COMPARISON OF LARVAL BIOASSAY AND CHEMICAL ANALYSIS FOR ASSESSING AERIAL SPRAY DEPOSITS

C. H. SCHAEFER,[1] A. F. GEIB,[2] W. H. WILDER [1] AND P. A. GILLIES [3]

During recent years low volume (LV) applications of insecticides, either as technical materials or as concentrate formulations, have had a tremendous influence on mosquito control operations. Reductions in time spent in insecticide loading and ferrying and increases in swath width have resulted in large increases in the acreage sprayed per load. As a result of low volume applications, up to 70 percent increases in operational efficiency have been reported; the impact of this methodology on mosquito control operations in California's San Joaquin Valley has recently been reviewed (Geib, 1969).

The production of wide swaths by application of concentrate formulations from relatively high altitudes has the potential of reducing operational costs and of rapid treatment of large areas, such as might be involved during an outbreak of a vector-borne disease. However, this drift-type of application, which allows wide swaths, may also result in increased detectable residues outside of the target area—a chief problem that limits the usefulness of this technique.

During the past year, low volume applications for mosquito control have been field-tested in California to determine swath widths and patterns and to compare recovery of applied materials by different methods (Akesson and Burgoyne, 1969). Past experience in California has indicated that insecticide deposits determined by bioassay methods give higher values as compared to chemical analyses of exposed surfaces, e.g., Mylar plastic sheets. The present paper describes field experiments in which LV applications were made and the spray deposits were compared following measurements by chemical and bioassay methods.

[1] University of California, Mosquito Control Research Lab., 5545 E. Shields Avenue, Fresno, California 93727.

[2] Kern Mosquito Abatement District, P.O. Box 907, Bakersfield, California 93302.

[3] California Department of Public Health, Bureau of Vector Control, 5545 E. Shields Avenue, Fresno, California 93727.

General Methods. All experiments were conducted in Kern County, California using a Piper Pawnee (PA–25–235) equipped with an electric motor-driven pumping system, and using Dursban® as the test insecticide. The evaluations reported here are restricted to determinations of insecticide reaching ground level, and, therefore, primarily pertain to larviciding operations. Deposits were measured at stations set out at various distances perpendicular to, and downwind from, the aircraft flight line.

For bioassay, 10-ounce waxed paper cups containing 250 ml. of water were exposed during spraying. *Culex pipiens quinquefasciatus* larvae were then added and the times of knockdown were compared to those of larvae exposed to a series of known insecticide standards; details of this technique have recently been published (Gillies *et al.*, 1968). The surface deposit from aluminum plates, exposed during spraying, were carefully washed, with acetone, into flasks (note: in contrast to this technique, a careful washing with n-hexane did not quantitatively remove Dursban deposits from the aluminum surfaces); the volume of acetone in each flask was reduced to about 0.1 ml. "in vacuo," and the residual contents were transferred, with n-hexane, into graduated tubes for gas-chromatographic analysis.

Glass petri dish covers (6-inch diameter), filled with water to within ⅛ inch of the brims, were also exposed to the application. The contents were then poured into bottles, and the sides and bottom of each dish were thoroughly rinsed into its respective bottle with chloroform; these samples were transported back to the laboratory for Dursban extraction and gas-chromatographic analysis. Petri dish covers were silanized prior to field use in order to prevent the plating-out of Dursban on the sides and bottom. Details of the methods for silanization Dursban extraction from water, and the subsequent gas-chromatographic analysis have recently been described (Schaefer and Dupras, 1969).

FEBRUARY 1968 TESTS—PROCEDURES AND RESULTS. These tests were conducted over dry, open lands in western Kern County. The aircraft was equipped with 22, #8002 fan-type nozzles, pressured at 40 p.s.i. and was flown at 90 m.p.h. Dursban was applied at .05 lb. in 3 ounces of Dowanol TPM per acre, for an estimated swath width of 3,000 feet when the application altitude was 1,000 feet, and for a 6,000-foot swath when the insecticide was applied from an altitude of 2,000 feet. For the test at the 1,000-foot application altitude, waxed cups and petri dishes containing water and aluminum plates were exposed simultaneously; stations were set out at 250-foot intervals for a total distance of 5,000 feet downwind from the flight line. The deposits indicated by the various sampling techniques are shown in Table 1. Between 1,500 and 3,750 feet downwind from the flight line, samples from the aluminum plates indicated that deposits were in the same range as those obtained by bioassay; beyond 3,750 feet, the depositions indicated by the aluminum plates were lower. Two petri dishes placed at the 3,000-foot station indicated deposits similar to those determined by bioassay or aluminum plates. However, at 4,500 feet the petri dish sample was similar to the bioassay results, and did not show a decline in deposit as indicated by the aluminum plates. As the distance from the flight line increases, finer spray particles predominate and the results indicate that these smaller droplets do not impinge onto aluminum plates to the same extent as into water samples.

