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AUTOMATIC MEASUREMENT OF DROPLET SIZE OF INSECTICIDAL AEROSOLS WITH A COULTER COUNTER®[1]

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ABSTRACT. Techniques were developed to use a Coulter Counter® (Model TA) to measure automatically the droplet size distribution of insecticidal aerosol samples collected in a liquid medium in the field. Samples of aerosols generated by ultralow volume (ULV) ground equipment were collected in a settlement chamber. Petri dishes (100 mm diameter) containing the collection liquid were placed on the chamber floor. A collection liquid consisting of a 45% sucrose/Isoton® solution plus 0.6% Nacconal 90F® as a surfactant was found to be satisfactory for technical malathion (diethyl mercaptosuccinate *S*-ester with *O,O*-dimethyl phosphorodithioate) (95%) and other insecticides with similar physical properties (specifically, technical fenthion (*O,O*-dimethyl *O*-[4-methylthio)-*m*-tolyl] phosphorothioate) and fenitrothion (*O,O*-dimethyl *O*-(4-nitro-*m*-tolyl)phosphorothioate)). A settlement time of 15 min was found to be adequate to obtain an aerosol sample. Droplet size measurements with the Coulter Counter were in close agreement with standard microscopic measurements of droplets collected by settlement onto Teflon®-coated glass slides. For 11 treatments with a malathion aerosol atomized at 4 psi, the volume median diameter (VMD) for microscope measurements averaged 18.7 μm (3.1 μm standard deviation) and accompanying Coulter readings averaged 18.2 μm (0.8 μm standard deviation). The procedures developed in this work provide for automatic measurement of droplet size distributions that can be used to facilitate extensive research on aerosol generators and the effects of different size distributions on insecticidal effectiveness.

Prior research has indicated that the droplet size of an insecticidal aerosol influences its efficiency in killing adult mosquitoes (LaMer et al. 1947, Latta et al. 1947, Yeomans 1949, Mount et al. 1968, Mount and Pierce 1972a, Lofgren et al. 1973). These reports suggest that research be continued to determine optimum droplet size distributions for new adulticide formulations and to estabish droplet size requirements for aerosol generators developed for use in mosquito control programs.

At the present time the most widely used methods for droplet size determinations of aerosols involve tedious and time consuming microscope measurements. Samples of aerosols are collected on glass microscope slides that are coated with either silicon (Yeomans 1949) or Teflon® (Anderson and Schulte 1971). These coatings are required to prevent excessive spreading of the droplets, thus giving a distinct border for measurement. Moreover, the determination of a "spread factor" is required to relate the actual droplet diameter to the measured diameter. Although these microscopic method are useful and are considered to be accurate, their labor requirements tend to limit research requiring droplet size determinations.

[1] This paper reflects the results of research only. Mention of a pesticide or a commercial or proprietary product in this paper does not constitute a recommendation or an endorsement by the U.S. Department of Agriculture nor does it imply registration under FIFRA as amended. The research reported was conducted in part with contract funds transferred from the Medical Research and Development Command, Office of the Surgeon General, U.S. Army.

A number of electronic systems have been developed to automatically determine particle or droplet size distributions (Cadle 1975). These systems include electronic scanners that measure particles collected on slides or cards, as well as optical instruments that use light scattering and laser holography techniques for direct particle size measurements.

Another system of instruments for automatic particle size analysis uses the current path interruption or Coulter® principle (Coulter 1956) which requires the particles to be suspended in an electrolytic solution. With this technique the particle suspension is drawn through a small aperture through which an electric current path has been established. As each particle passes through the aperture it changes the resistance to the current and results in a voltage pulse that is proportional to the particle volume within a certain size range depending on the aperture size. This technique was first developed for automatic blood cell counts and size analysis but is now used in many types of particle size work (Berg 1958, Silverman et al. 1971, Kinsman 1974). However, it has received little or no use for size analysis of liquid insecticide aerosols.

