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A Mathematical Approach Linking Aerosol Particles Obtained by Slit Sampling and Viable Organisms Obtained by Direct Inhalation Sampling

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Defence R&D Canada

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Abstract

Two different sampling methods were employed to measure a biological aerosol disseminated from a contaminated letter: slit samplers and a microfibre filter medium located at the inhalation port of a respirator canister worn by the individual who opened the letter. Measurements obtained from slit samplers may only be interpreted in terms of numbers of viable particles. Particles may be comprised of single or multiple organisms. Microfibre filter data are presented in terms of the number of colony forming units, which refer to individual viable organisms. We observed a significant difference between the number of viable particles and the number of colony forming units measured by these two techniques. The former was calculated to be 30975 *Bacillus atrophaeus* (BG) containing particles and the latter was determined to be 1072113 CFUs, assuming similar air flow sampling rates. At the simplest level, this suggests that each particle may contain 37 viable BG spores. It is far more likely however, that aerosolised BG consists of a distribution of particles of various sizes which, depending on the volume of the particle, would contain different numbers of spores. A model was developed to define a relationship between numbers of BG spore containing particle-aggregates measured by the slit samplers and numbers of individual viable spores comprising particles measured by entrapment on the microfibre filter medium. From this an estimate was derived of the total number of individual viable spores in a population of aerosol particles measured by slit samplers. The model assumes a relatively simple probability density function distribution for particle size and incorporates realistic assumptions regarding particle diameters, packing density and spore viability. The value predicted by the model, when corrected for aerosol sampling inefficiencies, differs by ~10% from the actual number of viable organisms measured by the microfibre filter at the inhalation port of the respirator filter canister.

R□sum□

On a employ□ deux m□thodes d□□chantillonnage diff□rentes pour mesurer un a□rosol biologique diss□min□ □ partir d□une lettre contamin□e. Ces deux m□thodes consistaient en des □chantillonneurs □ fentes et en un milieu de filtre en microfibres situ□ □ l□orifice d□inhalation d□une cartouche de respirateur port□ par l□individu qui ouvrait la lettre. Les mesures obtenues des □chantillonneurs □ fentes ne peuvent □tre interpr□t□s qu□en termes du nombre de particules viables. Les particules peuvent se comprendre d□un seul organisme ou d□organismes multiples. Les donn□es du filtre en microfibres se pr□sentent en termes du nombre de colonies formant des unit□s qui sont des organismes individuels viables. On a observ□ une diff□rence importante entre le nombre de particules viables et le nombre d□unit□s formant une colonie mesur□s par ces deux techniques. On a calcul□ que le premier □tait de 30975 particules contenant le *Bacillus atrophaeus* (BG) et on a d□termin□ que le dernier □tait de 1072113 CFU, en supposant des d□bits d□air similaires dans les taux d□□chantillonnage. Au niveau le plus simple, ceci sugg□re que chaque particule peut contenir 37 spores BG viables. Il est plus probable, cependant, que le BG mis en a□rosol consiste en une distribution de particules de tailles vari□es lesquelles contiendraient des nombres diff□rents de spores selon le volume des particules. On a mis au point un mod□le pour d□finir la relation entre le nombre de particules d□agr□gats contenant des spores BG mesur□es par les □chantillonneurs □ fentes et le nombre de spores individuelles viables comprises dans les particules mesur□es par pi□geage dans le milieu de filtre en microfibres. On a en d□riv□ une estimation du nombre total de spores individuelles viables dans la population des particules en a□rosol mesur□es par les □chantillonneurs □fentes. Le mod□le part du principe d□une fonction de densit□ relativement simple pour la taille de la particule et incorpore des hypoth□ses r□alistes concernant les diam□tres de particules, la masse volum□trique en vrac et la viabilit□ des spores. La valeur pr□dite par le mod□le, si corrig□e pour tenir compte de l□inefficience de l□□chantillonnage d□a□rosol, diff□re de ~10% du nombre actuel d□organismes viables mesur□s par le filtre en microfibres situ□ □l□orifice d□inhalation de la cartouche du respirateur.

