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LASER DETECTION AND MAPPING OF BIOLOGICAL
SIMULANTS III. DICHOTOMOUS SAMPLER
MEASUREMENTS OF AEROSOL CONCENTRATIONS AS
RELATED TO LIDAR SIGNALS.

by

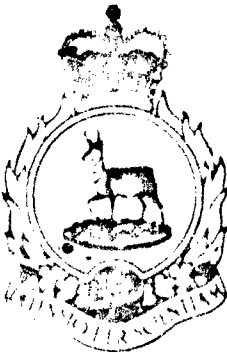
J. Ho, B.T.N. Evans* and G. Roy*



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* Defence Research Establishment Valcartier, P.O. Box 8800, Courcellette, P.Q.

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DICHOTOMOUS SAMPLER MEASUREMENTS OF
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ABSTRACT

In a continuing series of investigations on biological aerosol detection by the Laser Cloud Mapper (LCM), reliable measurement of biological aerosol concentrations were found to be critical to LCM data interpretations. The sampling method must give reproducible results under difficult field conditions of rapidly changing environmental parameters. A series of trials were conducted to evaluate the Dichotomous Sampler (DS) as a device for the sampling of biological aerosols and to assess its potential for the estimation of biological aerosol concentrations during LCM trials. Sampled biological aerosols were enumerated as viable cells and provided reliable estimates of viable aerosol concentrations with respect to time. Interpretation of DS and LCM data demonstrated a relationship between cell concentrations and depolarization signal ratios which suggests that this technique has potential for remote biological aerosol detection at the 200-400 viable spores/liter level.

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UNCLASSIFIEDIntroduction

A Laser Cloud Mapper (LCM), developed at DREV primarily for smoke measurements (1, 2), was evaluated during a series of field trials to determine if this instrument would be suitable for the detection of biological aerosols (3). In a more recent trial (4), it was found that by measuring the depolarized return signals, the LCM was capable of differentiating between biological aerosols and the contaminant particles associated with the media used for dispersion. However, there was insufficient data to positively correlate a wide range of biological aerosol concentrations with LCM data (4). The demonstration of such a relationship was considered essential prior to acceptance of the LCM technology for BW aerosol detection.

A series of trials with the following improvements were designed to assess the dichotomous sampler (DS) as a BW aerosol sampler and to attempt a correlation between the data generated by the DS and LCM.

a. In the previous trial, only one aerosol sampling instrument (DS) was used to sample the rapidly moving aerosol cloud. A second DS station, positioned adjacent to the first, was used to increase sampling reliability.

b. The original sampling interval of 2 min. was extended to 10 min. to permit measurements of lower biological aerosol concentrations.

c. The original spore suspension was diluted with water to provide source strengths of between 1 and 75% of original strength in order to estimate the applicable LCM range for BW aerosol detection.

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UNCLASSIFIEDMaterials and MethodsBW simulant

A spore suspension of *Bacillus subtilis* Var. *niger species globigii* (BG) was used as the simulant. Viability of the sample was 1×10^9 cells per ml (100% BG slurry). Lower concentrations used in the experiments were obtained by diluting the BG slurry with tap water. Untreated tap water was used to produce the control aerosol.

A vegetative simulant was used to provide an alternate aerosol source (5). Fresh cultures of *Erwinia herbicola* (EH) were grown on nutrient agar at 28 ° C for 4 days. Working cultures were kept viable on nutrient agar slants at 4 ° C for several months with periodic subculture to fresh media. Nutrient broth cultures were grown in 600 ml batches at 28 ° C for 4 day and used as an inoculum for larger batch cultures.

Large batches of EH cells were obtained by growing the organism in a 23 l fermenter (model Microferm, New Brunswick Scientific Co., Inc., Edison, N.J.) using nutrient broth at 28 ° C, pH 7.0. After 4 days, the harvest at 4×10^7 viable cells/ml was removed without further processing. Cells stored in this growth medium at 4 ° C retained viability for several months and this liquid was used as the source in aerosol generation.

Aerosol generator

A Micronair generator (Model AU7000, Micronair Limited, Bembridge Fort, Sandown, Isle of Wight, PO36 8QS, England) provided a continuous, polydisperse BG aerosol spray of about 1 to 8 μ m in diameter (4). This unit was factory equipped (special order) with a 110 VAC motor which drives a 18 cm dia. propeller at maximum speed (> 10000 rpm), giving wet droplets of about 30 μ m in

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diameter. The sample suspension was delivered at 0.6 L/min from a pressurized plastic bottle. This container was pressurized (0.6-0.7 atm) by a small adjustable air pump (Model MT3300, Campbell Hausfeld, Harrison, Ohio, 45030). Dispersion of the aerosol was achieved by the propeller, assisted by the prevailing wind. Electrical power to drive all the equipment was supplied by a 2500 watt Honda generator (Model E2500C, Honda Canada Inc., Scarborough, Ontario, M1B 2K8).

Biological aerosol sampling

The viable cell content is an essential piece of information for estimation of the aerosol concentration. This information is also useful in interpreting light scattering results by particles in general. In order to relate LCM results to biological aerosol content, it is necessary to estimate the numbers of viable spores. Two dichotomous samplers (DS) were used to collect particulate aerosols (Series 245, Sierra Instruments, Inc. Carmel Valley, CA 93924). This instrument was selected since it has been found to be highly efficient for inhalable particles (6). Subsequent testing of the DS with BG aerosols at DRES confirmed that its collection efficiency was equal to or better than standard glass impingers (7).

The aerosol was sampled at 1 meter above ground level, a constraint dictated by the height of the LCM scanning beam projection. Sample particles were drawn through a size exclusion intake manifold with an upper size limit of 15 μ m. Particulate samples were collected on filters held in a 20 slot carousel. Each slot contained two filters which corresponded to the two size groups sorted by a virtual impactor. The two size groups consist of small and large particles having diameters in the range 0.5-2.5 μ m (fine) and 2.5-15 μ m (coarse), respectively. Particles with diameters greater than 15 μ m were excluded by the intake manifold. Efficiency of collecting particles with diameters less than 0.5 μ m is a function of the type of filters employed. A cost effective borosilicate microfiber filter was selected for this purpose (Grade GA55, Cat. No. GA5537MM, 37 mm diameter, Micro Filtration Sys-

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tems, Dublin, CA 94568). This filter was chosen for its ease in resuspending collected particles in distilled water as well as for its collection efficiency for small particles.

Modifications to the electronic controls of the DS were made to speed up sampling times (selectable timing resolution in seconds rather than hours). External timing signals were provided by a programmable timer (Chronrol, Linburg Enterprises, Inc., San Diego, CA 92126). This timer was set to output a 110 volt AC pulse (1 sec) at 2 min intervals to a relay switch installed in the DS sample compartment. This provided a momentary contact closure of the sample position advance control switch. This action caused the sample tray to move forward one position. Actual sampling time under this condition was 85 seconds. The rest of the time (35 sec) was taken up by the slow mechanical movement of the sample change mechanisms.

For high aerosol concentrations (75% and 100% source strength), the 2 min interval timing was selected to allow collection of samples at optimal time resolution. Given the maximum of 20 sample slots, half of these are allocated to controls (five before the aerosol spray and five after) and the rest to samples. The duration of sample spray (20 min.) was dictated by economics of BG slurry expenditure. Preliminary testing indicated that the DS was able to collect sufficient viable spores within this timing interval, although for lower aerosol concentrations, a sampling time of 10 min was required.

Assay of viable cells

Particulate aerosol samples collected on filters were stored dry in capped glass tubes (nonsterile). These were transported back to DRES laboratories for microbiological assays. Distilled water (20 ml) was added to each sample tube containing a filter. The capped tubes were then shaken for 10 minutes by a wrist action shaker (model 75, Burrel Corp., Pittsburg, PA) to resuspend the particles. The glass

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fiber slurry was strained through a wire gauze disk to recover clarified filtrate containing biological particles. Viable organisms were enumerated by the spiral plating technique (8). Liquid samples were applied to standard nutrient agar plates with a spiral platter (model CU, Spiral Systems Instruments Inc., Bethesda, MD). The plates were incubated over night at 30° C. A laser-based spiral colony counter with an integrated data processor (model 500A and model 800 respectively, Spiral Systems Instruments Inc.) was used to calculate the viable spores in the original sample.

The Laser Cloud Mapper

Technical personnel from DREV operated the LCM and its associated systems. The technical details of the apparatus (1) along with the polarizer modifications (2) have been published. Briefly, the laser and its associated control electronics were housed in a large trailer. A beam of 1.06 μ m wavelength coherent light emitted by an Nd:YAG laser was directed through a series of output optics toward the target of interest. The scanning pattern of this beam was determined by preprogrammed parameters which control a moving mirror. Light scattered by particles was measured by a sensitive solid-state detector (silicon avalanche photodiodes). Analogue signals from this detector were first fed through logarithmic amplifiers in order to compress the large dynamic range, then were digitized and stored on disks. Data reduction and plotting were performed in the laboratory (DREV), and required several days as complex analytical procedures were required to extract the maximum information from the raw data. Further technical details are described in reference (3).

Experimental layout

Details of the test site at DRES were described in reference 3. The LCM scanned an angle of 90 degrees with a beam elevation of 10 degrees from horizontal (figure 1). The Micronair aerosol generator was located 100 meters upwind of the

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LCM. For collection of aerosol particles, the two DS units were located (adjacent to each other) 75 meters directly downwind from the aerosol source. As mentioned earlier, the aerosol intake of the sampler was about 1 meter above ground level.

At the earlier part of the trial week, the predominant wind direction was southwesterly so the samplers were located due east of the Micronair generator. Occasionally, the wind direction changed unexpectedly during a trial, causing abnormal sampling characteristics. During the later part of the trial period, the wind shifted to a northerly direction and so the samplers were relocated along a southerly axis with respect to the Micronair. Under these conditions, the LCM was also realigned accordingly to cover a north to south aerosol flow.

