A BIOASSAY METHOD AND RESULTS OF LABORATORY EVALUATION OF INSECTICIDES AGAINST ADULT MOSQUITOES [1, 2, 3]

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INTRODUCTION. In dealing with the insecticide resistance problem, the World Health Organization (W.H.O.) initiated in 1960 a long-term project for the comparative toxicological evaluation of potential insecticidal compounds. Under the terms of this project, a number of cooperating pesticide manufacturers submit outstanding new compounds to the W.H.O. who, in turn, channel them through several laboratories in various parts of the world, where they are screened against susceptible and resistant house flies and mosquitoes, evaluated for mammalian toxicity, studied under conditions of tropical storage, and tested for field performance. This is probably the first international cooperative effort in the methodical evaluation of insecticides, and the results will undoubtedly prove of immense benefit not only in the control of vector species but also in all phases of insect control by chemicals. This project is particularly timely in view of the increasing concern over the development of insecticide resistance which might jeopardize the success of mosquito eradication campaigns in various countries. By early 1960, the number of species of anopheline mosquitoes which are reported to have developed resistance to DDT and/or dieldrin had risen to 28 (Brown 1961). The assignment of stage I (laboratory screening) of this project to the University of California, Riverside, has presented the writers with the need for selecting or devising a bioassay method for testing the candidate compounds against adult mosquitoes.

Testing methods for house flies and mosquito larvae are fairly well standardized and generally accepted. There is, however, no satisfactory method for evaluating compounds against adult mosquitoes. Methods already published are designed mainly for small scale tests, or for the assay of resistance development. The present project calls for a method permitting the rapid evaluation of a large number of compounds of diverse chemical nature, with standardized equipment, solvent, and handling procedure. The applicability of the published methods to the present problem was thoroughly explored. The topical application method, which undoubtedly yields reproducible results independent of behavioral patterns of the test species, has been tried on mosquitoes (Ludvik, 1953; Hadaway and Barlow 1956; Ungureanu 1958) but never gained much popularity. One drawback of this method is that the insects must be handled individually, which, in the case of such fragile forms as mosquitoes, produces variable control mortalities dependent on the care and experience of the operator. Treatment by this means is of necessity slow. Other disadvantages are the ill effects of prolonged anesthesia, and the toxicity of acetone when used in volumes necessary (over 0.5 microliters per insect) for satisfactory topical application. Attention was, therefore, concentrated on contact methods of exposure.

Busvine and Barnes (1948) described a method involving exposure of mosquitoes to filter paper treated with acetone solu-

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tions of insecticides. The insects were confined between two petri dishes each lined with a filter paper. This method was subsequently modified (Busvine and Nash 1953) mainly by substituting a refined mineral oil for acetone as solvent. The mosquitoes were enclosed in cylinders made by rolling up the filter paper and closing the ends with glass discs. A roughly similar procedure was described by Fay *et al.* (1953) for the measurement of resistance to DDT. These authors utilized test chambers formed by rolling up treated papers in cylinders, and covering the ends with screen-wire caps. For treatment, the papers were dipped in a xylene solution of DDT, then drained vertically and dried in a horizontal position for 24–72 hours. Mathis *et al.* (1959) utilized the more important features of the methods of Busvine and Nash (1953) and of Fay *et al.* (1953), and designed an excellent testing kit, which has been incorporated in the widely used W.H.O. testing method for the measurement of insecticide resistance in adult mosquitoes. While the method ultimately decided upon as satisfactorily meeting the requisites of a laboratory screening program utilizes the essential principle of the W.H.O. testing method, it contains several modifications involving the type of solvent used, the exposure chamber and the handling procedure. The tests which led to these modifications are briefly discussed below.

