RELATIONSHIP OF MINIMUM LETHAL DOSE TO THE OPTIMUM SIZE OF DROPLETS OF INSECTICIDES FOR MOSQUITO CONTROL[1]

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The ultra-low-volume (ULV) technique of applying insecticides from aircraft (Lofgren *et al.,* 1967) and from ground equipment (Mount *et al.,* 1968) is a relatively new development and a highly effective method of controlling adult mosquitoes. With this technique, insecticides are applied either as technical insecticides or as highly concentrated solutions. For example, 95 percent technical malathion is now being applied aerially at rates as low as 3 to 6 fluid ounces per acre or with ground equipment at rates of 0.25 to 1 fluid ounce per acre.

The Insects Affecting Man Laboratory at Gainesville is therefore evaluating insecticides as ULV sprays or aerosols. However, at the same time, we are also studying the relationship between the size of a droplet and its effectiveness (Mount *et al.* 1970a, 1970b). Thus, in 1968, Mount *et al.* used ULV aerosols applied with ground equipment to demonstrate that the spectra of droplets of malathion having mass median diameters (mmd's) of 6 to 10 microns were more effective in killing adult mosquitoes than droplets with mmd's of 11–22 microns. In addition, Mount (1970) concluded that droplets with mmd's in the range of 10 to 25 microns may represent the optimum range of sizes for use in aerial applications.

Many complex factors including the effect of choice of equipment and technique; amount and kind of vegetative cover; drifting, settling, and impinging of droplets; and environmental and meteorological conditions ultimately determine the effectiveness of droplets of different sizes. However, the relationship between the minimum amount of insecticide needed to kill a mosquito and the size of droplet that contains this amount should provide basic information about the optimum size of droplets needed to control adult mosquitoes. This paper therefore reports the results obtained when tests were made to determine the minimum lethal dose (LD_{100}) of malathion for all adult mosquitoes exposed to contact sprays in a laboratory wind tunnel and the relationship between this minimal LD_{100} and the size of the droplets as it is related to the theoretical efficacy of ULV applications.

METHODS AND MATERIALS. Two separate experiments were made, one in June 1968 and another in December 1968. In each test, adults from our laboratory colony of *Aedes taeniorhynchus* (Wiedemann) (3 to 5 days old) were exposed to two concentrations of malathion (0.05 and 5.0 percent in deodorized kerosene) in a wind tunnel as described by Glancey *et al.* (1966). The lower concentration was used because it has proved to be the minimum concentration that kills 100 percent of these mosquitoes in our wind tunnel; therefore, the amount of malathion picked up by these mosquitoes represents the minimum LD_{100}. The larger dose was tested so we could compare the amount picked up at the two concentrations. In both tests, groups of 250 mosquitoes were exposed to provide sufficient residues for the chemical assays since we have observed no difference in effect in the wind tunnel when mosquitoes are exposed in groups of

[1] Mention of a pesticide or a proprietary product in this paper does not constitute a recommendation or an endorsement of this product by the U. S. Department of Agriculture.

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25 (Glancey *et al.* 1966) or in groups of 250. Immediately after the exposure, all treated mosquitoes were transferred to clean cages (250/cage). To determine mortality 1000 were held for 24 hours (four similar cages of unsprayed mosquitoes were held as controls), and another 1000 each treated and untreated were immobilized with cold within 30 minutes. These latter groups were then immersed in 10 milliliter (ml) of chloroform and stored over dry ice until the chemical analysis was performed.

CHEMICAL ANALYSIS. The external residues on the treated mosquitoes were recovered by transferring the chloroform and mosquitoes to a small glass funnel containing about 3 grams of anhydrous sodium sulfate supported in the funnel by a plug of glass wool. The container and the insects were successively rinsed twice with 5-ml portions of chloroform, and the combined filtrate was held for analysis.