Statistical analysis

Proper use of conventional (parametric) statistical analysis techniques on bacterial viable counts require a data transform (logarithm to the base 10). This procedure has been recommended (9) for treatment of non-normally distributed data before performing multiple means analysis (Student-Neuman-Keul or SNK test).

Output from standard SNK analysis were decoded in the form of a two dimensional matrix table. Listed on top and left hand column were labels of samples and their means sorted by their ascending values. By inspecting the point of convergence delineated from two samples of interest, the result of the null hypothesis could be determined (whether two means were significantly different). An "SD" indicated that the two samples were significantly different while "ND" denoted no difference. The results shown in each cell represented the analysis performed at the indicated significance level to illustrate the degree of confidence.

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UNCLASSIFIEDResults and DiscussionsViable biological aerosol concentrations

Blank DS samples were taken to determine background aerosol concentrations prior to release of BG spores. It was not unusual to find low background BG spore levels in the range (100 spores/l) as shown in figure 2. Possible sources of background contamination may include secondary aerosols from disturbing ground deposits from previous experiments or reaerosolization during sample handling. However, such background levels were sufficiently low so that interpretation of results was not affected.

The data in figure 2 represent viable spore numbers from the coarse fractions. Negligible numbers were collected on the fine fraction, also noted in previous observations (4). This observation was found in all other experiments at other source strengths which leads to the conclusion that the Micronair generator, with BG slurry as the spray source, produced particles predominately of diameter $>2.5 \mu\text{m}$.

Figure 2 also illustrates that from a 1% source strength, the measured aerosol contained about 200 to 1200 viable spores/l with this cloud being detected during the first 10 minute sampling period. This was consistent with the fact that the wind speed during the experiment was fairly strong (25-38 KPH, Table 1) thereby transporting the cloud to the samplers with little time lag. Both DS samplers produced consistent results (figure 2), suggesting that the aerosol cloud must have arrived at both samplers at the same time and that both instruments performed reliably.

Similar viable spore aerosol data were obtained from clouds produced from higher source strengths. As expected, higher source strength generally produced aerosol clouds of increasing concentrations. Figure 3 illustrate that the viable spore

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concentration from a 2% source strength was roughly two times higher than that from 1%. Indeed, increasing source strengths up to 50% resulted in corresponding detectable increases in viable aerosol concentrations (Figures 4, 5, 6 and 7). Exceptions were noted in two instances when the wind directions were unfavorable at the beginning of the spray (Table 1). Lower than expected concentrations (Figure 5 serial 80510 and figure 6 serial 80524) were observed.

For the highest source strength trials (75% and 100%, figures 8 and 9), 2 min. sampling times were selected. Apart from a considerable lag, shown in figure 9, these samples exhibited the higher aerosol concentrations with considerable sample concentration fluctuations, probably due to uneven aerosol puffs as previously noted (4).

Correlation between source strength and viable cells prior to spraying

Cell viability was estimated at the beginning of each trial taken from an aliquot of the source suspension just prior to dissemination as previous experience with BG stock revealed batch-to-batch variations (3). Figure 10 shows that increasing source strength was reflected by a linear increase in viable spores from the suspensions. The higher source strength samples (75 and 100%) showed greater deviations from the theoretical straight line fit. It has been observed that due to settling of BG Spores in the storage drums, inadequate mixing could cause some batch to batch variations in viable spore concentrations. Analysis of variance revealed that the data points were not random in their relationship (F value = 184.66, R SQ = 0.67). Thus it would be reasonable to expect that when these suspensions were used to produce aerosol clouds, their aerosol concentrations should reflect their increasing source strength.

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Correlation between source strength and viable cells in aerosol

As the viable spore count increases with the source concentration, it is expected that the aerosol densities derived from these suspensions should increase correspondingly. To demonstrate this relationship, a summary of all the viable spore concentrations in the sampled aerosols were plotted against source strength (figure 11). This figure shows that increases in source strength was accompanied by increases in aerosol concentrations up to the 50% level. Beyond this level, no higher aerosol concentrations could be detected. A means analysis (table 2) confirmed this conclusion that there was no significant difference between the mean BG aerosol concentrations associated with the higher source strengths (50, 75 and 100%). This observation could be partly due to batch variations in the BG source (figure 10), especially in the 100% material. But other factors like slurry viscosity could contribute towards the low aerosol yield at high source strength.

Correlation between viable spores in aerosol and LCM results

In a previous study (4), it was suggested that the LCM produced depolarized signal measurements (expressed as the ratio of circularly polarized return versus unpolarized out-going signal levels) that permitted differentiation between spherical and non-spherical particles and thus between water based particles (spherical) and those associated with BG spores (3, 4). Subsequent studies (10) with more data sets revealed that increasing source strength produced BG aerosols with increasing depolarized signal levels (figure 12 and 13; reproduced from ref. 10). However, it can be seen that the depolarization signals did not increase with the higher source concentrations beyond the 50% level. However, this phenomenon was also observed when plotting viable BG spores aerosol concentrations versus source strength (figure 11). By replotting the combined LCM and BG aerosol data as increasing viable spores in the aerosol versus depolarization ratios, a straight line relationship was obtained (figure 14). For the first time, there is good evidence to suggest that

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the LCM was actually measuring the presence of a biological aerosol, independent of contaminant particles associated with impurities in the water based carrier. The only other factor not considered in this relationship is particle size distribution and work in this area is in progress.

Morphology of biological particles in an aerosol

These observations generate a few intriguing questions. First, why do source concentrations greater than 50% fail to produce proportionally higher aerosol concentrations. The answer to this question could be the loss of BG particles due to production of overly large particles from high source strength. These large particles may have high enough settling velocities to have fallen to the ground 10 meters from the Micronair generator, as illustrated by the failure to detect higher laser returns associated with these samples (figure 12). Standard text book information states that particles greater than $220 \mu\text{m}$ (settling velocity 76 cm/sec) would mostly fall to the ground before traveling 10 m from a height of 2 m in a wind of 8 m/sec. Thus it can be assumed that the thick slurries (75 and 100% source strength) produced very large particles which rapidly dropped to the ground.

Second, mathematical calculations suggest that individual spores with an aspect ratio of about 2:1 cannot produce depolarization signals at the observed levels, 0.4 as shown in figure 14 (10). The solution may be more speculative in that it may be related to the actual morphology or particle size distribution of the particles which scatter the polarized light. One possibility may be the complex way by which polarized laser light is scattered by spore aggregates previously shown present in the aerosol (4). Perhaps the larger than expected depolarizations signal could be a result of particle aggregates with complex surface structures which may possess elongated projections that strongly depolarize light.

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This speculation may be tested in an experiment where the BG source suspension was treated with surfactants or detergents (2% of Triton and Pluronic 68 in the final liquid) to reduce spore aggregation. It was assumed that the resultant aerosol would contain fewer spore aggregates, resulting in lower depolarization signal levels. Preliminary results (figure 14, labeled as T) suggest that the presence of Triton apparently caused a drop in the depolarized signal levels at the corresponding measured viable spore concentration. However, Pluronic 68 (P) did not cause a change in the signal. Obviously further work must be done in this area to properly explore the phenomenon.

The LCM data is summarized in figure 15 in which the depolarization data has been analyzed by a nonparametric technique (Kolmogorov-Smirnov test ref. 9 and 10). This test compared the deviation of each treatment to the mean and as shown in the figure, all the spore aerosol samples showed greater depolarization than tap water (control). Also, viral (Newcastle Disease Virus, La Sota strain, NDV) and vegetative bacterial simulant (EH) aerosols were also detected, even though these aerosols were from relatively dilute source materials (5×10^6 /ml and 4×10^7 /ml respectively) compared to those of BG.

Conclusions

This report has shown that the DS is a reliable instrument for sampling biological aerosols. It has the advantage of providing sequentially timed sample collection producing results which reflect the dynamic properties of an aerosol in the real environment. This is a great improvement over traditional aerosol samplers which are mostly summation or time integration types which produce essentially one-dimensional results. Exploiting the capabilities of the DS during LCM trials yielded multiple data sets which could be subjected to statistical analysis.

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With the combined biological and LCM data, it was possible to demonstrate a relationship between viable spore aerosol concentrations and depolarization signal levels, suggesting that the concept and elements of the LCM have real potentials in the development of a future LIDAR-based BW detector. The findings here also confirm those of a previous report (4), which suggested that the LCM could detect at least 200-400 viable spores/liter aerosol. It is recognized that some of the measured scattered light may be due to a small fraction of nonviable spores. Studies are being carried out to determine the size and significance of this fraction. Colleagues at DREV have demonstrated that further detection sensitivity levels may be possible, as shown in figure 15, where advanced statistical analysis techniques (nonparametric Kolmogorov-Smirnov test, reference 10) could be used to compare the differences between depolarization signals from various aerosol types. In this set of results, there was evidence to suggest that the LCM could detect a viral aerosol and that of a vegetative bacterial simulant, *E. herbicola*.

In summary, DRES has demonstrated capabilities in setting up different types of biological simulant aerosols and measure their viable concentrations downwind with reliable samplers and then characterize prototype aerosol detection equipment in the field. This expertise will be invaluable in studying and assisting in the design of future BW aerosol detectors, for example the Biochemical Detector developed jointly by CA, UK and the US.

