EFFECT OF DROPLET SIZE OF MALATHION AEROSOLS ON KILL OF CAGED ADULT MOSQUITOES[1, 2]

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ABSTRACT. Experiments to determine the relationship between insecticidal droplet size and kill of adult mosquitoes were conducted using laboratory wind tunnel tests with monodisperse aerosols and field tests with ground ultra-low volume (ULV) aerosol generators.

Wind tunnel tests with uniform sizes of malathion droplets produced from a Berglund-Liu Monodisperse Aerosol Generator were conducted with caged *Aedes taeniorhynchus* females. Mortality was determined for 3 exposure times for 18 droplet sizes in the range from 2.8 to 32.8 μm. The results indicated that the optimum droplet size was in the range from 10 to 15 μm in diameter and that insecticidal efficiency decreased rapidly for sizes smaller than 5 μm and larger than 25 μm in diameter, but little difference in efficiency was noted for sizes from ca. 7 to 22 μm.

Field tests were conducted with malathion aerosols having a range of droplet sizes produced by ground ULV generators operated to provide volume median diameters (VMD's) of 5, 10, 15, 24 and 39 μm. Caged *Ae. taeniorhynchus* and *Anopheles quadrimaculatus* females were exposed in an open field under very selective atmospheric conditions. The results indicated that the 10 and 15 μm VMD aerosols were most efficient with 82% mortality each, compared to 67 and 72% mortality for the 5 and 24 μm VMD's. The 39 μm VMD aerosol was clearly less efficient than the smaller aerosols with a comparative mortality of 33%. The results of the field tests were consistent with laboratory tests.

INTRODUCTION

Currently, ultra-low volume (ULV) applications of adulticides represent one of the most widely used and effective methods for control of mosquitoes in urban and suburban areas. Previous research has shown that the effectiveness of these applications is fundamentally related to the particle or droplet size of the aerosol. This is particularly true for applications which depend on direct contact of the insecticide with the insect body.

Latta et al. (1947) conducted a laboratory wind tunnel study with uniform droplet sizes of DDT which indicated that 12 to 20 μm diameter was optimum for adult mosquito control with wind velocities of 2 to 8 mph. LaMer et al. (1947) indicated that the optimum droplet size for mosquito control was 15.8 μm in a theoretical analysis. Mount et al. (1968) reported that malathion aerosols with 6 to 10 μm volume median diameter (VMD) were more effective than aerosols with 11 to 22 μm VMD when applied with truck-mounted equipment in field tests with caged mosquitoes.

Weidhaas et al. (1970), in a study of the amount of insecticide required to kill a mosquito, indicated that the optimum size of malathion droplets should be below 25 μm. Mount (1970), in a review of previous work, concluded that 5 to 10 μm droplets may be optimum for ground aerosol applications. This review pointed out the need for experimental verification of the true optimum particle size for mosquito control. Lofgren et al. (1973), using electron microscopy, reported that

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[2] This paper reports the results of research only. Mention of a commercial or proprietary product does not constitute an endorsement of this project by the U.S. Department of Agriculture or the U.S. Army.

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particles in the range from 1 to 16 μm were most efficiently collected on the body of a mosquito.

Except for the work of Latta et al. (1947) and LaMer et al. (1947) with uniform sized particles of DDT, previous research on the optimum aerosol size for mosquito control has been conducted with aerosols having a wide range of droplet sizes. Verification of these results with chemicals presently used in ULV mosquito control applications has been limited due to the difficulty of producing a consistent uniform size. However, equipment for production of monodisperse aerosols having a high degree of size uniformity has been developed (Berglund and Liu 1973) and is commercially available, making additional studies feasible.

The objective of this research was to investigate further the relationship between droplet size and insecticidal efficiency in adult mosquito control. Laboratory wind tunnel experiments were conducted with various groups of uniformly sized droplets and field tests were conducted with current ground ULV aerosol generators operated to produce different levels of atomization.