TESTS LEADING TO DEVELOPMENT OF PRESENT METHOD. The present tests were performed on *Culex pipiens quinquefasciatus* Say, and *Anopheles albimanus* Wied. *C. p. quinquefasciatus* has been reared at the University of California, Riverside, for over 12 years without intentional exposure to chemicals, and is of normal susceptibility to the common insecticides. *A. albimanus* originated in Panama City, Panama, and is highly resistant to dieldrin (LC_{50} larvae 0.4 p.p.m., adults $>$ 100 cm^2). *C. p. quinquefasciatus* is maintained at a constant temperature of 75° F. and 60 percent r.h. and *A. albimanus* at 80° F. and 60 percent r.h. In both instances, a 12-hour photoperiod with one hour of twilight at 12-hour intervals, is observed. Unless otherwise indicated, the tests were carried out with 2- to 3-day old insects at 74 ± 2° F. The sexes were not segregated. After treatment, the insects were held at 60° F. and 60 percent r.h. and mortality was determined 24 hours later.

Anesthesia. In tests involving flying insects, it is desirable that these be briefly anesthetized so that they may be counted accurately and transferred with the minimum of handling. Various anesthetizing agents such as ether, chloroform or carbon dioxide have been utilized in handling mosquitoes. Testing methods intended for field use, or involving only a small number of insects, are designed so as to eliminate the need of an anesthetic (W.H.O. testing kit). Ether has been used satisfactorily in small scale tests (Ungureanu 1958), but for large scale operations in which over 1000 mosquitoes are handled daily, the use of carbon dioxide presents certain distinct advantages: (1) It is available in cylinders from which it can be discharged through a pressure regulating valve, and piped into a "knock-out" chamber, a counting dish, or elsewhere in the laboratory for as long as necessary; (2) provided the mosquitoes are not exposed to excessively high concentrations of the gas, or for unduly long periods of time, they will tolerate carbon dioxide anesthesia without ill effects; (3) most insecticide screening laboratories are equipped with CO_2-dispensing facilities, so that the existing set-up may be utilized for mosquito testing without substantial changes.

Tarshis (1957) has kept *Aedes aegypti* (Linn.) under CO_2 anesthesia for 2 hours without ill effects. CO_2 anesthesia was also employed in contact and topical application tests involving *Anopheles quadrimaculatus* Say (Ludvik *et al.* 1951; Ludvik 1952). The insects were placed in a CO_2 "knock-out" chamber (a battery jar) for 1 minute and then transferred to a counting dish through which a gentle stream of CO_2 was piped, and were exposed to the gas for periods varying from

3 to 30 minutes. One hour after this exposure, they were anesthetized again for 2 minutes and transferred to holding cages at 60° F. This double exposure to CO_2 was intended to simulate the type of anesthetization utilized in the method described later on in this paper. However, the 30-minute exposure tested is far beyond the few minutes required for normal handling.

After first exposures of 3, 10, 20 and 30 minutes for *C. p. quinquefasciatus* and 3, 5, 7, 10, 15, 20 and 30 minutes for *A. albimanus* there was 100 percent recovery of all specimens. After second exposure of 2 minutes for each of the above lots there was 95 percent to 100 percent recovery. Twenty-four hours after exposure the percentages of mortality, corresponding to the times of the first exposure periods tested above, were, respectively 0, 0, 3, 0 for *C. pipiens quinquefasciatus* and 8, 5, 0, 10, 5, 0 and 10 for *A. albimanus*.

Solvents. In dealing with hundreds of organic compounds of varying types of solubility, acetone has proved to be the most suitable solvent (Metcalf 1958), and standard w/v acetone solutions are used throughout the screening programs of this and most other laboratories. However, since oil has been suggested as solvent in filter paper contact tests, it was desirable to study some of the relative features of the two solvents.

Tests were carried out on the performance of insecticide deposits from acetone solution on dry filter paper, as compared with similar deposits on filter paper pretreated with various concentrations of refined mineral oil. Using the test method described below, the results in Table 1 were obtained with DDT, malathion and *m*-isopropylphenyl *N*-methylcarbamate. These indicate that the toxicity of DDT was slightly increased by low concentrations of oil but later decreased considerably as the concentration of oil was increased. Conversely, the toxicity of the carbamate was reduced by low concentrations of oil, and subsequently increased beyond the initial toxicity of the compound. The toxicity of malathion showed a steady increase with increasing concentration of oil.

