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**Slide Analysis Manual** 

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**Slide Analysis** is a companion program to Stainalysis, a freeware program from REMSpC that can be used to analyse deposit characteristics on scanned Kromekote cards. **Slide Analysis** is an analysis tool for calculating the droplet size distribution within a spray cloud. Spray-cloud sampling is achieved using rotating impinger glass slides that are Teflon coated for oil-based pesticides or coated with magnesium oxide for water-based pesticides. However, sampling by rotating impingers is inexact since

- 1. pesticide deposited on a slide surface will spread thereby requiring the measured drop diameter to be corrected using a spread factor. Spread factors are specific to the formulation being used and the type of surface on the slide.
- 2. the collection efficiency of rotating slides significantly reduces as drop size decreases. Therefore, to estimate the drop distribution in a spray cloud, the number of drops on a slide associated with different drop diameters (D) must be adjusted using a collection efficiency term  $(1/D^2)$ .
- 3. large drops (>50µm) may not be found due to shattering by the rotating slide or the small sampling area which leads to the few large drops being missed. Field data should always be compared to wind tunnel tests. Deposit cards should also be used when aerial swath-characterization trials at low heights are undertaken.

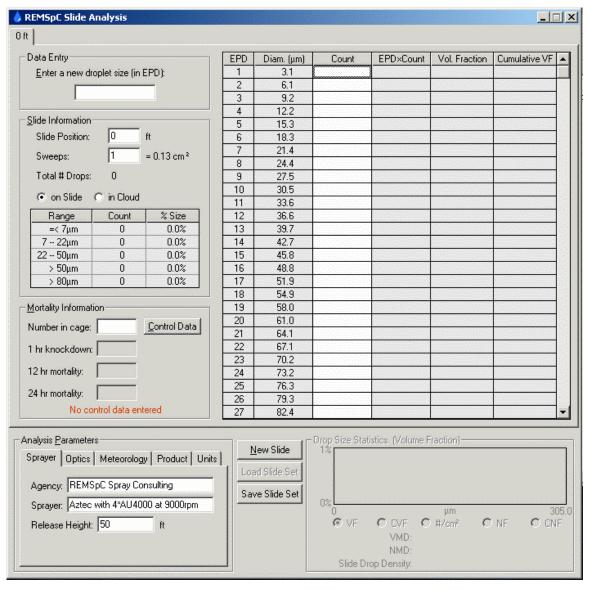
**Slide Analysis** produces Drop Size Statistics for both the slide and spray cloud. Drop density (# of drops/cm<sup>2</sup>) represents drops on the slide while Volume Fraction (VF), Cumulative Volume Fraction (CVF), Number Fraction (NF), Cumulative Number Fraction (CNF), Volume (Mass) Median Diameter (VMD = MMD) and Number Median Diameter (NMD) refer to drops within the spray cloud. For field trials involving multiple impinger sites (sample line for swath characterization), Composite Statistics provide an analysis of the bulked data set. Graphs summarize spatial differences of individual slide statistics in comparison to the bulked statistics. For field trials that include caged

mosquitoes, relationships are derived to compare caged-mosquito mortality with measured drop density. In all cases, measured mortality is adjusted using Abbott's formula to take account of mortality within a control sample. Questions should be directed to SlideAnalysis@REMSpC.com

## Installing the program

Slide Analysis is freeware that can be downloaded from <u>www.REMSpC.com</u>. Running the downloaded package will install the program onto your computer, typically in C:\Program Files\Slide Analysis\.

## Main Page



## **Analysis Parameters**

For each data set, certain parameters describing the field trial should be tabulated. Information describing a trial falls into 5 categories:

Sprayer	include information on
	Agency
	Sprayer – details such as type, nozzles, boom pressure, etc.
	Release Height – height of nozzles above ground

**Optics** description of how the slide analysis was performed

<u>Scan Length</u> – generally the slide is scanned across its width (1 sweep). Slides are typically 1 inch or 2.54cm wide.

 $\frac{\# \text{ of } \text{EPD } \text{ on reticule}}{\text{reticule of the microscope.}}$ 

 $\mu$ m/EPD – the number of microns that each EPD represents

<u>Spread Factor</u> – spread factor (SF – less than 1.0) is a correction applied to the measured drop diameter to allow for slumping of the drop on the slide surface. Spread factors are different for different formulations and different slide coatings (Teflon, magnesium oxide). The diameter of the measured drop is reduced by the spread factor to give the size of the drop in the spray cloud.

