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SECONDARY AEROSOL HAZARDS IN THE FIELD

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SUFFIELD REPORT

NO. 321

SECONDARY AEROSOL HAZARD IN THE FIELD (U)

by

D.E. Davids and A.R. Lejeune

Project No. 18-02-01

August 1981

DEFENCE RESEARCH ESTABLISHMENT SUFFIELD : RALSTON : ALBERTA

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Abstract

So // An area of ground was contaminated from an aerosol of spores of Bacillus subtilis var niger (BG). [Aerosol samples were collected in three stage samplers with 50 percent particle size cut-offs of 6 m in the second stage. These samples collected 45 percent of the spores in the top stage, 22 percent in the middle stage and 34 percent in the bottom stage. The ground contamination level 46 meters downwind was 3 x 10^5 particles or 2 x 10^7 spores per M^2 of terrain in all particle sizes. The aerosol concentration ranged from 6.2 to 7.6 x 10^4 spores min/liter.]

Secondary aerosols were produced by the activity of men moving and working within the contaminated area, and spores were recovered in standard samplers and on the filters of special samplers fitted to the opening of particulate canisters on respirators worn by the men. The

number of spores collected by each man was deemed to be representative of his respiratory intake had he been unmasked. After 24 hours, the respiratory dose varied from 100 to 1000 spores. This represents about 0.0020 to 0.12 percent of the original exposure dose. Even after 9 days, secondary aerosol was still being collected in a high volume sampler operating at 780 liters/min. [Comparison of standard samplers with the mask samplers indicates breathing rates from 13 to 64 liters/min.]

The clothing and boots of personnel became contaminated after exposure to both the primary and secondary aerosols. Respiratory hazard to men wearing clothing grossly contaminated from the primary aerosol, was an order of magnitude higher than that for men whose clothing was not so contaminated. //

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INTRODUCTION

One of the problems of medical support in a biological environment is concerned with secondary aerosols during the evacuation, undressing, decontamination and treatment of casualties in the field. Work carried out with men wearing Canadian protective clothing and exposed to high concentrations (10^6/L) of spores of Bacillus subtilis var niger (BG), showed that secondary aerosols in concentration high enough to be hazardous to unmasked men were produced, when the outer protective overgarment was removed during standard undressing procedures. When the clothing was wetted down with hypochlorous acid (activated solution of sodium hypochlorite, ASH), the secondary aerosol was considerably reduced.

Such decontamination procedures are valuable in situations where it is known that clothing is contaminated, however, little information

is available on the production of secondary aerosols per se, for example, what is the respiratory hazards to personnel carrying out a military task such as preparing a defensive position in a contaminated area?.

This report describes an experiment in which a guerrilla-like action was superimposed upon a standard troop exercise for the purpose of determining the following objectives.

1. Primary
   a. To determine the requirement for protective measures by troops occupying an area which has come under BW attack.
   b. To determine for how long the hazards requiring these procedures persist. This includes risk to personnel and degree of clothing contamination from secondary aerosol.

2. Secondary

To obtain further information on the amount of clothing contamination and the respiratory hazard to troops actually undergoing BW attack by a spray system.

PROCEDURES

1. A platoon (PPCLI), consisting of 36 men, was briefed on the objectives and methods to be used during the conduct of the trial. Each man was given a number and was issued with a set of protective clothing -mask, underclothing, protective overgarment, socks, rubber gloves and rubber boots.

INCIDENT C

2. On the morning of the trial, the platoon arrived at the layout

completely dressed except for masks. First, the area was inspected and then locations were selected for the trenches to be dug for a defensive position.

3. Group A, consisting of Platoon HQ and Rifle Section (15 men), donned masks and each canister was fitted with an external filter sampler which clips into the canister opening (Fig. 1). The men began preparing the defensive position (Fig. 2) and, during the next 20- to 25-minute period, were exposed to aerosols of Bacillus subtilis var niger spores released from an E-4 generator being towed by a truck at a speed of 5 mph. The spray line was 46 m upwind of the defensive position and each time the vehicle was about to pass by, the men in the platoon were instructed to stop work and stand upright facing the aerosol (Fig. 3). This was done soley to ensure uniformity of clothing contamination for sampling on the frontal area, and at the same time, position the external canister sampler at an angle of 90°to the face of the cloud as it went by. Three sets of aerosol samplers, 46 m apart and 14 m downwind of the men, were turned on at the start of emission and turned off when spraying had ceased.

After the 4th pass of the spray equipment, Group A formed up and marched off to a clean area where all external samplers were removed (Fig. 4) and duplicate samples were taken on the clothing of the arm (Fig. 5), mid-section, thigh (Fig. 6) and the side of one boot (Fig. 7).

4. Group A, now with contaminated clothing, and Groups B and C, comprised of Section 2 and 3 in clean clothing, were all fitted with new external canister samplers before taking up positions in the now contaminated area to continue preparing the defensive position during the first working period.

Group A resumed work at their original location 46 m downwind of

the release line, Group B began digging trenches in a line paralled to that of Group A, but 7.5 to 9 m downwind, and the four men of Group C walked abreast across the 137 m contaminated area up and down a line 7.5 to 9 m downwind of Group B.

These operations were continued for 30 minutes and were carried out to determine the amount of clothing contamination picked up by Groups B and C and to obtain a measure of the respiratory dose picked up by all personnel. Thus, after all personnel had formed up and marched out to a clean area, all external samplers were removed and contact samples were taken from the clothing and boots of Groups B and C.

During the 30-minute period, two sets of sampling devices were operated to determine the amount of secondary aerosol created and becoming airborne as a result of troop activity within the contaminated area.

5. This procedure was repeated twice, namely during the second and third working periods in the contaminated area, during which time the men continued to prepare the defensive position. The total time from the start of the primary aerosol to the taking of the last clothing samples was just under 3 hours.

6. The platoon, still fully dressed, returned to Camp Vacuum where a decontamination centre had been set up to remove all clothing for sterilizing and washing.

INCIDENT E

7. Incident C was followed up the next day by Incident E. The entire platoon, now dressed in a completely new set of clothing (coveralls, instead of protective overgarment), arrived at the layout at 0900. Clean vehicles were used for transportation. All personnel

donned masks, were fitted with external samplers, and then entered the area which had been contaminated 24 hours earlier. Half of the platoon proceeded to fill in the trenches dug the previous day, and the other half entered the area as if under fire and crawled along the ground for a distance of about 90 m along a line just upwind of the first line of trenches (Fig. 8). A high volume sampler (780 L/min), located just downwind, was operated to obtain a sample of any secondary aerosol particles. After 20 minutes of this activity, the platoon formed up and marched to a clean area where external samplers were removed, and clothing contact samples were taken as in Incident C. Masks were removed and the platoon returned to the decontamination centre at Camp Vacuum.

INCIDENT F

A week after Incident C had occurred, samples were taken in the contamination area to determine whether a secondary aerosol hazard still existed. The high volume sampler was set up in them middle of the contaminated area and turned on for 30 minutes to collect the airborne material stirred up by driving a truck back and forth along a line about 9 m upwind of the sampling location. Samples were taken 7, 8 and 9 days after the orgininal contamination ahd been laid down.

TEST SITE AND METEOROLOGICAL CONDITIONS

The trial was carried out on a level grassy layout, 137 x 137 m, located in an area of typical prairie terrain. The grass was dry, somewhat sparse, from 7.5 to 15 cm high, with most of the ripened, taller stalks bent over by the wind. Some light precipitation had fallen the night before; this, in fact delayed the time of zero to 0900 instead of 0500, as originally planned.

At the time of release of the primary aerosol, the sky was overcast, the temperature was 18°C and the relative humidity was 77%. The wind was south (180°), at 18.7 kph, in a slight inversion (+ 0.5°C temperature gradient).

The detailed meteorological report over the period is shown in Appendix A.

BIOLOGICAL MATERIAL AND DISSEMINATOR

A heat-shocked aqueous suspension of shoestring Bacillus subtilis var niger spores (BG) in peptone (1%) yeast extract (1%) broth (PYE) was sprayed from an E-4 disseminator head. The device was mounted on a stone boat and towed behind a vehicle, moving at 5 mph along a line 46 m upwind of the test site. At 250 psi of nitrogen, from cylinders carried in the vehicle, the disseminator liquid output is approximately 1 litre per minute. The viable count of the spore suspension was about 5×10^9 spores per mL of suspension which contained antifoam A emulsion to suppress foaming. This provided a source strength of 3.7×10^{10} spores per meter. Four emissions were made, each requiring 1 minute to complete. There was a 2- to 4-minute delay between emissions.

SAMPLING (see Appendix B for a more detailed description of samplers and methods)

a. Ground Contamination Samples

Open 150 mm petri dishes containing a solid nutrient medium were placed on the ground to collect particles which settled out of the primary and each secondary aerosol. Viable spores, on or within each particle, germinate and the cells multiply to form visible colonies which are counted to enumerate the total number of particles which settled out in the area of 176 cm^2.

b. Clothing Contamination Samples

Rodac plates (area 17 cm^2) were pressed to the areas of clothing to be sampled. Particles adhered to the agar surface and, on incubation, form visible colonies as described above. Pairs of plates were used, one of each pair was washed off to remove the particles and provide an estimate of the total number of spores.

c. Primary Aerosol Samples

Standard collecting devices were operated in the test area to determine the dosage to which troops were exposed. Samplers were set up in groups at each of three locations, slightly downwind of the test subjects and 47 m apart across the layout. Samplers used were all-glass impingers (AGI), 3-stage samplers (MRE) and Reyniers samplers. In addition, each member of the platoon wore his own external canister sampler.

d. Secondary Aerosol Samples

In addition to the samplers previously mentioned, Andersen samplers, for particle size data, and a high volume sampler, were used. Because of the large volume of air collected, the latter has great potential for obtaining assayable samples from aerosol concentrations below that required for samplers with lower flow rates. This sampler had not previously been used in the field.

