CONTACT TOXICITY OF INSECTICIDE DEPOSITS ON FILTER PAPER TO ADULT MOSQUITOES [1, 2]

G. P. GEORGHIOU AND FRANCES E. GIDDEN [3]
University of California, Riverside

Among the various laboratory methods available for the evaluation of the contact toxicity of insecticides against adult mosquitoes, two have been used rather extensively in recent years: one involves the use of oil-film deposits of the insecticides, and the other utilizes dry deposits of these compounds. In either case, the insecticide solution is applied to filter paper rather than glass, the former being a better substrate for mosquitoes. After the oil has spread evenly on the paper, or in the case of the dry deposit method, the volatile solvent has evaporated, the paper is rolled up and inserted into a vial or other suitable container which serves as the exposure chamber.

Although the principles involved in each method have been discussed (Busvine 1957), recent experience has emphasized certain outstanding features of the two methods which make them especially suitable in different situations. In the oil-film deposit method (Busvine and Nash 1953), uptake of dieldrin by the test insect was found to be a linear function of the concentration on the paper and also of the time of exposure (Pennell, Miskus and Craig 1964). Thus, the method is particularly suitable in the determination of insecticide resistance and has been widely accepted for this purpose (Pal 1964). On the other hand, the presence of oil on the substratum appears to affect the availability of various toxicants to the insect, probably due to differences in the solubility or stability of the compound in the type of oil used, and thus, the method does not give the true comparative toxicity of insecticides against a test population.

The dry residue method utilizes mainly acetone as a solvent, which, upon evaporation leaves a residue of crystals of the pure compound on the substratum. Since acetone is a nearly universal solvent it can be employed in tests with most compounds, so that no variable is introduced by differential rates of evaporation of various solvents. Although the type and size of crystal formation naturally varies with different compounds, insect exposure is to the pure compound, alone, without influence of additives such as oils. Thus, this method was found suitable for the screening and comparative evaluation of insecticides against adult mosquitoes (Georghiou & Metcalf 1961) and has been employed in tests with over 1,000 new compounds under the malaria eradication program of the World Health Organization. But this method is not suitable in the estimation of resistance, especially where the degree of resistance involved is elevated, since with certain compounds the uptake of insecticide is not proportional to that present on the substratum. This situation results in regression lines with plateaus or low slopes.

Because the two methods are best suited to different situations, it often becomes necessary to compare or convert data of authors who have used one or the other method. This paper presents comparative results obtained with two organochlorine, two organophosphorus and five carbamate insecticides by the use of the two testing methods. In addition, data are given which show that loss of activity

---

[1] Paper No. 1602, Citrus Research Center and Agricultural Experiment Station, University of California, Riverside.

This investigation was supported in part by Public Health Service Research Grant No. 11880-01 from the National Institute of General Medical Sciences.

[2] The able laboratory assistance of Mrs. Marilyn K. Hawley in the conduct of this investigation is gratefully acknowledged.

[3] Department of Entomology, University of California, Riverside.

of the carbamate residue is due to sorption by the cellulose fiber filter paper, and that such loss is minimized by use of glass fiber filter paper.

MATERIALS AND METHODS. All tests were performed on 3-day-old *Culex pipiens quinquefasciatus* adults from a susceptible laboratory strain. The procedure used in rearing and exposing the insects to the toxic residues has been previously described (Georghiou & Metcalf 1961).

The insecticides used were dieldrin, DDT, malathion, fenthion, B-39007 (*o*-isopropoxyphenyl methlycarbamate), B-37344 (4-methylthio 3,5-xylyl methylcarbamate), Upjohn U-12927 (6-chloro 3, 4-xylyl methylcarbamate), California Chemical RE-5030 (*m-tert-butylphenyl* methylcarbamate), and Isolan® (1-isopropyl-3-methyl-5-pyrazolyl dimethylcarbamate). All insecticides were of technical grade of the highest possible purity.

For the dry deposit tests, the insecticides were dissolved in acetone and applied at the rate of 1 ml of solution per No. 2 Whatman 9 cm filter paper disc. For the oil-film deposit tests with DDT and dieldrin, treated papers were kindly supplied by the World Health Organization. Papers with oil-film deposits of the remaining compounds were prepared in our laboratory shortly before use. The insecticide solutions were prepared with a mixture of petroleum ether and Risella Oil No. 921 (obtained from Shell Oil Co., Martinez, California) in a ratio of 2:1. As in the case of the acetone solutions, 1 ml of ether-Risella oil solution of the insecticide was applied per 9 cm filter paper disc. Exposure was for 1 hour, followed by a recovery period of 24 hours at 60° F.