Our purpose in this study was to develop methods to use a Coulter Counter® (Model TA) to measure automatically the droplet size distribution of ultralow volume (ULV) insecticidal aerosols used for adult mosquito control. This required (1) the selection of a suitable sampling liquid that was compatible with insecticide droplets from the standpoint of solubility and size distribution stability, and (2) the development of techniques to collect and suspend an aerosol sample in the liquid. The validity of Coulter size distribution measurements was determined by comparisons with standard microscopic measurements.

METHODS AND MATERIALS

COULTER COUNTER. The Model TA Coulter Counter used in the study reported here is a 16 channel particle analyzer. The volumetric size of the upper limit of each channel or size class is two times that of the lower limit for all 16 channels. The instrument automatically determines the percent of total volume in each size class. Differential and cumulative volume data are presented and can be plotted on an X-Y recorder. The total particle count is presented but the count in each channel is not presented with the TA system. A newer Model TA II Coulter Counter is capable of presenting both volume and count data for each channel.

In general, standard techniques recommended by the manufacturer were used for the Coulter measurements. A 200 or 280 μm aperture tube was used for our samples. The effective range of a tube is 2 to 40% of the aperture diameter; therefore the range for these tubes was 4 to 80 μm and 5.6 to 112 μm, respectively. Monosized polystyrene microspheres, 18.04 μm diameter, were used for calibrations which were performed daily. A concentration index (CI) meter provided an arbitrary indication of the particle concentration in a sample. With CI readings of $<5\%$, corrections for coincidence (more than one particle passing through the aperture at the same time) were not necessary. Field collected samples were diluted if necessary to provide a CI reading of ca. 5%. Typical size distribution curves produced from a Coulter analysis (200 μm aperture) of a technical malathion (diethyl mercaptosuccinate *S*-ester with *O,O*-dimethylphosphorodithioate) aerosol are presented in Fig. 1. Channel 1 was blanked to reduce background noise since it was out of the range of the aperture. The shape of the curves indicates that there was not a significant volume of particles below 4 μm in diameter. Channels 15 and 16 were also outside the range of the aperture but there was no provision for blanking the upper channels. These channels provide an indication if a significant number of excessively large particles are present in the sample. A low percent volume indication ($<2\%$) in channels 2, 15, and 16 indicated that the aperture size was appropriate for the aerosol.