At an application altitude of 2,000 feet, deposits as determined by bioassay of exposed waxed cups were consistently higher than those determined by gas-chromatographic analyses of deposits extracted from the surfaces of aluminum plates or Mylar plastic sheets (Akesson and Burgoyne, 1969). No petri dish samples were included in the latter test.

TABLE 1.—Deposits of Dursban in the February 1968 test from 1000 feet above ground with 3 to 6 m.p.h. winds.

Distance downwind (feet)	Method of sample collection and analysis								
	Al. plates—GLC			Waxed cup—Bioassay			Petri dish—GLC		
	No. samples	Total ft.^{2} sampled	Average $\mu g/ft.^2$	No. samples	Total ft.^{2} sampled	Average $\mu g/ft.^2$	No. samples	Total ft.^{2} sampled	Average $\mu g/ft.^2$
1500	2	.888	14.6	2	.143	25.2	1	.183	2.7
1750	1	.444	18.7	2	.143	21.0	..	...	....
2000	1	.444	8.1	2	.143	11.2	..	...	....
2250	1	.444	18.0	2	.143	8.4	..	...	....
2500	1	.444	36.0	2	.143	64.4	..	...	....
2750	1	.444	13.5	2	.143	92.4	..	...	....
3000	2	.888	48.9	2	.143	47.6	2	.366	99.4
3250	1	.888	91.8	2	.143	145.6	..	...	....
3500	1	.888	151.9	2	.143	156.8	..	...	....
3750	1	.888	101.7	2	.143	98.0	..	...	....
4000	1	.888	46.6	2	.143	151.2	..	...	....
4250	1	.888	48.4	2	.143	128.8	..	...	....
4500	2	.888	36.0	2	.143	72.8	1	.183	137.0
4750	1	.444	68.6	2	.143	109.2	..	...	....
5000	1	.444	12.6	2	.143	64.4	..	...	....

TABLE 2.—Comparisons for sampling units used at each spray sampling station in the June and July tests.

Sampling unit	Number of sampling units	Surface area per unit (ft.^{2})	Total surface area sampled (ft.^{2})
Waxed cup	8	.0715	0.572
Aluminum plate	3	.1914	0.574
Petri dish	3	.1826	0.548

JUNE 1968 TEST—PROCEDURES AND RESULTS. This test was conducted over dry, native pasture; while some low vegetation was present, samples were placed on open areas to prevent "screening-out" effects. The aircraft was equipped with 22, #8001 fan-type nozzles, pressured at 40 p.s.i., and was flown at 90 m.p.h. Dursban was applied at .05 lb. in 2.5 ounces of Dowanol TPM per acre, estimated to give a 660-foot swath when applied from 100 feet above ground. Winds were 3–4 m.p.h. at the time of the flight (about 9 a.m.). Sampling stations were established at 110, 220, 440, and 660 feet downwind.

For comparative purposes, the number of waxed cups, aluminum plates and petri dishes utilized at each sampling station was calculated to sample the same approximate surface area (Table 2). The insecticide deposits measured from the June experiment are shown in Table 3. Data for the 110-foot sampling station are omitted, since the deposits were negligible, i.e., the actual swath was downwind from this station. In all cases the deposits measured by bioassay of the waxed cups were significantly greater than those of the aluminum plates; there were no significant differences between waxed cups versus petri dish deposits, and in all cases the deposits from aluminum plates were significantly lower than either of the others. Thus, impingement of insecticide into any of the water samples was greater than onto the aluminum plate surfaces.