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Table 1. Summary of Environmental Conditions

Spray Material	LCM Serial #	Wind Direction & Speed (KPH)	Temperature °C	RH %
BG 1%	80516	SW38, W21, SW26	17.8	25
"	80521	SW25, SW29, S20	22.5	15
BG 2%	80517	SW25, W26, SW30	18.6	25
"	80522	SW20, SW37, SW34	22.7	15
BG 5%	80518	SW32, SW30, WSW21	18.6	25
BG 10%	80507	SW32, SSW21, SW25	17.4	40
"	80510	*SSE32, SW38, SSW28	22	25
"	80519	SW30, SW21, SW30	20.9	25
BG 25%	80511	SSW35, S33, SSW36	23.4	25
"	80524	*SSE40, SSE37, SSE50	27	30
BG 50%	80512	S50, S25, SSW32	23.4	25
"	80526	WSW19, WSW16, WSW24	21.4	55
BG 75%	80505	SW30, SW29, SW18	17.3	35
"	80513	S14, SSE21, SSW29	23.8	25
BG 100%	80502	SW20, W25, SSW18	14.8	40
E. herbicola	80541	N33, N40, NNW34	26.8	40

*Unfavorable wind directions causing abnormal sampling characteristics

LCM Serial #: Reference number corresponding to Laser Cloud Mapper experiment

Table 2. Means Analysis
BG Aerosol Concentration at Increasing Source Strength

Concentration/Mean*	1%: 2.492	2%: 3.292	5%: 3.562	10%: 3.609	25%: 3.951	100%: 4.056	75%: 4.126	50%: 4.174
1%: 2.942	--	SD @ 0.01	SD @ 0.01	SD @ 0.01	SD @ 0.01	SD @ 0.01	SD @ 0.01	SD @ 0.01
2%: 3.292	SD @ 0.01	--	SD @ 0.01	SD @ 0.01	SD @ 0.01	SD @ 0.01	SD @ 0.01	SD @ 0.01
5%: 3.562	SD @ 0.01	SD @ 0.01	--	ND	SD @ 0.01	SD @ 0.01	SD @ 0.01	SD @ 0.01
10%: 3.609	SD @ 0.01	SD @ 0.01	SD @ 0.01	--	SD @ 0.01	SD @ 0.01	SD @ 0.01	SD @ 0.01
25%: 3.951	SD @ 0.01	SD @ 0.01	SD @ 0.01	SD @ 0.01	--	SD @ 0.4	SD @ 0.35	SD @ 0.3
100%: 4.056	SD @ 0.01	SD @ 0.01	SD @ 0.01	SD @ 0.01	SD @ 0.4	--	ND	ND
75%: 4.126	SD @ 0.01	SD @ 0.01	SD @ 0.01	SD @ 0.01	SD @ 0.35	ND	--	ND
50%: 4.174	SD @ 0.01	SD @ 0.01	SD @ 0.01	SD @ 0.01	SD @ 0.3	ND	ND	--

* Concentration=Source strength in percent; Mean=average aerosol concentration in log(10) viable spores/liter; SD=significant difference determined at indicated significance level; ND=no difference

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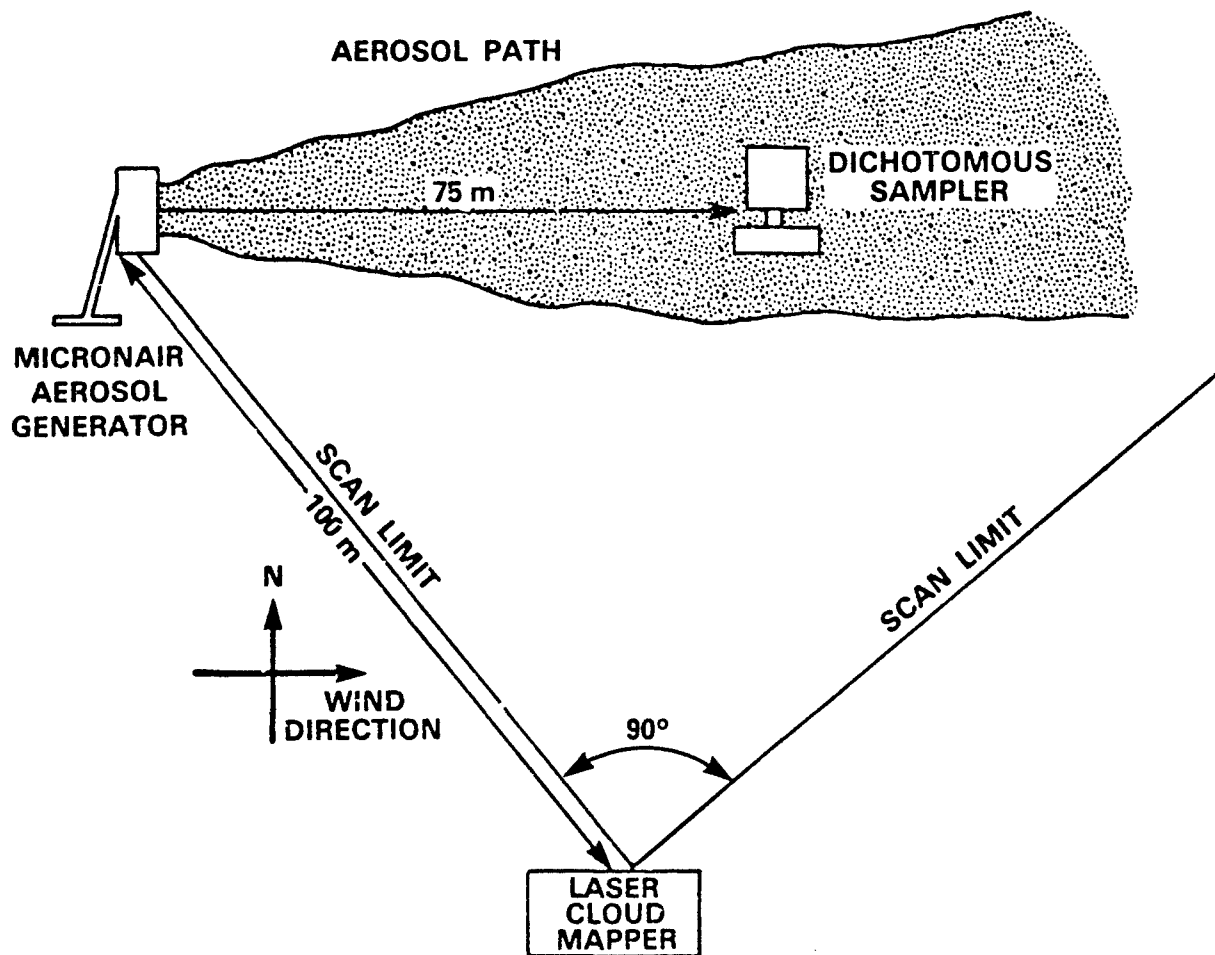