METHODS AND MATERIALS

LABORATORY TESTS WITH MONODISPERSE AEROSOLS. A laboratory wind tunnel system (Fig. 1) was developed to measure the effect of droplet size of monodisperse aerosols on insecticidal efficiency against caged adult mosquitoes. The wind tunnel was constructed with a cylindrical test section of Plexiglas®, 1.5 m (5 ft) long × 24 cm (9.5 in.) ID. Air was drawn through the test section by a shaded pole blower with its speed controlled by a variac to set air speed at 1 m/sec (200 fpm). Air speed was measured at the test section exit (center of cross section) with a hot-wire anemometer. The air was filtered by a fiberglas and charcoal filter to remove most of the insecticide before exhaustion to the outside. A screen cage was devised to hold insect samples at the exit of the test section. The test section could be separated from the fan section to change insect cages. A Berglund-Liu Monodisperse Aerosol Generator (Thermo-Systems Inc. Model 3050) was modified to introduce uniform size insecticide droplets into the test section inlet. A shutter mechanism which was controlled by a photographic cable release and timer was devised to control the time that the aerosol was introduced into the tunnel. This mechanism allowed variable exposure times down to 0.1 sec. Since the generator produced charged droplets, a radiation source (10 mCi of Kr-85) was placed in the tunnel to neutralize the aerosol.

This aerosol generator was developed by Berglund and Liu (1973) for production of primary aerosol standards in the range of 0.3 to 50 μm in diameter. The generator utilizes the vibrating orifice

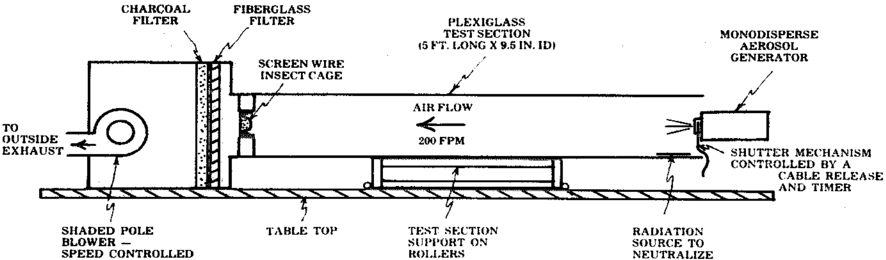

Fig. 1. Schematic diagram of wind tunnel for exposure of caged insects to monodisperse aerosols.

principle to uniformly break up a stream of liquid. The orifice is vibrated at a controlled frequency by a piezoelectric ceramic that is driven by a signal generator. The flow rate of liquid through the orifice is controlled by a syringe pump. With a known flow rate (Q) and frequency (f), the volume of each droplet (V) can be calculated ($V = Q/f$) and, therefore, the diameter (D) of each droplet can be calculated,

$$D = (6V/\pi)^{1/3}.$$

Droplet size can be varied by changing orifice size, flow rate, and frequency; however, variation of these parameters in practice is limited. With this system, the most effective method of obtaining various particle sizes is by dilution of a nonvolatile material in a volatile solvent with different concentrations (C). The diameter of the particle remaining after complete evaporation of the solvent can be determined by multiplying the original diameter by $C^{1/3}$. Although the droplets are generated uniformly, coalescence does occur resulting in droplets with multiple volumes of the primary droplet. This generator uses a turbulent air dispersion and dilution system to minimize this coalescence. In general, measurements of insecticide droplets collected on slides indicated that 90 to 95% of the droplets in the tunnel were the primary or uniform size.