BIOLOGICAL ASSAY

Gelatin saline fluid was used for dilution of all impinger samples. For washed plates and for suspending filter paper discs, the temperature of the fluid was 45°C. Dilutions for assay were plated on previously dried tryptose agar plates (with 0.125% phenylethylalcohol). All solid collecting media were tryptose agar, as above, but also containing polymixin B sulphate 1:350,000.

RESULTS AND DISCUSSION

All the data from the biological sample assessments are presented in appendices C to E. Details of sampling and assay procedures are given in Appendix B. Data were analyzed and reduced to provide summaries of results in three main areas. These are (a) ground contamination, (b) clothing contamination and (c) aerosol concentrations or dosages.

GROUND CONTAMINATION

The extent of ground contamination resulting from deposition from the primary aerosol and subsequent depositon of particles re-aerosolized during the three working periods is shown in Table 1. Deposition of particles on the ground from the secondary aerosols was 1 - 2 percent of that from the primary aerosols. The total number of spores deposited, and consequently, the number of spores per particle was more variable. Total spores per square meter ranged from 0.05 to 6 percent of the original deposition. There is no apparent trend to decreasing depositon with each successive working period. This suggests that, at least for the 3 hour period of this test, the amount of ground contamination which might be re-aerosolized by normal activities did not decrease appreciably.

CLOTHING CONTAMINATION

The clothing of men in the primary aerosol (Group A) was heavily contaminated on all areas sampled. Colonial growth was so heavy that it was necessary to grade each Rodac plate in order to differentiate between at the least (+) and the most heavily contaminted clothing areas (4+). Since a single colony develops from a single particle regardless of the number of spores contained therein, and since colony counts were

not obtainable, there is no estimate of the number of spores per particle. The duplicate plates which were washed off, diluted down and plated out, did, however, give an estimate of the total number of spores sampled.

Repeat sampling of the same group 90 minutes later, after the end of the third working period, indicated that the level of clothing contamination had been reduced by about two thirds on the arm and mid-section. Reduction was about 85 percent on the thigh and 90 percent on the boots. These results are shown in Table II.

Samples were also taken from Groups B and C who went into the contaminated area wearing clean clothing. Samples were taken at the end of the first 30 minute work period and after the third 30 minute work period. Contamination was very light on the arms and mid-sections and did not change appreciably between the first and third work periods. After the first working period, contamination on the thigh and boots was quite significant, but the level decreased considerably by the end of the third working period. These results are shown in Table III. It would apppear that the boots became contaminated directly from contaminated grass rather than from re-aerosolized particles. The results suggest that more attention should be paid to decontamination of boots and pants than to upper body clothing following transit through biologically contaminated terrain.

Twenty-four hours after the primary contamination, the platoon, dressed in clean clothing, returned to the area and were put to work filling the trenches dug the previous day. Half of the men, however, occupied the area as if under fire and crawled across the terrain in front of the first line of trenches. During the 24 hour period, there had been scattered clouds with sunny intervals, a condition which would have little or no effect on the viability of BG spores. Again, the contamination on the upper body clothing was very light. Most of the men had measurable amounts of BG spores on the thigh area but only the boots consistently showed moderate contamination. There was very

little difference in the level of contamination between those who were crawling those who were digging. These results are shown in Table IV. It appears that environmental factors do have some effect in reducing the level of ground contamination of BG spores over a 24 hours period.

AEROSOL CONCENTRATIONS

The primary aerosol concentrations were measured from the individual mask samples and from the MRE and AGI aerosol samplers. The Reynier samples indicated that each aerosol emission took 1 to 1.25 minutes to pass over the aerosol sampling position. The dosage measured by the MRE sampler was 7.6 x 10 spore min/L and by the AGI, 6.2×10^4 spore min/L. Individual doses from the mask samples ranged from 1.1 to 3.8×10^6 spores with a median of 1.9×10^6. Using an average aerosol sampler dosage of 6.9×10^4 spore min/L, the calcualted breathing rates of the men ranged from 16 L/min to 55 L/min with a median breathing rate of 27.5 L/min. These data are shown graphically in Fig. 9.

Similar aerosol sampling procedures were followed during each of the three 30 minute work periods in the area contaminated by the primary aerosol. The results for the three groups (A, B and C) are displayed in Figs. 10, 11 and 12. A summary of the median doses for each group and AGI dosages for each group is given in Table V. The doses received by the Group A men during the three work periods ranged from 2 to 7 times higher then those received by Groups B and C. The extra spores presumably came from the heavily contaminated clothing of Group A. Or, in other words, the dose from contaminated clothing alone may be as high as 1.2×10^4 spores during the first 30 minutes. The AGI dosages suggest that secondary aerosol concentrations decreased markedly and rapidly during the three work periods while the mask doses show very little decrease during this interval. Since particles shed from clothing appears to contribute the major portion of the dose received by group A,

it may be that a major portion of the doses received by Groups B and C come from particles trapped on their clothing being re-aerosolized by their activities. In other words, the contaminated clothing may serve as a source or generator for most of the respiratory dose received by these men over the 3 work periods. Re-examination of clothing contamination levels in Tables II and III suggest that this is not an unreasonable assumption. Furthermore, calculation of breathing rates from mask samples and AGI dosages during the second and third working periods indicate an impossible breathing rate of several hundred litres per minute.

Even after 24 hours of weathering, all of the men crawling through the contaminated area and five of eighteen men standing up and digging had mask sample doses of 1 x 10^3 spores or more after 20 minutes of activity. A large volume sampler operated at the same time downwind of this activity showed a dosage of 10 spore min/L. These results are shown in Fig. 13. Again, this suggests some of the secondary aerosol particles arise from a source other than a generalized secondary aerosol which drifts downwind for appreciable distances. Large volume aerosol samples taken downwind of a truck driving across the area 7, 8 and 9 days after the primary contamination produced dosages of 2.2, 1.7 and 0.6 spore min/L, respectively. Mask samplers in this instance might have shown a minimum dose of 22, 17 and 6 spores, respectively.

Using Andersen and MRE samplers, some measurements of the particle size distribution of the primary and secondary aerosols were made. In the primary aerosol, the MRE sampler which has a 50 percent diameter cut-off of 6 μm in the first stage and 3 μm in the second stage, collected 45, 22 and 34 percent of the aerosol particles in stages 1, 2 and 3, respectively. This distribution is typical of many operational type aerosols. A summary of the Andersen sampler data from secondary aerosols during the first three working periods is shown in Table VI. There appears to be a marginal decrease in very small particles during this test. However, considering the variability in most aerosol sampling

data, the significance of this apparent trend would be debatable.

CONCLUSIONS

A number of conclusions may be drawn from the above results.

1. Under the conditions of these tests, a respiratory hazard from secondary aerosols of a persistent agent would exist for at least 24 hours.

2. The particle size distribution of secondary aerosols does not differ appreciably from that of the primary aerosol and does not change significantly with time after the initial contamination.

3. Shedding of aerosol particles from contaminated clothing may be another source of a greater individual respiratory hazard than re-aerosolization of particles from contaminated terrain or equipment by the activity of surrounding personnel.

4. Twenty-four hours after contamination of terrain with aerosols of persistent agents, troops crawling over the terrain will likely be exposed to higher respiratory hazard than those carrying out other normal activities.

TABLE I

GROUND CONTAMINATION DATA

	Particles/M^2	Spores/M^2	Spores per Particle
Primary Aerosol	2.8×10^5	2.1×10^7	75 to 80
Secondary Aerosol			
1st working period	2.4×10^3	5.7×10^4 to 1.3×10^6	40 to 418
2nd working period	5.9×10^3	1.2×10^6	138 to 329
3rd working period	2.6×10^3	9.6×10^3 to 1.1×10^5	4 to 39

TABLE II

CLOTHING CONTAMINATION DATA

GROUP A

BG-bearing particles from clothing

Rodac plate area = 17 cm^2

Immediately after primary exposure

Arm	Mid-Section	Thigh	Boot
+ to 4+	+ to 4+	+ to 4+	+ to 4+
Total Spores per cm^2 of clothing			
590 to 8830	590 to 7660	590 to 13290	236 to 6490
Median			
2543	1965	1908	2023

90 minutes after primary exposure

Arm	Mid-Section	Thigh	Boot
+ to 4+	+ to 4+	+ to 4+	+
Total spores per cm^2 of clothing			
150 to 9900	59 to 1470	59 to 2950	59 to 1000
Median			
850	613	306	185

+ = colonies too numerous to count

TABLE III

SUMMARY OF CLOTHING CONTAMINATION DATA

SECONDARY AEROSOL

Rodac plate area - 17 cm^2

Group	Time Contaminated Area	Spores per cm^2 of clothing							
		Arm	Median	Mid-Section	Median	Thigh	Median	Boot	Median
B	30 min	0 to 53	4	2 to 36	15	4 to 114	24	77 to 450	167
C	30 min	0 to 14	6	0 to 2	2	32 to 320	11	385 to 725	441
B	90 min	0 to 151	8	0 to 20	5	0 to 23	2	11 to 720	60
C	90 min	1 to 5	3	0 to 3	1	11 to 27	22	28 to 92	59

Groups B and C - Started with clean clothing

TABLE IV

CLOTHING CONTAMINATION DATA*

Rodac plate area = 17 cm^2

Spores per cm^2 of clothing

DIGGERS				CRAWLERS			
Arm	Mid-Section	Thigh	Boot	Arm	Mid-Section	Thigh	Boot
0 to 2	0 to 2	0 to 13	2 to 25	0 to 2	0 to 3	0 to 29	2 to 30
MEDIAN							
0	0	2	4	0	0	4	8

\+ = colonies too numerous to count

* after 20 minutes in the area which had been contaminated 24 hours earlier

TABLE V

AEROSOL SAMPLING

AGI dosages* and median mask sample doses**

Group	Primary Aerosol	MASK DOSE First 30 min.	Second 30 min.	Third 30 min.
A	1.9×10^6	1.3×10^4	4.9×10^3	7.3×10^3
B	-	1.9×10^3	1.1×10^3	1.1×10^3
C	-	3.3×10^3	2.6×10^3	1.8×10^3
		AGI dosages		
	6.2×10^4	67	7.2	0

* AGI dosages in spore min/L.