To determine the effect of oil on insecticide deposits on glass surfaces, open-ended glass vials were obtained in which the ends were slightly constricted so that the vial could hold at least 1 ml. of solution when lying on its side. One-half ml. of acetone solution of the insecticide and one-half ml. of acetone solution of the oil were introduced and the solvent was evaporated by rotating the vials on an electrically operated roller mill. The results (Table 2) show that while the toxicity of malathion and carbamate was somewhat increased in the presence of oil, that of DDT was again decreased. In the tests with malathion and carbamate, there appeared to be an optimum concentration of oil beyond which the performance of the insecticide was reduced. Hoskins *et al.* (1952) suggested that high concentrations of oil affect adversely the normal activity of house flies on glass surfaces

TABLE 1.—Toxicity of insecticides on filter paper impregnated with various concentrations of refined mineral oil, as measured against *Culex pipiens quinquefasciatus*

Compound and concentration	24-hour percent mortality						
	Concentration of oil (γ/cm.^{2})						
	0	1.6	4.7	16	47	160	470
DDT 7.8 γ/cm.^{2}	67	71	76	84	60	12	0
Malathion 6.3 γ/cm.^{2}	10	12	30	62	59	63	66
AC 5727* 0.24 γ/cm.^{2}	48	18	28	54	71	70	84

* *m*-isopropylphenyl *N*-methylcarbamate.

TABLE 2.—Toxicity of insecticides in glass vials, treated with various concentrations of refined mineral oil, as measured against *Culex pipiens quinquefasciatus*

Compound and concentration	24-hour percent mortality					
	Concentration of oil (γ/cm.^{2})					
	0	0.19	1.9	5.7	19	95
DDT 0.19 γ/cm.^{2}	59	49	49	24	24	6
Malathion 0.038 γ/cm.^{2}	75	65	88	85	48	8
AC 5727* 0.0019 γ/cm.^{2}	75	78	90	78	100	48

* *m*-isopropylphenyl *N*-methylcarbamate.

and consequently the extent of their exposure to the toxic residue. Such an effect was apparent in the tests reported here with mosquitoes. It is obvious from these data that the use of oil in comparative screening tests would enhance the performance of some insecticides while suppressing that of others, thus creating an erroneous impression of the intrinsic toxicity of the pure insecticide deposit. These difficulties with oil-insecticide surfaces led us to discard the idea of glass surfaces in favor of impregnation of the compound on filter paper from acetone. Application of the insecticide to filter paper rather than to bare glass presents certain distinct advantages: the paper can be treated more evenly by saturating it with the volatile solvent, and there is no problem with supersaturated droplets or induced crystallization; the insects have a firm foot hold and thus excessive activity, which in bare glass vials results in considerable physical injury to the test insects, is avoided; exposure is for the most part through the tarsi, thus approaching the type of contact obtained under field conditions.

Interval between Treatment of Paper and Exposure of Insects. In the W.H.O. testing kit the oil-treated papers may be used several months after impregnation (Busvine 1958; Mathis *et al.* 1959). In the present work it was observed that when acetone is used as solvent there is a negligible loss in toxicity during the first four hours (Table 3), which, however, becomes greater with time, at rates varying with the different compounds. The percentage of loss in toxicity as measured against *Culex p. quinquefasciatus* adults, 12 days after application was as follows: dieldrin 20; parathion 29; *m-sec*-butylphenyl *N*-methylcarbamate 39; *m*-isopropylphenyl *N*-methylcarbamate 41; and DDT 57. In practice, it is recommended that insects be exposed to the toxic residue within 2 hours after application. Numerous tests run within this period have shown no greater variation than what may be attributable to day-to-day differences in the susceptibility of the test population.

