INITIAL AND RESIDUAL ACTIVITY OF CIDIAL® (PHENTHOATE) AGAINST MOSQUITOES AS COMPARED WITH THAT OF OTHER WELL-KNOWN PRODUCTS [1]

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The broad spectrum and the high level of the activity shown by Cidial® ("phenthoate"; = ethyl O, O-dimethyldithiophosphoryl-1-phenylacetate) against phytophagous pests damaging crops (Anon. 1962, 1964) have already been verified. Preliminary tests have also revealed some definitely practical properties of this product as regards its possible use in mosquito control.

On the strength of the data obtained, this study has been designed to investigate deeply the activity limits of Cidial for use against mosquito larvae and adults. Insecticides with a low mammalian toxicity are especially required against these pests. Cidial with a high degree of purity could serve this particular purpose very well.

EVALUATION OF LARVICIDAL ACTIVITY. *Initial activity*. The tests were carried out in an air-conditioned room (24–25° C.; 65–75 percent RH; 12 hrs' daily lighting) on third and fourth instar larvae of *Culex pipiens* L., *Aedes aegypti* L., *Anopheles gambiae* G. and of a strain of *Anopheles albimanus* W. resistant to DDT-dieldrin, following the dip method recommended by the W.H.O. (W.H.O., 1963) in which some slight alterations were made. The test hydro-acetonic solutions were prepared by adding 1 ml. of acetonic solution, at various concentrations of each technical product (with a high degree of purity) under examination, to 99 ml. of tap water (7.3–7.4 pH) contained in paper cups. One ml. of acetone was also added to the 99 ml. of water of the untreated controls.

About 10 to 15 min. after preparation, 50 larvae were transferred to these solutions where they were left for 24 hrs. Mortality counts were then made and the LC 50 and LC 95 were determined (Table 1).

Activity tests were performed with the same technique on various stages of *C. pipiens* (egg; second and fourth instar larvae; pupae). The death rates were recorded after 24 hrs. in the case of larvae and after 48 hrs. in the case of eggs and pupae (Table 2).

Residual activity. The tests were carried out in an air-conditioned room (24–25° C.; 65–75 percent RH; 12 hrs' daily lighting) by using polythene basins (40 x 30 x 20 cm), each containing 10 lit. of tap water and a 2-cm earth layer. The reference products (malathion, fenthion, dieldrin, heptachlor) were used at the rate of 1 ppm a.i., whereas Cidial was also tried out at other concentrations. The chemicals, suitably formulated, were evenly distributed all over the water surface by means of a pipette.

The bioassays for determination of the residual activity were conducted on water samples taken from the basins at various intervals after treatment and transferred to paper cups. In order to avoid sampling portions of water from the upper layer (where the product concentration could be different), a glass tube was placed, before treatment, upright into each basin, one end dipped into the liquid for about 4–5 cm, which allowed samples to be taken by means of a pipette. Fifty *C. pipiens* larvae (third and fourth instar) were then transferred to each cup and mortality counts were made after 24 hrs. (Table 3, Fig. 1).

[1] Report presented at the VI[th] International Congress of Plant Protection, Vienna 30.8–6.9.1967.

TABLE 1.—Initial activity of Cidial and of other insecticides on third and fourth instar larvae of various mosquito species.

Insecticides	*Culex pipiens* L.		*Aëdes aegypti* L.		*Anopheles gambiae* G.		*Anopheles albimanus* W.	
	LC 50 (ppm)	LC 95 (ppm)	LC 50 (ppm)	LC 95 (ppm)	LC 50 (ppm)	LC 95 (ppm)	LC 50 (ppm)	LC 95 (ppm)
Cidial	0.008	0.016	0.016	0.034	0.006	0.018	0.018	0.052
parathion	0.004	0.007	0.009	0.021	0.005	0.008	0.007	0.015
methylparathion	0.004	0.009	0.010	0.028	0.017	0.033	0.011	0.025
malathion	0.050	0.098	0.016	0.360	0.070	0.130	0.170	0.370
Sumithion	0.005	0.009	0.014	0.033	0.024	0.049	0.036	0.066
fenthion	0.003	0.008	0.005	0.010	0.007	0.015	0.022	0.047
DDVP	0.030	0.060	0.043	0.075	0.064	0.130	0.140	0.220
DDT	0.056	0.230	0.026	0.066	0.056	0.210	0.740	14.0
dieldrin	0.029	0.330	0.027	0.120	0.008	0.022	>10.0	>10.0

TABLE 2.—Cidial activity against eggs, larvae and pupae of *Culex pipiens* L.