Figure 1
EXPERIMENTAL LAYOUT

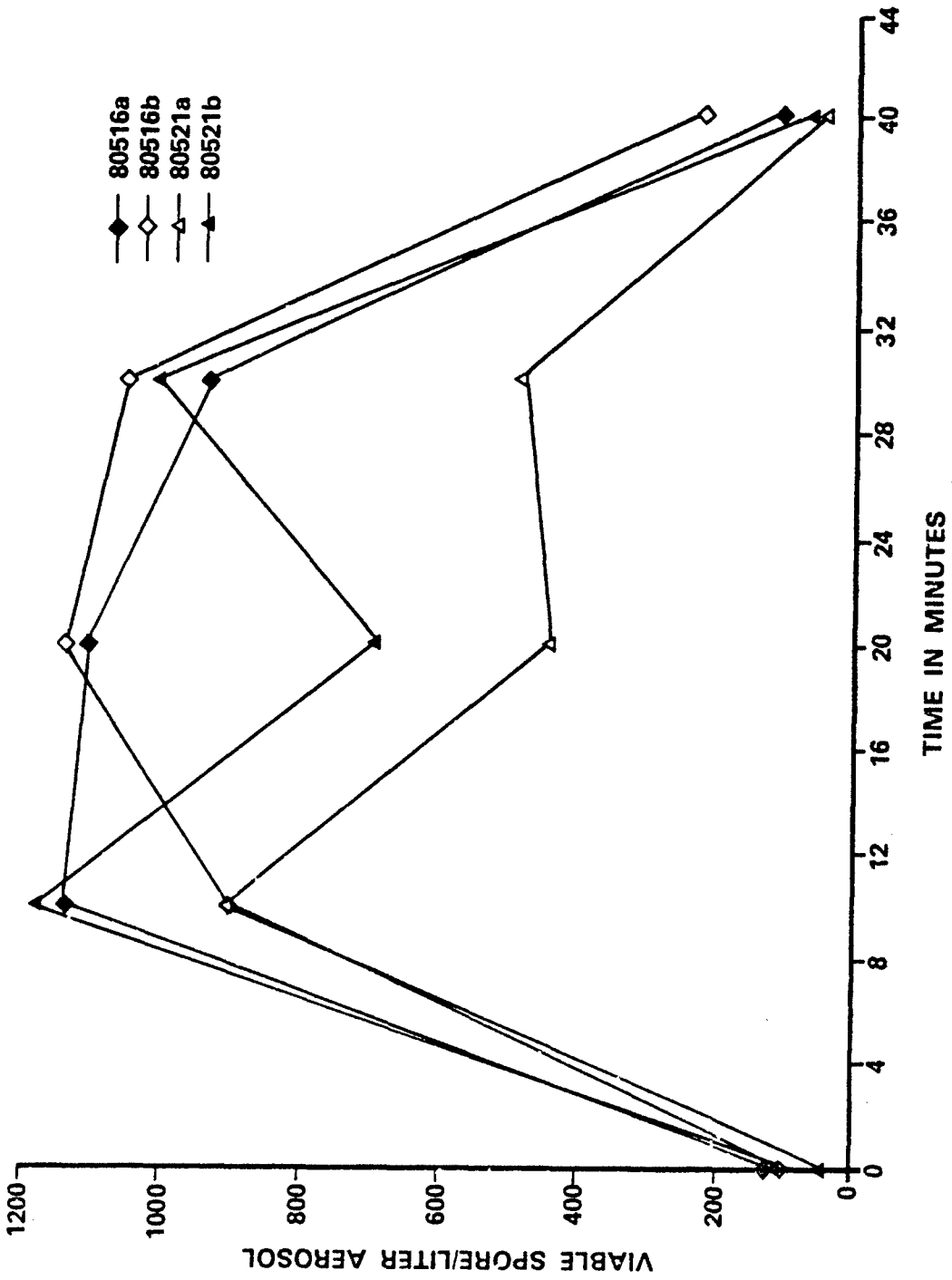


Figure 2
BG AEROSOL FROM 1% SOURCE

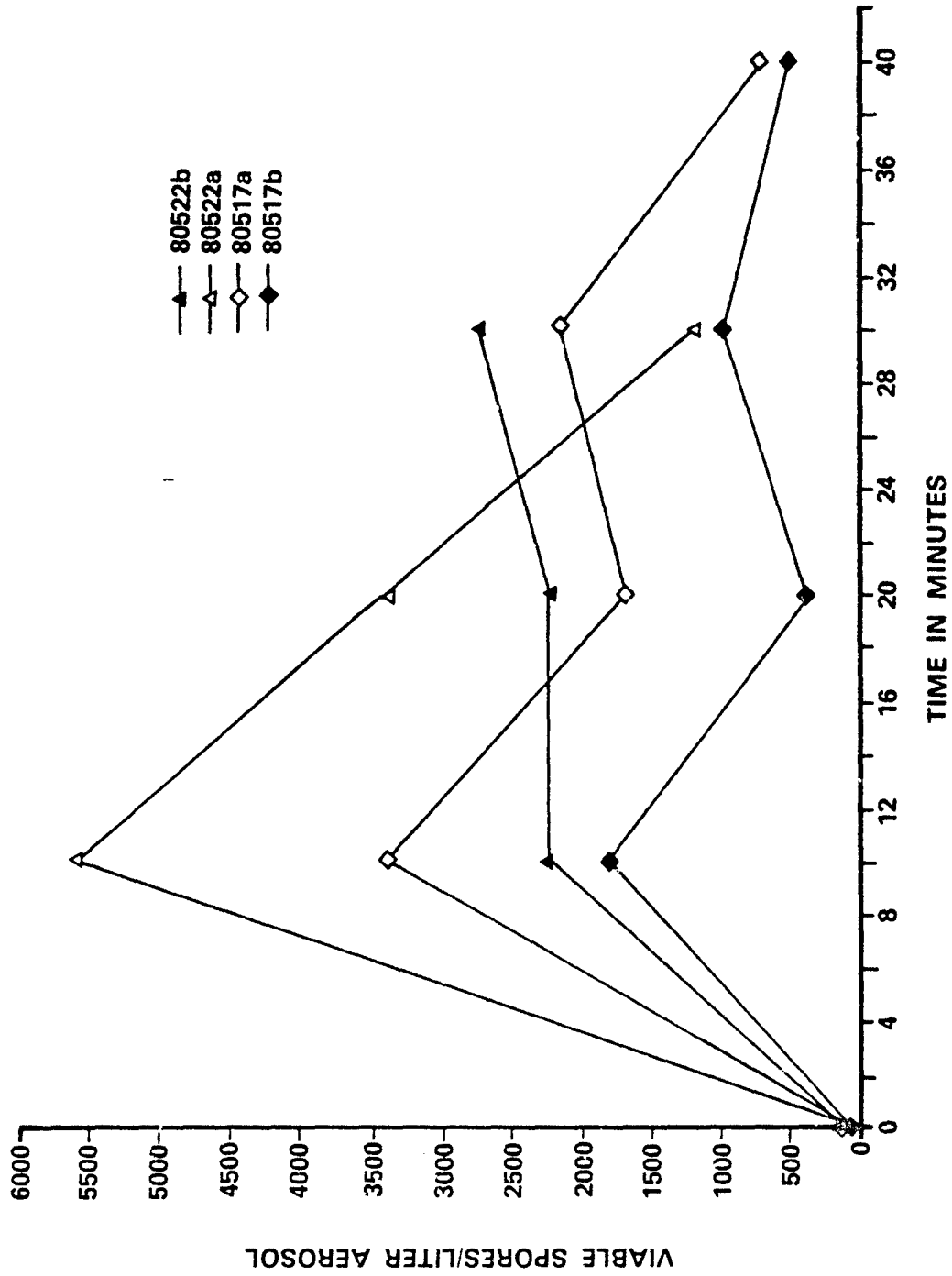


Figure 3
BG AEROSOL FROM 2% SOURCE

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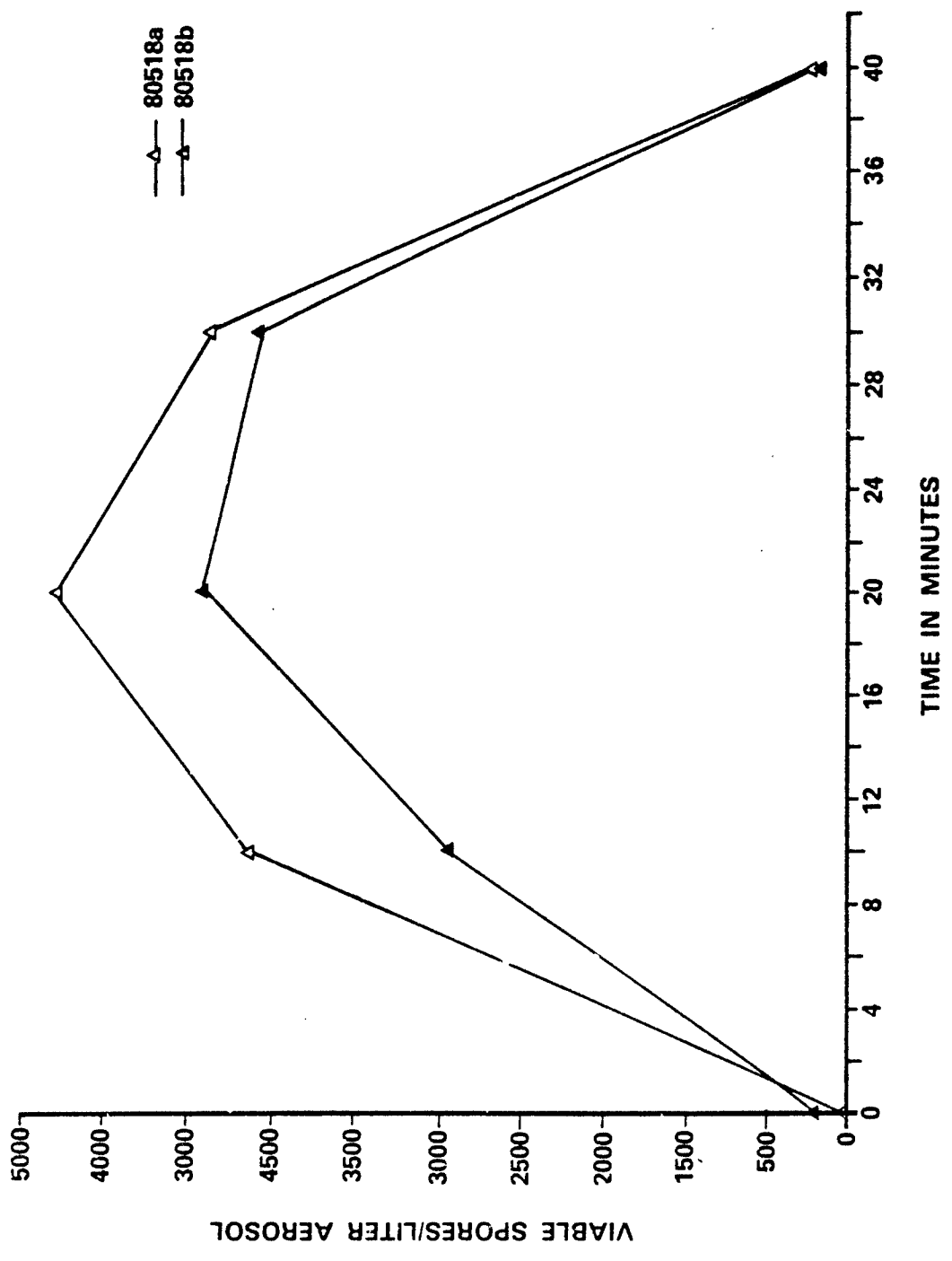