Exposure Chambers. Various types of exposure chambers were investigated in an effort to find one which would ensure virtually continuous contact of the insects with the treated surface. These included petri dishes, combinations of half petri dish and powder funnel, glass tubes open at both ends and shell vials open at one end.

TABLE 3.—Persistence of activity of insecticides applied to filter paper from acetone solutions, as measured against *Culex pipiens quinquefasciatus*

Compound	Concentration (γ/cm.^{2})	24-hour percent mortality*						
		Interval between treatment of filter paper and exposure of insects (hours)						
		0.25	0.5	1	1.5	2	3	4
DDT	11	88	90	83	83	82	88	78
Baytex	1.26	100	98	87	97	88	92	93
AC 5727**	0.28	85	85	77	75	82	83	82

* Average of 3 replications.
** *m*-isopropylphenyl *N*-methylcarbamate.

Petri dishes and petri dish-powder funnel combinations were inconvenient and frequently resulted in high insect mortality during handling. When petri dish-powder funnel combinations were used, the mosquitoes tended to settle on the walls of the funnel rather than on the treated dish or filter paper surface. This avoidance reaction was more pronounced during the late afternoon and early evening hours. Coating the funnel with a light film of vaseline forced the mosquitoes to remain on the insecticide-treated surface, but this procedure was rather tedious. Shell vials (2.1 x 8.4 cm.) were found to be the most satisfactory: they can be handled in groups without danger of crushing the insects, they can be accommodated in relatively small anesthetization chambers, and are readily available at reasonable cost. Treated filter paper can easily be rolled up and placed into the vial. The treated area thus comprises 89 percent of the total area of the exposure chamber, the two ends of the vial remaining untreated. As the insects move about inside the vial, they are exposed to the residue. The very rare mosquito which remains on the untreated surface throughout the period of exposure does not materially affect the LC_{50} or LC_{90} values obtained.

Holding Chambers. Davidson (1958) used ½-pint unwaxed paper cups fitted with a net cover, as post-exposure holding chambers. In the present tests, such cups were found very convenient, especially when fitted with a snap-on transparent plastic cover, which permits unobstructed examination of the insects. The cups are provided with a small piece of cotton roll moistened with 10 percent sucrose solution and are discarded after use.

THE TEST PROCEDURE. The pupae are collected either with a hand strainer, a pupal separator, or by some other means, depending on the species. Approximately 300 pupae are placed in emergence cages consisting of 1-gal. ice cream containers with cloth sleeves and a screen end. The pupae are moved daily to new emergence cages so that mosquitoes of known, uniform age are available. A waxed paper cup filled with cotton soaked with 10 percent sucrose solution is placed in each cage to provide nourishment for the adults. Since it is more convenient to avoid blood feeding, the adults are used on the second or third days after emergence. The sexes are not segregated.

The insecticide is applied in standard w/v acetone solution to Whatman No. 2 9-cm. diameter filter paper placed horizontally on pin points. One ml. of the liquid (which completely saturates the paper) is pipetted onto the paper spirally and allowed to evaporate completely. After about 5 minutes, a section approximately 0.5 cm. wide at the widest point is trimmed off one side of the paper and the latter is rolled up into a shell vial (2.1 x 8.4 cm.) lining the sides almost completely. For the sake of uniformity, the insects are exposed to the treated paper within one hour and in no case later than two hours after treatment.

The insects are anesthetized for 1–2 minutes by placing the emergence cage in the "knock-out" chamber, separated into groups of 20 with a camel's hair brush in a counting dish through which CO_2 is being piped, and transferred to the test vials with an aspirator. The vials are covered at the open end with cheesecloth, and placed flat on the side under standard conditions of temperature (74° F.) and lighting. The insects normally recover within 5 minutes from the onset of anesthesia. Slight jarring of the vials speeds up the recovery period. One hour after initiation of exposure the insects are lightly anesthetized and transferred into holding cages (described above). These are kept in the dark at 60° F. and 60 percent r.h. to minimize activity, and mortality is determined after 24 hours.