Biological stage	LC 50 (ppm)
egg	>1.0
2nd instar larva	0.003
4th instar larva	0.008
pupa	0.240

TABLE 3.—Residual activity of Cidial 50 L (°) in water (8.2–8.4 pH), compared with that of other insecticides, against *Culex pipiens* L. larvae. Initial a.i. concentration: 1 ppm.

Insecticides	Residual activity (days) at the following levels:	
	LC 95	LC 50
Cidial	4	5
malathion	2	3
heptachlor	<1	3
dieldrin	2	17
fenthion	18	24

(°)—Cidial 50 L is a commercial, emulsifiable liquid formulation at 50% technical grade and 50% carriers and emulsifiers.

The residual activity of Cidial was also field tested, in a locality in the Milan area, in ditches (2 x 1.5 x 0.60 m) (Fig. 3) lined with polythene sheets and containing a 3–4 cm earth layer. Each ditch was filled with 400 lit. of water and during the experiment care was taken to maintain the initial level by adding further water to make up for possible wastes due to evaporation. Throughout the test period (June, July, August), the water pH in the various ditches fluctuated from 8.3 to 9.0. The treatment was carried out by evenly applying 100 ml of a Cidial aqueous emulsion all over the surface of the water contained in each ditch, so as to obtain the concentrations required (Fig. 2). At different intervals following treatment, water samples were taken from each ditch by means of a glass tube, as previously described for the laboratory tests, and were used in the laboratory to evaluate the residual activity on *C. pipiens* larvae, according to the method already outlined. The results obtained are shown in Fig. 2.

BEHAVIOUR OF DIFFERENTLY FORMULATED CIDIAL. The tests were performed in the laboratory, in an air-conditioned room (24–25° C., 65–75 percent RH; 12 hrs' daily lighting), on third and fourth instar larvae of *C. pipiens*, by following the same method used for residual activity tests, and by using Cidial in different formulations. All the formulations were employed at the rate of 0.1 ppm a.i. Throughout the whole of the test period the water pH was 7.6–8.3 (Table 4).

Some formulations based on mineral oils, DDT and Cidial were also tried out with a view to investigating the possibility of enhancing, with Cidial, the insecticidal

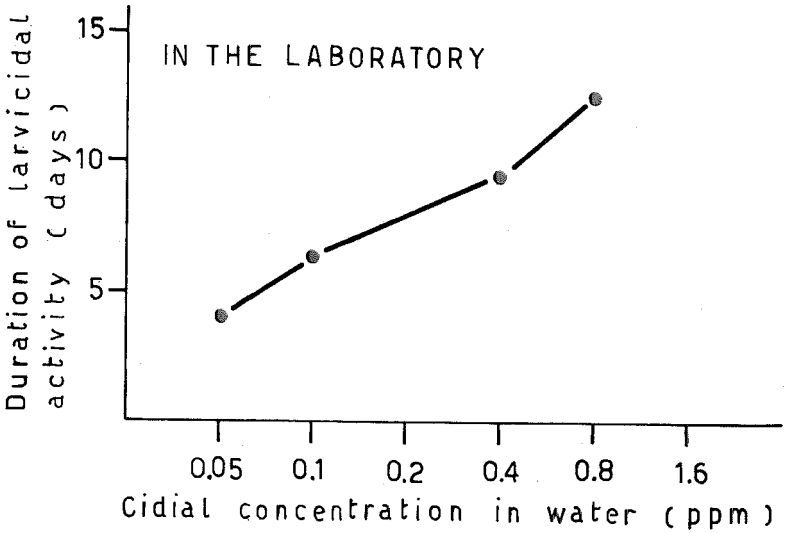

FIG. 1.—Persistence against *Culex pipiens* L. larvae shown by Cidial (Cidial 50 L formulation) at various concentrations in water (8.2–8.4 pH).

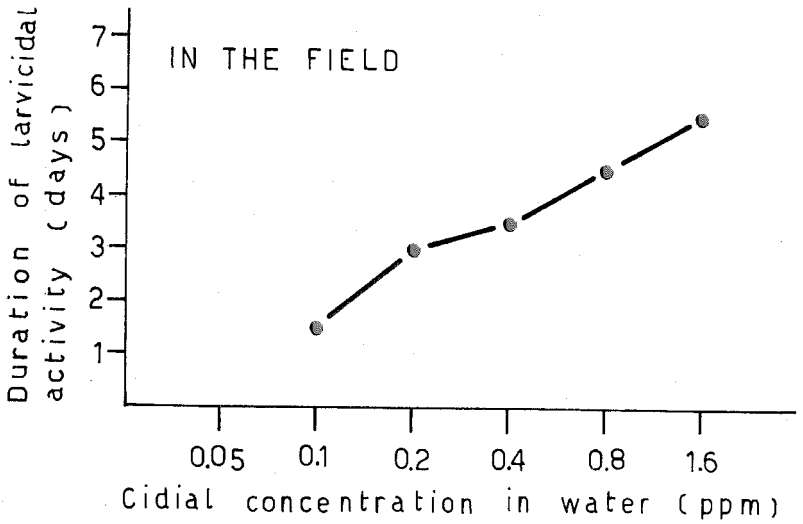

FIG. 2.—Cidial persistence against *Culex pipiens* L. larvae observed in the laboratory on water samples (8.3–9.0 pH) taken from ditches placed in the open and treated with Cidial 50 L at various concentrations.