Note:

Drop density  $(drops/cm^2) = \frac{\text{number of drops read}}{\text{area scanned}}$ 

Area scanned  $(cm^2)$  = scan length (cm) × number of sweeps × ×[# of EPD on reticule ×  $\mu m$ /EPD × 0.0001 (cm)] where 1 $\mu m$  = 0.0001cm or 1cm = 10000 $\mu m$ 

Drop diameter (in cloud) = SF × measured Drop Diameter on slide where measured Drop Diameter =# of EPD ×  $\mu m$ /EPD

Meteorology	meteorology at site during trial
	<u>Height</u> – height of met sensors
	<u>Air Temperature</u>
	<u>RH</u> – Relative Humidity
	Wind Speed
	Wind Direction – wind direction relative to spray line
	$0^{\circ}$ – spray line into wind
	$90^{\circ}$ – crosswind with wind striking right-hand side of vehicle
	$180^{\circ}$ – spray line with the wind
	270° – crosswind spray with wind striking left-hand side of the vehicle
Product	Product – product name, manufacturer
	<u>Spray Rate</u> – total volume rate being applied. For tank mixes this includes diluent (water, oil). Product rate should be included in brackets
	ie. $3(0.6)$ oz/ac indicates a total spray rate of 3 oz/ac with 0.6 oz/ac being product (2.4 oz/ac is diluent)
Units	Metric – metres, litres, hectares
	$\underline{\text{US}}$ – feet, US oz, acres
	Note: changing units converts only the slide width in order to maintain

Note: changing units converts only the slide width in order to maintain proper drop density calculations per  $cm^2$ . All other values (ie. temperature, application rate, slide position, etc.) remain unchanged, only the units change.

## Data Entry

#### Load Slide Set

Data from previous Slide Analysis sessions can be loaded by clicking the **Load Slide Set** button. Slide set files contain all of the trial parameters as well as all of the drop and mortality data previously entered, so re-entering data is unnecessary.

#### <u>N</u>ew Slide



Sweeps:

Slides are added one-by-one to a set (either a new set, or one loaded from a file) by clicking **New Slide** which then requires entry of the slide's position relative to the spray line. Distances to pilot's right of the spray line are positive, to the left are negative; the spray line being marked by position 0. Only one slide can be associated with each position. A slide's position can be changed at any time in the **Slide Position** box.

#### Drop-size data can be entered either

– Data Entry	
<u>E</u> nter a r	ew droplet size (in EPD):

 as a slide is being read through a microscope. As the size (EPD) of each drop is entered, the spread sheet is automatically updated.

EPD	Diam. (µm)	Count	EPD×Count	Vol. Fraction	Cumulative VF	
1	3.1	9	9	0.0	0.0	
2	6.1	15	30	0.2	0.2	
3	9.2	6	18	0.1	0.3	
4	12.2	2	8	0.0	0.3	1
5	15.3	2	10	0.1	0.4	
6	18.3					]
7	21.4	3	21	0.1	0.5	1
8	24.4	3	24	0.1	0.6	1
9	27.5	2	18	0.1	0.7	
10	30.5	4	40	0.2	0.9	
11	33.6					
12	36.6	1	12	0.1	1.0	
13	39.7					
14	42.7					]
15	45.8					]
16	48.8					1
17	F1 0					1

 on the spread sheet where count for each EPD can be entered when slide-scanning is completed.

 $10 = 1.27 \text{ cm}^2$  When a slide is completed, enter the number of **Sweeps** (scans across a slide) needed to produce the data set. The scanned area in cm<sup>2</sup> (based on microscope optics) is updated.

0 m 50 m 100 m 150 m 200 m -50 m As new slides are added to the set, each slide is tagged by its distance from the spray line. Slide data from each position can be viewed by clicking on the appropriate distance tab.

## **Mortality Information**

Control Data		
- Mortality Informatic	n	
Number in cage	20	
12 hr mortality:	0 :	= 0.0%
24 hr mortality:	1	= 5.0%
	Accept	Cancel

– <u>M</u> ortality Informati	on	
Number in cage:	100	<u>C</u> ontrol Data
1 hr knockdown:	5	= 5.00%
12 hr mortality:	27	= 27.00%
24 hr mortality:	56	= 53.68%

If the field trial includes caged mosquitoes at each of the slide positions, control mortality must be entered before information on 1-hr knockdown, 12-hr mortality and 24-hr mortality at each slide site. Good field practice should limit control mortality (handling stress) to less than 10%. Caged-mosquito mortality is corrected by control mortality using Abbott's formula. These data are used to develop a relationship between slide drop density (surrogate for dose) and cagedmosquito mortality. Slide data taken during operational sprays can be used to infer potential peak mortality

during operational treatment programs (spray loss by canopies, mosquito inactivity can reduce mortality).