We used the volume median diameter

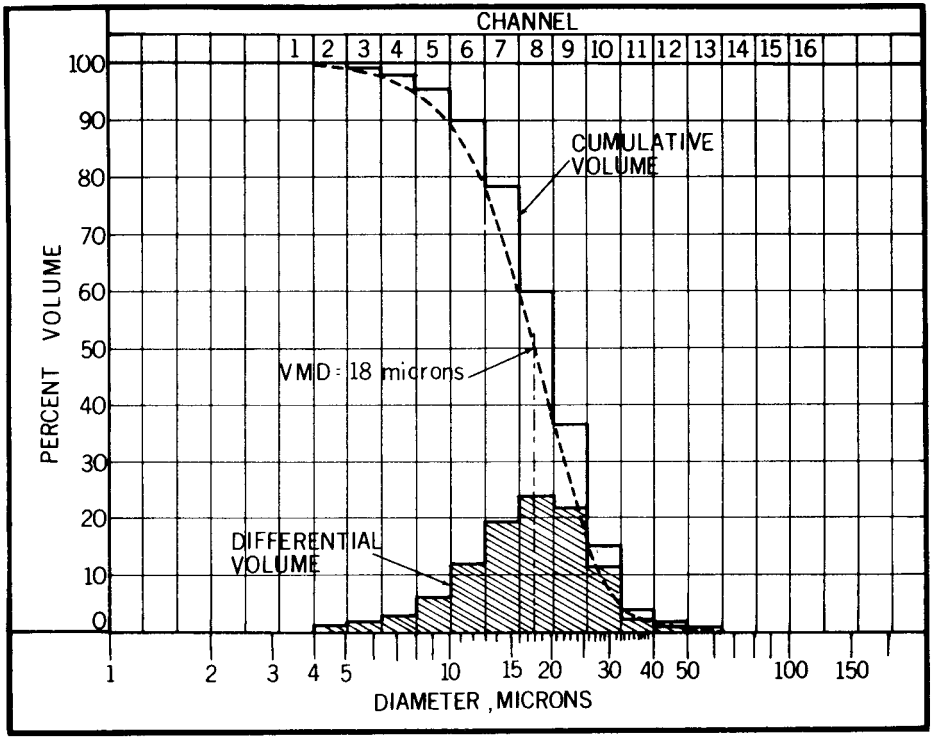

Fig. 1. Example of Coulter Counter graphs (differential and cumulative) for a malathion aerosol (4 psi, 4.3 fl oz/min, 200 μm aperture tube).

(VMD) as the primary parameter to compare size distributions. The procedure for graphical determinations of VMD is illustrated on the cumulative curve in Fig. 1. The accumulation is started with the largest size channel, therefore the lower edge of each channel is used to establish the true curve (dotted line) and the point where the cumulative volume reaches 50%. Other percentage points can be determined in the same manner from the curve. The diameter scale of this graph is logarithmic, therefore the resolution is less as the size increases. Graphical estimates of size were made to the nearest ½ μm in the range from 10 to 30 μm. This range should include the VMD for most ground ULV aerosols.

INSECTICIDES AND AEROSOL GENERATION. Aerosols of technical malathion (95%) were used as a standard to develop the basic techniques for use of the Coulter Counter. A limited number of size measurements were made with aerosols of other materials to demonstrate some of the capabilities and limitations of the Coulter system. The other materials included technical fenthion (*O,O*-dimethyl *O*-[4-(methylthio)-*m*-tolyl] phosphorothioate) (93% Baytex® ULV), naled (1,2-dibromo-2, 2 - dichloroethyl dimethyl phosphate) (Dibrom® 14), fenitrothion (*O,O*-dimethyl *O* - (4 - nitro - *m* - tolyl) phosphorothioate) (93%), and a white mineral oil (Klearol®). All aerosols were produced with a truck-mounted Leco® ULV aerosol generator (Model HD).

DROPLET COLLECTION. Exploratory tests were required to select suitable sampling liquids and a basic procedure to collect and disperse an aerosol sample into the liquid.

We chose a settlement chamber ap-

proach (Yeomans 1949, Mount and Pierce 1972b) to collect droplet samples since this method allowed simultaneous collections for both the Coulter and microscope readings. With sufficient settlement time, droplets of different sizes can be sampled with equal efficiency in a closed chamber.

A plywood building was constructed with 3 chambers, each 3.7 x 3.7 x 2.4 m (12 x 12 x 8 ft) high so that 3 aerosol samples could be collected in a series. Water was sprayed on the ground outside the building and inside the chambers just prior to running a test to minimize collection of dust particles. An aerosol was blasted through the doorway, 1.2 m (4 ft) wide, of the chamber by slowly driving the truck mounted generator past the door (Fig. 2). After closing the door, the aerosol was allowed to settle onto Teflon-coated glass microscope slides and disposable petri dishes (100 mm diameter) containing ca. 10 ml of a sampling liquid. This provided a layer of liquid that was slightly more than 1 mm deep in each dish. Generally, 10 dishes/test provided an adequate amount of sample for a Coulter reading. However, the number of dishes could be varied depending on the amount of aerosol introduced into the chamber. This amount was dependent on the truck speed and insecticide flow rate. Truck speeds of 2.5 and 5 mph were used in our tests to provide different droplet concentrations.

Preliminary observations indicated that the settlement time must be kept as short as possible to prevent coalescence of the droplets while they were on the liquid surface. The droplets tended to hang on the surface due to surface tension even when they were heavier than the sampling liquid. A settlement time of 45 min was used for the initial tests; subsequent tests were conducted to select the most appropriate time.