Executive summary

A Mathematical Approach Linking Aerosol Particles Obtained by Slit Sampling and Viable Organisms Obtained by Direct Inhalation Sampling

Scott Duncan; Jim Ho; DRDC Suffield TM 2007-250; Defence R&D Canada Suffield.

Introduction: There is renewed concern within military and civilian agencies, now faced with addressing an emerging global asymmetric threat, regarding the use of *Bacillus anthracis* (anthrax) as a weapon of choice by individuals or groups contemplating a bioterrorist act. Anthrax may be readily disseminated as an aerosol and as it disperses via air currents and subsequently settles out it contaminates materials and surfaces. Disturbing these will result in re-suspension of the aerosol. Biological aerosols are known to consist of both single organisms and particle-aggregates containing multiple organisms. However, not every organism is viable, ie, capable of causing an infection. Accordingly, from an infectivity perspective, the relationship between the number of viable particles and the number of viable single organisms in a biological aerosol is of interest. A model has been developed that will provide researchers with the tools to consider total infectious organisms in a bioaerosol challenge and thus provide the military and first responder with an improved understanding of the hazard associated with bioaerosol exposures.

Results: Two different sampling methods were employed to measure a biological aerosol of *Bacillus atrophaeus* (BG) spores disseminated from a contaminated letter: slit samplers and a microfibre filter medium located at the inhalation port of a respirator canister worn by the individual who opened the letter. The slit sampler measured aerosol particles (aggregates of spores) whilst the microfibre filter measured individual organisms (spores). We observed a significant difference between the number of viable particles (30975) and the number of viable organisms (1136600) measured by these two techniques. A model was developed to define a relationship between numbers of BG spore containing particle-aggregates measured by the slit samplers and numbers of individual viable spores comprising particles measured by entrapment on the microfibre filter medium. The model assumes a relatively simple probability density function distribution for particle size and incorporates realistic assumptions regarding particle diameters, packing density and spore viability. The value predicted by the model, when corrected for aerosol sampling inefficiencies, differs by ~10% from the actual number of viable organisms measured by the microfibre filter at the inhalation port of the respirator filter canister.

Significance: Primate studies suggest that the LD_{50} for humans (lethal dose sufficient to kill 50% of the persons exposed to it) is 2,500 to 55,000 inhaled anthrax spores. The issue of viable particles versus viable individuals has wide implications, particularly in terms of estimated lethal dose. In this study, the slit sampler data, which is a measurement of viable particles only, falls within the estimated LD_{50} range for anthrax. However, the data obtained from the microfibre filter at the inhalation port of the respirator canister shows that the number of individual viable organisms exceeds the upper end of the LD_{50} by over an order of magnitude. Clearly this is cause

for concern. Particle-aggregates of multiple spores may be far more resilient and aggressive point-sources of acute infection and far fewer particle-aggregates will be necessary to push respiratory exposures into the lethal range. The model demonstrates that it becomes extremely important to factor in particle size, spore aggregation and spore viability into aerosol particle measurements.

Future Plans: Viable versus non-viable organisms in biological aerosols is not limited to laboratory investigations. The viability of organisms that make up naturally occurring bioaerosols (including pathogenic vectors), as well as those comprising aerosols of biological warfare agents disseminated with criminal intent, is not well understood. Future work is aimed at characterizing viability and its relationship to infectivity in natural settings (indoor and outdoor) in order to provide advice on managing and minimizing the spread of infection.

Sommaire

A Mathematical Approach Linking Aerosol Particles Obtained by Slit Sampling and Viable Organisms Obtained by Direct Inhalation Sampling

Scott Duncan; Jim Ho; DRDC Suffield TM 2007-250; R & D pour la défense Canada – Suffield.