The ability to load a previous data set into Slide Analysis allows for mortality information to be entered at a later date, after the slide data has been recorded. Since only a single mortality data set can be associated with a slide, sites with collocated cages should be bulked and entered as a single data set.

## **Analysis Output**

As slide data are entered, drop statistics are continuously updated. Statistical output is presented in three forms:

### **Range Statistics**

Range Statistics divide the measured drop sizes into categories that reflect current thinking as to drop sizes that are important for mosquito control (7-22 $\mu$ m) and drop sizes that could result in rapid deposit and hence loss to adult mosquito control (Diameter > 50 $\mu$ m and 80 $\mu$ m). Other ranges (<7 $\mu$ m, 22-50 $\mu$ m) are tabulated to complete the information on the drop distribution. Range statistics are available for drops on the slide or drops in the cloud (slide data corrected for collection efficiency).

💿 on Slide	🔿 in Cloud	
Range	Count	% Size
=< 7μm	10	14.7%
7 22μm	36	52.9%
22 50µm	22	32.4%
> 50µm	0	0.0%
> 80µm	0	0.0%

**On-slide** data include

- 1. number of drops found in each range
- 2. percentage of total drops associated with range

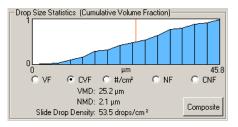
⊙ on S	lide 🤇	in Cloud	l
Rang	le	% Mass	% Size
=< 7µ	ım	5.2%	81.1%
7 - 22	ım	38.5%	17.1%
22 50	ım	56.3%	1.8%
> 50	ım	0.0%	0.0%
> 80	ım	0.0%	0.0%

In-cloud data include

- 1. percentage of volume (mass) associated with the range
- 2. percentage of total drops associated with the range

**Notes:** In-cloud statistics will always show a larger fraction of sub- $7\mu$ m drops than were actually measured reflecting the lower collection efficiency of these smaller drops on the rotating slides.

### **Drop Size Statistics**



In the **Drop Size Statistics** panel, the contribution of individual drop sizes to volume fraction (VF), cumulative volume fraction (CVF), number fraction (NF) and cumulative number fraction (CNF) within the cloud can be viewed by clicking

the appropriate radio button (O). The drop density ( $\#/cm^2$ ) provides information about drops collected on the slide.

Listed below the graph are

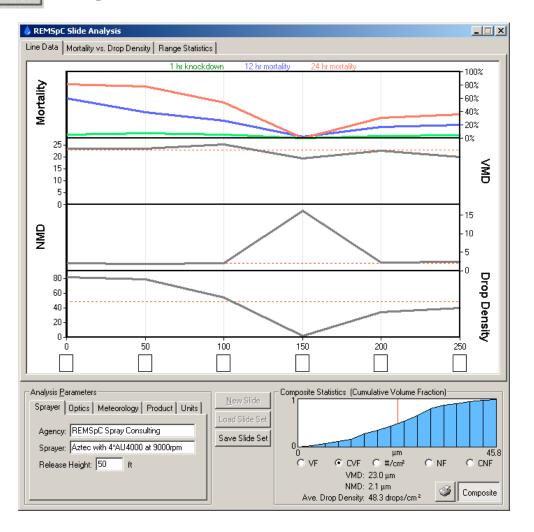
VMD – volume median diameter in the cloud: defined as the drop diameter above which 50% of the cloud volume is found in drops with larger diameters and 50% of the cloud volume is associated with drops of smaller diameter. VMD is the same as MMD, mass median diameter.

NMD – number median diameter in the cloud: defined as the drop diameter above which 50% of the drops have a larger diameter. Generally, the NMD of mosquito adulticide sprays is less than 5 $\mu$ m indicating that most of the drops are smaller than the 'ideal range' of 7 – 22 $\mu$ m.

Slide Drop Density (#/cm<sup>2</sup>) – number of drops/cm<sup>2</sup> found on the slide. Drop density includes all drop sizes.