The internal residues were recovered from the treated mosquitoes by using a 50-ml tube (Corning no. 8240) and a glass rod (10-millimeter diam) as mortar and pestle for trituration after the grinding surfaces of both were etched with coarse carborundum powder to increase their efficiency. First, the mosquitoes were finely ground in the presence of 3 grams of anhydrous sodium sulfate, then, the mixture was further triturated three times with 10-ml portions of chloroform. Each portion of supernatant chloroform was successively decanted through the funnel, and the combined filtrate was reserved for analysis. Thereafter, the chloroform extract was either concentrated, diluted, or analyzed directly for malathion and for the oxygen analog, mala-oxon, with a gas chromatograph in a manner similar to that described by Corley and Beroza (1968). However, we used a 5 percent DC-200 column instead of the 2 percent DEGS column described by Corley and Beroza (1968) because it completely resolved the two compounds, yielded sharp symmetrical peaks, and required less than 4 minutes per analysis.

A Hewlett-Packard (Avondale, Pa.) Model 5750 chromatograph equipped with a Melpar flame photometric detector (available from Tracor, Inc., Austin, Texas) was operated in the phosphorus mode (526-mμ filter). The 90-cm glass column (4-mm ID) contained 5 percent DC-200 (w/w) on 80–100 mesh Gas Chrom Q (Applied Science Lab., State College, Pa.) and was operated isothermally at 160° C. The temperatures of the injection port, the detector (external), and the line connecting the oven to the detector were 180, 170, and 180° C., respectively. Flow rates of the gases (ml/minute) were: nitrogen (carrier) 160, hydrogen 200, and oxygen 40.

For the analysis of residue at the stated conditions, we injected 5 microliters of sample. The retention times for mala-oxon and malathion were found to be 2.10 and 2.80 minutes, respectively. Once the column was conditioned to the two compounds (Corley and Beroza, 1968), the response (peak height) was proportional to concentration to at least 250 nanograms.

Recoveries from untreated insects each spiked with 5 nanograms of both compounds were 95 percent or better. Extracts of untreated insects contained nothing that interfered with the analysis. Figure 1 is a typical chromatogram of mosquito extract spiked with both compounds.

RESULTS AND DISCUSSION. No malathion or mala-oxon was detected in the unsprayed mosquitoes used as biological and analytical controls, and untreated mosquitoes survived the 24-hour posttreatment holding period with no mortality. All treated mosquitoes died in less than 24 hours. The analyses of the spray indicated that the 0.05 percent concentration actually contained 0.045 percent malathion and that the 5 percent concentration contained 4.91 percent; also, the malathion used in the test spray contained 99.3 percent malathion and 0.68 percent mala-oxon. Then, since the ratio of malathion to mala-oxon found in treated mosquitoes was about the same as the ratio found in the spray, the amount of malathion found on and in the mosquitoes was considered to be represen-

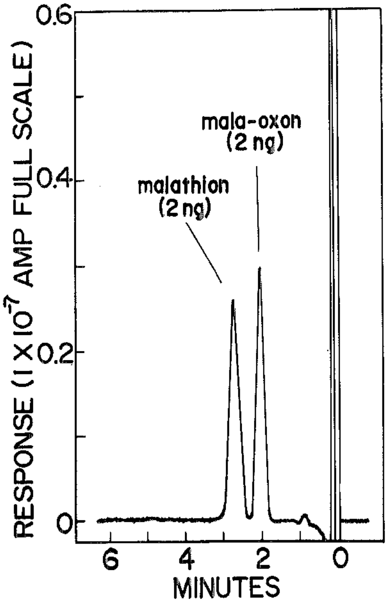

FIG. 1.—Chromatogram of raw extract equivalent to 500 μg of adult mosquitoes spiked with 2 nanograms each of malathion and mala-oxon.

tative of the amount picked up by the mosquitoes and of the minimum LD_{100}.

The amounts of malathion found on and in the treated mosquitoes are given in Table 1. The nanograms of malathion/individual adult in the replicates and in the two tests were in good agreement. (We did not calculate standard error because we were interested primarily in an aproximation of the minimum LD_{100}.) When the results of the two tests were averaged, the values obtained were about 8.77 and 1147 nanograms/adult for the 0.05 and 5.0 percent concentrations. Then, since a 100-fold increase in the concentration of the spray resulted in about a 100-fold (actually 130-fold) increase in the amount of malathion picked up by the mosquitoes, the technique appeared valid, and a dose of 10 nanograms/adult was set as the minimum LD_{100} for *A. taeniorhynchus*.