Tests were conducted to establish the relationship between dose and mortality for a range of malathion droplet sizes with *Aedes taeniorhynchus* (Wiedemann) females (4–6 days old). The droplet sizes were established by using 3 generation frequencies and 6 different concentrations of malathion diluted in isopropanol. A 20 μm orifice was used with a constant flow rate of 0.139 ml/min for each concentration. With this orifice and flow rate, generator frequencies of 25, 49, and 80 khz could be used to produce a consistent and stable stream of uniform droplets for all concentrations. To establish equivalent doses for the different concentrations, the exposure times were changed with concentration. Table 1 shows the droplet sizes produced for each combination of frequency and concentration and the exposure time required to establish different doses for each concentration. The droplet size in these tests ranged from 2.8 μm to 32.8 μm. Larger sizes were not possible because the higher concentrations required would result in dose rates that were too high (causing 100% mortality), since exposure time was limited to a minimum of 0.1 sec. After each exposure, the insects were knocked down with CO_2, transferred to a clean cage and held overnight for mortality determinations. The insects were fed during the holding period from a cotton pad soaked in a 10% sugar/water solution. Each treatment was replicated 6 times and each replication consisted of exposure of 2 cages of mosquitoes containing 25 females each. Due to the number of treatments, 2 days were required to com-

Table 1. Droplet sizes and required exposure times for different insecticide concentrations in isopropanol (20 μm orifice, 0.139 ml/min solution).

Chemical concentration % volume	Diameter, μm, at each frequency, hz			Required exposure time sec, for each relative dose[a]			
	80000	49000	25000	1X	2X	4X	6X
0.04	2.8	3.3	4.1	25.0	50.0	100.0	150.0
0.2	4.8	5.6	7.1	5.0	10.0	20.0	—
1.0	8.2	9.7	12.1	1.0	2.0	4.0	—
5.0	14.0	16.5	20.7	0.2	0.4	0.8	—
10.0	17.6	20.8	26.0	0.1	0.2	0.4	—
20.0	22.2	26.3	32.8	—	0.1	0.2	0.3

[a] 1X Dose = 0.066 μg A.I. (91% technical malathion).

plete one replication. All tests for one replication with 3 concentrations (.04, 1.0 and 10.0%) were completed on one day and the remaining concentrations (0.2, 5.0 and 20.0%) were tested on the next day to complete the replication.

FIELD TESTS. Field tests were conducted to measure the effect of aerosol droplet size by exposing caged mosquitoes to aerosols with different VMD's produced by commercially available ground ULV generators. Air pressure and nozzle components were varied to obtain different levels of atomization. Coulter Counter® readings were used to establish the VMD for each size treatment. This technique for droplet size measurements was described by Haile et al. (1978). Technical malathion was applied with a flow rate of 29.6 ml/min (1 oz/min) and a truck speed of 8 km/hr (5 mph) for all tests. This application rate was used to provide a low discriminating dose so that any effects due to size could be measured. Laboratory reared females (4 to 6 days old) of 2 species (*Ae. taeniorhynchus* and *Anopheles quadrimaculatus* Say) were exposed in standard WHO test cages (4.5 cm diam. × 15 cm long cylindrical screen cage) at 3 distances (45.7, 91.4 and 183 m or 150, 300 and 600 ft) downwind from the line of travel of the aerosol generator. Two cages for each species with 25 female mosquitoes per cage were exposed at each distance for each treatment. A minimum of 4 unexposed cages were used to determine natural mortality. The control mortality was generally less than 5% and the entire test was discarded if control mortality exceeded 15%, therefore, no adjustments were made for control mortality. Tests were conducted in an open field with cages placed 1.2 m (4 ft) above ground on stakes. All tests were conducted at dusk during the spring in Alachua County near Gainesville, Florida. Tests were conducted only on days when environmental conditions were acceptable for ground ULV application. Treatments were not made if wind speed was less than 3.2 km/hr (2 mph) or gusting above 16 km/hr (10 mph). Also, temperature measurements at 2.4 m (8 ft) and 9.8 m (32 ft) above ground indicated either stable or inversion conditions before tests were started.

Three sets of field tests were conducted. The first series was conducted to see if a difference in aerosol size could be detected with available bioassay techniques. The second and third series used the same procedures but the range of aerosol sizes was extended to enhance differences due to size.