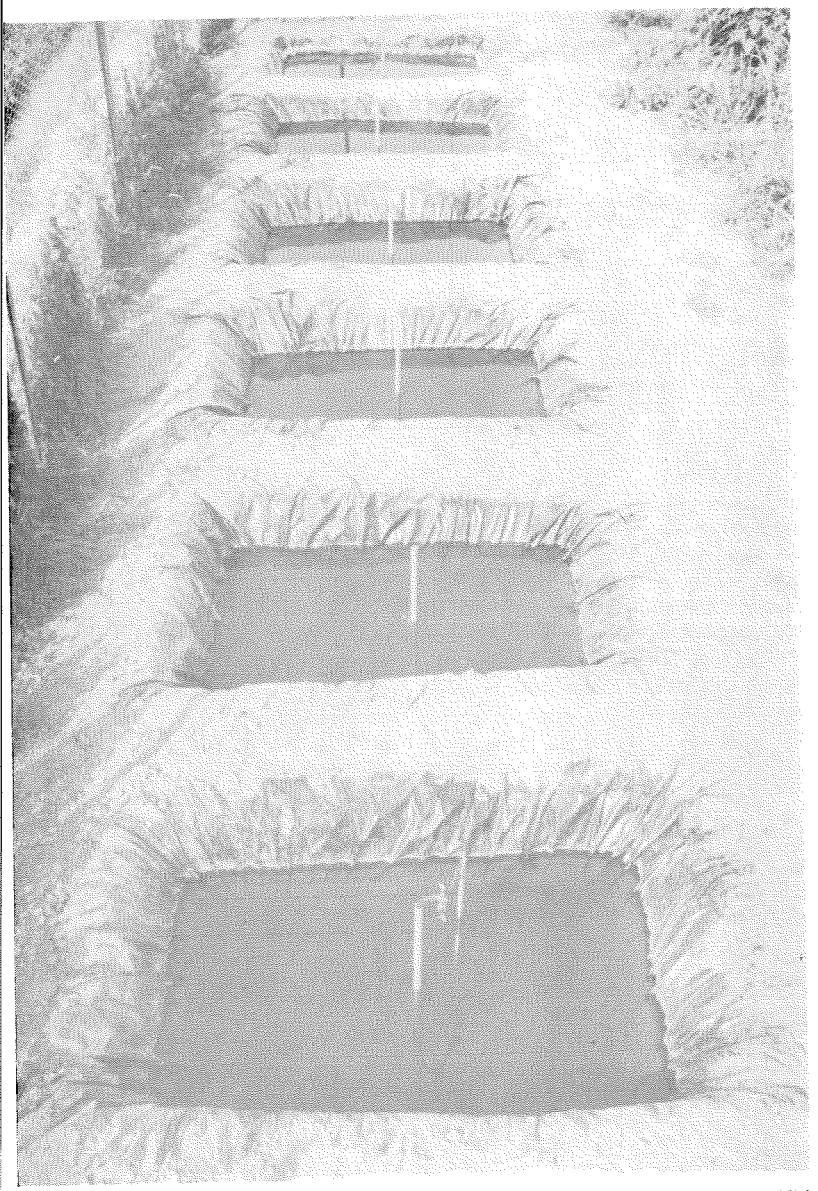

FIG. 3.—Ditches (2 x 1.50 x 0.60 m.) lined with polythene sheets, used to carry out the tests on Cidial persistence against *Culex pipiens* L. larvae.

TABLE 4.—Influence of the formulation on the activity and persistence of Cidial at a rate of 0.1 ppm a.i. in water (7.6–8.3 pH), against *Culex pipiens* L. larvae.

