



ULV Droplet Analysis ¹

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This chapter was developed as a companion to the videotape "ULV Droplet Analysis," available from the Florida Medical Entomology Laboratory (FMEL) for \$10. The video script closely follows the text in this chapter. Follow the text as you view the tape, pausing after each topic for the best understanding of this subject.

Professional mosquito control typically includes ground adulticiding as part of a comprehensive control program. In fact, a large segment of the public recognizes mosquito control only as ground adulticiding.

The federal law, enforced nationally by the Environmental Protection Agency (EPA) and in the state of Florida by the HRS Office of Entomology, clearly states that the label of an insecticide is the law. You must adhere to what the label says as to dosage rates, application techniques and, in the case of ultra low volume (ULV) applications, to the droplet sizes specified. Each product has a specific set of rules for droplets on its label and each must be followed to the letter for proper compliance. This chapter is designed

to give you the basic knowledge to comply with each label.

Foggers, or thermal aerosols, were used for many years to dispense pesticides. Traffic hazards from the dense fog cloud and the rising cost of fog oil contributed to the development of the ultra low volume cold aerosol technique, commonly called ULV. This technique was developed by Dr. Gary Mount in the early 1960s.

ULV insecticide sprayers produce a cloud of minute particles through a shearing action at the nozzle. The droplets, although small, will vary greatly in size, usually from one micron to 100 microns. Droplet size affects efficiency, economy and safety.

Very large droplets can settle out of the air stream quickly and may fall uselessly to the ground. Droplets that are too large will contain more than enough insecticide to kill the target insect. In other words, if one oversize drop hits the mosquito, all of the chemical beyond that required to kill it, is wasted. In addition to this problem, very large droplets of some insecticides cause automotive paint spotting. On the other hand, very small droplets contain only a fraction

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of the insecticide necessary and may pose potential human respiratory problems.

Droplet size relationships are not direct. Decreasing the diameter of a droplet 10 times will increase the number of droplets by a thousandfold. For example, a single 40-micron droplet has the same volume as 1,000 four-micron droplets. Droplet settling velocity increases one hundred times with only a 10-fold increase in droplet diameter. Droplets in the 10- to 15-micron range appear to be the most effective for mosquito control, considering chances of impingement, ability to remain airborne, potential killing dosage and avoidance of paint spotting.

Collecting a representative sample is a very important element in determining the size and spectrum of the insecticide droplets. There are several collection devices that can be used to sample liquid particles. Each device is capable of sampling a given range of sizes and is dependent upon various physical properties of the droplets.

Some of these methods require complicated equipment best suited to the laboratory. These include photometers, centrifuges, electrostatic precipitators and lasers. Two techniques simple enough to be used in the field are settling and impaction. Both will cover the size range in which we are interested.

Droplets settling by gravity onto a slide in a closed chamber is the most accurate method of collection, because all of the particles eventually settle onto the slide and are represented in their true numbers. However, this method presents many problems in analyzing the slide and will not be considered at this time.

One of the simplest methods of droplet collection is the impaction principle of the waved slide technique. It was developed in the early 1950s by A.H. Yoemans and is the technique we will consider in this chapter.

Equipment and Supplies

Most of the equipment needed for droplet determinations may be purchased from scientific supply houses and some can be easily made. You will need a specially equipped compound microscope, a

wand or stick to wave the slides, teflon-coated slides and acetone, data sheets, graph paper and a calculator. Detailed equipment specifications have been provided along with a list of possible suppliers.

Microscope

Measurement of droplets is made with a monocular or binocular compound microscope that must have a mechanical stage that moves easily in both directions for scanning slides. Ideally, the scope should be equipped with substage illumination and a focusing condenser. For sufficient resolution and ease of use, you will want an objective lens with a magnification close to 40x. Additional objectives of 4x and 10x in the nosepiece turret will add to the scope's ease of use and versatility.

One of the eyepieces, which should be wide-angle 10x units, must be fitted with an ocular micrometer. This is a small glass disk with an etched scale of 100 numbered divisions that fits into the eyepiece's base.

A stage micrometer must be used to calibrate the ocular scale in the eyepiece. This is an ordinary glass slide that has a millimeter divided into 100 parts of 10 microns each. This is indicated on the slide by markings that read ".01 mm."

Slides

The droplets are collected on microscope slides that have been coated with a clear teflon film. This oil-repelling coating prevents excessive and irregular spreading of the droplets and helps to keep them round. The slides, available commercially, are provided with a small frosted area on the same side as the teflon coating. This area can be used to number the slides. Use pencil because most ink cannot be removed for slide reuse.