The samples were collected by pouring the contents of the petri dishes into a common container (200 ml glass jar). This process suspended the droplets within the sampling liquid. After being collected, the

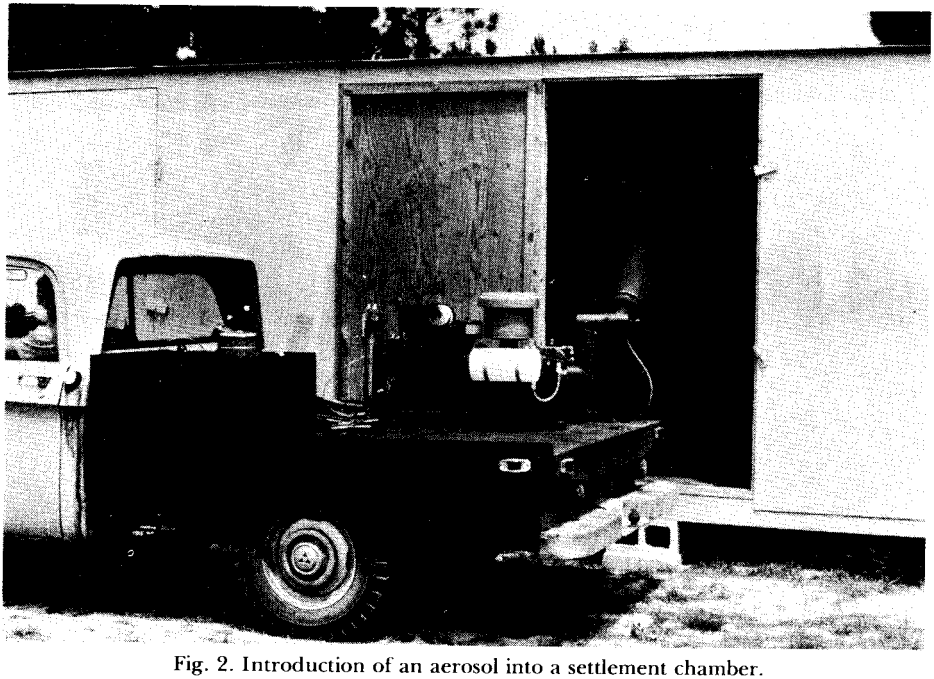

Fig. 2. Introduction of an aerosol into a settlement chamber.

samples were taken to the laboratory for analysis (transport time was ca. 30 min).

Basic considerations for choosing an electrolytic solution as a sampling liquid required:

(1) insolubility with the insecticide droplets.
(2) addition of a surface active agent (surfactant) to allow the droplets to partially penetrate the liquid surface and permit dispersal of the droplets without further breakup.
(3) a density and viscosity that would help prevent settlement and coalescence of the droplets.

Isoton®, an electrolytic solution consisting of water and ca. 1% salts (sodium chloride, 7.9 g/liter; sodium phosphate, 2.1 g/liter; potassium chloride, 0.4 g/liter) was used as the basis for our sampling liquid since malathion and other technical insecticides are not soluble in water. An anionic surfactant, Nacconal 90F®, was used at a rate of 0.06%. Preliminary tests with Isoton plus the surfactant with malathion droplets indicated that the droplets settled rapidly after suspension and that coalescence occurred either while droplets were in suspension or after they had settled to the bottom of the container. To increase the viscosity and density of the mixture we added either glycerol or sucrose (cane sugar). The following two mixtures were selected for further investigation:

(1) 40% glycerol/Isoton (v/v) plus surfactant (40% GLY/ISO)
(2) 45% sucrose/Isoton (w/v) plus surfactant (45% SUC/ISO)

These liquids were filtered through membrane filters having a pore size of 0.45 μm.