Introduction: Il existe un souci renouvelé parmi les agences militaires et civiles, actuellement confrontées au problème émergeant d'une menace asymétrique globale concernant l'utilisation du *Bacillus anthracis* (charbon), que cet agent devienne l'arme préférée d'individus ou de groupes en quête d'actes terroristes. Le charbon, qui peut être facilement disséminé en aérosol, se disperse dans les courants d'air et se dépose sur des matériaux et des surfaces qui deviennent alors contaminés. Le fait de déranger les particules déposées provoque une nouvelle suspension en aérosol. On sait que les aérosols biologiques consistent à la fois en des organismes simples comme en des particules d'agrégats contenant de multiples organismes. Chaque organisme n'est cependant pas toujours viable ni capable, par exemple, de causer une infection. En ayant l'infectiosité comme perspective, on s'intéresse par conséquent à la relation entre le nombre viable de particules et le nombre viable d'organismes simples existant dans un aérosol biologique. On a développé un modèle qui donne aux chercheurs les outils pour examiner la totalité d'organismes infectieux dans un test de provocation d'aérosol organique ce qui par conséquent procure à l'armée et aux premiers intervenants une meilleure connaissance des dangers associés à l'exposition aux aérosols organiques (bioaérosols).

Résultats: On a employé deux méthodes d'échantillonnage différentes pour mesurer les spores *Bacillus atrophaeus* (BG) en aérosol biologique disséminé à partir d'une lettre contaminée. Ces deux méthodes consistaient en des échantillonneurs à fentes et en un milieu de filtre en microfibres situé à l'orifice d'inhalation d'une cartouche de respirateur porté par l'individu qui ouvrait la lettre. L'échantillonneur à fentes mesurait les particules en aérosol (agrégats de spores) alors que le filtre en microfibres mesurait les organismes individuels (spores). On a observé une différence importante entre le nombre de particules viables (30975) et le nombre d'organismes viables (1136600) mesurés par ces deux techniques. On a mis au point un modèle pour définir la relation entre le nombre de particules d'agrégats contenant des spores BG mesurées par les échantillonneurs à fentes et le nombre de spores individuelles viables comprises dans les particules mesurées par piégeage dans le milieu de filtre en microfibres. Le modèle part du principe d'une fonction de densité relativement simple pour la taille des particules et incorpore des hypothèses réalistes concernant les diamètres de particules, la masse volumétrique en vrac et la viabilité de la spore. La valeur prédite par le modèle, si corrigée pour tenir compte de l'inefficience de l'échantillonnage d'aérosol, diffère de ~10% du nombre actuel d'organismes viables mesurés par le filtre en microfibres à l'orifice d'inhalation de la cartouche du respirateur.

Portée des résultats: Les études sur les primates suggèrent que la DL_{50} pour les humains (dose létale suffisante pour tuer 50 % des personnes qui y sont exposées) est de 2,500 à 55,000 spores inhalées de charbon. La problématique des particules viables par rapport aux spores individuelles

viables a d importantes implications surtout en termes de l estimation de la dose l tale. Dans cette tude, les donn es d chantillonnage fentes qui sont une mesure des particules viables seulement, sont dans les limites de DL_{50} estim e pour le charbon. Les donn es obtenues du filtre en microfibres l orifice d inhalation d une cartouche de respirateur indiquent cependant que le nombre d organismes individuels viables exc de la limite sup rieure de la DL_{50} de plus d un ordre de grandeur. Ces r sultats sont videmment inqui tants. Les particules d agr gats contenant de multiples spores peuvent tre des sources d infections graves beaucoup plus r silientes et agressives et beaucoup moins de particules d agr gats seraient n cessaires pour pousser les expositions respiratoires dans la zone l tale. Le mod le indique qu il devient extr mement important de tenir compte de la taille des particules, de l agr gation des spores et de la viabilit des spores pour mesurer les particules en a rosol.

Perspectives d avenir: La comparaison entre les organismes viables et non viables contenus en a rosols biologiques ne se limite par aux tudes en laboratoire. On ne comprend pas encore bien la viabilit des organismes qui se forment naturellement en bioa rosols (y compris les vecteurs pathog niques) ni la viabilit de ceux compris dans les agents de guerre biologiques en a rosol diss min s dans une intention criminelle. l avenir, les travaux cibleront la caract risation de la viabilit et la relation de cette derni re avec l infectiosit dans un endroit naturel (l int rieur ou l ext rieur) pour tre en mesure de procurer des conseils concernant la gestion et la minimisation de la diss mination de l infection.