TABLE 3.—Deposits of Dursban (micrograms/ft.^{2}) measured during the June 1968 test

Distance downwind (feet)	Method of Collection and Analysis: Paper cup—Bioassay	Petri dish—GLC	Al. plate—GLC
220	198.9 (8)* ±73.0	127.1 (3) ±29.3	44.7 (3) ±23.0
	yes (Paper cup — Al. plate)		
significance**	no (Paper cup — Petri dish)		
		yes (Petri dish — Al. plate)	
440	125.1 (8) ±58.0	120.2 (3) ±24.6	11.8 (2) ± 9.3
	yes (Paper cup — Al. plate)		
significance**	no (Paper cup — Petri dish)		
		yes (Petri dish — Al. plate)	
660	119.2 (8) ±40.3	106.9 (3) ± 9.9	38.8 (3) ± 9.9
	yes (Paper cup — Al. plate)		
significance	no (Paper cup — Petri dish)		
		yes (Petri dish — Al. plate)	

* Number in parenthesis refers to number of replicates.

** Comparisons of pairs of means by t-test (5% level).

JULY 1968 TEST—PROCEDURES AND RESULTS. The same aircraft "setup" was used as in the June test. The test field was an irrigated pasture with an average water depth of 2 inches and the sampling devices were placed within 1 inch of the water surface. Additionally, 1 by 3 inch microscope slides, coated with MgO layers, were placed at each sampling station so that spray particle size distribution could be determined. Larval density in the field was estimated just prior to treatment. The aircraft flight line was 100 feet upwind from the beginning of the sampling line, as wind conditions were similar to those of the June experiment in which no deposit "settled" at the 110-foot station; such operational adjustments are very important in utilizing LV techniques, but they must be made only after careful consideration of the prevailing meteorological conditions. Dursban was applied at .05 lb. in 2.5 ounces of Dow polyglycol 400 per acre.

Results of the July experiment are shown in Table 4. It is apparent that good deposits were obtained 110 feet downwind from the edge of the target area; posttreatment counts of *Aedes nigromaculis* larvae showed excellent control over the entire 660-foot swath. In all cases, as for the June experiment, deposits as measured by waxed-cup bioassay were significantly greater than those obtained from aluminum plates. The deposits from petri dishes were not significantly different from either of the other sampling units at the 220 or the 440 foot stations, where the spray particle sizes were essentially the same (Figure 1). At the 110-foot station, the deposits showed the greatest differences, as would be expected, since drop sizes are largest and the inclusion or ab-

TABLE 4.—Deposit of Dursban (micrograms/ft.^{2}) measured during the July 1968 test.

Distance downwind (feet)	Method of Collection and Analysis: Waxed cup—Bioassay	Petri dish—GLC	Al. plate—GLC
110	167.0 (8)* ±28.7	108.7 (3) ±59.5	79.2 (3) ±50.3
significance** (Waxed cup–Al. plate)	yes		
(Waxed cup–Petri dish)	no		
(Petri dish–Al. plate)		no	
220	136.5 (8) ±18.6	100.0 (2) ±40.7	52.9 (3) ± 6.1
significance (Waxed cup–Al. plate)	yes		
(Waxed cup–Petri dish)	no		
(Petri dish–Al. plate)		no	
440	66.8 (8) ±25.3	35.8 (3) ± 7.4	27.8 (3) ± 6.0
significance (Waxed cup–Al. plate)	yes		
(Waxed cup–Petri dish)	no		
(Petri dish–Al. plate)		no	
660	29.3 (8) ± 9.5	18.1 (3) ± 4.0	8.7 (3) ± 2.5
significance (Waxed cup–Al. plate)	yes		
(Waxed cup–Petri dish)	no		
(Petri dish–Al. plate)		yes	

* Number in parenthesis refers to number of replicates.
** Comparisons of pairs of means by t-test (5% level).

sence of a relatively small number of drops in a given sample could appreciably affect deposit; this becomes readily apparent when it is considered that drop volume increases in relation to the cube of diameter increase. At the 660-foot station, the drop sizes were much smaller (Figure 1), e.g., the mmd. was 25 microns as compared to twice this value at 220 and 440 feet and about three times this value at 110 feet; thus, at 660 feet the average diameter drop contained only 1/27th of the volume of that at 110 feet and ⅛th of the volume of that at 220 or 440 feet! The data for the 660 foot station agree with that for all of the stations in the June experiment, i.e. impingement of spray into water is significantly greater than onto aluminum plate surfaces. Unfortunately, no spray particle size information is available for the June experiment; it is quite possible that the degree of spray atomization was quite different, especially since the formulations utilized different solvents.

