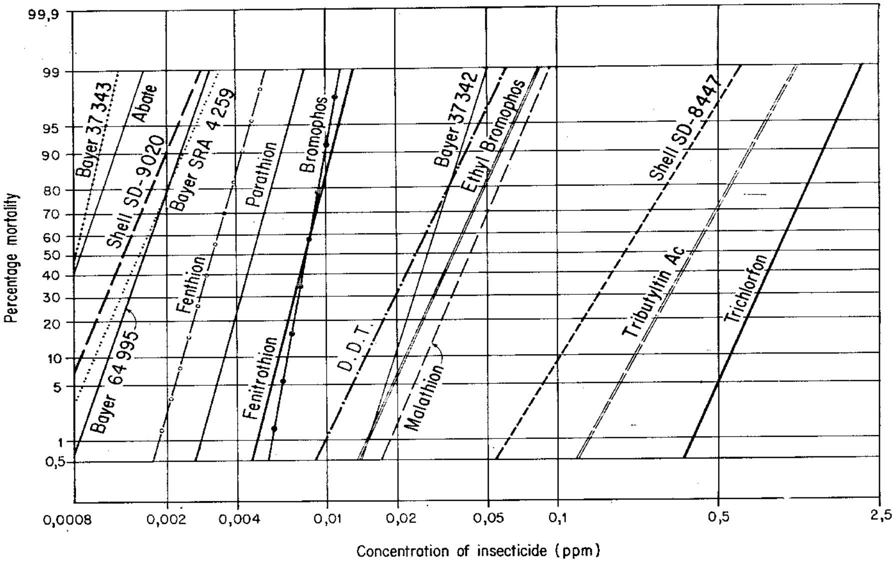

Fig. 1.—Dosage reponse lines of several organo-phosphorus compounds to 4th instar larvae of a susceptible laboratory strain of *Culex pipiens pipiens L.* (autogenous). D.D.T. and Tributyltin acetate included for comparison.

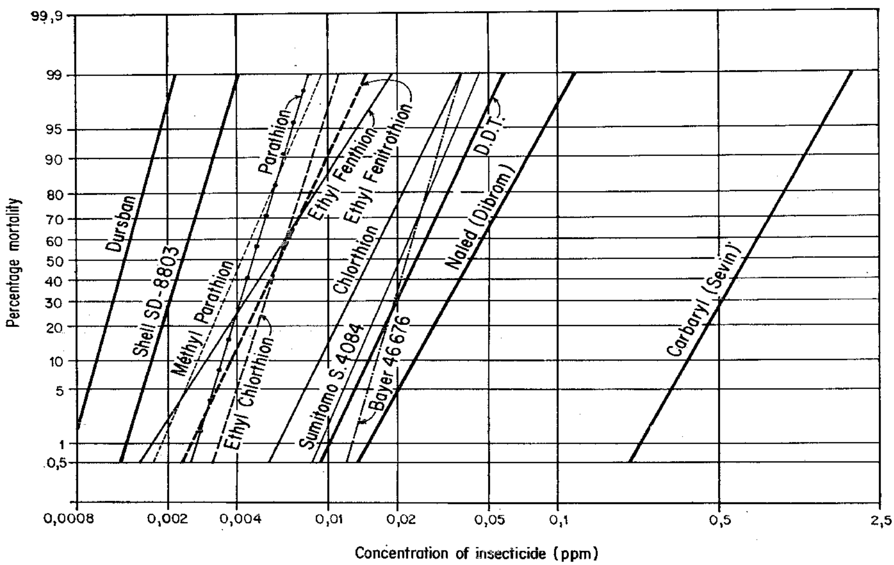

Fig. 2.—Dosage reponse lines of several organo-phosphorus compounds to 4th instar larvae of a susceptible laboratory strain of *Culex pipiens pipiens L.* (autogenous). D.D.T., Parathion and Carbaryl included for comparison.