Experimental formulations			% mortality at various intervals after treatment				
Code name	Type	a.i. content	1 day	2 days	3 days	6 days	7 days
		Percent					
1920	granules	1	99	97	92	3	0
2028	granules	1	100	100	98	1	0
1921	floating granules	1	100	100	96	3	0
1922	floating granules	1	100	100	100	22	3
1923	floating granules	1	100	100	99	2	0
1924	floating granules	1	100	100	100	2	0
1925	dry dust	3	99	97	95	0	0
1926	wettable powder	10	100	100	98	11	12
1928	emulsifiable liquid	20	100	100	100	7	0
719/64	emulsifiable liquid (commercial)	50	100	100	100	27	1

activity of already available commercial formulations based on oil and DDT, the action of which is dependent upon the formation of an oily film on the water surface. These tests were performed in the laboratory by using 1,000-ml. beakers (18 cm high; 9 cm Ø) each holding 1 lit. of tap water. The chemicals were evenly distributed all over the water surface (64 sq. cm.) by means of an Agla microsyringe. The formulations containing Cidial were compared at the concentration of 0.02 ppm Cidial, i.e. at a rate slightly exceeding the LC 95. Just after treatment, each beaker was filled with 50 third and fourth instar larvae of *C. pipiens*. In order to prevent the insects from coming into contact with the product, a glass tube placed upright for about 4–5 cm into the beaker was used to let them into the water. The mortality counts were made 24 hrs. later (Table 5).

Further laboratory tests were performed for the purpose of verifying the diffusion in water of Cidial formulated with mineral oils alone (Larviol C) and with mineral oils plus DDT (Larviol F). The formulations were applied by means of an Agla microsyringe over the surface (240 sq. cm.) of the water held in 5-lit.

TABLE 5.—Initial activity of Cidial 50 L against *Culex pipiens* L. larvae as compared with that of formulations containing Cidial, DDT and mineral oil.

Formulations	Cidial ppm	DDT kg/ha	Formln. kg/ha	% mortality after 24 hrs.
Cidial 50 L	0.02	....	...	100
Larviol C[a]	0.02	....	2.5	100
Larviol F[b]	0.02	0.05	2.5	100
Larviol [c]	....	0.05	2.5	0
Larviol	....	0.5	25	76

[a] Commercial, non emulsifiable, liquid formulation containing 1 percent technical Cidial, 92.1 percent gas oils derived from oil-refining surplus, 6.9 percent solvents and dispersing agents.

[b] Commercial, non emulsifiable, liquid formulation containing 1 percent technical Cidial, 2 percent technical DDT and 97 percent solvents and dispersing agents and gas oil derived from oil-refining surplus.

[c] Commercial, non emulsifiable, liquid formulation containing 1 percent technical Cidial, 2 percent technical DDT, 8 percent solvents and dispersing agents and 90 percent gas oils derived from oil-refining surplus.

Mariotte bottles in the amount of 4,000 ml.

At various intervals after treatment, water samples were taken from the bottom of the bottles, transferred to paper cups and tested on third and fourth instar larvae of *C. pipiens*, according to the method described under residual activity (Table 6).

samples taken and by using third and fourth instar larvae of *C. pipiens* as test animals. The latter were immersed in the dilutions for 1 hour and, after numerous dips in distilled water to get rid of possible insecticide residues, they were transferred to 100-ml. paper cups containing 100 ml. of distilled water. The

TABLE 6.—Diffusion in water of Cidial in different formulations, biologically assessed with *Culex pipiens* L. larvae.

Formulations	Cidial ppm	DDT kg/ha	Formln. kg/ha	% mortality at various intervals after treatment								
				3 hrs.	19 hrs.	2 days	10 days	11 days	12 days	14 days	15 days	20 days
Cidial 50 L	0.06	...	...	100	100	100	100	100	99	61	77	9
Larviol C	0.06	...	10	0	100	100	100	98	28	18	3	0
Larviol F	0.06	0.2	10	0	100	100	100	100	10	6	7	0
Larviol	....	0.5	25	0	0	0	0	0	0	0	0	0
Larviol	....	1.0	50	0	0	0	0	0	0	0	0	0

INFLUENCE OF THE WATER PH ON CIDIAL RESIDUAL ACTIVITY. Cidial in acetonic solution was diluted at the rate of 1 ppm a.i. in five portions of water prepared previously and buffered at 6–7–8–9–10 pH. Samples were taken at various intervals after dilution. The Cidial concentration in the water was determined biologically, that is by suitably diluting the water

mortality counts were taken 24 hrs. later and the concentration of biologically active substances, expressed as Cidial, was assessed each time by comparison with a standard regression line.

The data obtained and given in Fig. 4 indicate that Cidial degradation is influenced by the water pH and is remarkable at a very alkaline pH.