We want to emphasize, however, that this is an average figure based on a large number of mosquitoes and the toxic dose for each individual mosquito may vary considerably. In addition, the 10 nanograms/adult dose was obtained by applying a dose that killed all mosquitoes; thus some mosquitoes received more than the minimum dose required to kill. As a result, 10 nanograms may or may not be sufficient to kill the most malathion-tolerant mosquito. Despite these qualifications, we were now ready to relate the established minimum LD_{100} to the amount of malathion contained in droplets of different sizes. We therefore calculated (1) the volume of single droplets ranging in diameter from 100 to 0.1 microns (2) the weight of 95 percent technical malathion (specific gravity of 1.23) contained in these single droplets, and (3) the number of these droplets required to kill one adult (Table 2).

Ultra-low-volume space sprays and aerosols actually used to control mosquitoes are composed of droplets of varying sizes with mmd's ranging from 30 to 60 microns (Mount *et al.* 1970) and usually less than 50 percent of the total volume of insecticide is in droplets less than 25 microns in diameter. It was particularly interesting that a droplet 25 microns in diameter contained 10 nanograms of technical malathion—the amount required to kill one mosquito. Moreover, in space applications in which the droplets are deposited on the mosquitoes, one droplet cannot impinge on more than one mosquito, but more than one droplet can reach one mosquito. Thus, droplets containing more insecticide than is necessary to kill one mosquito are wasteful (Latta *et al.*, 1947), and droplets of malathion 50 or 100 microns in diameter contain about 8 to 64 times as much insecticide as necessary to kill *A. taeniorhynchus*. Below 25 microns the number of droplets required to kill one mosquito increases rapidly and reaches 1.56 million (1.56 X 10^6) at 0.1 micron. However, we did not know whether such small droplets were more efficient. It was possible that the surface area of a single mosquito could not ac-

TABLE 1.—Results of analyses of mosquitoes treated with 0.05 or 5.0% malathion.

	Malathion/adult mosquito (ng) after treatment with—							
	0.05% concentration				5.0% concentration			
	Test 1		Test 2		Test 1		Test 2	
	Replicate 1	2	Replicate 1	2	Replicate 1	2	Replicate 1	2
External rinse	7.55	7.50	8.66	9.09	945	975	1250	1190
Internal homogenate	0.54	0.59	0.54	0.59	43	51	65	67
Total	8.09	8.09	9.20	9.68	988	1026	1315	1257
Avg by test	8.09		9.44		1007		1286	
Avg by concentration	8.77				1147			

commodate all of the large number required to kill. Therefore, we calculated the area which would be required to receive the number of these droplets necessary to kill one mosquito (see Table 2) from the area of the squares that would circumscribe a circle with a diameter equal to the diameter of the sphere of the droplet. The calculations demonstrated that even 1.56 million droplets (0.1 micron diam) could be deposited on the body of one mosquito even with a considerable spread factor for the malathion droplets. Although we do not know the surface area of an adult mosquito, it is certainly much greater than the area of 0.155 (1.55×10^{-1}) mm^2 (Table 2) that is needed.

Finally, we determined the number of droplets of various diameters that could be obtained from 0.25 pound of technical malathion and then calculated the density per ml for each size (assuming they were evenly distributed 30 feet high over an acre. Such a situation is obviously artificial since space sprays and aerosols do not contain droplets of only one size and since droplets in the field do not distribute themselves equally within a given volume of space. However, this type of reasoning could provide information about the probability of droplets of different sizes contacting a mosquito.

The number of droplets obtained from 0.25 pound of malathion and the number per ml of space increases, of course, with decreasing droplet diameter as shown in Table 2. For example, in 0.25 pound of malathion, there are 64 times more droplets of 25 micron-diameter than 100-micron diameter; also, only one 100-micron droplet will occur in every 193 ml of space compared with one 25-micron droplet in every 3 ml of space. Thus, with larger droplets, fewer large particles are available to kill, and the odds of a single mosquito contacting a lethal dose appear to be lessened. Also, large droplets fall at a greater rate; thus they remain airborne for a shorter period so the chances that they will contact a mosquito would be decreased.