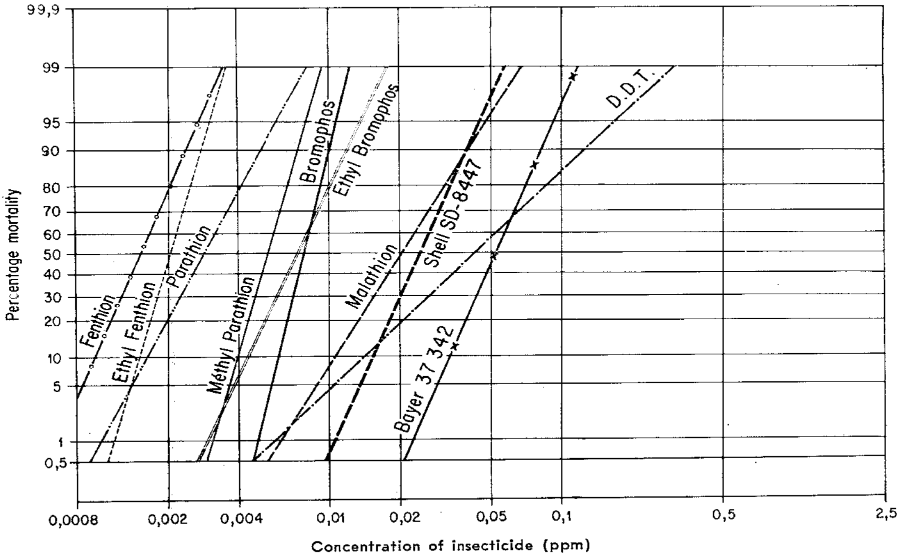

FIG. 3.—Susceptibility levels of several organo-phosphorus compounds to 4th instar larvae of field strain of *Aedes (O.) detritus*. D.D.T. included for comparison.

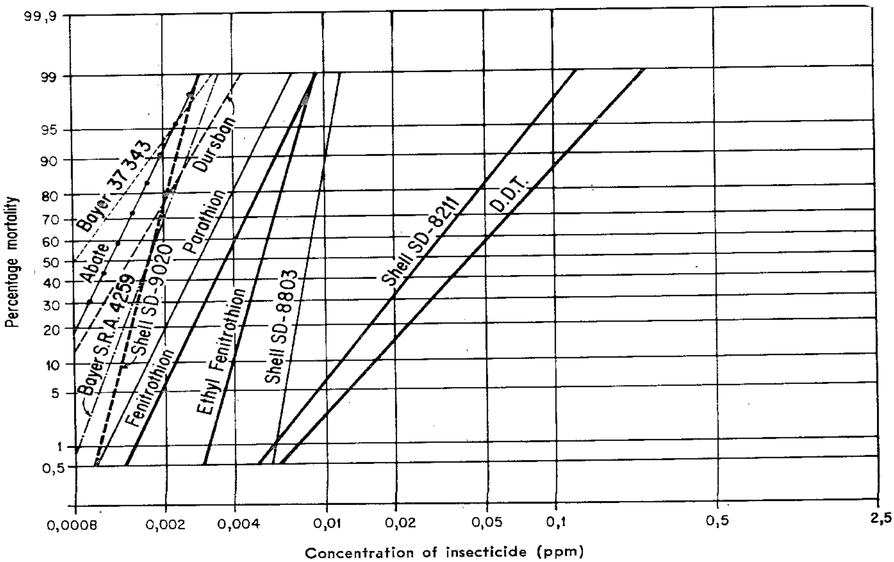

FIG. 4.—Susceptibility levels of several organo-phosphorus compounds to 4th instar larvae of field strain of *Aedes (O.) detritus*. D.D.T. and Parathion included for comparison.

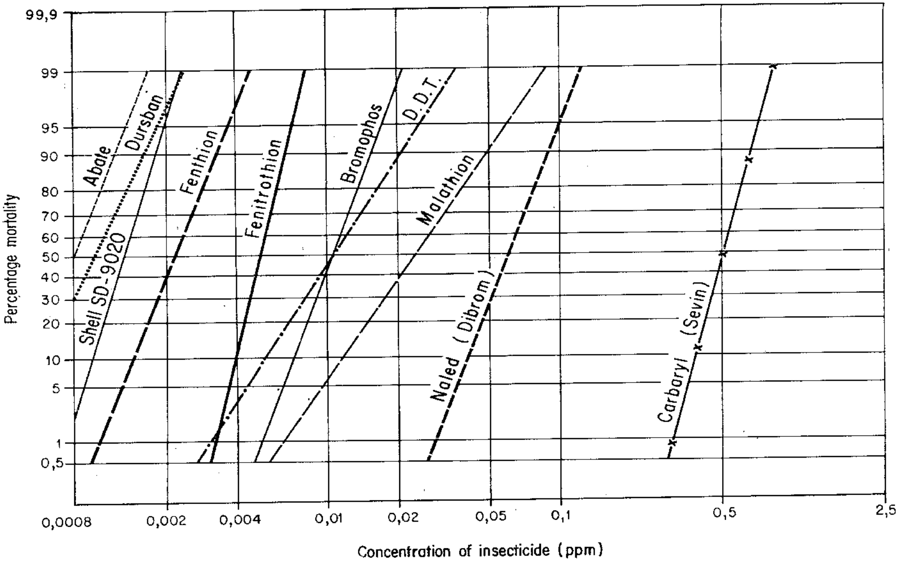

FIG. 5.—Susceptibility levels of several organo-phosphorus compounds to 4th instar larvae of field strain of *Aedes (O.) caspius*. Carbaryl and D.D.T. included for comparison.

proved highly active against the three species studied. Bromophos is also very active, although at a lower level.

Among those compounds, Abate and bromophos have a favorable mammalian toxicity and are likely to be widely used as larvicides. Although much more toxic, Dursban may also be of great interest. Lewallen and Peters (1966) have found that, unlike Abate, Dursban retained its activity against strains of *Aedes nigromaculis* resistant to parathion and fenthion.