MICROSCOPIC ANALYSIS. Standard techniques described by Mount and Pierce (1972b) were used for microscopic analysis of droplets collected on Teflon slides in a settlement chamber. Spread factors used for malathion, fenthion, and Klearol were 0.70, 0.64, and 0.60 respectively. In general, ca. 100 drops/slide were measured with 3 slides/test. We made one slight variation in the normal measurement procedure for these tests. From both Coulter and microscope measurements of aerosols in these tests (VMD$\geq$15 μm) we noted that droplets <4 μm in diameter contained a negligible percentage of the total volume. Therefore, we ignored these particles while making the microscope readings to give a somewhat better sample of the more important larger droplets.

RESULTS AND DISCUSSION

The initial comparisons of VMD's determined by the Coulter Counter and microscope methods (Table 1) indicated close agreement between the two methods for both sampling liquids and two atomization pressures with malathion aerosols (45 min settlement time). Microscope measurements were made for each Coulter measurement but the overall average of the microscope measurements was used for comparison in Table 1. In these tests the microscope VMD readings were intermediate between the Coulter readings for the two collection liquids. The 40% GLY/ISO solution gave the highest readings. These results, plus the observation that the aerosol sample settled to the bottom with the 40% GLY/ISO solu-

Table 1. Comparison of average VMD determinations (μm) by the Coulter Counter with 2 collection liquids and microscopic analysis for malathion aerosols at 2.5 and 4 psi (4.3 fl oz/min flow rate, 2.5 mph truck speed, 45 min settlement time).

	4 psi		2.5 psi	
Method of analysis	No. of Determinations Averaged	VMD	No. of Determinations Averaged	VMD
Coulter 40% GLY/ISO*	3	22.7	2	32.5
Coulter 45% SUC/ISO*	2	18.5	2	26.0
Microscope	5	20.4	4	29.8

* 280 μm aperture tube.

tion and required resuspension before being read led us to conclude that some coalescence was occurring with this liquid. This settlement resulted from the difference in density between malathion (1.23 g/ml) and the 40% GLY/ISO solution (1.10 g/ml). Settlement of the aerosol droplets was not observed in the 45% SUC/ISO solution since its density (1.21 g/ml) was more closely matched to that of malathion. Therefore, the sucrose solution was used for all further tests.

Size measurements were made with different settlement times in the aerosol collection chamber (Table 2) for malathion atomized at 4 psi. These results indicated that after ca. 2 hr the Coulter readings increased with increasing settlement time while the microscope readings did not show a definite trend to increase or decrease. Therefore, the Coulter readings indicated that coalescence was occurring in the collection liquid with the longer settlement times. The microscope readings indicated that the very small drops that settled last did not contain a sufficiently significant volume to affect the VMD.

Further Coulter measurements were made with settlement times of 15, 30, and 45 min since shorter times would be more desirable in actual operations. In these tests there was practically no difference between the VMD's determined with these settlement times for the 4 psi malathion aerosol at 2 initial concentrations (obtained by using truck speeds of 2.5 and 5 mph). Two replications were made for each treatment and the VMD (overall) ranged from 19.0 to 20.0 μm with an average of 19.2 μm and standard deviation of 0.4. These data showed that settlement times as short as 15 min could be used and still obtain an adequate sample of the aerosol droplets for analysis by the Coulter Counter. Shorter settlement times might be possible, however, we concluded that 15 min was as short as practical for our purposes. These data indicated a slight increase in the VMD compared to the previous Coulter readings with 1 hr or less settlement time (Table 2) which averaged 18.2 μm (range 16.5 to 19 μm, standard deviation 0.8). This change apparently resulted from replacement of the generator nozzle cone during routine machine maintenance.

Table 2. Coulter and microscope VMD determinations for different settlement times in the aerosol collection chamber for malathion atomized at 4 psi (4.3 fl oz/min, 2.5 mph truck speed).

Settlement Time (hr)	No. Replications	VMD (μm)	
		Coulter*	Microscope
½	1	18.0	16.7
¾**	2 & 5	18.5	20.4
1	2	17.3	16.3
2	1	19.0	22.1
4	1	20.5	17.5
8	1	27.0	16.6

* 45% SUC/ISO collection liquid and 200 μm aperture tube.