Figure 4
BG AEROSOL FROM 5% SOURCE

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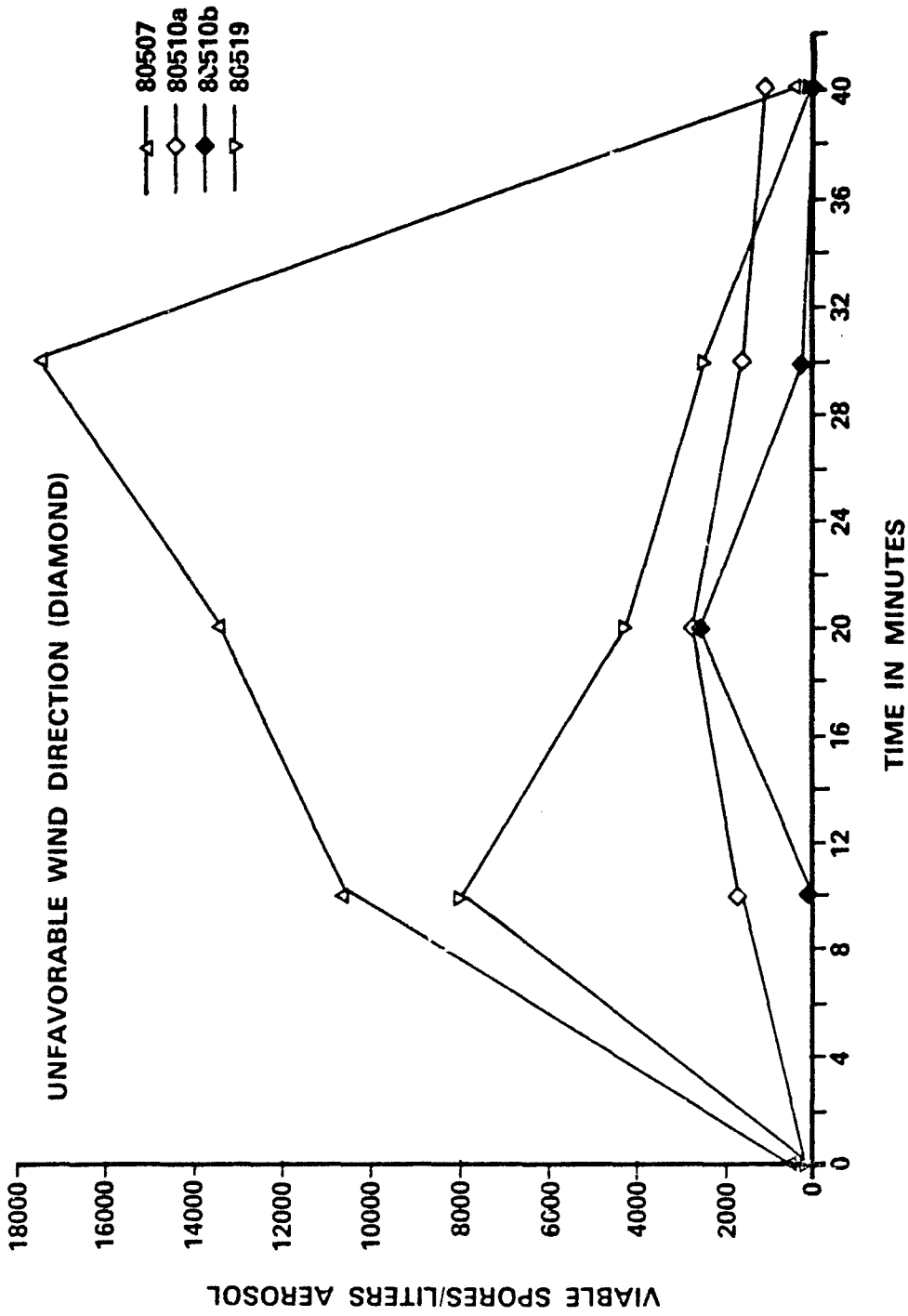


Figure 5
BG AEROSOL FROM 10% SOURCE

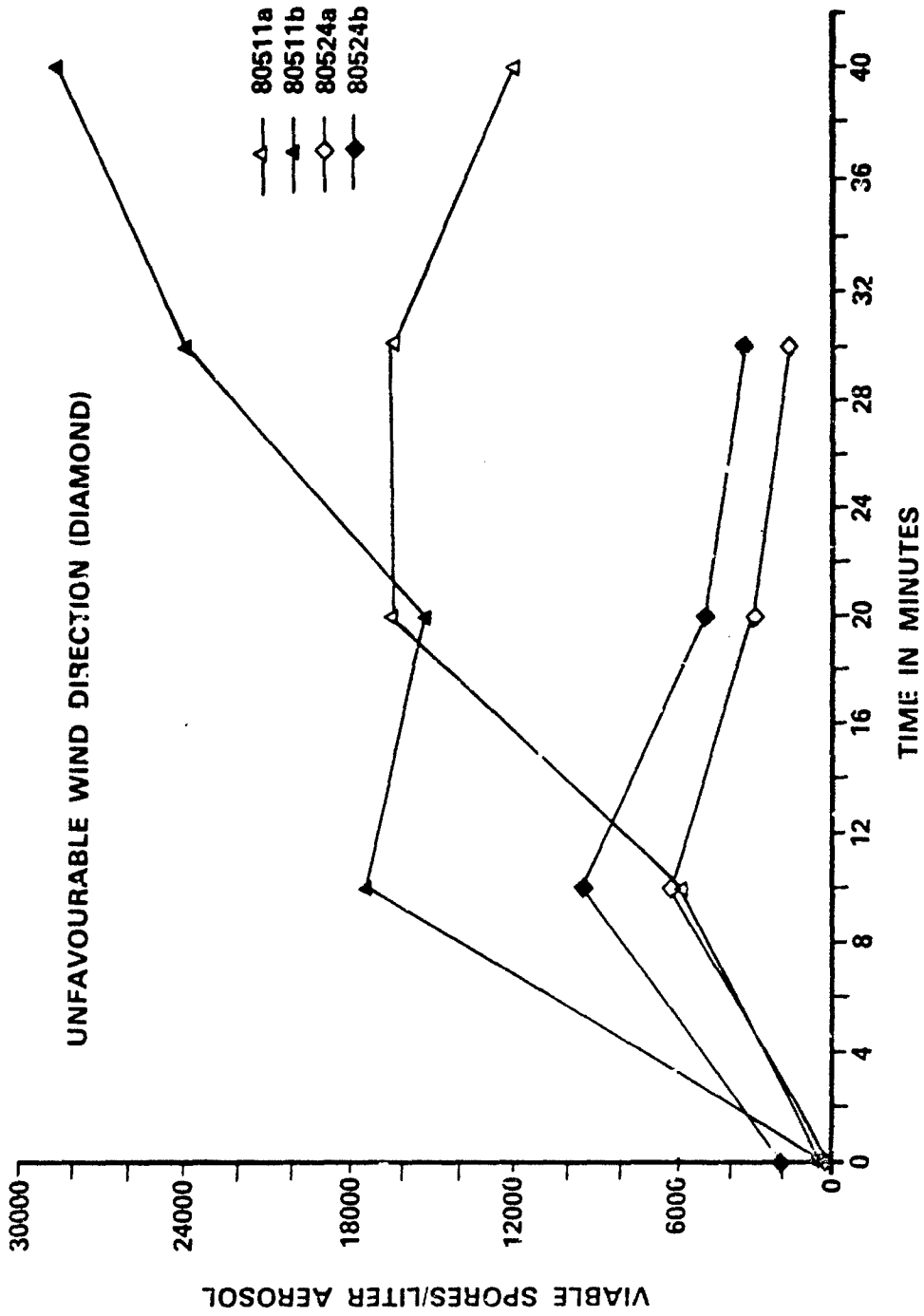


Figure 6
BG AEROSOL FROM 25% SOURCE

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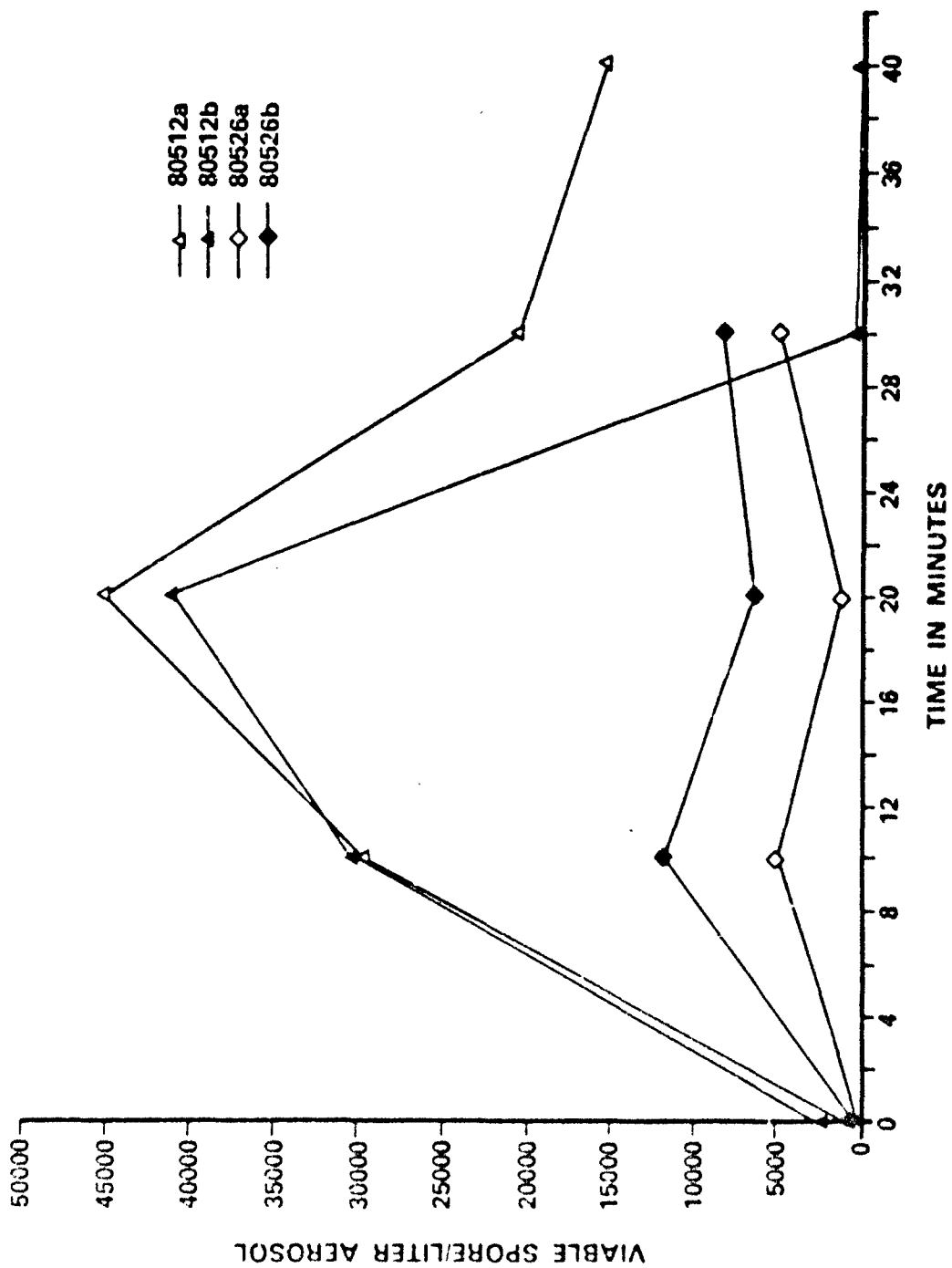