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1 Introduction

The infectivity of a disease causing microorganism such as *Bacillus anthracis* (anthrax) is of concern to researchers in aerobiology, medicine and protection/detection. There is renewed concern within military and civilian agencies, now faced with addressing an emerging global asymmetric threat, regarding the use of anthrax as a weapon of choice by individuals or groups contemplating a bioterrorist act [1,2,3]. The infectious vector for anthrax is a spore. It is particularly virulent for respiratory exposures [4,5]. If the disease goes undiagnosed, and in the absence of antibiotics, death may occur within 36 h from respiratory failure and bacteremia- and toxemia-related systemic complications [6,7,8]. The spores are extremely resilient and can remain infectious for decades [8,9]. Particle size also impacts on infectivity. Individual spores are ovoid in shape with the long axis approximately 1 μm in length. The most effective particle size for inhalation and retention in the lung alveoli is between 1 and 5 μm in diameter. Particles smaller than this are not retained, and those larger are trapped in the upper respiratory pathway [10]. Primate studies suggest that the LD_{50} for humans (lethal dose sufficient to kill 50% of the persons exposed to it) is 2,500 to 55,000 inhaled anthrax spores [11]. These values are similar to estimates of aerosol hazards to man resulting from "anthrax letter" terrorist incidents [12].

In recent years an increasing number of letters alleging to contain anthrax have been sent to health clinics, government offices and other sites in various countries around the world. Until October 2001 in the United States, all such "anthrax letter" incidents were hoaxes. The first Canadian "anthrax letter" incident occurred on 30 January 2001 at a federal government office in Ottawa, Ontario. Following this incident, a study was completed at Defence R&D Canada – Suffield (DRDC Suffield) to assess the hazard of an "anthrax letter" [13]. Envelopes containing a letter contaminated with non-pathogenic *Bacillus atrophaeus* (BG; formerly known as *B. subtilis var niger*, and *B. globigi*) spores, a recognised simulate for anthrax, were employed in the study. The setup and protocol were an attempt to mimic what might occur in an office, mail room or central registry environment if an envelope containing anthrax spores was received and opened. Slit samplers and filters were used to measure and track the aerosol release following the opening of the envelope. Although the opening of such an envelope was considered a "passive" form of dissemination, the results showed the dispersion to be far more effective than previously suspected.

The study presented addresses biological aerosols disseminated from letters contaminated with BG. Aerosols of BG are known to consist of both single organisms and particle-aggregates containing multiple organisms ranging in size from 1 μm to 9 μm [14,15]. However, not every individual spore, in the case of BG, is viable [14]. Thus, in a particle-aggregate comprised of many spores only a percentage may be viable. Accordingly, the relationship between the number of particles and the number of viable spores in a biological aerosol is of interest. We present results from two different methods of measuring aerosols resulting from opening contaminated letters: slit samplers and glass microfibre filter medium. The slit sampler is attractive because the approach, which uses nutrient growth medium for particle measurement, is linked to infectivity. To be labelled infectious, each individual particle must grow and replicate either in a host or in an artificial medium. One of the issues with slit samplers is that they measure the concentration of the aerosol specifically, and it is not possible to ascertain whether colony growth on the nutrient medium is the result of a single viable BG spore or a particle-aggregate containing multiple viable spores. In the second methodology, we placed glass microfibre filters at the inhalation port of a

respirator canister to measure the number of viable individual spores that would have been inhaled had a respirator not been worn. When the two data sets were compared, dramatically fewer BG-containing particles were collected by slit samplers than viable individual spores measured using the microfibre filter for the same exposure duration and aerosol challenge. The large discrepancy illustrates the need for establishing the relationship between number of aerosol particles and number of individual viable spores. From the perspective of hazard assessment and respiratory protection, the potential for infectivity will depend greatly on whether the aerosol is considered to consist of particles that are discrete, individual organisms, or particles that are comprised of aggregates of organisms. In this study we propose a model that links number of aerosol particles and number of individual viable spores through a relatively simple probability density function distribution for particle size, and which incorporates realistic assumptions regarding particle diameters, packing density, and spore viability.