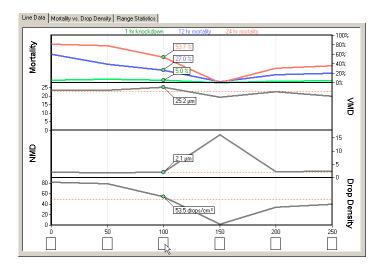
#### Composite

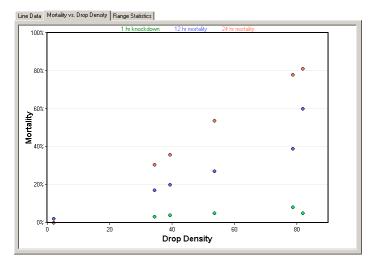
#### **Composite Statistics**



Activating the composite button provides statistics on the complete data set. While drop density (#/cm<sup>2</sup>) reflects the average of all slides, number (NF, CNF, NMD) and volume (VF, CVF, VMD) cloud statistics are derived from bulking all slide data into a single data set and applying collection efficiency. Weighting of individual slide data accounts for different areas that may have been read on each slide. In the **Composite Statistics** panel, the contribution of individual drop sizes to total volume fraction (VF), total cumulative volume fraction (CVF), total number fraction (NF) and total cumulative number fraction (CNF) for the full data set can be viewed by clicking the appropriate radio button (). Listed below the graph are the cloud VMD and NMD for the total data set and the average drop density of the slide set.

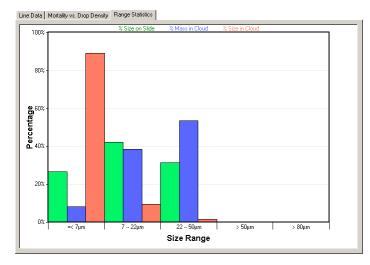
Graphs on composite statistics are available by clicking the appropriate tab:





Line Data graph provides the spatial variation of individual slide/cloud statistics and cagedmosquito mortality. Composite statistics are represented by the dashed red lines. Mouse-over on a slide located at the bottom of the graph shows statistics for that location.

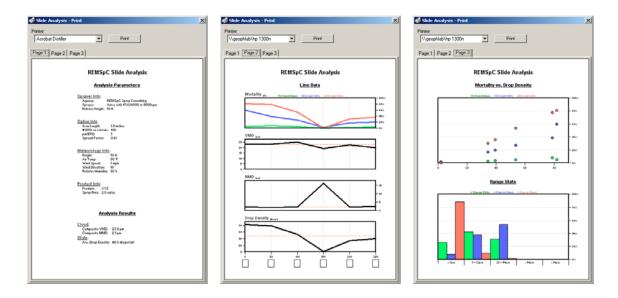
Mortality versus Drop Density highlights the relationship between these two parameters. Knockdown (1hr), 12hr mortality and 24hr mortality are plotted. Mortality data are corrected for mortality in the controls.



**Range Statistics** provide a comparison of the size and mass distribution of drops on the entire set of slides and in the cloud.



Clicking the print button opens a Print window, in which the desired printer can be chosen (if more than one printer is installed on the system or network). A print preview of each page can be seen by clicking the appropriate tab.



## Save Slide Set

Saves the slide set for future retrieval. Slide Analysis saves files as Comma-Separated Values files (CSV), a common file format that can also be loaded into virtually any spreadsheet program (ie. MS Excel). An example is given in Appendix 1. The output file contains analysis parameters, raw and statistical data for each of the slides and statistics for the composite data set.