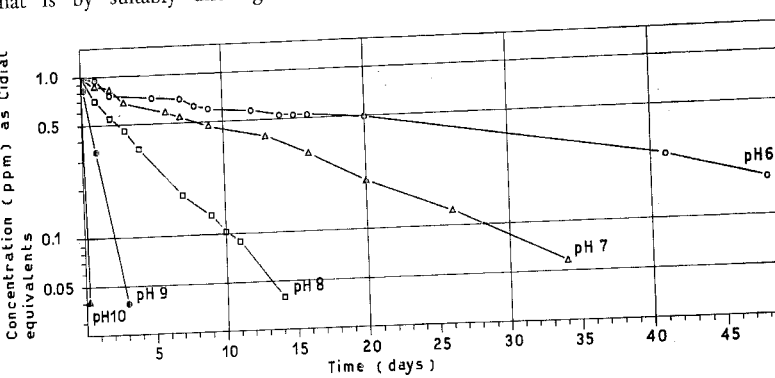

FIG. 4.—Cidial break-down in water (at 25° C), with a different pH, biologically determined by using *Culex pipiens* L. larvae.

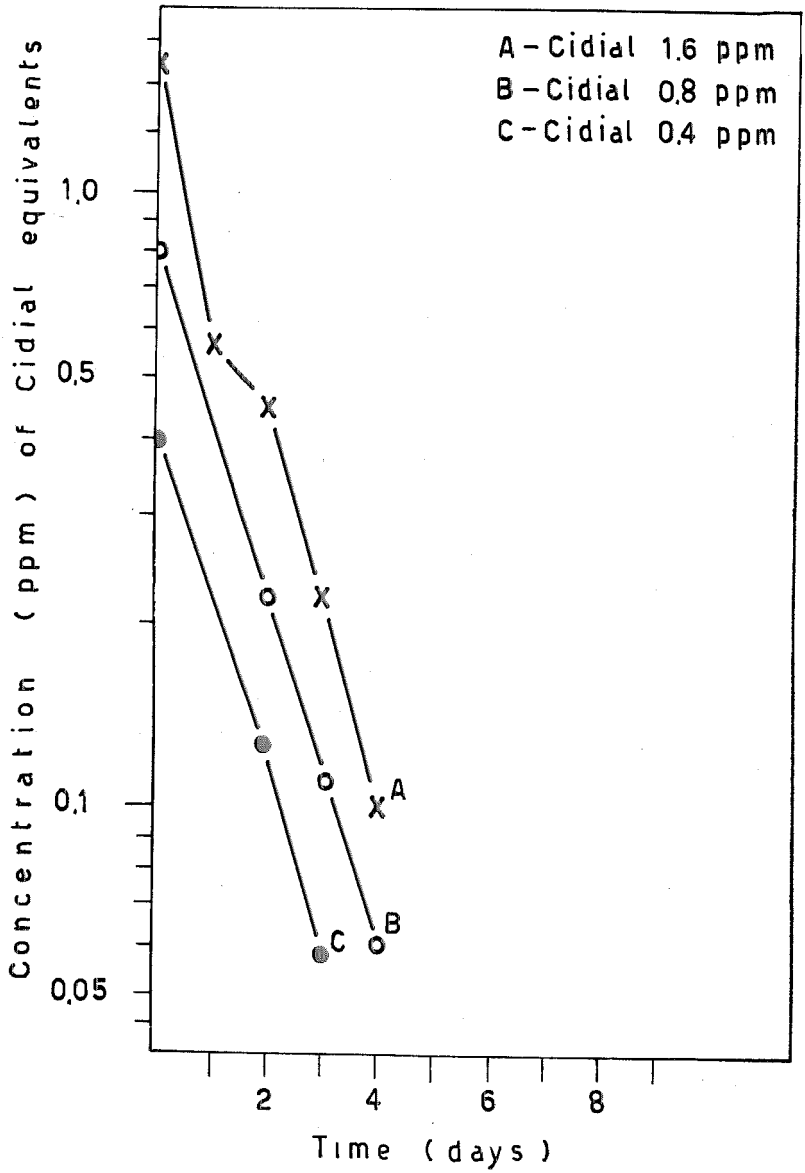

FIG. 5.—Cidial break-down in the water (8.2–8.4 pH) of three ditches treated with Cidial 50 L in the field. Bioassay with *Culex pipiens* L. larvae.

To confirm the break-down trend, in the long run, of the Cidial at a higher pH, a field test was carried out by operating in ditches (Fig. 3) filled with water (8.2–8.4 pH) treated with three different concentrations of Cidial (1.6; 0.8; 0.4 ppm) at the end of August.

Water samples were taken from the ditches at various intervals after treatment and the concentration of biologically active substances, expressed as Cidial, was determined each time on third and fourth instar larvae of *C. pipiens* (Fig. 5), following the procedure already illustrated for the laboratory experiments.