Each material was tested by each method at five or more concentrations. Dosage-mortality regression lines were determined by probit analysis (Finney 1952) on a programmed IBM 1620 computer. Classes with expected values below 5 were pooled with adjacent classes prior to calculating X^2 values. The regression sum of squares was tested for significance against X^2/k where k is the number of degrees of freedom associated with the calculated X^2 and equals n-2-m, where m is the number of classes absorbed.

RESULTS AND DISCUSSION. The results obtained with each insecticide by the two testing methods are summarized in Table 1. These include LC_{50} values and their 95 percent fiducial limits, slopes (b) and their standard error, degrees of freedom and X^2 values for each ld-p-line. The last column indicates the quotient of the LC_{50} for oil-film/LC_{50} for dry deposit of each compound.

The organochlorine insecticides dieldrin and DDT were considerably more toxic (10.17- and 8.26-fold, respectively) as dry deposits than as oil-film. The organophosphorus insecticides malathion and fenthion were also more toxic when applied in acetone than in oil, but by a lower factor, i.e., 1.54- and 2.38-fold, respectively. The term "dry" deposit is not appropriate in this case since both compounds are oily liquids in the technical grade used. However, the addition of Risella oil obviously reduced the availability of the toxicants to the insect.

In contrast to the above, the contact toxicity of the five carbamates was enhanced by the oil-film. At the LC_{50} levels, dry deposits were from 0.17 to 0.96 times as toxic as oil-film deposits.

All tests produced straight ld-p lines, except in the case of the dry deposits of DDT. Although at low concentrations this compound was more toxic in dry form than in oil-film, uptake of the toxicant appears to have reached maximal levels at a dry residue concentration of approximately 15 $\mu g/cm^2$ (Fig. 1). Increasing the dry residue concentration to 300 $\mu g/cm^2$ did not produce an increase in mortality.

The slope of the ld-p lines has not been greatly influenced by the presence of oil except in the case of dieldrin for which $b = 3.95$ for the oil-film residue and 5.68 for the dry residue. This is consistent with the higher toxicity of this compound in the pure crystalline form. Variability of results with a given concentration of organochlorine or carbamate insecticide was more pronounced in the dry than in

TABLE 1.—Comparative toxicity of several insecticides tested on cellulose filter paper as dry and oil-film residues against *Culex pipiens quinquefasciatus*

Insecticide	Residue	LC_{50} ($\mu g/cm^2$)	95% fiducial limits	Slope $b \pm$ S.E. b	Degrees of freedom	X^2	Ratio LC_{50}s Oil/Dry
Dieldrin	Oil	8.892	8.022—9.857	3.95±0.42	2	0.892	10.17
	Dry	0.874	0.763—0.974	5.68±0.76	6	19.076	
DDT	Oil	55.953	49.592—63.130	3.19±0.30	3	2.884	8.26
	Dry	6.775	3.986—9.821	2.00±0.31	8	59.231	
Malathion	Oil	9.501	8.738—10.168	6.77±0.77	3	7.761	1.54
	Dry	6.158	6.562—7.332	7.33±0.95	3	2.186	
Fenthion	Oil	2.746	2.515—2.968	5.55±0.65	3	1.881	2.38
	Dry	1.153	1.071—1.259	4.91±0.59	3	1.235	
B-39007	Oil	0.822	0.717—0.942	2.67±0.30	4	8.235	0.17
	Dry	4.864	2.707—7.204	1.92±0.29	5	17.288	
B-37344	Oil	3.275	2.943—3.532	7.24±1.25	1	0.116	0.34
	Dry	9.667	8.808—10.479	6.97±1.11	4	0.689	
U-12927	Oil	3.102	2.717—3.457	4.40±0.51	2	2.670	0.96
	Dry	3.238	2.758—3.665	3.15±0.37	6	12.254	
RE-5030	Oil	0.438	0.393—0.488	5.20±0.55	3	2.093	0.62
	Dry	0.703	0.615—0.776	4.92±0.68	2	3.106	
Isolan ®	Oil	4.824	4.086—5.509	4.10±0.59	2	3.128	0.48
	Dry	9.970	3.651—26.713	3.42±0.72	2	6.418	

[a] All compounds crystalline in technical grade except malathion, fenthion and Isolan.

the oil-film residues. This is also evident in the X^2 values given in Table 1 which are greater for the ld-p lines of the dry residues of these compounds than the oil-film. In general, a large number of replications is necessary in the dry residue tests in order to produce ld-p lines with acceptable X^2 values. This, however, is compensated by the greater convenience and speed which is possible in the dry residue tests.