** Total number of BG spores in mask sample.

TABLE VI

SECONDARY AEROSOL

PARTICLE SIZE DATA FROM ANDERSEN SAMPLERS

Stage	d_{50}*	% Proportion by Stage		
		1st Period	2nd Period	3rd Period
Top	9.5	53	65	67
1st	6.5	12	14	14
2nd	4.6	10	8	7
3rd	3.0	7	10	3
4th	1.7	9	7	4
5th	1.0	9	6	5

* The 50 percent cut-off diameter in μm for each stage.

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APPENDIX A

METEOROLOGICAL OBSERVATIONS

FIELD EXPERIMENT LOCAL TRIAL 427 - "C"

DATE August 31, 1971 ZERO 0910 M.S.T. MET. O.P. 25 yards at 240 degrees of working site

TIME		WIND		T.G.	TEMP.		SKY	
HOUR	MINS	DRCN	SPEED (MPH)	4 - ½ m	AIR	RH	CLOUDS	SUN
(MST)	(Z+)	(°T)	2 m	(°F)	(°F)	(%)		
0910	0	180	11.6	+.5	64.1	77	10 Sc	obscured
0915	5	180	10.8	+.5	64.0		"	"
0920	10	180	9.0	0	64.0		"	"
0925	15	185	12.0	-.2	64.0		"	"
0930	20	185	10.5	-.3	64.1	77	"	"
0935	25	180	10.1	-.5	64.3		"	"
0945	35	175	11.5	-.5	63.3	80	"	"
1000	50	180	12.6	-.5	64.7		"	"
1015	65	180	12.3	-.7	64.0		"	"
1030	80	200	13.4	-.8	65.3	75	"	"
1045	95	180	13.4	-1.1	66.2		"	"
1100	110	185	15.5	-1.1	65.7	75	8 Sc	"
1115	125	180	10.4	-1.3	65.3		7 Sc	"
1130	140	205	11.1	-1.3	65.1		6 Sc	"
1145	155	185	13.6	-2.7	69.5	67	6 Sc	moderate

NOTES: 1) IN GENERAL OBSERVATIONS ARE INSTANTANEOUS AT MOMENT GIVEN EXCEPT WIND SPEED WHICH IS MEAN OF INTERVAL SINCE PREVIOUS OBSERVATION.

2) STANDARD METEOROLOGICAL ABBREVIATIONS ARE USED (SUCH AS FOR "CLOUDS").

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APPENDIX B

SAMPLING

1. Ground Contamination Samples

An estimate of particle fallout was obtained by using 150 mm petri dishes (area 176 cm^2) containing a solid tryptose agar nutrient medium. The open plates were spread out on the ground to collect particles which settled out of the primary aerosol and a new set was used for each of the three subsequent working periods. After 18 hours incubation at 37°C, visible colonies grew in the medium, and the number of colonies represented the total number of particles retained on the agar surface. There is, however, no enumeration of the total number of viable spores per particle, since a single colony develops from either a very small particle containing a single spore, or a very large particle which may contain up to hundreds of spores. Some differentiation is possible, however, by setting out duplicate plates, one of which has been painted with a sterile solution of warm diluent (gelatin (4%) glycerol (35%), which solidified into a thin sticky layer over the surface. After exposure, this plate is washed with 20 mL of warm dilutent (gelatin saline) to liquefy the gelatin, remove the particles, and thereby suspend individual spores. The suspension is then serially diluted and biologically assessed. The total number of colonies formed by the released spores, divided by the total number of colonies obtained from the unwashed plates, is an approximate measure of the average number of spores per particle.

2. Clothing Contamination Samples

The number of particles per unit area of clothing of men exposed to either the primary or secondary aerosols was estimated by pressing to the clothing surface a specially designed Rodac plate (area 17 cm^2) which contains a solid nutrient medium. Particles adhere to the agar surface and, upon incubation, the spores within each particle germinate and grow to form a single colony. The approximate number of viable spores per particle is obtained as previously described by using paired Rodac plates, one of the pair having a gelatin glycerol layer which is washed off for assay.

Duplicate Rodac contact samples were taken on the right shoulder, mid-section, right thigh, and the side of the right boot as follows:

Group A	- after exposure to the primary aerosol
Group B and C	- after first sortie in the contaminated area
Groups A, B and C	- on completion of the experiment (after 3rd sortie)

3. Primary Aerosol Samples

Standard collecting devices were operated in the test area to determine the "dosage" to which the troops were exposed. Samplers were set up in groups at each of three locations, slightly downwind of the test subjects and 46 m apart across the layout.

a. All-Glass Impinger (AGI)

Each contains 20 mL of glycerol gelatin milk phosphase (GGMP) and draws 12.5 litres of air per minute. The curved inlet tube is

washed down to remove particles which may have impacted on it. The total number of viable spores per sampler, divided by the flow rate, is an estimate of the dosage in spore/min per litre. Dosage, multiplied by man's breathing rate (approximately 10 L/min at rest) indicates the dose, or the number of spores which could have been inhaled.

b. MRE 3-Stage Sampler (with shield)

Operates at 55 litres per minute and fractionates the aerosol according to particle size as it moves down through each of the stages. Thus, particles larger than 6 microns are retained in 10 mL of GGMP in stage 1, particles 3 to 6 microns are retained in 9 mL in stage 2, and particles less than 3 microns are retained in 10 mL in stage 3. Dosage in cell minutes per litre is similarly calculated but, in this case, it is also possible to determine the approximate relationship of the number of spores which came from particles is the given size ranges.

c. External Canister Sampler

This device consists of a special type of filter paper disc held in a plastic holder which snaps into the opening of a man's respiratory canister. All air breathed by the individual passes through the paper at a rate determined by his activity - this slight increase in resistance has little effect on breathing and causes no undue stress. Retention of particulate matter (1 to 15 microns) is of the order of 90 to 99%, respectively. The device is man-operated, thus the total number of spores recovered is a realistic measure of what the man would have inhaled had he been unmasked.

d. Reyniers Sampler

Operates at 28:3 L/min (1 cfm) drawing the aerosol through a slit opening onto the surface of a nutrient medium where most of the particles are impacted. The medium is contained in a petri dish held on a platform which rotates by means of a clockwork mechanism at the rate of 1 complete revolution in 60 minutes. Thus, the colonies, which form after incubation, represent the number of particles per unit volume of air sampled, and the area covered by the deposit indicates when the aerosol arrived, and for how long it persisted. The quantitative aspects of the device, however, depend on the particle concentration - if this is too high, vast numbers of particles are impacted in close proximity one to the other and, as the colonies form, they coalesce to form a confluency from which no accurate count can be made.

e. Andersen Sampler

Operates at 28.3 L/min (1 cfm) drawing the aerosol through holes of diminishing size drilled in a series of 6 stages which contain plates of solid nutrient culture medium. The particles impact out onto the surface of the culture medium according to their size, in the range greater than 9 μm to 1 μm. A single colony forms from each BG-bearing particle.

f. High Volume Sampler

Operates at 780 L/min and collects particles in the 1 to 15 micron size range. Airborne particles are concentrated, in a small

cyclone chamber, into a few millilitres of collecting fluid. The sampler was constructed here at DRES from a design supplied by Mr. H. Decker, U.S. Army Biological Defence Research Laboratory, Fort Detrick, Maryland.