2 Methodology

The letters contaminated with BG were prepared according to the procedure of Kournikakis *et al*.[13] 1.0 g of dry BG spores was placed in the middle of an 8.5 x 11 inch (21.6 mm x 27.9 mm) sheet of writing paper, which was then folded in thirds and inserted into a standard business envelope (10.5 mm x 23.5 mm) and sealed by wetting the adhesive strip on the flap of the envelope. The spore stock (donated to our laboratory by the US Department of Defense, Dugway Proving Ground, Utah), is in dry powder form. It has a concentration of $\approx 1x10^{11}$ cfu gm^{-1}. The BG contaminated letter was opened using a typical letter-opener knife which was inserted under the flap at the corner of the envelope and slit across the top. The letter was withdrawn from the envelope and unfolded as if it was to be read. As the BG spilled on to the desk the individual who opened the envelop dropped the letter and left it and the envelope on the desk, and thereafter remained motionless until the experiment was concluded. Opening of the letter was defined as time = 0 for the aerosol challenge. It is important to note that the release and dispersion of BG was a dynamic event that took place over time. It was not a singular point source release. Thus it is to be expected that the spatial aerosol concentration gradient will vary initially as the aerosol cloud develops and begins to disperse from the desk.

The experiments with BG contaminated letters were conducted in a typical office in a building located at DRDC Suffield. The office contained a desk and chair where the individual who opened the letter sat. During the experiment the door to the office remained open and the heating-ventilation system was operating. Two techniques were used in this study to measure the amount of BG aerosolised as a result of opening a contaminated letter. The first involved high resolution (HR) serial slit samplers to measure the concentration of BG in the air. Serial slit sampling is a simple concept that describes a set of electronically networked instruments, each with an address that can be activated in sequence or any combination thereof by means of a computer or timer [16]. The system permits continuous air sampling for biological particles over short to long time spans. The slit samplers currently used by DRDC Suffield are the result of several design changes based on specific biological aerosol sampling requirements. This instrument was used previously in the Brentwood postal station to sample anthrax aerosol [17]. Ten HR slit samplers were co-located on a 1 m^2 table approximately 1.5 m to the immediate right of the person who opened the contaminated letter. The slit samplers were connected in series to run sequentially for 2 min each

for a total run time of 20 min. During the 2 min sampling period of an individual slit sampler the nutrient medium collection plate makes one full revolution. The plate is divided into 30 sectors of 4 s and colony growth is determined in each, for a total of 30 data points per 2 min sample period. The average air flow sampling rate for the slit samplers was 28 L min^{-1}. Four replicate experiments were completed for this study. Prior to the BG-contaminated letter experiments, a full series of slit samplers was run to collect background aerosol data in the office. The nutrient agar plates from the HR slit samplers were incubated overnight at 37°C. Bacterial colonies were counted using an optical counter (Model CASBA 4, Spiral Biotech Inc., Norwood, MA, USA) to determine the number of colonies on the plate. Slit sampler data is typically presented in terms of agent containing particles per litre of air (ACPLA). The sampling efficiency of slit samplers has been shown to be 50% □70% for aerosol particles in the 1 to 5 μm range [18,19,20].

The second method involved collecting BG aerosol at the inhalation port of the filter canister on the air purifying respirator worn by the person who opened the letter. The collection medium was a glass microfibre filter (Whatman, GF/A). It was held in place by a holder that fit into the inhalation port of the filter canister. This trapped BG aerosol that would have been inhaled by the person opening the letter had they not been wearing a respirator. Clark et al. have demonstrated that similar button-type inhalation aerosol samplers have sampling efficiencies exceeding 90% [21]. In contrast to the constant air flow sampling of the slit samplers, the air sampling process in this method was tied to the respiratory cycle of the individual both in terms of the mechanism and the volume of air sampled; deposition of aerosolised BG in the microfibre filter was possible only as air was drawn through the filter during inhalation. Exhaled air does not pass back through the canister but exits the respirator at the front through a separate valve. BG aerosol was collected in this manner at the desk location for 30 min. Five replicate experiments were completed for this study. At the conclusion of the experiment the microfibre filters were placed in tubes and transferred to a wrist-action shaker. A known volume of distilled water was added to each and they were shaken for 15 min. This step was completed in order to break up particles comprised of aggregates of spores into individual organisms. The wrist action shaker method has been verified by plating aliquots of the BG spore suspension for different periods of shaking time until there was no change in the number of recovered viable organisms. Clark et al. [21] investigated both shaker agitation and ultrasonic agitation methods and found that the former resulted in higher total microbial counts in the extraction fluids. The supernatants were diluted in a 10-fold and 100-fold dilution series. Aliquots of these were plated using standard microbiology techniques. The plates were incubated and counted similar to the slit sampler plates. The results reflect growth of individual spores and are expressed in terms of colony forming units (CFU).