Slide Holder

Attach a large, spring paper clip onto the end of a broom handle or three-quarter inch dowel with a screw. Place adhesive tape on the jaws of the clip to prevent the slide from slipping (Figure 1).

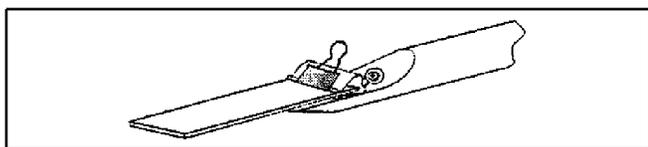


Figure 1 .

Other Items

- acetone
- cotton balls
- data sheet
- probability graph paper
- calculator
- tally counter
- clear plastic 6" or 12" ruler

Microscope Calibration

Before droplets can be measured, the ocular micrometer of the microscope must be calibrated. We must find out how many microns are represented by each eyepiece division.

The stage micrometer scale is clipped into the mechanical stage, making sure that the little, raised cover glass is on top. Rotate the objective lens turret in order to use the 10x objective lens. Locate and focus on the stage micrometer scale. Now rotate the objective turret to the 40x lens and refocus, if necessary.

The eyepiece containing the micrometer may be inserted into either eye tube if you have a binocular scope, depending on what is most comfortable. The scale is oriented horizontally during this calibration to match the horizontal stage micrometer. After focusing the stage micrometer scale, move the stage until the stage scale is under the eyepiece scale. Align the zero ends of each scale, one on top of the other.

Then look for the stage scale line that is at or just below the 100 line of the eyepiece micrometer. Count the divisions of the stage scale. Usually it is about 25. Remember that each stage division is 10 microns, thus 25 divisions is equal to 250 microns.

Divide the eyepiece divisions (there are 100) into the microns covered on the stage (250 in this case) to obtain the microns per eyepiece division. In this case each eyepiece division is equal to 2.5 microns. You may have slightly different figures with various combinations of lenses.

Spread Factor

A droplet of liquid impinging on a flat surface will spread out to resemble a small lens. This spread is the ratio of the diameter of an airborne spherical droplet to the diameter of the flattened lenslike drop on the slide, and is called the spread factor.

The amount that the drop spreads is dependent upon the surface material of the slide, the surface tension of the liquid, the density of the liquid and the droplet's diameter. For operational purposes, we will assume certain parameters with teflon-coated slides:

- The spread factor of average-sized droplets of malathion and similar thick compounds is assumed to be 0.7.
- Materials like the synthetic pyrethroids will have spread factors of about 0.6.
- Very thin oils and solvents will be around 0.5.

The spread factor is usually provided on the label by the insecticide's manufacturer.

Correction Factor

To obtain the final correction factor for each material, one must multiply the number of microns per eyepiece division as found in the eyepiece calibration, times the spread factor.

For example, if we found:

- the microns per eyepiece division to be **2.5**, and
- we are measuring an insecticide with a spread factor of **0.7**, then
- the correction would be **1.75** microns per eyepiece division.

The microscope has now been calibrated for this combination of lens, slide coatings and insecticide.

We will only need to do this once for this combination.

Sprayer Preparation

The equipment needed to calibrate ULV machines has not been included because of widely differing procedures. Flow calibration is usually outlined in the equipment manual. It is necessary, however, to check the insecticide flow rate before collecting droplets for size analyses, as flow rate will directly affect particle size.

To properly calibrate a piece of equipment, it is important that the flow control be set at the highest rate at which the unit will be normally operated. In other words, if a truck unit is to be operated at a maximum of 20 miles per hour, then the droplet output should be checked at the 20-mile-per-hour flow rate.

Choose a time and place for sampling droplets where the air is fairly calm. Make sure that the spray cloud will not blow into an area where it may cause a problem. Face the vehicle directly into the wind using the spray as an indicator. The sprayer nozzle is then loosened and rotated into a horizontal position, if possible. If you are not able to do this, make some provision to stand high enough behind the sprayer to sample across the center of the spray cloud.

Start the unit and allow it to warm up. Check the pressure and cycle the flow control or spray switch to be sure that any trapped air is removed and the spray is not pulsating. Make sure that the output is insecticide and not flushing solution.

Slide Preparation

The insecticide droplets will be collected on teflon-coated slides. Slides should never be handled by their flat surfaces except at the frosted end.

For an accurate collection, the teflon-coated surface must be cleaned before the slides are used.

1. Soak a cotton ball with ordinary acetone, available at most paint stores. Remember that acetone is very flammable.
2. Gently wipe both sides of the slide. Finish the slide by slowly drawing the acetone-soaked

cotton ball over the teflon surface lengthwise. This prevents the surface from spotting.