APPENDIX C

CLOUD MONITORING DATA

PRIMARY AEROSOL

BG collected by various samplers

	Total spores or particles	Average	Average Dosage	Dose per man at rest (10 L/min)
AGIs 12.5 L/min	8.4) 7.5) x 10^5 7.2)	7.7×10^5	6.2×10^4 spore min/L	6.2×10^5 spores
MREs 55 L/min	3.6) 4.2) x 10^6 3.4)	3.8×10^6	7.6×10^4 spore min/L	7.6×10^5 spores
Reyniers Samplers 28.3 L/min	TNTC* TNTC TNTC	Indicated a passage to time of 1 to 1.25 minutes for each aerosol "shot"		
Ground Settling Plates	4770) 5140) 4770)	4893 Particles	2.8×10^5 particles per m^2	
Duplicate Plates Washed	4.0) 3.2) x 10^5 3.9)	3.7×10^5	2.1×10^7 spores per m^2	

MAN EXTERNAL SAMPLERS - Actual Dose (Cont'd)

2.2)		
3.8)		
1.1)		
2.5)		
1.7)		
2.3)		
2.6)		
1.5)	x 10^6	Actual Dose = 1.1 x 10^6 to 3.8 x 10^6 spores
1.9)		Median Dose = 1.9 x 10^6
1.1)		
2.0)		
2.2)		
1.8)		
1.9)		
1.8)		

* TNTC = too numerous to count

CLOUD MONITORING DATA - FIRST WORKING PERIOD

SECONDARY AEROSOL

BG collected by various samplers

	Total spores or particles	Average	Average Dosage	Dose per man at rest (10 L/min)
AGIs 12.5 L/min	830 spores	830	67 spores min/L	670 spores
Andersen Samplers 28.3 L/min	142 particles 113 particles	127.5	4.5 particle min/L	45 particles
Reyniers Samplers 28.3 L/min	80 particles 85 particles	82.5	2.9 particle min/L	29 particles
Ground Settling Plates	54 particles 30 particles	42	2.4×10^3 particles per m^2	
Duplicate Plates	2.3×10^4	418 spores/particle	1.3×10^6 spores per m^2	
Washed	0.1×10^4	40 spores/particle	5.7×10^4 spores per m^2	

MAN EXTERNAL SAMPLERS (Cont'd)

GROUP A -
previously contaminated clothing

0.5)
0.9)
0.96)
0.89)
2.8)
1.6)
1.2)
1.3) x 10^4 spores — Actual dose = from 0.5 to 3.1 x 10^4 spores
1.6)
2.0) — Median dose = 1.3 x 10^4
1.1)
3.1)
1.5)
1.2)
1.8)

GROUP B -
clean clothing

1.0)
1.7)
1.5)
3.0)
1.3)
1.3)
2.1)
2.3) x 10^3 spores — Actual dose = from 0.5 to 3.4 x 10^3 spores
1.8)
0.5) — Median dose = 1.9 x 10^3
1.1)
3.4)
2.0)
2.7)
3.2)
1.97)

GROUP C -
Walkers -
clean clothing

4.8)
2.0) x 10^3 — Actual dose = from 2.0 to 4.8 x 10^3 spores
2.4) — Median dose = 3.3 x 10^3
4.1)

CLOUD MONITORING DATA - SECOND WORKING PERIOD

SECONDARY AEROSOL

BG collected by various samplers

	Total spores or particles	Average	Average Dosage	Dose per man at rest (10 L/min)
AGIs 12.5 L/min	90 spores	90	7.2 spore min/L	72 spores
Anderson Samplers 28.3 L/min	106 particles 161 particles	133.5	4.7 particle min/L	47 particles
Reyniers Samplers 28.3 L/min	83 particles 41 particles	62	2.2 particle min/L	22 particles
Ground Settling Plates	131 particles 78 particles	104.5	5.9×10^3 particles per m^2	
Duplicate Plates	1.8×10^4 spores	138 spores/particle	1.9×10^6 spores per m^2	
Washed	2.5×10^4 spores	329 spores/particle	1.4×10^6 spores per m^2	

MAN EXTERNAL SAMPLERS (Cont'd)

GROUP A

5.3)
8.4)
2.2)
16.0)
8.3)
3.7)
4.9) x 10^3 spores
4.8)
4.1)
22.9)
1.1)
2.4)
0.97)
8.6)
6.2)

Actual dose = from 1 to 23 x 10^3 spores
Median dose = 4.9 x 10^3

GROUP B

1.1)
0.63)
0.13)
0.77)
0.47)
2.8)
0.93)
0.83) x 10^3 spores
2.2)
0.07)
1.0)
1.4)
1.5)
2.6)
1.9)
2.2)

Actual dose = from 0.7 to 2.8 x 10^3 spores
Median dose = 1.05 x 10^3

GROUP C

3.1)
3.6) x 10^3 spores
2.0)
1.6)

Actual dose = from 1.6 to 3.6 x 10^3 spores
Median dose = 2.6 x 10^3

CLOUD MONITORING DATA - THIRD WORKING PERIOD

SECONDARY AEROSOL

BG collected by various samplers

	Total spores or particles	Average	Average Dosage	Dose per man at rest (10 L/min)
AGIs	0	0	-	-
12.5 L/min	0			
Anderson Samplers	126 particles	123	4.3 particle min/L	43 particles
28.3 L/min	120 particles			
Reyniers Samplers	83 particles	30,5	1.1 particle min/L	11 particles
28.3 L/min	41 particles			
Ground Settling Plates	42 particles	46	2.6×10^3 particles per m^2	
	50 particles			
Duplicate Plates	170 spores	4 spores/particle	9.6×10^3 spores per m^2	
Washed	1940 spores	39 spores/particle	1.1×10^5 spores per m^2	

MAN EXTERNAL SAMPLERS (Cont'd)

GROUP A

2.8)		
8.9)		
1.7)		
3.5)		
9.5)		
7.8)		
8.7)	x 10^3 spores	Actual dose = from 1.7 to 14.5 x 10^3 spores
4.2)		Median dose = 7.3 x 10^3
10.6)		
14.5)		
8.6)		
7.3)		
6.7)		
7.2)		
7.3)		

GROUP B

1 filter lost

1.2)		
0.5)		
1.1)		
0.4)		
0.6)		
0.7)		
0.8)	x 10^3 spores	Actual dose = from 0.4 to 3.6 x 10^3
2.3)		Median dose = 1.1 x 10^3
0.7)		
1.4)		
2.5)		
1.4)		
2.0)		
3.6)		
0.97)		

GROUP C

1.7)		
1.97)	x 10^3 spores	Actual dose = from 1.5 to 2.5 x 10^3 spores
2.5)		Median dose = 1.8 x 10^3
1.5)		

APPENDIX D

CLOTHING CONTAMINATION DATA

PRIMARY EXPOSURE*

Total spores recovered from contact clothing samples
Washed Rodac plate area - 17 cm^2

Arm (x 10^{-4})	Mid-Section (x 10^{-4})	Thigh (x 10^{-4})	Boot (x 10^{-4})
1.5	0.7	1.3	0.4
1.6	1.8	1.7	0.6
2.5	2.5	2.3	1.1
2.9	2.9	2.4	1.1
2.9	2.9	2.4	3.1
3.4	3.1	2.5	3.3
4.3	3.2	3.2	3.4
4.4	3.4	3.3	3.5
6.3	3.9	3.4	3.6
6.4	4.1	3.7	3.7
6.8	4.5	9.5	4.1
8.5	6.4	9.6	5.0
10.4	6.9	9.8	5.9
11.1	10.2	23.0	7.8
15.3	13.3	lost	11.1
Number of spores per cm^2			
590 to 8830	590 to 7660	590 to 13290	236 to 6490
Median			
2543	1965	1908	2023

*GROUP A - 15 men exposed to 4 aerosol releases

CLOTHING CONTAMINATION DATA

Total spores remaining after 90 minutes*
Washed Rodac plate area - 17 cm^2

Arm ($\times 10^{-3}$)	Mid-Section ($\times 10^{-3}$)	Thigh ($\times 10^{-3}$)	Boot ($\times 10^{-3}$)
2.6	0.85	1.1	0.59
3.2	1.5	1.5	1.9
3.4	1.6	1.8	1.9
4.9	2.9	1.8	2.3
8.5	3.6	2.0	2.3
8.8	7.7	4.2	2.7
9.3	9.9	5.0	3.0
20.0	10.6	5.3	3.2
20.8	10.6	6.1	4.3
25.6	11.7	7.2	4.4
26.6	11.7	8.2	5.1
37.6	14.1	15.2	7.7
42.9	14.1	36.8	8.8
168.0	14.6	42.6	12.8
lost	24.8	48.8	17.3
Number of spores per cm^2			
153 to 9900	59 to 1470	59 to 2950	59 to 1000
Median			
850	613	306	185

*GROUP A - 15 men exposed to 4 primary aerosol releases, then 90 minutes of activity in a contaminated area

CLOTHING CONTAMINATION DATA
SECONDARY AEROSOL - AFTER FIRST WORKING PERIOD
BG-bearing particles from clothing (Rodac plate area - 17 cm^2)

	Arm	Mid-Section	Thigh	Boot
GROUP B - dressed in clean clothing after 30 mins working in contaminated area				
	6	1	48	4+
	8	27	54	4+
	10	37	102	4+
	17	48	+	4+
	18	55	+	4+
	22	108	+	4+
	27	110	+	4+
	30	110	+	4+
	32	120	+	4+
	67	130	+	4+
	80	+	+	4+
	85	+	+	4+
	98	+	+	4+
	+	+	+	4+
	+	+	+	4+
	+	+	+	4+
GROUP C - dressed in clean clothing, after 30 mins walking in the contaminated area				
	25	6	+	4+
	30	10	+	4+
	80	12	+	4+
	+	18	4+	4+

+ = 150 to 200 colonies

CLOTHING CONTAMINATION DATA
SECONDARY AEROSOL - AFTER FIRST WORKING PERIOD

Total spores recovered from contact clothing samples washed Rodac plate area 17 cm^2

	Arm	Mid-Section	Thigh	Boot
GROUP B - dressed in clean clothing after 30 mins working in a contaminated area				
	0	33	66	1300
	13	53	66	1626
	13	53	93	2186
	16	80	186	2386
	26	106	306	2413
	40	133	316	2453
	53	160	373	2466
	66	213	400	2840
	80	293	413	2933
	80	306	440	3080
	133	320	493	4360
	183	440	533	4493
	186	466	560	4650
	200	493	946	4653
	813	573	1386	6653
	893	616	1946	7586
Number of spores per cm^2	0 to 53	2 to 36	4 to 114	77 to 450
Median	4	15	24	167
GROUP C - dressed in clean clothing, after 30 mins walk downwind of Groups A and B				
	0	0	533	6533
	53	26	1533	7533
	160	40	2453	7733
	233	40	5466	12166
Number of spores per cm^2	0 to 14	0 to 2	32 to 320	385 to 725
Median	6	2	115	441