On the basis of the time (biologically determined on *C. pipiens* larvae) taken by Cidial break-down in water in relation to the pH, the values relating to the insecticide half-life and the correlation between the latter (expressed as Cidial equivalents) and the water pH were worked out. The experimental knowledge of such a correlation is extremely useful as it allows forecasts to be made of the larvicidal residual activity of the product at various concentrations and in relation to the water pH (Fig. 6–7).

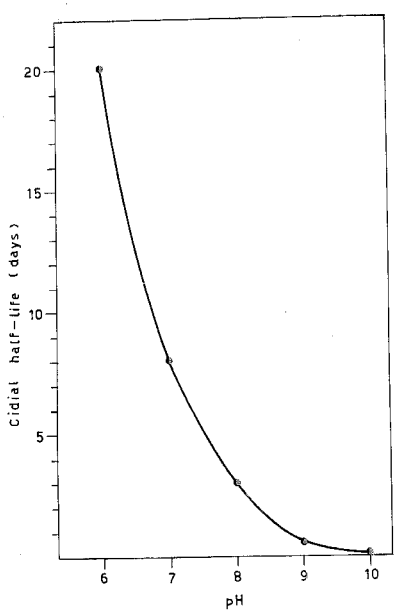

FIG. 6.—Correlation between Cidial half-life, biologically determined by using *Culex pipiens* L. larvae, and water pH.

ADULTICIDAL ACTIVITY. *Initial activity.* The tests were performed in an air-conditioned room (24–25° C.; 65–75 percent RH, 12 hrs' daily lighting) by following, as a general rule, the tarsal contact method recommended by the W.H.O. (W.H.O., 1963).

Females, 48–72 hrs. old, of *C. pipiens, A. gambiae* and of a strain of *A. albimanus* resistant to DDT and dieldrin, reared in the laboratory and fed on guinea-pigs 5–6 hours before treatment, were placed into contact for 1 hour with square pieces of no. 1 Whatman filter paper, 13 cm side length, treated with 1.7 ml of an ethanol solution of the test chemicals at various concentrations, applied in drop form all over the surface. After 1 hour's contact with the treated surface, the adults were transferred to an untreated surface and fed on sugar and water for 24 hrs., after which period mortality counts were taken (Table 7).

Residual activity. The tests were carried out in an air-conditioned room (24–25° C.; 65–75 percent RH; 12 hours' daily lighting) according to the tarsal contact method and on various substrata (fir-wood, poplar plywood, plastered panels) treated with an aqueous dilution of the test product, at a concentration of 2 percent a.i., at a rate of 50 ml./sq.m. surface (1 g a.i./sq.m.). The liquid was evenly applied all over the surface by means of a De Vilbiss sprayer, at a pressure of 0.4 Atm.

Use was made, in this case too, of 48- to 72-hrs. adult blood-fed females of *C. pipiens, A. gambiae* and of a strain of *A. albimanus* resistant to DDT and dieldrin. At various intervals after treatment, groups of these females were placed into contact for 30 min. with the above-mentioned treated surface. To do this,