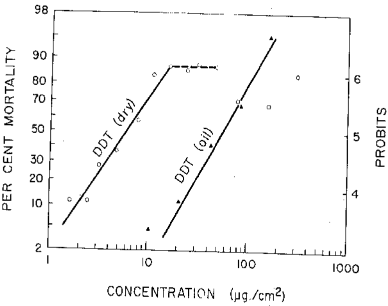

FIG. 1. Dosage-mortality regression lines for dry and oil-film residues of DDT against *Culex pipiens quinquefasciatus.*

The differences in the toxicity of dry vs. oil-film deposits of insecticides are due most likely to differences in the availability of the active ingredient to the test insect. The results obtained with organochlorine and organophosphorus insecticides parallel data presented by Hadaway *et al.* (1963) who found that wettable powders of DDT, dieldrin, malathion and fenthion were more toxic than emulsions on plywood and dried mud bricks against *Anopheles stephensi* and *Aedes aegypti*.

The results with the carbamates are interesting inasmuch as dry residues of these compounds show lower toxicity than oil-film residues. This could be due to greater sorption of the carbamate crystals into the fibers of the filter paper, resulting in a reduction in the residue available to the insect. It may also be due to low solubility of the carbamates in the tarsal waxes, so that the rate of penetration of the pure compound into the insect is relatively slow. In this case, the presence of oil on the substratum may have the effect of increasing the uptake of toxicant by enhancing the rate of penetration and/or of contamination of the insect by the residue. This, however, may be of only minor importance since previous results by Georghiou & Metcalf (1961) show that mineral oil-film residues of AC-5727 (*m*-isopropylphenyl methylcarbamate) in glass vials are not more toxic to *C. p. quinquefasciatus* than dry residues of this carbamate.

The possibility of differential loss in activity of dry vs. oil-film residues of carbamates due to sorption was investigated as follows: filter papers were treated with B-39007 (*o*-isopropoxyphenyl methylcarbamate) in acetone solution, at a rate producing a dry residue of 9.5 $\mu g/cm^2$ and in Risella oil/ether solution producing a residue of 1.6 $\mu g/cm^2$. These concentrations were selected to give 80 to 90 percent mortality. There were 10 replications per treatment. New papers were treated on different days, stored at 80° F. and 60 percent relative humidity, and tested in one operation when the residues were 2 hours, 1, 3, 6, and 10 days old.

TABLE 2.—Effect of aging on toxicity of dry and oil-film residues of B-39007 on cellulose filter paper, to *Culex p. quinquefasciatus*.

	24-hour mortality (%) [a]	
Age of Residue	Dry residue (9.4 $\mu g./cm^2$)	Oil-film residue (1.6 $\mu g./cm^2$)
2 hrs	90.7±2.7	81.0±3.7
1 day	36.4±6.2	78.0±4.5
3 days	20.4±3.9	34.9±4.3
6 days	10.6±5.5	17.2±5.3
10 days	20.0±4.3	4.8±1.6

[a] Average of 10 replications ± standard error.

The results presented in Table 2 and Fig. 2 show that the toxicity of both dry and oil-film residues to *C. p. quinquefasciatus* declined sharply during the first 6 days after treatment. The loss within the first 24 hours was more pronounced in the dry residue and amounted to 60 percent of the initial (2-hour) toxicity. Since this insecticide is known to be chemically stable for several months (Gahan *et al.*, 1962) there can be little doubt that the rapid loss in toxicity on filter paper is due to sorption of the compound by the cellulose fiber of the filter paper.

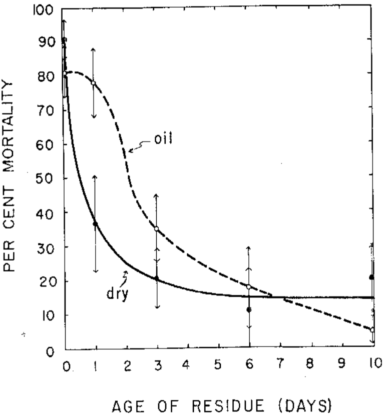

FIG. 2. Comparative loss of toxicity of dry and oil-film residues of B-39007 on cellulose filter paper, evaluated against *Culex pipiens quinquefasciatus*. (Arrows indicate 95% confidence intervals.)