CLOTHING CONTAMINATION DATA
SECONDARY AEROSOL - AFTER THIRD WORKING PERIOD
BG-bearing particles from clothing (Rodac plate area - 17 cm^2)

	Arm	Mid-Section	Thigh	Boot
GROUP B	7	15	44	+
	12	25	46	+
	16	27	49	+
	18	29	52	+
	19	42	64	+
	19	42	76	+
	30	50	98	+
	31	54	123	+
	45	65	129	+
	70	68	133	+
	75	94	161	+
	115	114	189	+
	163	115	219	+
	184	131	236	+
	186	151	+	+
	198	184	+	+
	Median 38	60	126	+
GROUP C	17	1	99	+
	17	3	130	+
	18	20	+	+
	20	21	+	+

+ = colonies too numerous to count

CLOTHING CONTAMINATION DATA

SECONDARY AEROSOL - AFTER THIRD WORKING PERIOD

Total spores recovered from contact clothing samples

samples - washed Rodac plate area 17 cm^2

	Arm	Mid-Section	Thigh	Boot
GROUP B				
	0	0	0	186
	13	13	0	226
	26	26	0	320
	80	26	0	320
	93	26	0	600
	93	66	13	693
	120	66	26	960
	120	66	40	1013
	146	93	40	1066
	186	133	66	1083
	253	173	93	1120
	283	186	106	1400
	413	213	160	2240
	520	213	240	2413
	1013	240	293	5973
	2573	333	386	12133
Number of spores per cm^2	0 to 151	0 to 20	0 to 23	11 to 720
Median	8	5	2	60
GROUP C				
	16	0	186	480
	53	13	346	666
	53	33	400	1383
	80	53	450	1546
Number of spores per cm^2	1 to 5	0 to 3	11 to 27	28 to 92
Median	3	1	22	59

APPENDIX E

CLOTHING CONTAMINATION DATA

INCIDENT E
(24 hours after Incident C)

DIGGERS				CRAWLERS			
Arm	Mid-Section	Thigh	Boot	Arm	Mid-Section	Thigh	Boot
BG-bearing particles in 17 cm^2							
0	0	3	6	1	2	9	10
0	0	4	13	1	4	10	13
0	0	5	20	1	5	10	29
0	0	9	34	2	7	16	38
1	0	10	38	2	10	16	54
1	0	12	42	3	11	18	81
1	1	17	45	3	11	20	94
1	1	18	55	3	11	22	135
1	2	18	56	3	13	23	160
2	2	20	87	6	13	24	168
2	3	22	109	6	13	26	190
2	3	24	130	6	14	34	195
3	3	26	134	6	17	39	196
3	3	33	217	6	19	60	314
3	4	42	+	11	23	63	+
4	5	56	+	17	23	79	+
6	9	67	+	17	26	86	+

CLOTHING CONTAMINATION DATA

INCIDENT E
(24 hours after Incident C)

DIGGERS				CRAWLERS			
Arm	Mid-Section	Thigh	Boot	Arm	Mid-Section	Thigh	Boot
Total spores from 17 cm^2 plate							
0	0	0	26	0	0	0	26
0	0	0	26	0	0	0	40
0	0	0	26	0	0	13	80
0	0	0	26	0	0	13	93
0	0	0	26	0	0	26	106
0	0	0	40	0	0	26	120
0	3	13	53	0	0	66	120
0	3	13	66	0	0	66	133
0	13	40	80	0	13	80	146
0	13	53	93	0	13	80	146
13	13	80	106	13	13	93	160
13	26	80	120	13	26	120	200
13	26	120	120	13	26	146	200
13	26	146	120	13	40	200	280
26	26	160	386	26	40	213	386
26	26	213	426	26	53	493	506
Spores per cm^2							
Range							
0-2	0-2	0-13	2-25	0-2	0-3	0-29	2-30
Median							
0	0	2	4	0	0	4	8