In standard practice, the compounds are tested at 0.1 percent concentration (16 $cm.^2$), and those producing less than 50 percent mortality are excluded. The remaining are tested further for the determination of LC_{50} values. Each LC_{50} is

TABLE 4.—Dosage mortality data for Baytex and DDT against *Culex pipiens quinquefasciatus* adults

Compound	Concentration (γ/cm.^{2})	Percentage mortality in replications[a] I	II	III	IV	V	Average	LC_{50} (γ/cm.^{2})	Slope
Baytex									
	0.47	10	0	0	0	0	2	..	..
	0.63	20	25	0	0	5	10	..	..
	0.78	30	60	5	0	5	10	..	..
	0.94	85	65	5	35	20	42	..	..
	1.26	95	85	35	40	45	60	..	..
	1.6	95	95	40	50	85	73	1.1	5.2
DDT									
	2.4	..	5	5	10	0	5	..	..
	3.1	10	10	5	20	5	10	..	..
	4.7	45	35	10	15	25	26	..	..
	6.3	..	..	35	30	35	33	..	..
	7.8	85	80	40	40	60	61	..	..
	12.6	..	..	..	60	90	75	6.9	3.3

[a] Performed on different dates.

based on about 5 concentrations of the insecticide replicated on 3 or 4 different days.

A skilled person may treat approximately 1000 mosquitoes within 3 to 4 hours. The cost of supplies, mostly cups and lids, is estimated at 73 cents per LC_{50} determination, if 5 concentrations are replicated 4 times.

Although the present method was designed on the basis of tests performed on *C. p. quinquefasciatus* and *A. albimanus,* it is believed to be applicable to other species of mosquitoes as well.

RESULTS AND DISCUSSION. A set of typical data obtained with the method described, is given in Table 4. The steepness of the slope of the dosage-mortality regression lines manifests a high degree of sensitivity and correlation between concentration and mortality. Table 5 shows the percentage of mortality and the coefficient of variation for replicated treatments of DDT. The variation is satisfactorily small and comparable to that encountered in topical application data with house flies. Day to day variation is likely to be greater (see Table 4) and will depend largely on the degree of standardization of the rearing method and on the homogeneity of the test population.

The importance of several other sources of error in technique was also investigated. Varying the period of exposure to CO_2 immediately prior to exposure to the toxic residue did not significantly alter the degree of mortality (Table 6). Increasing the length of exposure to the residue from 45 to 75 minutes resulted in corresponding increases in mortality (Table 7).

TABLE 5.—Variation in mortality of adult mosquitoes in replicated treatments of same date

Species	Compound and concentration	Percentage of mortality (Replications)					Ave.	Standard deviation	Coefficient of variation
Culex pipiens quinquefasciatus	DDT (3.1 γ/cm.^{2})	65	65	75	55	65	63.5	6.25	0.098
		60	65	70	60	55			
Anopheles albimanus	DDT (0.24 γ/cm.^{2})	25	20	25	20	30	24	4.18	0.174

TABLE 6.—Effect of CO_2 anesthesia immediately preceding exposure to toxic residue, on mortality of *Culex pipiens quinquefasciatus*

Length of exposure to CO_2 (minutes)	24-hour percent mortality* with insecticide indicated: DDT (7.8 γ/cm.²)	AC 5727** (0.24 γ/cm.²)	Control
5	28	43	..
8	17	40	..
12	22	27	..
20	38	57	0
28	53	53	2
36	28	30	3

* Average of three replications.
** *m*-isopropylphenyl *N*-methylcarbamate.