** Values for ¾ hr settlement time were taken from Table 1.

All the previous microscope readings for the 4 psi aerosol were combined and the cumulative data were plotted along with a typical Coulter curve for the same aerosol (ca. 18 μm VMD) as a further comparison of the 2 methods (Fig. 3). The 2 graphs were very similar with essentially the same VMD and range although the microscope data indicated a slightly "flatter" distribution. The microscope data included measurements of a total of 3171 droplets from 11 aerosol collections. The calculated VMD for the combined microscope data was 18.6 μm and the average VMD for the 11 separate collections was 18.7 μm with a standard deviation of 3.1 μm (range 15.0 to 24.9 μm). A comparison of these data with the average for Coulter readings stated previously (18.2 μm average, 0.8 μm standard deviation, and 16.5 to 19.0 μm range) again indicates no significant difference in VMD. However, the standard deviations and range indicate that the Coulter readings were less variable than the individual microscopic determinations. This was expected because of the large difference in the number of droplets sampled for the individual de-

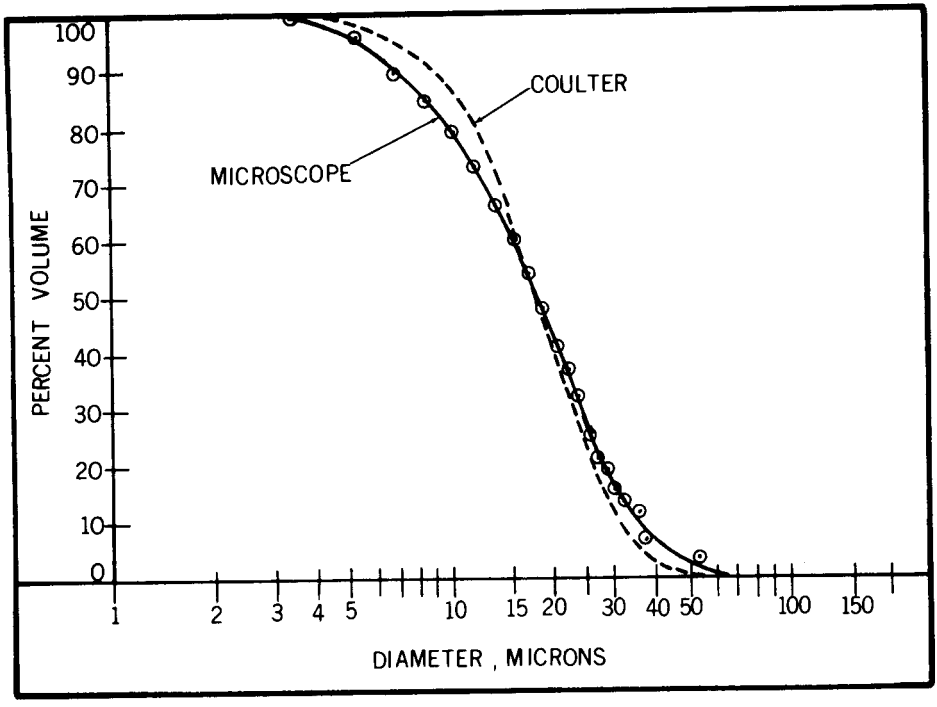

Fig. 3. Cumulative size distribution curves for combined microscope measurements and a typical Coulter reading for a malathion aerosol produced by a Leco HD generator at 4 psi and 4.3 fl oz/min flow rate.

terminations (ca. 300 for the microscope readings compared to between 50,000 and 100,000 for the Coulter readings). In general, we concluded that the 2 methods of analysis yielded similar results and the differences were well within the accuracy of either system.

Coulter readings made on the same sample were almost always repeatable within a time of ca. 2 hr. After this time a decrease in concentration and an increase in VMD began to be noticeable. This indicated that the stability of the aerosol in the 45% SUC/ISO solution was limited and after ca. 2 hr coalescence began to affect the reading.