3. Once the slides are cleaned they should be returned to the box.
4. Before the actual droplet collection, number the slides with a pencil. They can be marked with the sprayer number or a number referenced on a data sheet.
5. This data sheet should contain the date, sprayer number, insecticide formulation, flow rate, collection technique, nozzle pressure, the name of the person taking the collection and air temperature.
6. After the slide is marked, attach it to the spring clip on the stick. Make sure the teflon coating and the frosted writing surface on the slide are facing out when it is clipped to the stick. Make sure the slide is secure in the jaws of the clip and won't fly off when the stick is waved.

Droplet Collection

The collection should be made about 15 feet behind the nozzle or that distance specified on the product label. The label will also specify any protective clothing or equipment that should be worn.

1. Wave the slide rapidly through the insecticide spray with a forward and upward motion similar to swinging at a baseball. Although the waved slide impaction method we are using will not collect many droplets under 3 to 4 microns, the droplet spectrum will be more representative with a rapid wave.
2. Check the slide to be sure there are particles on it. It should have a slightly cloudy appearance when viewed through the reverse side over a dark surface. Several waves through the spray cloud may be required for low flow rates. This will not cause many droplets to impinge on each other, and even if they do, the effect on droplet diameter is negligible.
3. After the slide has been exposed to the spray, it is returned to the box for protection. It's a good idea to collect two slides from each unit in case one is unuseable.

Data Form

A form is used to tabulate the information and simplify calculations. A blank sample form that you can copy and use for your droplet analyses is provided (Figure 2). It is an example of a completed data sheet that we will describe in detail later (Figure 3).

Figure 2 .

Figure 3 .

At the top of the sheet, spaces have been provided to record the date, sprayer number, insecticide formulation, flow rate, collection method, nozzle pressure, the name of the person taking the collection, and air temperature.

Eyepiece Divisions (D)

We have listed consecutive numbers from 1 to 24 down the first column. These represent eyepiece scale divisions of droplets we will measure with the scope. While the hash marks on page TDA-10 are shown as vertical marks, as is typically done, horizontal marks (=) make it easier to count by fives and less likely that // will be inadvertently read as 11 rather than two.

Hash Counts

For each droplet in any size range, we will enter a hash mark in the second column to the right of the corresponding size.

Number of Droplets (N)

This is the number of hash marks in the second column and will be added vertically. This column should total close to 200.

D x N

The fourth column is a calculation: D multiplied by N. For any given eyepiece measurement D, we will multiply this measurement by the number of droplets N that we found in that size class. The numbers in this column are then added to give Total D x N.

Percent of Total

The fifth column, Percent of Total, is calculated by dividing the D x N number for each row by the Total D x N, and then multiplying the result by 100.

Accumulative Percent

The last column is a running subtotal of the numbers in the Percent of Total column working down the sheet. The last number should be very close to 100.

Slide Examination

To help you learn the methods described, we will walk through an example (Figure 3).

1. The first step in determining the size of the droplets is to carefully put the exposed slide onto the mechanical stage of the microscope. Be careful not to touch the teflon-coated portion of the slide, as this will smudge the surface. Make sure the treated surface is facing up. Remember the frosted area for writing is on the same side as the exposed teflon surface.
2. Rotate the objective lens turret to use the 10x objective lens. Locate and focus on the droplets that will appear as a series of different-sized rings.
3. Now rotate the lens turret to use the 40x lens and refocus, if necessary.
4. Rotate the ocular micrometer until it is positioned vertically.