Figure 7
BG AEROSOL FROM 50% SOURCE

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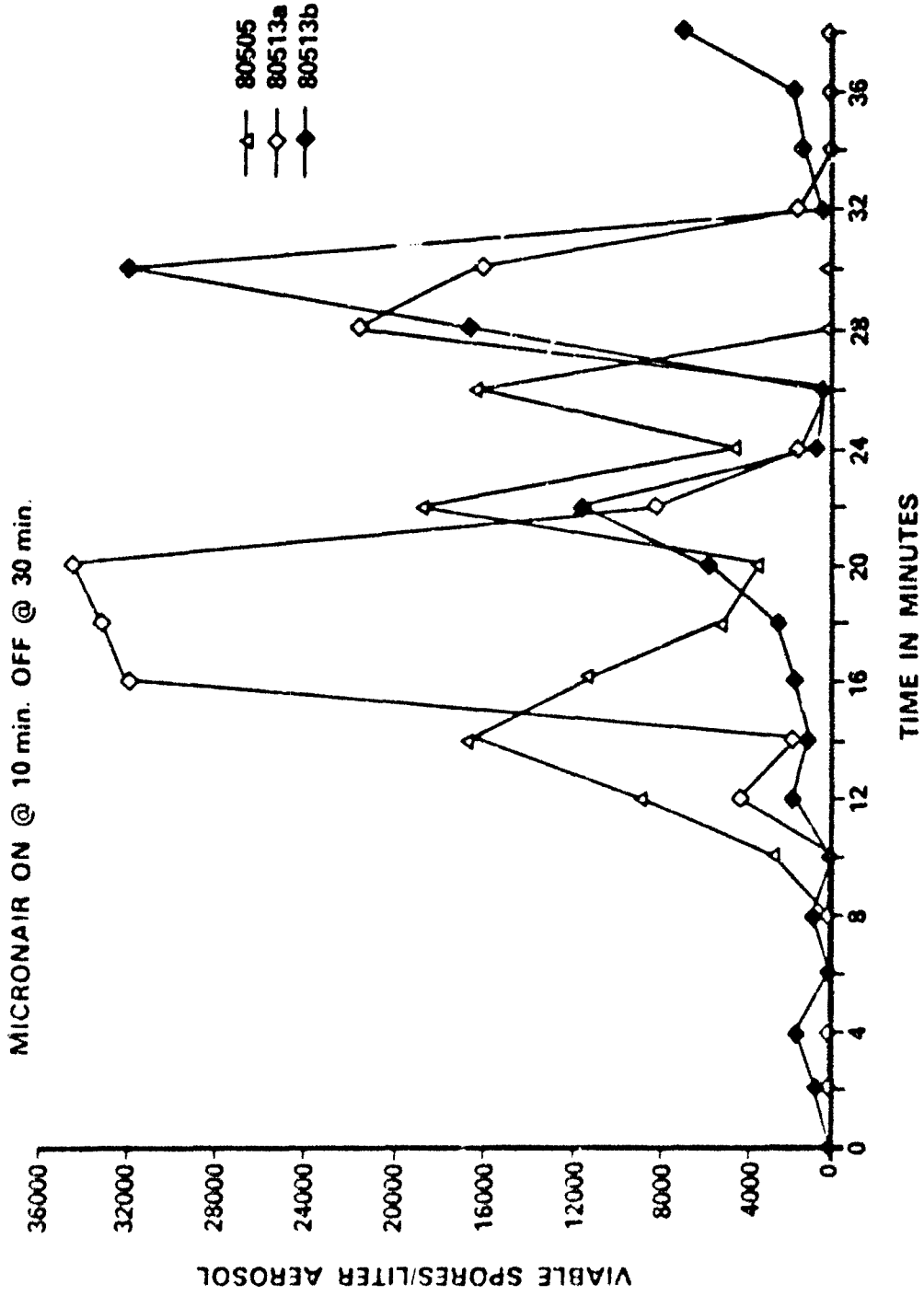