TABLE 7.—Influence of length of exposure to insecticide-treated filter paper on mortality of *Culex pipiens quinquefasciatus* adults

Length of exposure to insecticide residue (minutes)	24-hour percent mortality* with insecticides indicated: DDT (4.7 γ/cm.²)	Baytex (0.78 γ/cm.²)	AC 5727** (0.2 γ/cm.²)
45	57	58	38
50	48	73	45
55	63	83	50
60	70	90	58
65	68	93	73
70	68	95	77
75	75	95	63

* Average of three replications.
** *m*-isopropylphenyl *N*-methylcarbamate.

Holding the treated insects at 80° F. instead of 60° F. produced lower mortalities (Table 8), indicating a more efficient detoxication mechanism for the particular insecticide at the higher temperature. These results point out the great importance of rigidly standardized procedures in an insecticide evaluation method.

TABLE 8.—Influence of post-exposure holding temperature on mortality of adult mosquitoes

Species	Treatment	24-hr. percent mortality: 60° F.	80° F.
Culex pipiens quinquefasciatus	AC 5727* (0.2 γ/cm.²)	42	34
	Control	0	2
Anopheles albimanus	AC 5727* (0.16 γ/cm.²)	33	17
	Control	0	0

* *m*-isopropylphenyl *N*-methylcarbamate.

As a further test of the validity of the present method, the results obtained with DDT, Baytex, *m*-isopropylpheny *N*-methylcarbamate and Dow Chemical (*O*-methyl *O*-(2,4,5-trichlorophenyl) ethylphosphoramidate) by the contact and topical application methods were compared (Table 9). In the topical treatments, the materials were applied to the nota of the test mosquitoes in 0.3 microliter drops with a micrometer-driven microsyringe. CO_2 exposure during treatment was kept at a minimum by turning the gas on and off as necessary, and control mortality due to CO_2 and acetone was less than 5 percent. The results indicate a very close agreement in the relative toxicity of DDT and Baytex by the two methods. The small divergence in the results obtained with *m*-isopropylphenyl *N*-methylcarbamate may

TABLE 9.—Susceptibility of *Culex pipiens quinquefasciatus* to various insecticides as determined by the contact and topical application methods

Compound	Topical [a]: LD_{50} (γ/gm.)	Slope	Contact: LC_{50} (γ/cm.²)	Slope
DDT	6.99	3.4	6.9	3.3
Baytex	1.11	3.6	1.1	5.2
AC 5727 [b]	0.09	2.8	0.2	5.6
Dow Chemical [c]	39.7	3.3	>16	..

[a] Average weight 1.26 mg./mosquito.
[b] *m*-isopropylphenyl *N*-methylcarbamate.
[c] *O*-methyl *O*-(2,4,5-trichlorophenyl)ethylphosphoramidate.

TABLE 10.—Comparative toxicity of several insecticides to larvae and adults of *Culex quinquefasciatus* and *Anopheles albimanus*