The first series of tests was conducted with aerosol VMD's of 30, 15, 13 and 8 μm. These aerosols were produced with a Leco-HD® aerosol generator operated at 1, 3, 4 and 7.5 psi (6.9, 20.7, 27.6 and 51.7 kpa). Tests for each size were replicated 5 times, except for the 8 μm size, which was replicated only 3 times. The second series of field tests was conducted with VMD's of 39, 24, 15, 10 and 5 μm (5 replications each). The 4 largest sizes were produced by a Leco-HD at 0.5, 1.5, 3 and 5 psi (3.5, 10.3, 20.7 and 34.5 kpa). The smallest size (5 μm VMD) was produced by a London-aire® aerosol generator operated at 90 psi (620 kpa). The third series was a replication of the second series except that only one species (*Ae. taeniorhynchus*) was used in the tests.

RESULTS

LABORATORY TESTS WITH MONODISPERSE AEROSOLS. The average mortality obtained in the wind tunnel tests for each uniform size-dose combination (6 replications) is presented in Table 2. A probit analysis of these data was calculated using both log-dose and then dose (untransformed) in the Statistical Analysis System (SAS) programs. In general, both methods gave similar results, however, the chi-square values were generally lower with the untransformed doses and the LD-50's calculated from that analysis are presented in Table 2 along with the 95% confidence limits. Considerable variation is evident in the results for the different droplet sizes, however, a clear trend toward reduced mortality or effi-

Table 2. Percent mortality[a] vs. Dose and LD-50 for *Ae. taeniorhynchus* females exposed to different uniform droplet sizes of malathion.

Droplet diameter, μm	Relative dose[b]				LD-50[c]	95% confidence limits for LD-50	
	1X	2X	4X	6X		Lower	Upper
2.8	2.7	15.7	19.0	36.8	7.4	6.4	9.2
3.2	4.0	4.0	22.3	46.0	6.2	5.7	7.0
4.1	12.7	20.6	56.0	70.4	4.1	3.7	4.5
4.8	8.0	12.3	16.3	—	11.4	4.5	—[d]
5.6	4.0	11.3	35.3	—	4.8	3.8	19.9
7.1	15.0	31.3	64.3	—	3.2	2.3	5.5
8.2	20.0	32.0	70.6	—	2.9	1.9	4.7
9.7	16.3	28.6	72.3	—	2.9	2.1	4.4
12.1	20.3	35.3	68.7	—	2.9	1.9	5.0
14.0	19.7	43.0	75.7	—	2.5	1.6	3.8
16.5	16.3	30.3	66.3	—	3.1	2.2	5.2
17.6	15.7	39.3	63.7	—	3.0	2.0	5.8
20.7	18.6	30.0	60.0	—	3.4	2.3	8.6
20.8	10.3	18.3	59.7	—	3.6	2.7	5.9
22.2	—	35.0	51.0	77.0	3.6	0.8	5.6
26.0	16.0	37.3	44.3	—	4.3	2.6	—[d]
26.3	—	21.3	45.0	67.3	4.5	2.9	7.5
32.8	—	25.0	28.7	43.0	7.7	4.5	—[d]

[a] Mortality values represent the average of 6 replications (2 cages/replication and 25 females/cage).

[b] Relative dose and LD-50 are in arbitrary units (1X = 0.0066 μg A.I. of 91% technical malathion).

[c] Calculated by the probit analysis program of the Statistical Analysis System (SAS) 79.5.

[d] Uncalculated by SAS program, indicating large variation.

ciency is indicated for the smallest and largest droplet sizes tested. The data for 4.8 μm droplets were clearly faulty due to the unusually high LD-50 and variability between replications; therefore, this point was ignored for further data analyses. The relationship between droplet size and insecticidal efficiency represented by these data is best shown by a plot of LD-50 vs size (Fig. 2). The trend of the curve in Fig. 2, which was estimated by eye, indicates that the optimum uniform droplet diameter is between 10 and 15 μm; however, little difference in efficiency is indicated for sizes from ca. 7 to 22 μm. A rapid reduction in efficiency is shown for sizes smaller than 5 μm as a result of reduced droplet impingement. A less-rapid reduction in efficiency is indicated for sizes larger than 25 μm as a result of reduced droplet concentration and fallout in the tunnel.