5. Scan the slide from one side to the other, beginning about one-quarter inch in from the side. For the most accurate results, never measure the droplets close to the edges because the edges tend to have more small droplets than normal.
6. Once the scale is over a droplet, count the divisions on the scale from the top to the bottom of the droplet.
7. In our example the first droplet falls between 23 and 34 for a total of 11 divisions. This measurement is then recorded as a hash mark on the data sheet next to 11, and the tally counter is pushed one time.
8. The second droplet falls between 24 and 31, or seven divisions. This measurement is then recorded like the first as a hash mark on the data sheet next to the seven and the tally counter is pushed again.
9. The third droplet is 11 divisions. We will then continue to work across the slide until we have measured 200 droplets. If there are not enough in one pass, move the slide up or down about two fields of view.
10. After about 200 droplets are measured, the hash marks are added across each eyepiece division row and the number noted in the Number of Droplets column.
11. In our example, there are two droplets measuring two eyepiece divisions. There are three droplets measuring three divisions. There are six droplets in the four-division row.
12. This calculation is completed for each of the rows with hash marks and the column of numbers must be added if a tally counter is not used.
13. The next step is to multiply the number of droplets (N) times the size in eyepiece divisions.
14. The first is the two-division row with two droplets. Therefore, two times two equals four, which we enter in the D times N column.
15. The next row is three divisions with three droplets. Three times three equals nine.
16. Continue down the column multiplying D times N.
17. When this is completed, the next step is to add the D times N column of numbers.
18. In our example, the total sum is 1,635.
19. Next we divide each number in the D times N column by the Total D times N.
20. The first number is four and the total is 1,635; four divided by 1,635 is 0.0024. This is then multiplied by 100 and the result, 0.24, is entered under Percent of Total.
21. The next number, nine, divided by 1,635 and the result multiplied by 100 equals 0.55.
22. The next number, 24, divided by 1,635 and the result multiplied by 100 equals 1.47.
23. Continue this process for all eyepiece divisions.
24. The last column is a running subtotal of the numbers in the previous column.
25. The first is 0.24, the second is $0.24 + 0.55 = 0.79$, the third is $0.79 + 1.47 = 2.26$, etc.
26. Plot the points on a piece of probability graph paper. A blank sheet of probability paper can be photocopied for your ULV droplet analyses (Figure 4).
27. Plot the Eyepiece Divisions (D) on the vertical axis and the Accumulative Percent on the horizontal axis. Plot only the numbers between 10 percent and 90 percent, as found in the Accumulative Percent column.
28. In our example, the smallest eyepiece division with an accumulative percent greater than 10 percent is six divisions. Its accumulative percentage is 14.01 percent.
29. The largest eyepiece division with an accumulative percent below 90 is 11. Thus, plot only the six points for eyepiece divisions six through 11 (Figure 5).

30. After all the data points have been plotted, take a clear straight edge and draw a single line that best connects all the dots on the graph. Some dots will necessarily fall below and some above the line. The majority should be very close to the line.
31. Locate the 50 percent point on the horizontal scale. Follow it to the line you have just drawn. At this point draw a line across to the left or Eyepiece Divisions scale.
32. This point, which is 8.25 in the example, is the Volume Median Diameter (VMD), but is still in eyepiece divisions.
33. The VMD is the point where one-half the volume of the particles measured will be found in droplets smaller than this, and the other half will be found in larger droplets. These numbers are still eyepiece divisions and need to be converted to microns.
34. To convert to microns, you will recall the conversion factor that was determined when the ocular micrometer was calibrated.
35. For our example, the conversion factor was 1.75. Multiply the number of eyepiece divisions, 8.25, by the conversion factor for a VMD of 14.4. Some of the pesticide labels specify a certain percentage of droplets must fall between given diameters. This is easily shown with the graph.
36. Simply divide the diameters listed on the label by the spread factor to find the eyepiece divisions.
37. Then note the eyepiece divisions on the vertical axis of the graph.
38. Draw a horizontal line from the eyepiece divisions to the line we plotted.
39. From this point, draw a horizontal line to the Accumulative Percent scale.

There are computer programs into which you can input the count of eyepiece divisions and they will calculate the VMD. These will greatly speed the calculations, but you should become familiar with the longhand method. Whatever the measurement

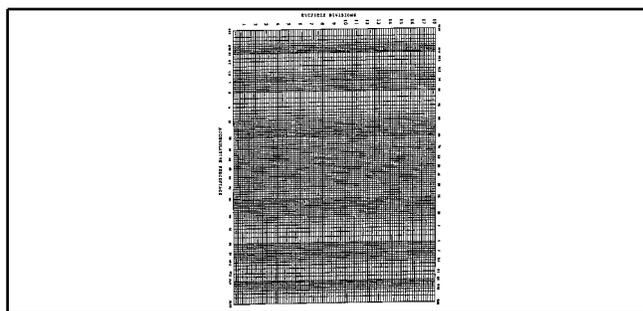


Figure 4 .

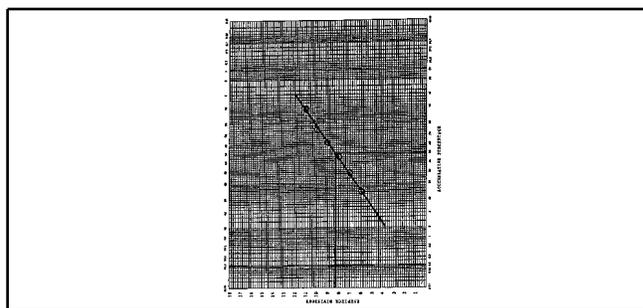


Figure 5 .

process, collecting the sample is still the most important step in droplet analysis.

All the information that has been collected should be filed together. You may be asked to produce these tests as evidence of checking your machinery.