Coulter Counter readings of aerosols of materials other than malathion gave varied results depending mainly on the density of the materials. All the other materials tested were nonsoluble in water. Results with fenthion (Table 3), having a density of 1.25 g/ml (near that of malathion), showed excellent agreement between Coulter and microscope VMD determinations. With Klearol (density 0.83 g/ml) the Coulter readings were low (Table 3), indicating that the larger drops were rising toward the surface of the sampling liquid (and away from the aperture) faster than the smaller droplets due to the lighter density of the oil. A thickened solution with a density of less than 1 g/ml would probably be required to obtain an accurate Coulter reading with a low density oil.

We attempted to use the Coulter procedure for aerosols of naled, which has a density of 1.82 g/ml. No reading could be obtained with 45% SUC/ISO apparently because the droplets settled and coalesced rapidly or the two liquids were otherwise incompatible.

Coulter measurements were possible for fenitrothion aerosols collected in the

Table 3. Coulter and microscope readings (VMD, μm) for fenthion and Klearol atomized at 4 psi (2.5 mph speed, 15 min settlement time).

Material	Flow Rate (fl oz/min)	Rep.	VMD (μm) Coulter*	VMD (μm) Microscope
Fenthion	1	1	14.5	14.4
		2	14.0	14.0
		3	11.0	10.8
		Average	13.2	13.1
Klearol	4.3	1	13.5	18.3
		2	15.0	19.2
		Average	14.3	18.8

* 45% SUC/ISO collection liquid and 200 μm aperture tube.

45% SUC/ISO mixture. A reading of 15 μm VMD was obtained with a flow rate of 2 oz/min at 4 psi. No microscope measurements were made for fenitrothion in this study but this reading was in the range of microscope measurements from previous studies (unpublished data). The density of fenitrothion (1.31 g/ml) was only slightly greater than that of malathion, and we concluded that the Coulter measurements should be accurate.

We concluded from these results that the Coulter technique can be used to measure droplet size of ULV insecticidal aerosols. Coulter Counter readings of aerosol samples collected by settlement into a 45% sugar-Isoton mixture were in close agreement with microscopic measurements for malation and fenthion. The same procedures should be usable for aerosols of other materials that are not soluble in water and have densities close to that of malathion. Use of the Coulter technique with materials having different physical properties should be possible with the development of other suitable sampling liquids.

The procedures developed in this study for the Coulter Counter provide for automatic measurement of droplet size distributions that can be used for extensive research on aerosol generators and the effects of different size distributions on insecticidal effectiveness. Use of this technique for general field measurements may be limited because exact procedures must be followed in collecting, handling, and reading the liquid samples. General use may also require a means of stabilizing and preserving the droplet samples.

Literature Cited

Anderson, C. H. and W. Schulte. 1971. Teflon® as a surface for deposition of aerosol droplets. Mosquito News 4:499–504.

Berg, R. H. 1958. Electronic size analysis of subsieve particles by flowing through a small liquid resistor. Symposium on Particle Size Measurement, ASTM, STP No. 234. pp. 245–255.

Cadle, Richard D. 1975. The measurement of airborne particles. John Wiley and Sons, New York.

Coulter, W. H. 1956. High speed automatic blood cell counter and cell size analyzer. Presented at National Electronics Conference, Chicago, Illinois, October 3, 1956.

Kinsman, S. 1974. Instrumentation for filtration tests. Chem. Eng. Prog. 70:48–51.

LaMer, V. K., S. Hochberg, K. Hodges, I. Wilson, J. A. Fales and R. Latta. 1947. The influence of the particle size of homogenous insecticidal aerosols on the mortality of mosquitoes in confined atmospheres. J. Coll. Sci. 2:539–49.

Latta, R., L. D. Anderson, E. E. Rogers, V. K. LaMer, S. Hochberg, H. Lauterbach and I. Johnson. 1947. The effect of particle size and velocity of movement of DDT aerosols in a wind tunnel on the mortality of mosquitoes. J. Wash. Acad. Sci. 37:397–407.