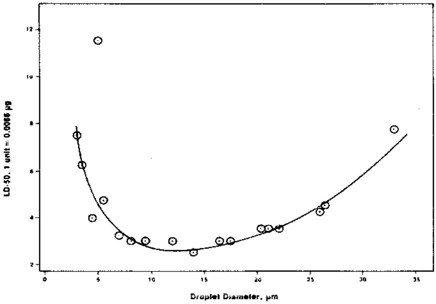

Fig. 2. Relationship between LD-50 and droplet diameter from wind tunnel tests exposing caged *Ae. taeniorhynchus* mosquitoes to monodisperse aerosols of malathion.

Field tests. The mortality data from the first series of field tests are presented in Table 3. A statistical analysis of vari-

Table 3. Percent mortality[a] of caged mosquitoes in field tests with different aerosol sizes of technical malathion dispersed at 1 oz/min and 5 mph truck speed—First series.

Species[b]	Distance, ft	VMD, μm 30	15	13	8
Q	150	83	83	86	77
	300	63	82	53	62
	600	44	58	81	49
Average		63	75	74	63
T	150	87	78	88	75
	300	73	82	51	81
	600	36	48	49	42
Average		65	69	63	65
Overall average		64	72	68	64

[a] Average of 5 replications with 2 cages/replication and 25 mosquitoes/cage except for 8 μm VMD where only 3 replications were made.

[b] Q = *An. quadrimaculatus.*
T = *Ae. taeniorhynchus.*

ance (AOV) for these data indicated a significant decrease in mortality with increased downwind distance (0.01 level), but no significant difference was noted for aerosol size, species or any interaction. Even though the differences between aerosol sizes in these tests were statistically nonsignificant, the trend of the data indicated a reduction in kill at the upper (30 μm VMD) and the lower (8 μm VMD) ends of the size range.

The results from the second and third series of field tests with an extended range of sizes are presented in Table 4. An AOV for the second series showed, again, that the effects of species and all interactions were nonsignificant, but the effects of distance, as well as aerosol size, were highly significant (0.01 level). A comparison of the overall (second series) means for each VMD using Duncan's Multiple Range Test (0.05 level), showed that the largest size (39 μm VMD) was significantly different (lower mortality) from the other VMD's and that there were no significant differences among the other VMD's, even though the 10 and 15 μm VMD's showed the highest mortalities (Table 2). A separate AOV for the third series data with only one species gave similar results except that the smallest size (5 μm VMD) was significantly different from 10 and 15 μm VMD's (Table 2). However, an AOV for the combined data of the second and third series resulted in a statistically significant separation of the VMD's into 3 groups as follows: 1) 10 and 15 μm VMD with the highest mortality (82% for both), 2) 5 and 24 μm VMD with lower mortalities of 67 and 72%, respectively, and 3) 39 μm with the lowest mortality of 33%. In the combined AOV the only significant effect other than aerosol size was downwind distance; there were no differences between species (with the third series included as a separate species) and no significant interactions. These results clearly show that the optimum aerosol size for ground ULV applications should be between 10 and 15 μm VMD.

CONCLUSIONS

The results from the monodisperse aerosol study and the field tests are in agreement and give a clear indication that the optimum droplet size of malathion for adult mosquito control is in the range from 10 to 15 μm in diameter. This is in general agreement with most previously published research. Both types of tests in this study also indicate that there is a considerable range of sizes for which insecticidal efficiency changes only slightly. In general, this range can be stated as ca. 5 to 25 μm diameter. Efficiency begins to drop steadily for sizes outside of this range. The laboratory tests indicate that uniformity of sizes in itself would not significantly improve efficiency over an aerosol with most of the insecticide in the 5 to 25 μm range. Present commercial ground ULV aerosol generators are capable of producing aerosols that are substantially within this range. A typical mosquito control aerosol with a 13 μm VMD has ca. 92% of the insecticide in the range from 5 to 25 μm with 2% of the volume below 5 μm and 6% above 25 μm (Coulter Counter reading, unpublished

Table 4. Percent mortality[a] of caged mosquitoes in field tests with different aerosol sizes of technical malathion dispensed at 1 oz/min and 5 mph truck speed—Second and third series with expanded VMD range.