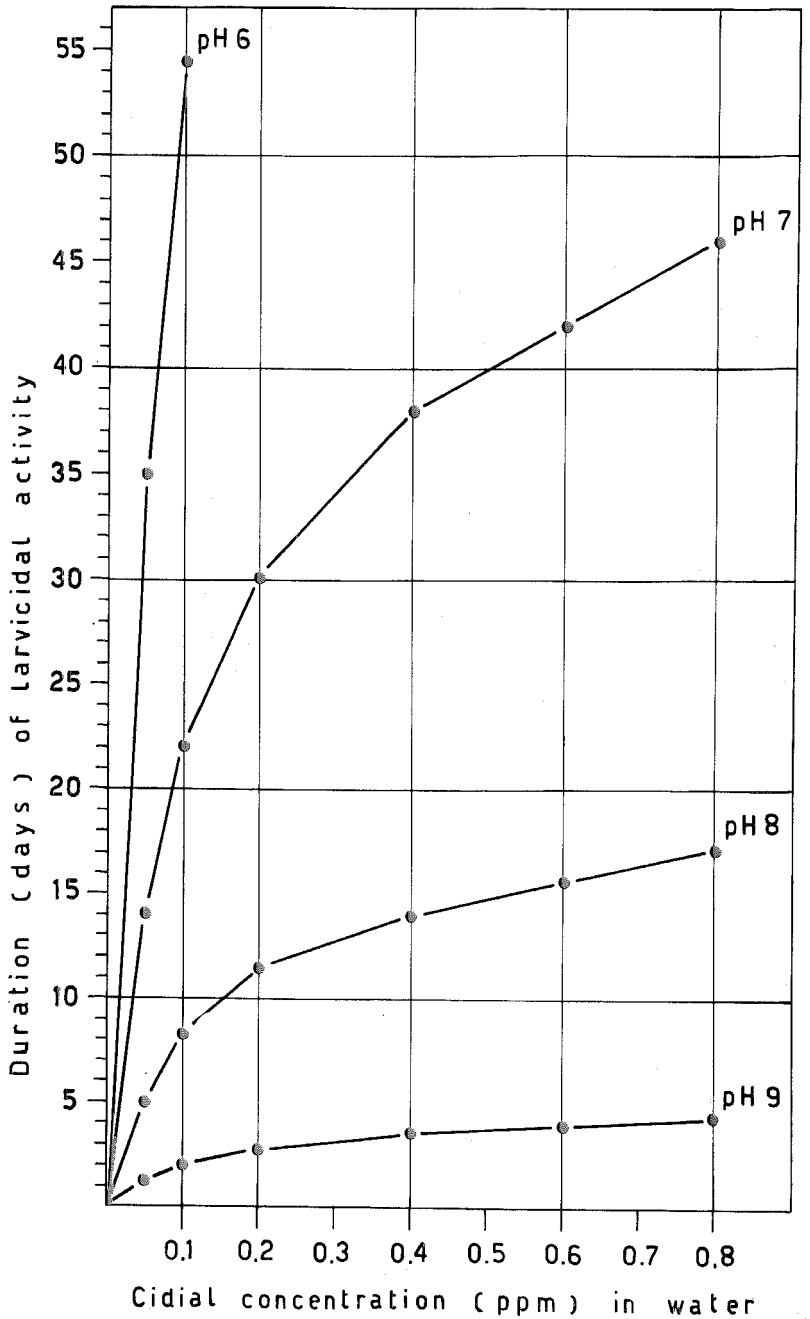

FIG. 7.—Correlation (determined by interpolation of the data shown in fig. 5) between duration of the larvicidal activity of Cidial at LC 95 level (0.015 ppm) and Cidial concentration in water (at 25° C) at various pH.

TABLE 7.—Initial activity of Cidial and of other insecticides on females, 48–72 hrs. old, of three mosquito species.

Insecticides	*Culex pipiens* L.		*Anopheles gambiae* G.		*Anopheles albimanus W.*	
	LD 50 g/sq.m.	LD 95 g/sq.m.	LD 50 g/sq.m.	LD 95 g/sq.m.	LD 50 g/sq.m.	LD 95 g/sq.m.
Cidial	0.017	0.035	0.14	0.25	0.2	0.34
malathion	0.15	0.62	0.23	0.6	0.56	1.4
DDT	>5	>5	[a]	[a]	>10	>10
dieldrin	0.014	0.66	0.019	0.08	>10	>10
fenthion	0.08	0.18	0.06	0.16	0.15	0.23

[a] The mortality obtained was not appreciable due to the excessive knock-down which stopped the contact in advance.

plastic cones adopted by the W.H.O. for residual activity field tests on adult mosquitoes were employed. The insects were then transferred to untreated surfaces and fed with sugar and water for 24 hrs., after which period mortality counts were taken (Fig. 8).

CONCLUSIONS. The data resulting from laboratory and field experiments and relating to the activity of Cidial against mosquito larvae and adults allow the following conclusions:

1) Cidial provides an extremely high initial activity against the larvae of *C. pipiens, A. aegypti, A. gambiae* and *A. albimanus* (Table 1). Among the products tested, Cidial has shown itself to be more effective than DDT, dieldrin, malathion, dichlorvos and slightly inferior to fenthion (Baytex 50), parathion, methylparathion and Sumithion against the two first species mentioned above. The control of *A. gambiae* given by Cidial was slightly inferior to that of parathion whereas it proved equivalent to or better than that of all the other chemicals tested. Against *A. albimanus,* Cidial was inferior to parathion and methylparathion only.

2) In the case of *C. pipiens,* Cidial was successful against the larvae of all instars and, when used at a higher dosage rate, also against pupae. No control of eggs was obtained even with the 1 ppm dose (Table 2).

3) The residual activity of Cidial, at LC_{95} level, against *C. pipiens* (operating in water at 8.2–8.4 pH) was found to be higher than that of malathion, heptachlor and dieldrin and inferior to that of fenthion (Table 3).

4) The different Cidial formulations tried out (granules, dust, wettable powder and emulsifiable liquids) did not exercise any significant influence on the residual activity against *C. pipiens* larvae (Table 4).

5) The larvicidal activity of the oil formulations (oil + Cidial and oil + Cidial + DDT) is due, within certain limits of doses and time, to the presence of Cidial in the formulation itself. Furthermore, it has been observed that Cidial does not remain in the oily film which develops on the water surface but that it spreads in water just a few hours after the oil coformulation has been applied.