	Compound	Chemical name	Larval LC_{50} (p.p.m.)		Adult LC_{50} (γ/cm.²)	
			Culex	*Anopheles*	*Culex*	*Anopheles*
1.	DDT	2,2-*bis* (*p*-chlorophenyl)1,1,1-trichloroethane	0.07	0.015	6.9	0.23
2.	Lindane	gamma-hexachlorocyclohexane	0.025	0.21	0.14	0.2
3.	Dieldrin	1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo-exo*-5,8-dimethanonaphthalene	0.0078	0.4	0.95	>16
4.	Allethrin	*dl*-allylrethronyl *dl-cis-trans*-chrysanthemate	0.14	0.96	11.0	3.3
5.	LB–95–61	2,4-dimethyl benzylchrysanthemate	0.09	0.1	1.9	1.7
6.	Malathion	*S*-(1,2-dicarbethoxyethyl)*O,O*-dimethylphosphorodithioate	0.081	0.12	5.9	4.4
7.	Ethyl malathion	*S*-(1,2-dicarbethoxyethyl)*O,O*-diethylphosphorodithioate	0.3	0.43	>16	ca. 10
8.	Bayer 16450	*O,O*-diethyl-1-acetyl-1-carbethoxy methylphosphonate	0.11	0.28	5.4	1.8
9.	Dibrom	1,2-dibromo-2,2-dichloroethyl dimethylphosphate	0.11	0.16	1.1	0.92
10.	Guthion	3-(4-ketobenzotriazine)-methyl *O,O*-dimethylphosphorodithioate	0.025	0.22	>16	>16
11.	DDVP	*O,O*-dimethyl-*O*-2,2-dichlorovinyl phosphate	0.075	0.1	0.088	0.094
12.	Bayer 24882	*O*-methyl *O*-(1-chloromethylethyl)-*O*-2,2-dichlorovinyl phosphate	0.036	0.1	1.0	1.2
13.	Bayer 22684	*O*-methyl *O*-2-chloroethyl-*O*-2,2-dichlorovinyl phosphate	0.07	0.17	0.4	0.3
14.	EPN	ethyl-4-nitrophenyl phenylphosphonothionate	0.0045	0.0084	14.0	12.0
15.	Methyl parathion	*O,O*-dimethyl *O*-4-nitrophenyl phosphorothionate	0.018	0.096	0.33	0.52
16.	Parathion	*O,O*-diethyl *O*-4-nitrophenyl phosphorothionate	0.0032	0.0047	1.2	0.46
17.	*n*-propyl parathion	*O,O*-di-*n*-propyl *O*-4-nitrophenyl phosphorothionate	0.011	0.064	7.2	11.0
18.	Dicapthon	*O,O*-dimethyl *O*-(2-chloro-4-nitrophenyl) phosphorothionate	0.027	0.093	5.7	7.1
19.	Chlorthion	*O,O*-dimethyl *O*-(4-nitro-3-chlorophenyl) phosphorothionate	0.026	0.13	8.0	ca. 16.0
20.	Ethyl Dicapthon	*O,O*-diethyl *O*-(2-chloro-4-nitrophenyl) phosphorothionate	0.0062	0.02	2.2	2.6
21.	Dow	*O*-propyl *O*-(4-nitrophenyl)methylphosphoramidothioate	0.023	0.062	ca. 16	>16
22.	Bayer S-5660	*O,O*-dimethyl *O*-3-methyl-4-nitrophenyl phosphorothionate	0.0058	0.011	2.8	3.3
23.	Bayer 30237	*O*-methyl *O*-4-methylthiophenyl methylphosphonothionate	0.0034	0.015	0.47	0.68
24.	Bayer 38107	*O*-methyl *O*-4-methylthiophenyl ethylphosphonothionate	0.0031	0.0096	0.38	1.5
25.	Bayer 38104	*O*-ethyl *O*-4-methylthiophenyl methylphosphonothionate	0.0043	0.007	0.98	0.64
26.	Bayer 32384	*O,O*-dimethyl *S*-4-methylthiophenyl phosphorodithioate	0.0086	0.025	11.0	7.7
27.	Bayer 34098	*O*-(3-methyl-4-methylthiophenyl)dimethylphosphinothionate	0.025	0.12	6.2	9.4
28.	Bayer 33333	*O*-methyl *O*-(3-methyl-4-methylthiophenyl)methylphosphonothionate	0.011	0.024	10.0	3.2
29.	Bayer 38108	*O*-methyl *O*-(3-methyl-4-methylchiophenyl)ethylphosphonothionate	0.0027	0.012	0.78	1.0
30.	Baytex	*O,O*-dimethyl *O*-(3-methyl-4-methylthiophenyl)phosphorothionate	0.0045	0.016	1.1	1.2
31.	Bayer 29492	*O,O*-diethyl *O*-(3-methyl-4-methylthiophenyl)phosphorothionate	0.011	0.015	1.7	2.7
32.	Bayer 34042	*O*-ethyl *O*-(3-methyl-4-methylthiophenyl)*N*-methylphosphoramidothionate	0.0035	0.0095	7.6	6.0
33.	Bayer 30468	*O*-ethyl *O*-(*p*-ethylthiophenyl)methylphosphonothionate	0.0028	0.011	1.0	0.33