Considering droplets 25 microns and smaller, one might assume that an increase in the number of droplets or the number per unit volume with decreasing diameter could increase efficiency or effectiveness of space sprays. Although droplets smaller than those containing a minimum lethal dose may be more effective, we do not believe that an increased effectiveness would be associated simply with increased numbers or number/unit volume. With diameters of 25 microns, as illustrated in our calculations, 1.13×10^{10} droplets would be obtained from 0.25 pound of malathion and would be distributed in our theoretical volume of space at the rate of 1 droplet per 3 ml of space. With a drop-

TABLE 2.—Theoretical calculations of some factors related to size of droplets of malathion.

Diameter	Volume/ droplet (ml)	Wt of malathion/ droplet (ng)	No. of droplets required to kill 1 adult assuming 10 ng/ adult is lethal	Area occupied by no. of droplets required to kill 1 adult (mm^2)	No. of droplets from 0.25 lb of malathion	No. of droplets/ml in a 30 ft of space above 1 acre from 0.25 lb malathion
100	5.23×10^{-7}	6.43×10^{2}			1.76×10^{8}	5.2×10^{-3}
50	6.54×10^{-8}	80.4			1.41×10^{9}	4.2×10^{-2}
25	8.13×10^{-9}	10.0	1.0	6.25×10^{-4}	1.13×10^{10}	3.33×10^{-1}
20	4.19×10^{-9}	5.15	1.9	7.77×10^{-4}	2.20×10^{10}	6.55×10^{-1}
15	1.77×10^{-9}	2.18	4.6	1.04×10^{-3}	5.21×10^{10}	1.55
10	5.23×10^{-10}	6.43×10^{-1}	15.5	1.55×10^{-3}	1.76×10^{11}	5.24
5	6.54×10^{-11}	8.04×10^{-2}	1.24×10^{2}	3.11×10^{-3}	1.41×10^{12}	42.
2	4.19×10^{-12}	5.15×10^{-3}	1.94×10^{3}	7.77×10^{-3}	2.20×10^{13}	6.55×10^{2}
1	5.23×10^{-13}	6.43×10^{-4}	1.56×10^{4}	1.55×10^{-2}	1.76×10^{14}	5.2×10^{3}
0.1	5.23×10^{-16}	6.43×10^{-7}	1.56×10^{7}	1.55×10^{-1}	1.76×10^{17}	5.2×10^{6}

let diameter of 1 micron, 1.76 X 10^{14} droplets (15,500 times as many as with droplets of 25 mass diameter) and 5,200 droplets/ml of space would be obtained. However, 15,500 times as many droplets would have to be delivered to obtain the minimum LD_{100} which would still be present in 3 ml of space.

Since our wind tunnel measures the relative toxicity of insecticides, we should now be able to estimate the minimum LD_{100} for other insecticides used in ULV applications. For example, the LC_{100} for naled and fenthion in our wind tunnel is 0.025 percent, one half that for malathion. Thus, 5 nanograms of either insecticide is the LD_{100}. In ULV applications, 5 nanograms of naled (85 percent technical and specific gravity of 1.9) or 5 nanograms of fenthion (93 percent technical and specific gravity of 1.25) would be contained in droplets of 20 and 17.5 micron diameter, respectively.

Many factors are involved in the successful outdoor application of space sprays; however, our results provide guidelines for the optimum size of droplets for contact sprays for control of adult mosquitoes. Insecticides such as malathion, fenthion, and naled should be atomized to droplets of 25 microns or less if maximum efficiency is to be obtained from ground and aerial ULV sprays.

Summary. When adult *Aedes taeniorhynchus* (mixed sexes) were exposed to space sprays of malathion (technical 95 percent) in a laboratory wind tunnel, the lethal dose (LD_{100}) was found to be about 10 nanograms/adult. This LD_{100} in ULV contact sprays would be contained in one droplet about 25 microns in diameter. Larger droplets would be undesirable because some of the insecticide would be wasted (droplets 50–100 micron in diameter would contain about 8 to 64 times more than the lethal dose). The relationship of lethal dose and droplet sizes (0.1–100 microns) to effectiveness of space sprays for mosquito control was considered.

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