Figure 1

FITTING EXTERNAL CANISTER SAMPLER

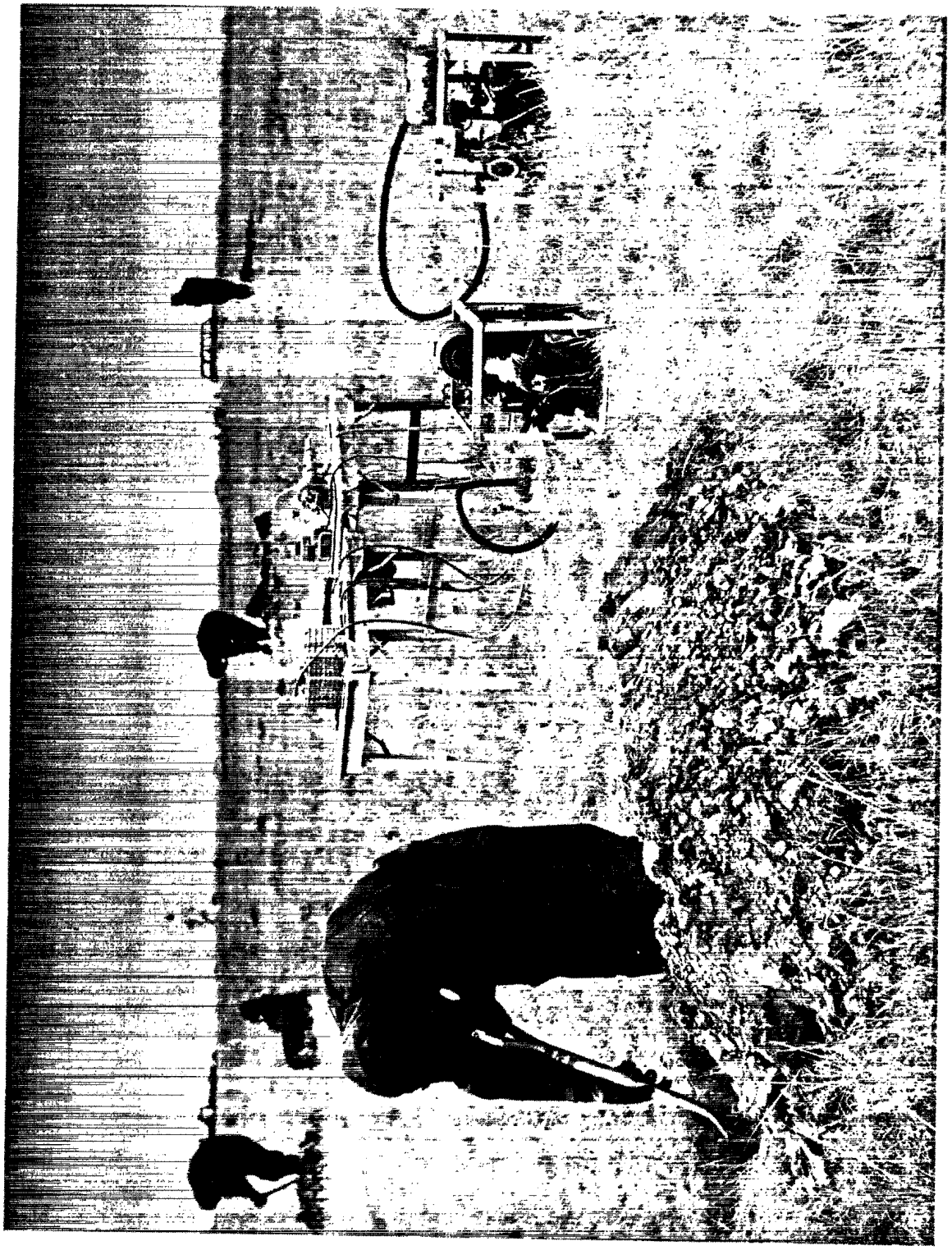

Figure 2

PREPARING A DEFENSIVE POSITION NEAR A SAMPLING STATION

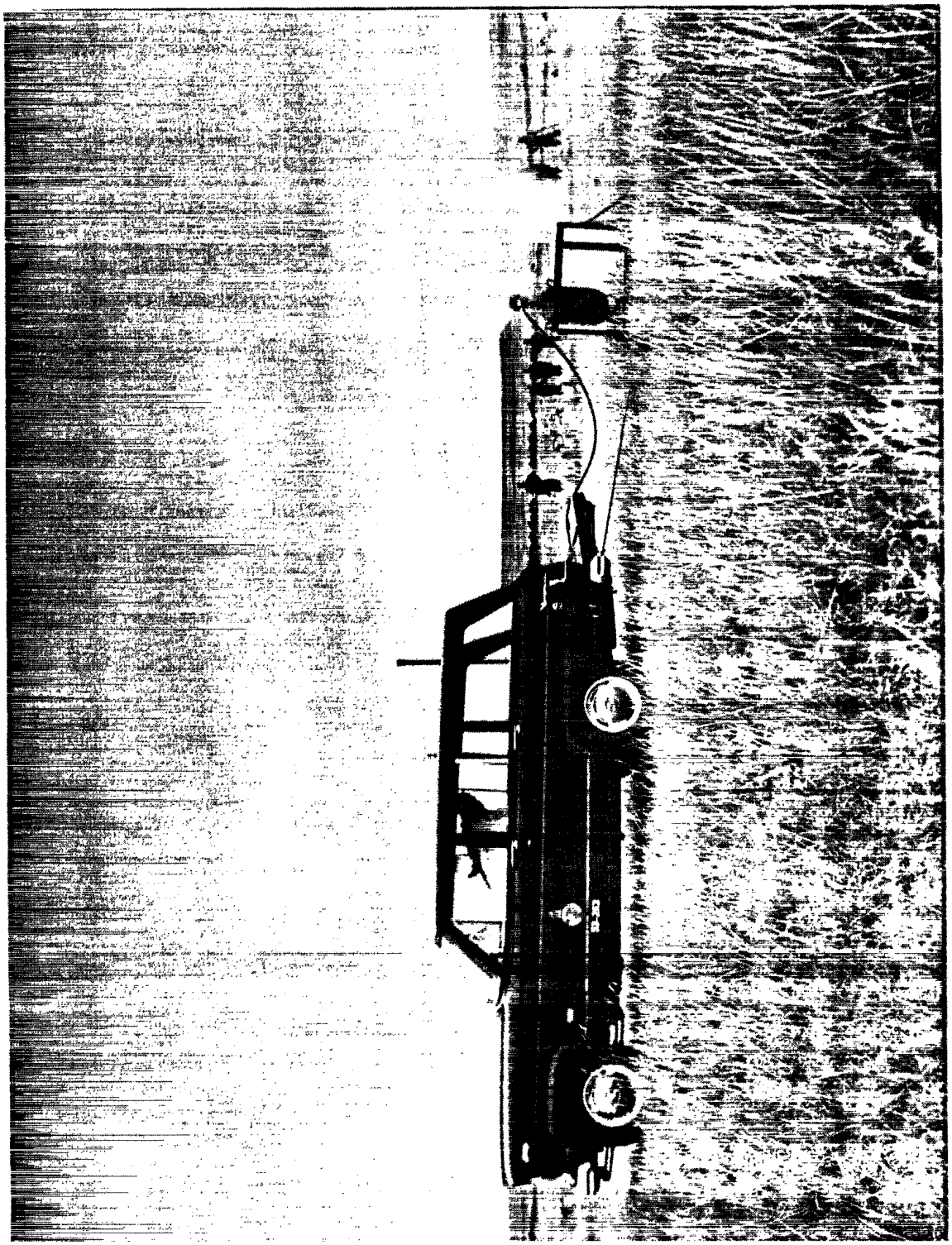

Figure 3

E-4 GENERATOR IN ACTION

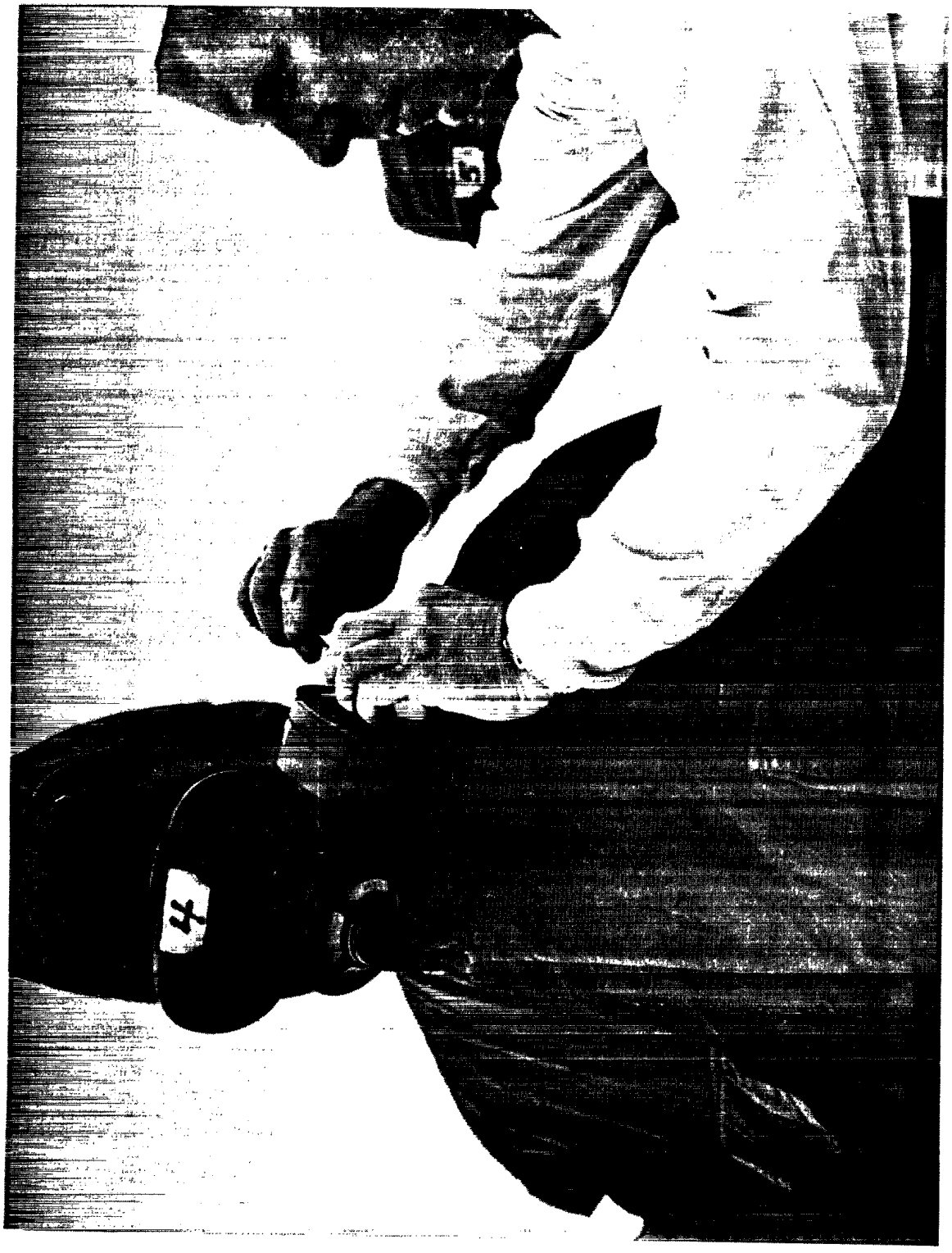

Figure 4

REMOVING CANISTER SAMPLER

Figure 5

RODAC-PLATING THE ARM

Figure 6

RODAC-PLATING THE THIGH

Figure 7

RODAC-PLATING THE BOOT

Figure 8

TROOPS CRAWLING, INCIDENT "E"