TABLE 10.—Continued

	Compound	Chemical name	Larval LC_{50} (p.p.m.)		Adult LC_{50} (γ/cm.^{2})	
			Culex	*Anopheles*	*Culex*	*Anopheles*
34.	Bayer 29491	*O,O*-dimethyl *O*-(3-chloro-4-methylthiophenyl)phosphorothionate	0.0018	0.0044	1.0	1.4
35.	Bayer 37343	*O,O*-diethyl *O*-(3,5-dichloro-4-methylthiophenyl)phosphorothionate	0.0015	0.0016	1.2	1.3
36.	Bayer 30554	*O*-methyl *O*-(4-methylsulfinylphenyl)methylphosphonothionate	0.027	0.11	3.4	7.2
37.	Ronnel	*O,O*-dimethyl *O*-(2,4,5-trichlorophenyl)phosphorothioate	0.03	0.066	0.61	2.8
38.	Dow	*O*-methyl *O*-(2,4,5-trichlorophenyl)methylphosphoramidothioate	0.03	0.23	>16	>16
39.	Dow	*O*-methyl *O*-(2,4,5-trichlorophenyl)ethylphosphoramidothioate	0.02	0.15	12	>16
40.	Dow	*O*-methyl *O*-(2,4,5-trichlorophenyl)isopropylphosphoramidothioate	0.025	0.22	12	>16
41.	Dow	*O*-ethyl *O*-(2,4,5-trichlorophenyl)methylphosphoramidothioate	0.043	0.22	>16	16
42.	Dow	*O*-ethyl *O*-(2,4,5-trichlorophenyl)ethylphosphoramidothioate	0.025	0.096	9	11
43.	Dow	*O*-isopropyl *O*-(2,4,5-trichlorophenyl)methylphosphoramidothioate	0.032	0.052	>16	>16
44.	Dow	*O*-isopropyl *O*-(2,4,5-trichlorophenyl)ethylphosphoramidothioate	0.038	0.028	>16	>16
45.	Dow	*O*-isopropyl *O*-(2,4,5-trichlorophenyl)isopropylphosphoramidothioate	0.043	0.023	12	13
46.	Dow	*O*-methyl *O*-(2,4-dichlorophenyl)ethylphosphoramidothioate	0.064	0.064	9.5	15

indicate a differential in the rates of absorption of the compound by the two alternative routes of entry.

Over 250 compounds have already been tested by the present method and the results have been made available to W.H.O. Some of the more active mosquito adulticides and larvicides encountered in the present work are given in Table 10. Data on several commercially available insecticides have been included for comparison. The results indicate the outstanding larvicidal activity of a number of methyl- and chloro-substituted *p*-methylthiophenyl or *p*-ethylthiophenyl phosphorus esters (compounds 23–25, 29, 30, 32–35), and also the high adulticidal activity of certain aryl *N*-methylcarbamates. The latter have been listed in other papers (Georghiou and Metcalf 1961, 1962). Similarly, cyclodiene compounds are dealt with separately (Metcalf *et al.* 1962). Several chloro-substituted phosphoramidothioates (compounds 38–46) which have been found to be remarkably toxic to susceptible and resistant strains of the house fly (unpublished data), are rather ineffective against mosquitoes. It is interesting that some of the very active organophosphorus larvicides (compounds 14, 26, 32) are less active as adulticides in comparison with some aryl *N*-methylcarbamates (Georghiou and Metcalf 1961). Conversely, the latter show poor larvicidal and outstanding adulticidal activity. This evident lack of consistent correlation between larvicidal and adulticidal activity in a number of insecticides points out the need for evaluating candidate compounds against both stages of mosquitoes.

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