Figure 8
BG AEROSOL FROM 75% SOURCE

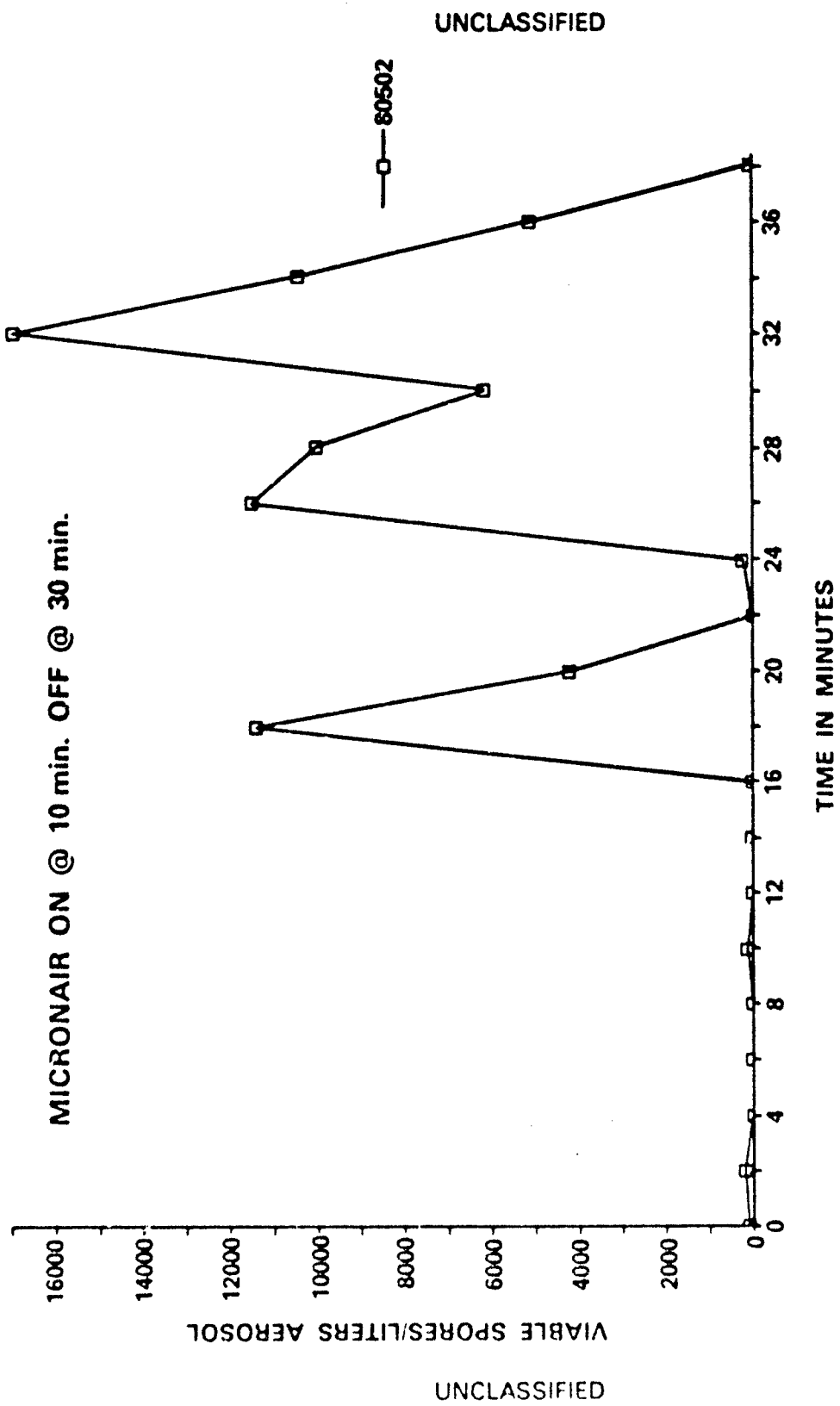


Figure 9
AEROSOL FROM 100% SOURCE

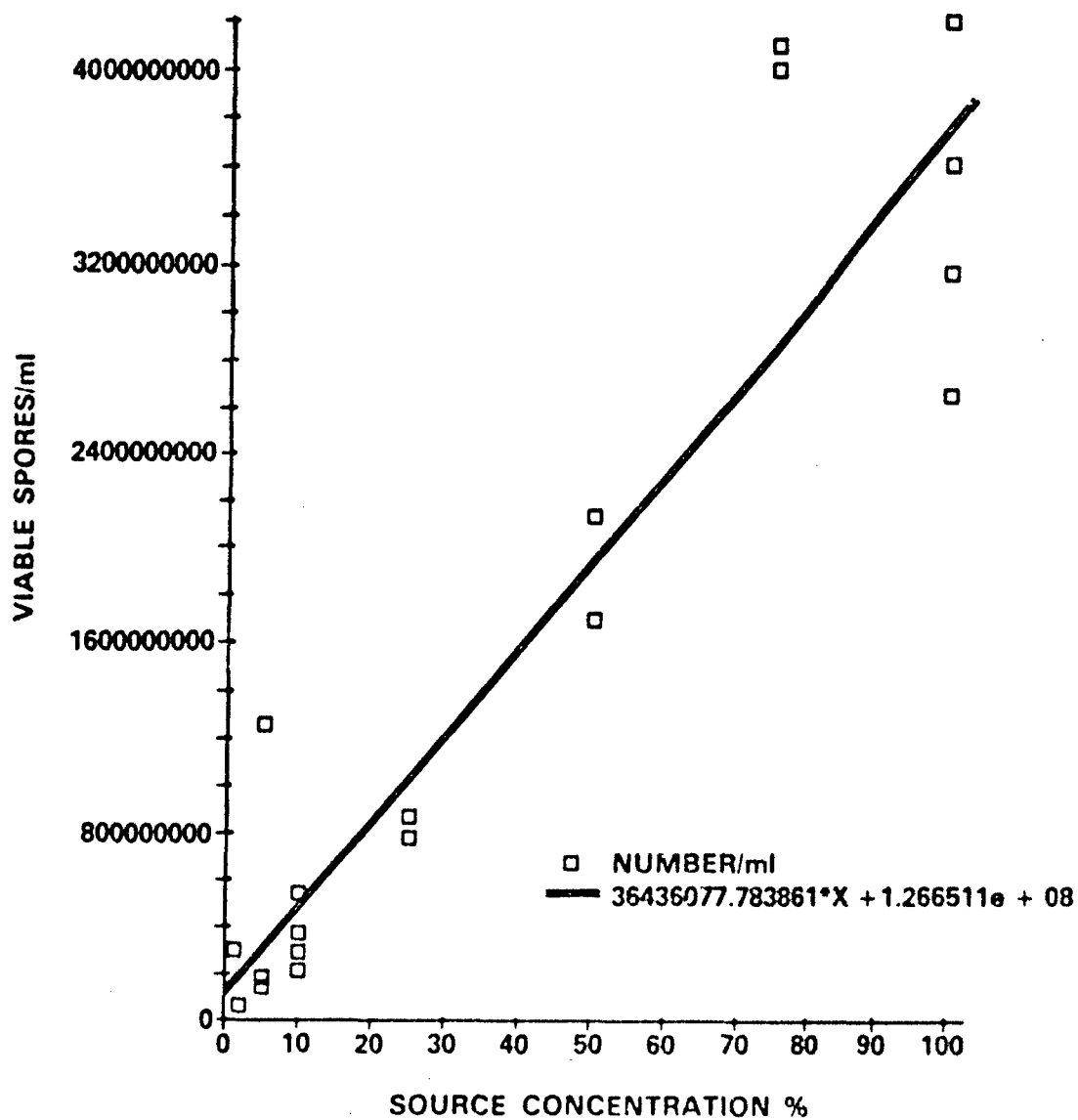


Figure 10
VIABLE SPORES IN SOURCE MATERIAL

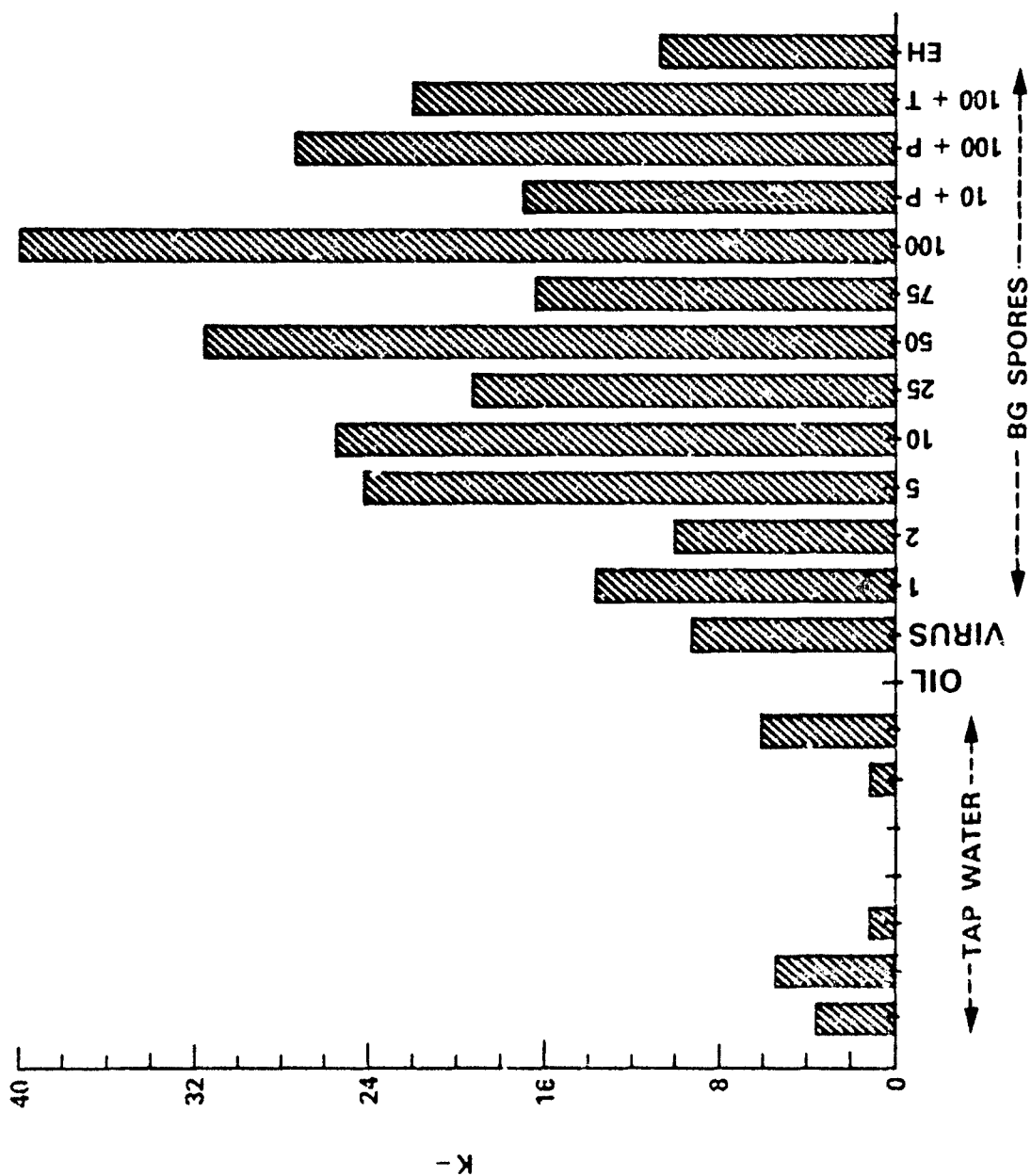
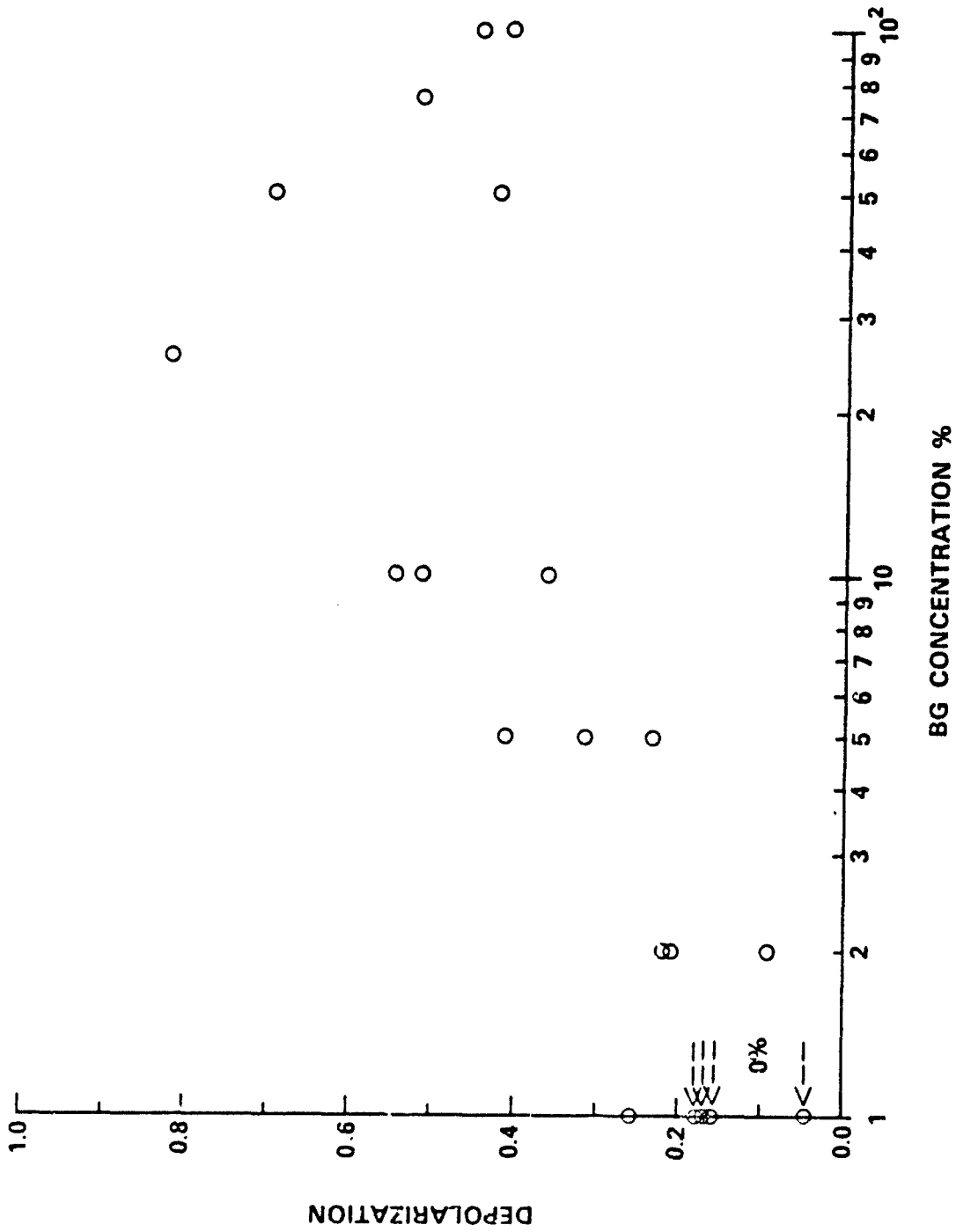