This point was substantiated by comparing the persistence of toxicity of dry deposits of B-39007 on cellulose filter paper vs. glass fiber paper (Whatman G/F A 9.0 cm glass paper circles). Two-hour old residues of B-39007 were 6.5 times more toxic on glass paper than on cellulose paper (LC_{50} 0.75 and 4.86 p.p.m., respectively). The degree of persistence of toxicity of residues on glass and cellulose paper is shown in Table 3 and Fig. 3. There was only negligible loss of toxicity on glass paper while on celluose paper the

TABLE 3.—Comparative sorption by cellulose and glass fiber papers of dry residues of B-39007 evaluated against *Culex p. quinquefasciatus*.

	24-hour mortality (%) [a]	
Age of Residue	Cellulose paper (9.4 $\mu g./cm^2$)	Glass paper (3.2 $\mu g./cm^2$)
2 hours	81.5±2.7	95.0±2.1
1 day	20.0±6.1	93.5±2.1
3 days	20.5±5.7	88.0±2.1
6 days	17.0±5.1	90.5±3.3
10 days	19.4±3.6	86.0±3.5

[a] Average of 10 replications ± standard error.

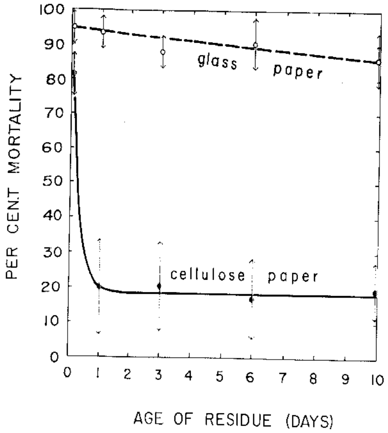

FIG. 3. Differential sorption of B-39007 residues by glass fiber and cellulose fiber paper, evaluated against *Culex pipiens quinquefasciatus*. (Arrows indicate 95 percent confidence intervals.)

loss was considerable and closely approximated the earlier results (Fig. 2). Further tests have indicated that extraction of 10-day old "inactive" residues of B-39007 with acetone from cellulose filter papers and their transfer to glass fiber papers re-establishes fully the toxicity of the residue to the adult mosquitoes.

It is likely that some carbamates may be sorbed less extensively than others by cellulose filter paper. This work has demonstrated, however, that sorption of both dry and oil-film residues of B-39007 may cause a considerable reduction in the amount of residue available to the test insect. In order to avoid the variables introduced by differential sorption of the test compounds, it is suggested that glass fiber papers be used in lieu of cellulose filter papers. The former should prove useful not only in laboratory screening and bioassay tests, but also in the field resistance tests introduced by the World Health Organization.

References Cited

BUSVINE, J. R. 1957. A critical review of the technique for testing insecticides. Commonwealth Institute of Entomology, London, 208 pp.

——— and NASH, RUTH. 1953. The potency and persistence of some new synthetic insecticides. Bull. Ent. Res. 44:371–376.

FINNEY, D. J. 1952. Probit Analysis, 2nd ed. Cambridge Univ. Press, England.

GAHAN, J. B., LABRECQUE, G. C., and WILSON, H. G. 1962. Relative toxicity of selected compounds as residual sprays against adults of *Anopheles quadrimaculatus* Say. Mosquito News 22 (4):333–336.

GEORGHIOU, G. P., and METCALF, R. L. 1961. A bioassay method and results of laboratory evaluation of insecticides against adult mosquitoes. Mosquito News 21(4):328–337.

HADAWAY, A. B., BARLOW, F., and DUNCAN, J. 1963. Some effects of formulation on the initial contact toxicity of insecticides to adult mosquitoes. Bull. Wld. Hlth. Org. 28:129–132.

PAL, R. 1964. Test methods for resistance. Unpublished document, WHO/Vector Control/87. 12 pp.

PENNELL, J. T., MISKUS, R., and CRAIG, R. 1964. The use of gas chromatography for the quantitative determination of micro-amounts of insecticides picked up by mosquitoes. Bull. Wld. Hlth. Org. 30:91–95.

CHARGES FOR EXTRA PAGES and REPRINTS

Please refer to pages 227, 237 and 239 for this important information. See especially the last paragraph in the second column on page 239.