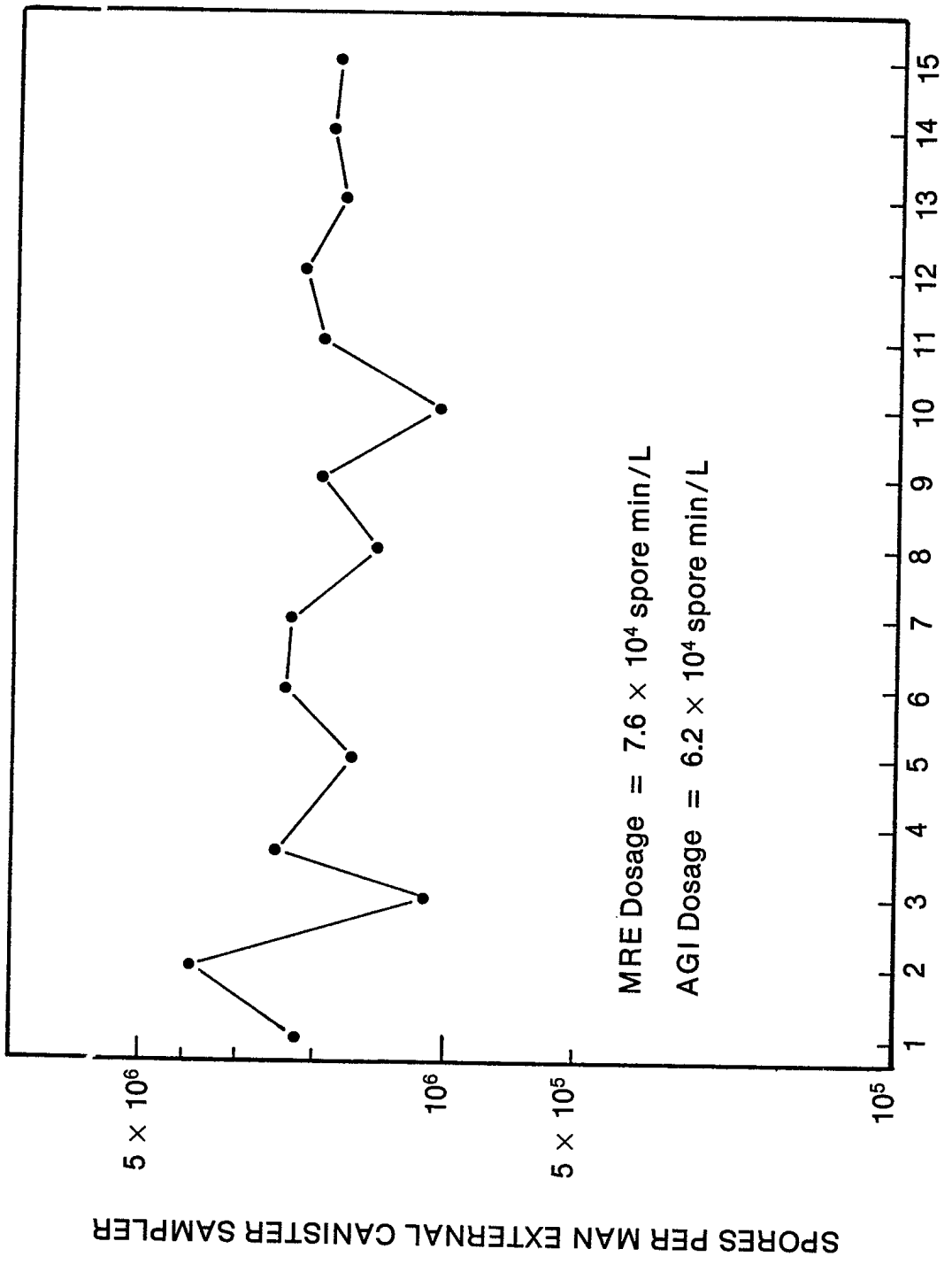

Figure 9

THE NUMBER OF BG SPORES COLLECTED ON EXTERNAL CANISTER SAMPLERS DURING PRIMARY AEROSOL EXPOSURE

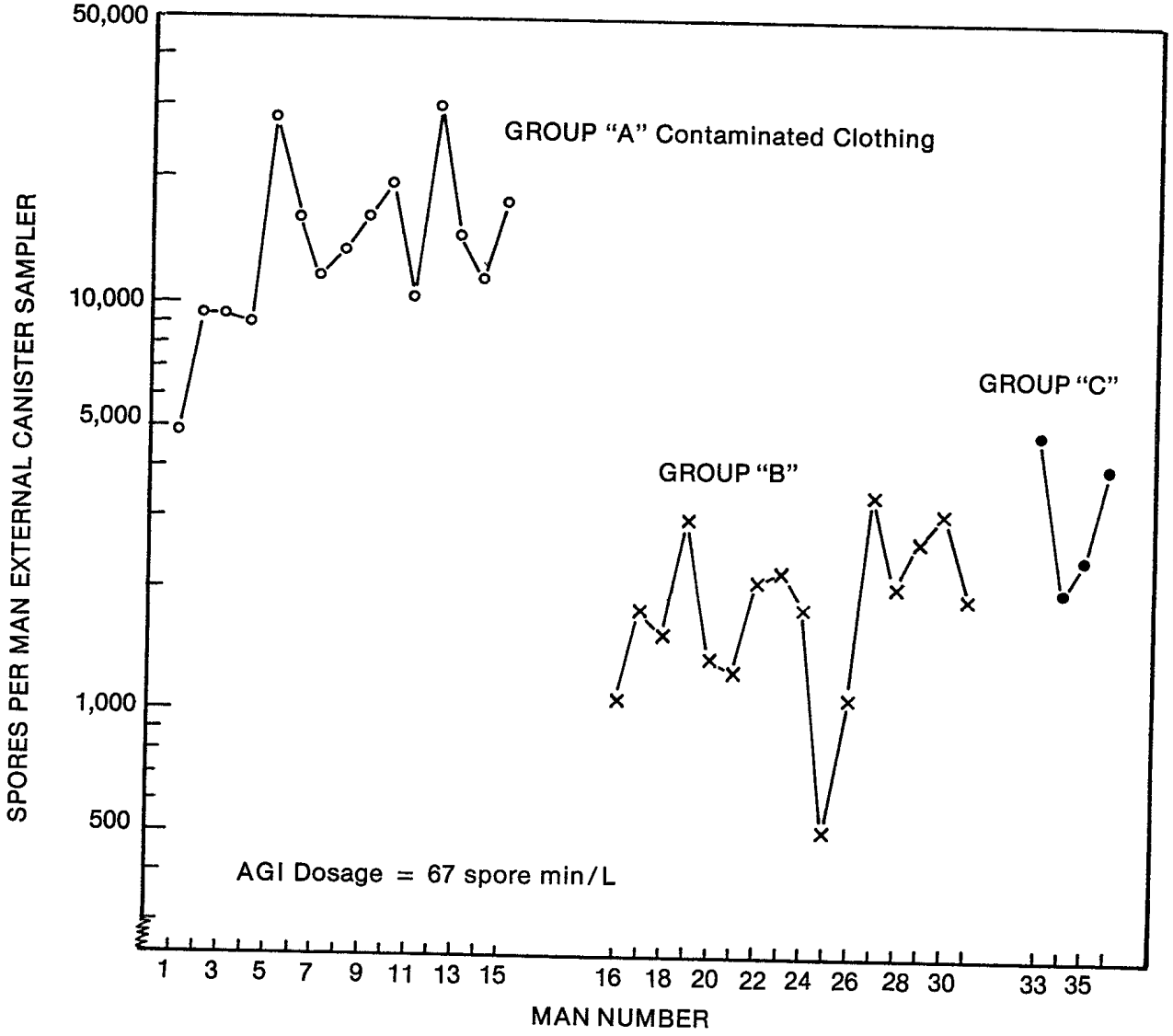

Figure 10

THE NUMBER OF BG SPORES COLLECTED ON EXTERNAL CANISTER SAMPLERS DURING THE FIRST WORKING PERIOD IN A CONTAMINATED AREA

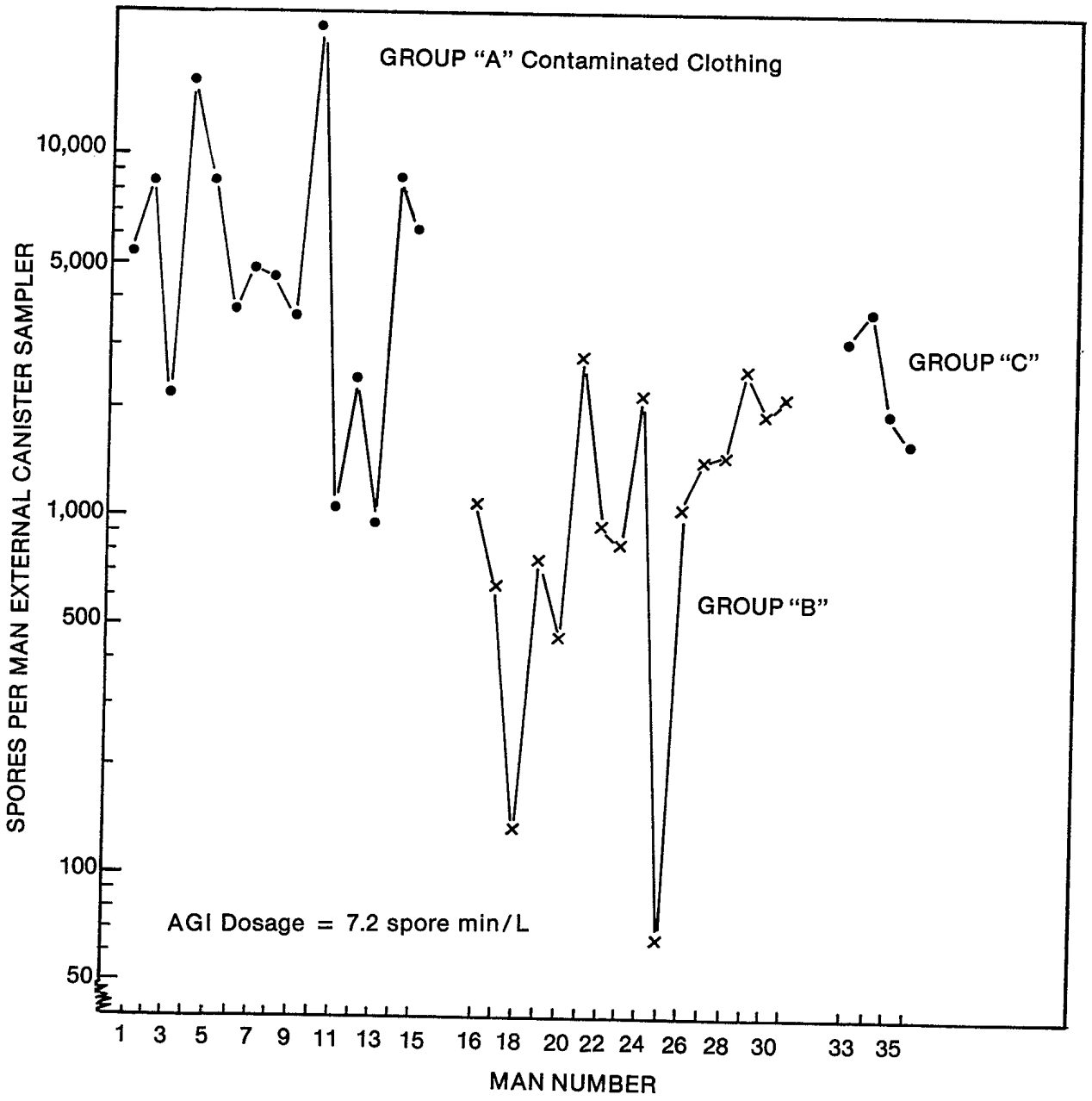

Figure 11

THE NUMBER OF BG SPORES COLLECTED ON EXTERNAL CANISTER SAMPLERS DURING THE SECOND WORKING PERIOD IN A CONTAMINATED AREA

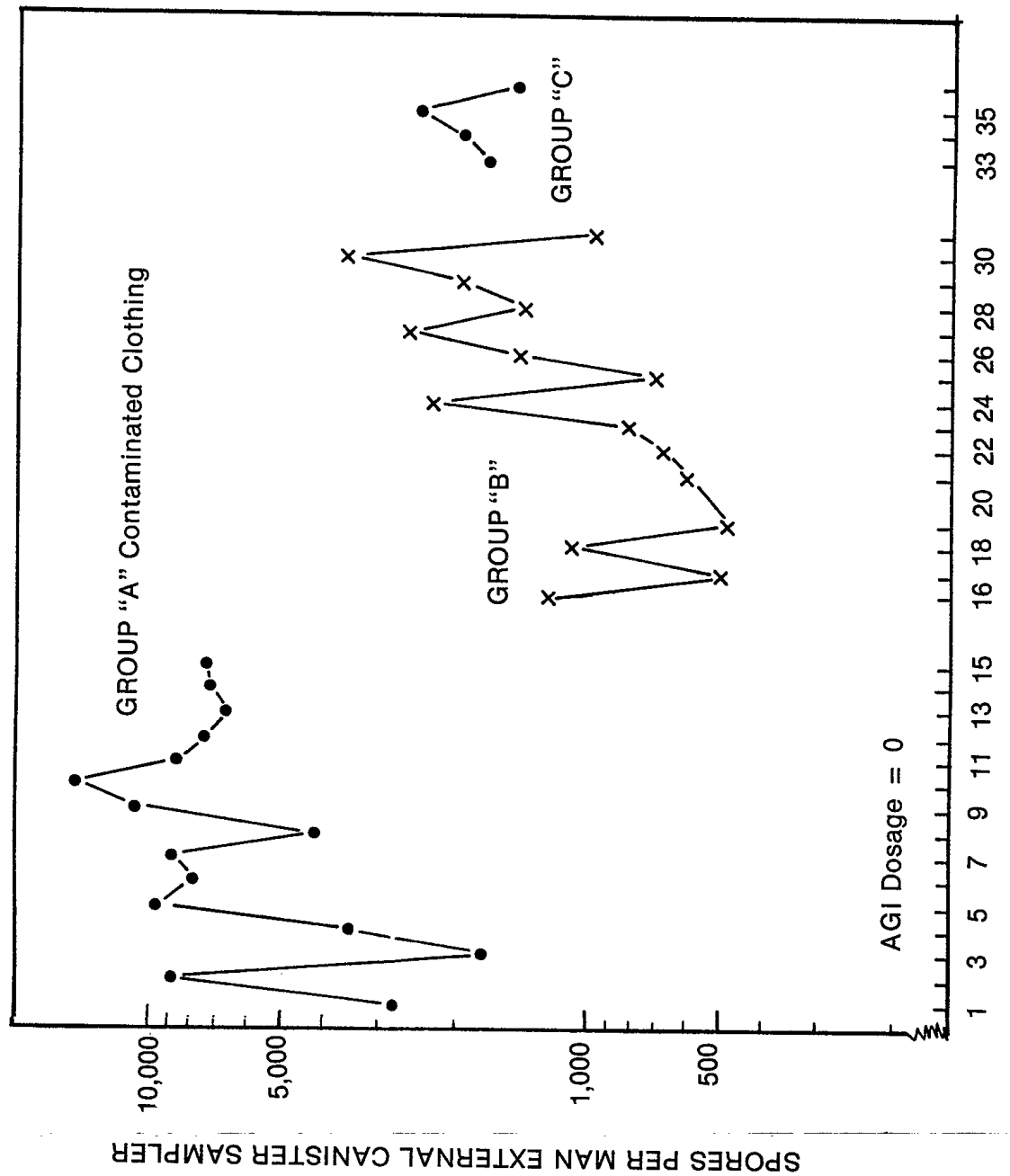

Figure 12

THE NUMBER OF BG SPORES COLLECTED ON EXTERNAL CANISTER SAMPLERS DURING THE THIRD WORKING PERIOD IN A CONTAMINATED AREA

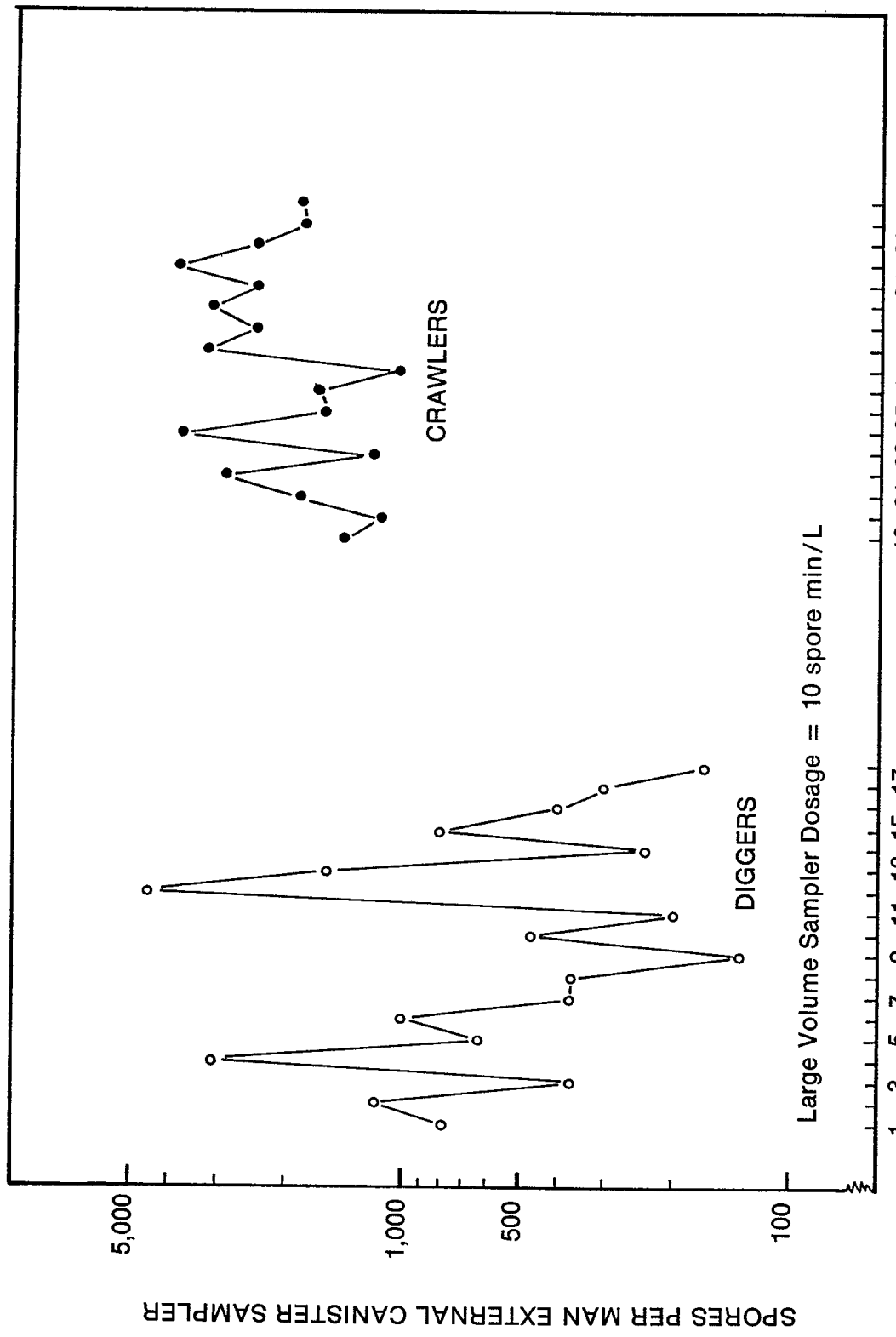

Figure 13

THE NUMBER OF BG SPORES COLLECTED ON EXTERNAL CANISTER SAMPLERS DURING A WORKING PERIOD IN AN AREA CONTAMINATED 24 HOURS PREVIOUSLY

UNCLASSIFIED

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1 ORIGINATING ACTIVITY

DEFENCE RESEARCH ESTABLISHMENT SUFFIELD

2a. DOCUMENT SECURITY CLASSIFICATION

UNCLASSIFIED

2b. GROUP

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SECONDARY AEROSOL HAZARDS IN THE FIELD (U)

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SUFFIELD REPORT NO. 321

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8b. CONTRACT NO.	9b. OTHER DOCUMENT NO.(S) (Any other numbers that may be assigned this document)

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11. SUPPLEMENTARY NOTES	12. SPONSORING ACTIVITY