Species[b]	Distance, ft	VMD, μm 39	24	15	10	5
	Second series					
Q	150	51	95	92	87	87
	300	33	68	85	81	69
	600	13	60	56	69	56
Average		32	74	77	79	70
T	150	52	89	94	93	76
	300	35	69	82	85	76
	600	22	45	65	64	67
Average		37	68	81	81	73
Overall average[c] (Second series)		35A	71B	79B	80B	72B
	Third series					
T	150	43	88	98	89	59
	300	36	75	91	94	64
	600	13	56	75	72	47
Average[c]		31A	73BC	88B	85B	57C
Overall average[c] (Second and third series)		33A	72B	82C	82C	67B

[a] Average of 5 replications with 2 cages/replication and 25 mosquitoes/cage.

[b] Q = *An. quadrimaculatus*.
T = *Ae. taeniorhynchus*.

[c] Means followed by the same letter are not significantly different by Duncan's Multiple Range Test (0.05 level).

data). With only 8% of the insecticide outside the optimum range, a narrower droplet spectrum would improve efficiency less than this amount.

The results of this research should be applicable to all mosquito adulticide applications regardless of the target species and insecticide used. This is particularly true for ground ULV aerosols, however for aerial applications the atomization capability of the dispersal equipment and factors affecting transport of the aerosol to the target area must be considered.

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EVALUATION OF NEW PYRETHROIDS AGAINST IMMATURE MOSQUITOES AND THEIR EFFECTS ON NONTARGET ORGANISMS

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ABSTRACT. In the laboratory, 5 new pyrethroids were screened against larvae and pupae of *Culex quinquefasciatus*. Two of the materials proved highly active, yielding 90% mortality in larvae at 0.07–0.46 ppb. Three of the materials were highly active against pupae, yielding 90% mortality at 1–4 ppb.

Two of the most active pyrethroids (cypermethrin and S–3206 or fenpropathrin: α-cyano-3-phenoxybenzyl 2, 2, 3, 3-tetramethyl cyclopropanecarboxylate) were evaluated under field conditions against larvae and pupae of *Cx. tarsalis* and *Psorophora columbiae*. Cypermethrin produced 90–100% mortality in larvae of the former species at 3–5 g AI/ha while S–3206 yielded 90–100% control at 27–55 g AI/ha. Both materials were equally effective against the second species, yielding high level to complete control of larvae at the low rates of about 6–11 g/ha. These rates also produced high level to complete control of pupae. The activity of these materials against pupae provides a decided advantage for use of these materials in mosquito control programs.

Under field conditions, both cypermethrin and S-3206 showed no adverse effects on diving beetle adults. Cypermethrin was also relatively innocuous to dragonfly naiads and ostracods at effective larvicidal rates. However, this material reduced mayfly naiads to a very low level and recovery was not noticeable 2 weeks later. S-3206 adversely affected both mayfly and dragonfly naiads, but had no effects on ostracods except for a short period. The affected organisms recovered in 2 weeks after treatment.

INTRODUCTION

During the past few years a number of photostable pyrethroids have become available for the control of a variety of insect pests. Some of these materials have proven to be more active than some of the most highly active organophosphorous compounds such as temephos, chlorpyriphos, malathion, fenthion, and naled against several groups of insects of public health importance, such as mosquitoes and chironomid and biting midges (Ali and Mulla 1980, Darwazeh et al. 1978, Kline et al. 1981, and Mulla et al. 1980). Decamethrin and permethrin, for example, produced excellent reduction in the number of endophilic mosquitoes, *Anopheles gambiae* s.l. and *An. funestus* Giles for 24 weeks when applied as emulsifiable or suspension concentrates to interior surfaces of houses in Kenya, and the reduction in the number of resting adults on treated surfaces is attributed partly to repellent effects; the residual sprays also reduced biting activities of

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