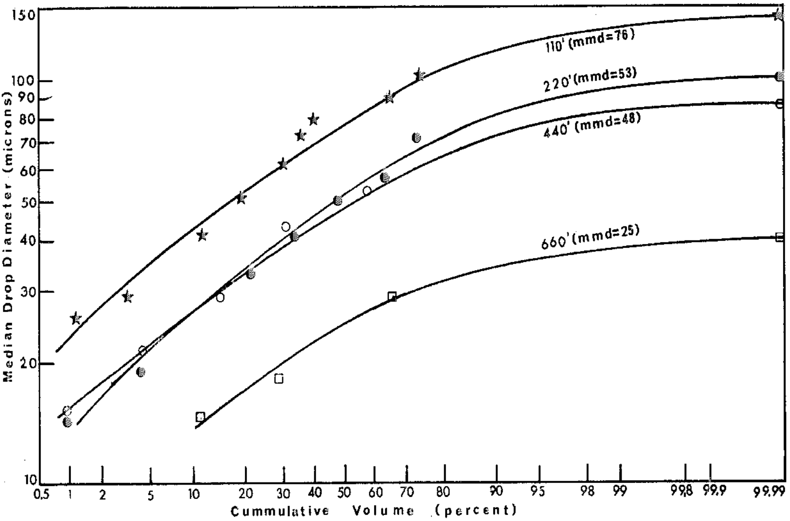

FIG. 1.—Distribution of drop sizes at 110, 220, 440, and 660 feet from the flight line.

It is interesting to compare the impingement of the fine spray into water with the recent report in *Science* of the VX nerve gas accident that occurred in Utah following ULV applications (Boffey, 1968). In this instance a cloud of very fine particles of nerve gas moved over two ranges of mountains and affected sheep about 40 miles from the aircraft drop area. It was reported that scattered rain showers developed in the early evening after the test and "one of these rain showers could have washed out this airborne VX out of the air and deposited it on vegetation and the ground." Could a high degree of impingement of fine spray particles into the rain droplets have been of great importance in the Utah incidence?

SUMMARY. It is apparent that there are real differences between spray deposits as determined by bioassay of water in waxed cups versus chemical analyses of the surface deposits from aluminum plates or Mylar plastic sheets; we have no data to explain the cause of these differences. The biggest problem in conducting such experiments is that the number of samples which can be dealt with practically is small in comparison to the variations encountered under field conditions. It appears that finer spray particles, in particular, have a greater tendency to impinge into water, rather than onto a solid surface; it is quite apparent that further and more basic research should be conducted to ascertain the cause of this relationship.

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EVALUATION OF THE ULTRA-LOW VOLUME AERIAL SPRAY TECHNIQUE BY USE OF CAGED ADULT MOSQUITOES

CARLISLE B. RATHBURN, JR., ANDREW J. ROGERS, ARTHUR H. BOIKE, JR. AND ROBERT M. LEE

West Florida Arthropod Research Laboratory, Florida State Board of Health, Panama City, Florida

Since the introduction of the ultra-low volume (ULV) aerial spray technique (Messenger, 1963), a number of reports have been published on the effectiveness of this procedure against natural populations of mosquitoes, evaluation being by pre- and posttreatment landing rate counts in most instances. (Stevens and Stroud, 1966, 1967; Glancy *et al.*, 1965, 1966; Mount and Lofgren, 1967; Knapp and Pass, 1966a, b; Knapp and Roberts, 1965; Knapp and Gayle, 1967; Knapp and Rogers, 1968.)

Early trials by this research group, using caged mosquitoes to evaluate results in two different habitats, were disappointing (Rathburn *et al.*, 1968). It was felt that distribution and behavior of the small droplets produced by this technique were the important factors affecting results. Rogers *et al.* (1965) gave some insight into the problem when they demonstrated the effect of habitat on the penetration of spray droplets and subsequent kill of mosquitoes. Cutkomp *et al.* (1950) also realized the importance of a study of factors contributing to the successful control of insects by aerial sprays when they demonstrated the effect of air temperature, spray density, flight altitude and droplet size on the deposition of spray droplets from aircraft. Probably the most comprehensive single treatment of the behavior of aerial sprays is offered by Johnston *et al.* (1947), who list droplet spectrum and the number of droplets reaching the mosquito habitat, the type and height of vegetation and meteorological conditions as some of the more important considerations in the aerial dispersion of insecticides. Many other research workers also have added to our knowledge in this area; however, much of this research is agriculturally oriented and therefore the problems are not necessarily the same as those encountered in vector control.

The objective of the study reported here was to evaluate the ULV technique with special emphasis on size, number and distribution of droplets in the target area, using caged adult mosquitoes as the principal means of evaluation.

METHODS. All tests were conducted with