6) The residual activity of Cidial is greatly affected by the water pH: at 6 pH, the 0.1 p.p.m. concentration is successful against *C. pipiens* larvae for about 55 days; at 7 pH, control is given for about 22 days, at 8 pH for about 8 days and at 9 pH for only 2 days.

7) Cidial also displays a marked initial activity against 48- to 72-hr. females of *C. pipiens, A. gambiae* and *A. albimanus.* This control has been found to be superior to that given by fenthion, malathion, DDT and dieldrin in the case of *C. pipiens,* by malathion and DDT in the case of *A. gambiae* and by malathion, DDT and dieldrin in the case of *A. albimanus* (Table 7).

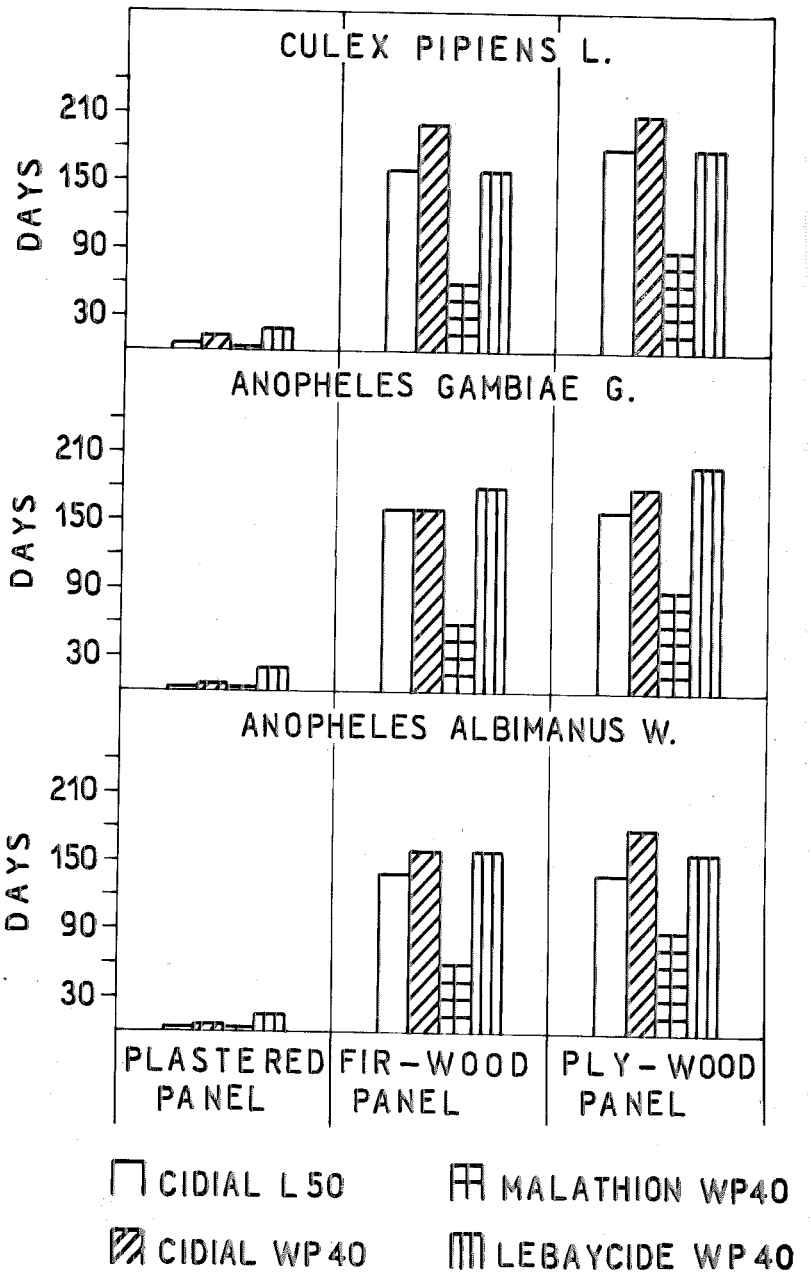

FIG. 8.—Persistence of adulticidal activity on substrata treated at the rate of 1 g active/sq.m. (30 min. contact).

8) The residual activity of Cidial, applied to fir-wood and poplar plywood panels, against 48- to 72-hrs. females of *C. pipiens, A. gambiae* and of a strain of *A. albimanus* resistant to DDT and dieldrin proved very high (140–210 days) and was found to be as good as that of fenthion (Lebaycide WP 40) and much superior to that of malathion (Fig. 8).

9) All the experimental products tested on plastered panels showed a low degree of residual activity (3 to 20 days) against the three above mentioned species. A formulation especially adopted for use on porous substrata could allow decidedly better results.

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