Lofgren, C. S., D. W. Anthony and G. A. Mount. 1973. Size of aerosol droplets impinging on mosquitoes as determined with a scanning electron microscope. J. Econ. Entomol. 66:1085–88.

Mount, G. A. and N. W. Pierce. 1972a. Adult mosquito kill and droplet size of ultralow volume ground aerosols of insecticides. Mosquito News 32:354–57.

Mount, G. A. and N. W. Pierce. 1972b. Droplet size of ultralow volume ground aerosols as determined by three collection methods. Mosquito News 32:586–89.

Mount, G. A., C. S. Lofgren, N. W. Pierce and C. N. Husman. 1968. Ultralow volume nonthermal aerosols of malathion and naled for adult mosquito control. Mosquito News 28:99–103.

Silverman, Leslie, Charles E. Billings and Melvin W. First. 1971. Particle Size Analysis in Industrial Hygiene. Academic Press, New York and London.

Yeomans, A. H. 1949. Directions for determining particle size of aerosols and fine sprays. USDA Bur. Entomol. Pl. Quar. ET-267.

OBSERVATIONS ON THE LABORATORY BIOLOGY AND MAINTENANCE OF *AEDES TRIVITTATUS*[1]

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ABSTRACT. Data on the biology and laboratory maintenance of *Aedes trivittatus* (Coq.) are reported. Methods used to obtain large numbers of *Ae. trivittatus* for laboratory experimentation with minimum effort involve the collection and storage of eggs from field-collected adults. Each field-collected female produces an average of 62 eggs and nearly 50% hatch, even after storage of up to 1 year. Eighty % survival of larvae and 97% survival of pupae are obtained by using the rearing methods described. Fifty % of adult females survive for more than 26 days, with maximum survival for any mosquito being 60 days. Problems and precautions involved in maintaining *Ae. trivittatus* in the laboratory are discussed.

Aedes (Ochlerotatus) trivittatus (Coquillett) is a medium sized, floodwater mosquito that is widely distributed in North America. It has been reported from 39 of the continental United States (Carpenter and LaCasse 1955; Carpenter 1968, 1970), southern Canada (Carpenter and LaCasse 1955, Trimble 1972), and parts of Mexico and Panama (Howard et al. 1917). *Ae. trivittatus* is found throughout Iowa (Wong et al. 1970, Pinger and Rowley 1972) and can be the most abundant species in certain areas (Christensen and Andrews 1976). It has been reported as the second most abundant mosquito collected in CO_2-baited CDC light traps set primarily near urban areas of Iowa (Wong et al. 1970) and the third most abundant species collected in New Jersey light traps in rural Iowa (Pinger and Rowley 1972).

Ae. trivittatus has been reported to be a vector of several mosquito-borne diseases. Trivittatus virus (TVT) was originally isolated from a pool of *Ae. trivittatus* collected in North Dakota (Hammon et al. 1952), and this species has since been incriminated as the natural vector of this virus (Wong et al. 1970, Rowley et al. 1973, Watts et al. 1976). In addition to TVT virus, arboviruses isolated from field-collected *Ae. trivittatus* include western equine encephalomyelitis, Bunyamwera, Jamestown Canyon, and Flanders viruses (Wong et al. 1970, 1973, Rowley et al. 1973, Anslow et al. 1969, Thompson et al. 1972, Sudia et al. 1971, Rowley, unpublished data). *Ae. trivittatus* also has been reported as a natural vector of dog heartworm, *Dirofilaria immitis*, in central Iowa (Christensen 1977).

Although some data are available on the biology of *Ae. trivittatus* (Abdel-Malek 1948 a, b, Horsfall et al. 1958, Wright and Knight 1966, Pinger and Rowley 1975), most of this information concerns field observations. A paucity of information is available on the laboratory biology and maintenance of this mosquito. *Ae. trivittatus* has been used routinely in our laboratory in studies with TVT virus and *D. im-*

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