Other compounds, such as chlorthion, ronnel, Bayer 37342, Shell 8447 and 8211, are less active. Some of these, however, such as Bayer 37342, Shell 8211 and ronnel, have proved better larvicides in the field than could have been expected after the laboratory tests (Mulla, 1966; Lofgren

TABLE 6.—The toxicity of methyl and ethyl analogues of some organophosphorus compounds to 4th—instar larvae of *Culex pipiens pipiens* L.

	Methyl analogues		Ethyl analogues		Toxicity of methyl/ethyl	
Compounds	CL 50	CL 90	CL 50	CL 90	CL 50	CL 90
Parathion	0,0042	0,0067	0,0047	0,0064	1,1	0,95
Chlorthion	0,015	0,026	0,0060	0,0085	0,40	0,33
Fenitrothion	0,0084	0,011	0,0060	0,010	0,71	0,90
Bromophos	0,0083	0,0102	0,035	0,059	4,2	5,9
Fenthion	0,0032	0,0044	0,0058	0,011	1,8	2,5
Shell-SD–9020	0,0013	0,0020	0,0023*	0,0032	1,7	1,6

* Shell SD–8803.

: *al.*, 1966). It will be necessary, there- ›re, not to reject compounds, especially ıose of low mammalian toxicity, before aving made tests in the field.

The persistence of larvicides in water ıd in the ground, their eventual accumu- tion in aquatic organisms, their toxicity man, and chiefly their activity against rains having acquired resistance to ma- rials generally used, are some of the pri- ary considerations to be taken into ac- unt in the south of France when the fficult decision of selecting a new in- cticide for mosquito control becomes perative.

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References Cited

BROWN, A. W. A. 1958. The World Health ganization test kit for detection of resistance mosquito larvae. Mosq. News 18:128.

FINNEY, D. J. 1952. Probit analysis: a statis- al treatment of the sigmoid response curve. ıd edit. 318 p. Cambridge Univ. Press London, ew York.

GRAS, G. 1964. La démoustication en Cali- rnie. Organisation et moyen de lutte. Bull. c. Pharm. Montpellier 24:5–35.

GRAS, G. 1966. Toxicité de fenitrothion pour *edes (O.) detritus* (Haliday). *Gambusia affinis* (Baird et Girard) *Anguilla anguilla* (L.) et *Pelodytes punctatus* (Daudin). Bull. Soc. Pharm. Montpellier 26:375–404.

GRAS, G., and RIOUX, J. A. 1965. Relation entre la structure chimique et l'activité insecticide des composés organiques de l'étain. Essais sur larves de *Culex pipiens pipiens* L. Arch. de l'Institut Pasteur Tunis 42:9–22.

GRAS, G., and RIOUX, J. A. 1966. Sensibilité de base d'*Aedes (O.) caspius* (Pallas) et d'*Aedes (O.) detritus* (Haliday) à quelques insecticides organophosphorés. Bull. Soc. Path. Exot. 59:898–903.

GRAS, G., and RIOUX, J. A. 1967. Les insecticides organo-phosphorés dans la lutte contre les moustiques. Problèmes théoriques et pratiques posés par l'utilisation de ces composés comme larvicides. XXVII[e] Congrès de l'Assoc. Fr. pour l'Avancement des Sciences Bordeaux 8–12 juillet 1967.

KEPPLER, W. J., KLASSEN, W., and KITZMILLER, J. B. 1965. Laboratory evaluation of certain larvicides against *Culex pipiens*, Linn., *Anopheles albimanus* Wied. and *Anopheles quadrimaculatus* Say. Mosq. News 25:415–419.

LEWALLEN, L. L., and PETERS, R. F. 1966. Control of California mosquitoes resistant to organophosphorus insecticides. Mosq. News 26:328–330.

LOFGREN, C. S., PENNINGTON, N., and YOUNG, W. 1966. Evaluation of insecticides against two species of *Culex* mosquitoes on Okinawa. Mosq. News 26:52–59.

MULLA, M. S., AXELROD, H. and ISAAK, L. W. 1961. Effectiveness of new insecticides against mosquito larvae. Mosq. News 21:216–224.

MULLA, M. S., METCALF, R. L., and GEIB, A. F. 1966. Laboratory and field evaluation of new mosquito larvicides. Mosq. News 26:236–242.

MULLA, M. S., METCALF, R. L., and ISAAK, L. W. 1962. Some new and highly effective mosquito larvicides. Mosq. News 22:231–238.

MULLA, M. S., METCALF, R. L., and KATS, G. 1964. Evaluation of new mosquito larvicides with notes on resistant strains. Mosq. News 24:312–319.

RIOUX, J. A., CROSET, H., GRAS, G., and JUMINER, B. 1964. Priorité aux méthodes antilarvaires dans la lutte contre les *Aedes* halophiles en Languedoc-Roussillon. Arch. de l'Institut Pasteur, Tunis, 41:385–396.