Figure 15
KOLMOGOROV-SMIRNOV TEST COMPARISON OF DIFFERENT
AEROSOL SOURCES

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Figure 12

DEPOLARIZATION SIGNAL RATIO FROM INCREASING SOURCE STRENGTH (10 m from source)

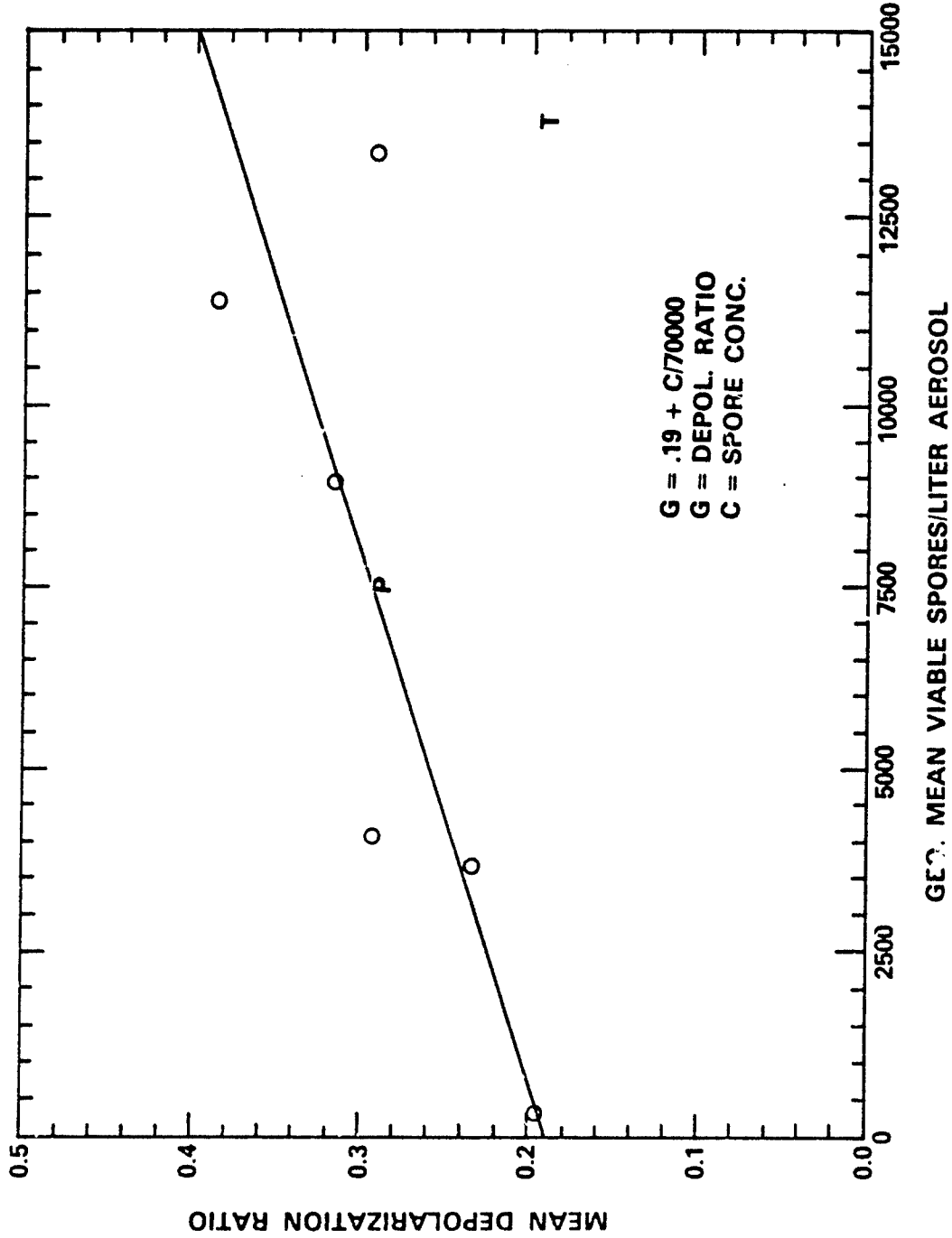
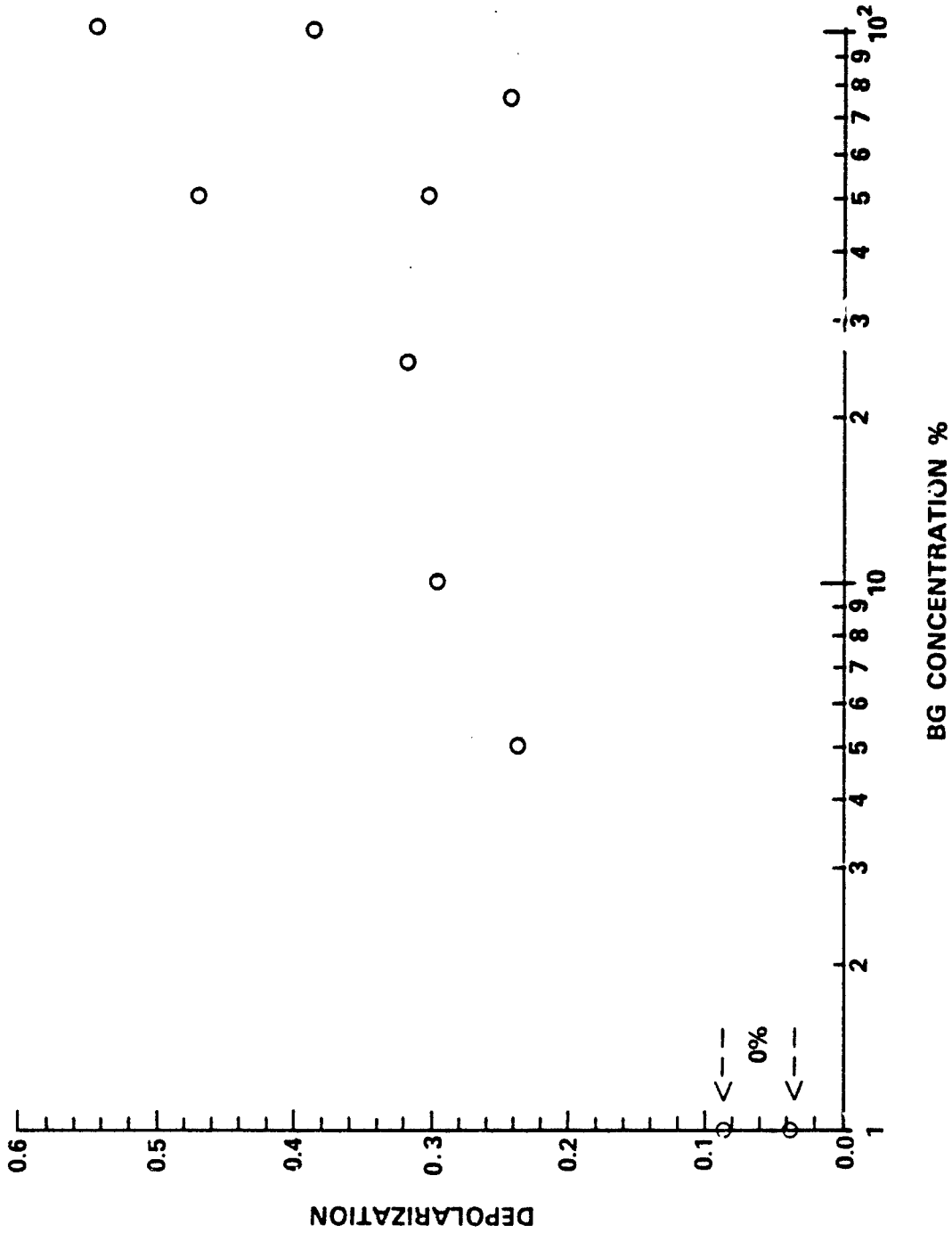


Figure 14
MEAN DEPOLARIZATION SIGNAL RATIO AT INCREASING AEROSOL CONCENTRATIONS (70 m from source)

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Figure 13

DEPOLARIZATION SIGNAL RATIO FROM INCREASING
SOURCE STRENGTH (80 m from source)

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In a continuing series of investigations on biological aerosol detection by the Laser Cloud Mapper (LCM), reliable measurement of biological aerosol concentrations were found to be critical to LCM data interpretations. The sampling method must give reproducible results under difficult field conditions of rapidly changing environmental parameters. A series of trials were conducted to evaluate the Dichotomous Sampler (DS) as a device for the sampling of biological aerosols and to assess its potential for the estimation of biological aerosol concentrations during LCM trials. Sampled biological aerosols were enumerated as viable cells and provided reliable estimates of viable aerosol concentrations with respect to time. Interpretation of DS and LCM data demonstrated a relationship between cell concentrations and depolarization signal ratios which suggests that this technique has potential for remote biological aerosol detection at the 200-400 viable spores/liter level.

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Keywords: Laser; LIDAR; biological aerosol; BW simulant; dichotomous sampler; concentration measurement; depolarization; CANADA. ✓ DS ✓

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