# Appendix 1

Sample of Slide Analysis File

------REMSpC Slide Analysis ------

#### -----Analysis Parameters -----Units: U.S. Units: U.S. Agency: REMSpC Spray Consulting

Agency: REMS		onsulting						
Sprayer:	xyz							
Release Height:	50ft							
Scan Length:	1 inche	s						
# EPD on reticule:	100							
um/EPD:								
1	5µm							
Spread Factor:	0.61							
Height:	50ft							
Air Temp.:	80°F							
Wind Speed:	5mph							
Wind Direction:	10°							
Rel. Humidity:	80%							
_								
Product:	XYZ							
Spray Rate:	2oz/ac							
Mortality Control Dat	ta							
Number in cage:	20							
-								
12 hr mortality:	0							
24 hr mortality:	1							
Slide Data								
Number of Slides:	6							
	-							
Slide 1 Position:	0ft							
Number of Sweeps:	10							
Cloud VMD:	23.4µm	L						
Cloud NMD:	2.1µm							
Slide Drop Density:								
Mortality Info:	01.09410	207 Ciii						
-	100							
Number in cage:								
1 hr knockdown:								
12 hr mortality:								
24 hr mortality:	82 = 81.0	)5%						
Drop Info:		on Slid	e			in	Cloud	
#EPD Microns		orop Densit			•	CVF		CNF
1 3.1		10.236						
	10	7.874 4.724	0.096	0.221	0.032	0.053	0.133	0.00
3 9.2								
4 12.2	8	6.299	0.077	0.356	0.051	0.133	0.028	0.925
5 15.3	14	11.023	0.135	0.49	0.112	0.244	0.031	0.956
6 18.3	8	6.299	0.077	0.567	0.077	0.321	0.012	0.968
7 21.4	10	7.874	0.096	0.663		0.433	0.011	0.979
8 24.4	8	6.299	0.077	0.74	0.102	0.535	0.007	0.986
9 27.5	7	5.512	0.067			0.636	0.005	0.991
10 30.5	8	6.299	0.077	0.885	0.128	0.764	0.004	0.995
11 33.6	5	3.937	0.048	0.933	0.088	0.851	0.002	0.998
12 36.6	1	0.787	0.01	0.942	0.019	0.871	0	0.998
13 39.7	3	2.362	0.029	0.971	0.062	0.933	0.001	0.999
14 42.7	3	2.362	0.029	1	0.067	1	0.001	1

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Range Statistics				
	Count	% Size	% Mass	% Size
=< 7µm	23	22.1	6.1	87.1
7 22µm	46	44.2	39.3	11
22 50µm	35	33.7	54.5	1.9
> 50µm	0	0	0	0
> 80µm	0	0	0	0

Slide 2 Position:50ftNumber of Sweeps:10

etc

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Composite Data

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Cloud VMD:	23µm
Cloud NMD:	2.1µm
Ave. Slide DD:	48.3drops/cm <sup>2</sup>

#EPD Microns Drop Density VF CVF NF CN   1 3.1 7.2 0.027 0.027 0.733 0.   2 6.1 5.6 0.042 0.068 0.142 0.	733 875
	875
2 6.1 5.6 0.042 0.068 0.142 0.	
3 9.2 4.1 0.046 0.114 0.047 0.	922
4 12.2 3 0.044 0.158 0.019 0.	941
5 15.3 6.3 0.117 0.275 0.026 0.	966
6 18.3 3.2 0.072 0.347 0.009 0.	975
7 21.4 3.7 0.096 0.443 0.008 0.	983
8 24.4 3.6 0.107 0.55 0.006 0.	989
9 27.5 3.4 0.113 0.663 0.004 0.	993
10 30.5 3.8 0.14 0.803 0.004 0.	997
11 33.6 2 0.08 0.883 0.002 0.	999
12 36.6 0.5 0.023 0.906 0 0.	999
13 39.7 1.1 0.052 0.958 0.001 1	
14 42.7 0.5 0.027 0.985 0 1	
15 45.8 0.3 0.015 1 1	
Range Statistics	
% Size % Mass %	Size
=< 7µm 26.5 8.2 88	3.9
7 22μm 42.0 38.4 9.	5
22 50μm 31.5 53.4 1.	6
> 50µm 0 0 0	
> 80µm 0 0 0	
Line Statistics	
Cloud Slide Mortality	
Position(ft) VMD NMD DD 1hr KD 12hr Mort. 24hr	r Mort
0 23.4 2.1 81.9 5 60	81.1
50 23.3 1.9 78.7 8 39	77.9
100 25.2 2.1 53.5 5 27	53.7
150 19.2 16.2 2 0 2	0
200 22.5 2.2 34.4 3 17	30.5
250 19.9 2.4 39.4 4 20	35.8

Appendix 2

Work Sheet for Slide Data

Length of scan	
# of EPD on redicule	Test:
microns/EPD	
Product	Spread Factor

Slide #		Distance			Slide #		Distance		
EPD	Sweeps			Total Cnt	EPD	Sweeps			Total Cnt
1					1				
2					2				
3					3				
4					4				
5					5				
6					6				
7					7				
8					8				
9					9				
10					10				
11					11				
12					12				
13					13				
14					14				
15					15				
16					16				
17					17				
18					18				
19					19				
20					20				
21					21				
22					22				
23					23				
24					24				
25					25				
26					26				
27					27				
28					28				
29					29				
30					30				
	Canad Maaguitaaa					agad Maagui	tooo	Total #	
Caged Mosquitoes Total #						aged Mosqui	IDES	10tal #	
1hr Knockdown 12 hr Mortality						nr Knockdown	ļ		
						24hr Mortality			
24hr Mortality						24hr Mortality			