13. ABSTRACT An area of ground was contaminated from an aerosol of spores of Bacillus subtilis var niger (BG). Aerosol samples were collected in three stage samplers with 50 percent particle size cut-offs of 6 m in the second stage. These samples collected 45 percent of the spores in the top stage, 22 percent in the middle stage and 34 percent in the bottom stage. The ground contamination level 46 meters downwind was 3×10^5 particles or 2×10^7 spores per M^2 of terrain in all particle sizes. The aerosol concentration ranged from 6.2 to 7.6 $\times 10^4$ spores min/liter.

Secondary aerosols were produced by the activity of men moving and working within the contaminated area, and spores were recovered in standard samplers and on the filters of special samplers fitted to the opening of particulate canisters on respirators worn by the men. The number of spores collected by each man was deemed to be representative of his respiratory intake had he been unmasked. After 24 hours, the respiratory dose varied from 100 to 1000 spores. This represents about 0.0020 to 0.12 percent of the original exposure dose. Even after 9 days, secondary aerosol was still being collected in a high volume sampler operating at 780 liters/min. Comparisonof standard samplers with the mask samplers indicates breathing rates from 13 to 64 liters/min.

The clothing and boots of personnel became contaminated after exposure to both the primary and secondary aerosols. Respiratory hazard to men wearing clothing grossly contaminated from the primary aerosol, was an order of magnitude higher than that for men whose clothing was not so contaminated.

DSIS
79-079

KEY WORDS

Aerobiology
Aerosols
Biological aerosols
Baccillus subtilis
Spores
Contamination
Clothing
Sampling
Secondary emission
Hazards
Respiratory system
Terrain
Range grasses

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