An aerodynamic particle sizer was used to collect particle size data on the BG aerosol released from the letter. This instrument was located approximately 1.5 m from the desk on which the BG spilled. Aerosol sampling commenced some time before the BG containing envelop was opened to confirm the background aerosol in the room and continued for the duration of the 30 min experiment.

3 Results and Discussion

Opening an envelope and removing and unfolding a letter contaminated with dry BG powder resulted in an immediate and significant dispersion of BG aerosol. Figure 1 presents the mean number of agent containing particles per litre of air (ACPLA) measured by the series of HR slit samplers over a period of 18 min from the time that the envelope was opened. The number of experimental replicates was 4. The data define a concentration versus time plot. Blank regions are due to a slit sampler that malfunctioned. Statistical variability in the bioaerosol concentration is evident, but not unexpected in a dispersing aerosol. Notwithstanding this, the trend in the concentration is well defined over time. There was a sharp rise in ACPLA within seconds of the envelope being opened which peaked at approximately 3.0 min. Thereafter, the number of ACPLA decreased with time following an exponential decay as shown by the fitted curve, which has the form

$$ACPLA = a \exp\left[-0.5 \left(\frac{\ln\left(\frac{t}{b}\right)}{c} \right)^2 \right] \qquad (1)$$

where t is the sampling time and a, b, c are constants defining the shape of the curve with values 128, 3.0 and 1.35 respectively. These data are consistent with a one-time dissemination event of particles from a common source and is caused by the dilution of the aerosol concentration as the particles disperse over a larger volume and settle-out by gravity and physical contact with surfaces. The area under the curve (AUC) for a total sampling period of 18 min was determined to be AUC=1510 particle min L^{-1}. This represents the mean dosage of viable BG-containing particles measured in the air due to opening a contaminated letter. Multiplying this value by the average flow rate of the HR slit samplers (28 L min^{-1}) gives an average total number of BG-containing particles collected by the slit samplers of 42,320 after 18 min.

The above particle measurements pertain specifically to the air sampling flow rates for the HR slit samplers. By comparison, the flow rate through the microfibre filter attached to the inhalation port of the filter canister was the pulmonary ventilation rate of the individual who opened the letter. Louhevaara *et al*. [22] measured the pulmonary ventilation on individuals sitting at rest wearing half face piece respirators with organic/particulate filtration canisters at approximately 10 L min^{-1}. This value was observed to increase to 24 L min^{-1} at low to moderate work levels. The results of a study by Jett□ *et al*. [23] on physiological effects related to wearing a military respirator at high aerobic work levels suggests that pulmonary ventilation may be somewhat higher for full face piece military respirators such as the one worn by the individual who opened the letter in our study. We were not able to measure the pulmonary ventilation rate of the person wearing the respirator. Therefore, based on the aforementioned literature studies, for a person sitting at rest we chose a slightly higher pulmonary ventilation value of 15 L min^{-1} to reflect an effective air flow through the inhalation port of the full face piece military respirator and canister system used in this study. Assuming that the number of BG particles collected by the HR slit

samplers is linearly related to the air sampling flow rate, the number of BG particles that would have been measured by the HR slit samplers at the more physiologically relevant air flow sampling rate of 15 L min^{-1} was calculated by multiplying the area under the curve in Figure 1 for a 30 min measurement period (mean estimated dosage equals 2065 Agent Containing Particles min L^{-1}) by the pulmonary ventilation of 15 L min^{-1} to give an estimated total number of BG-containing particles of 30,975.

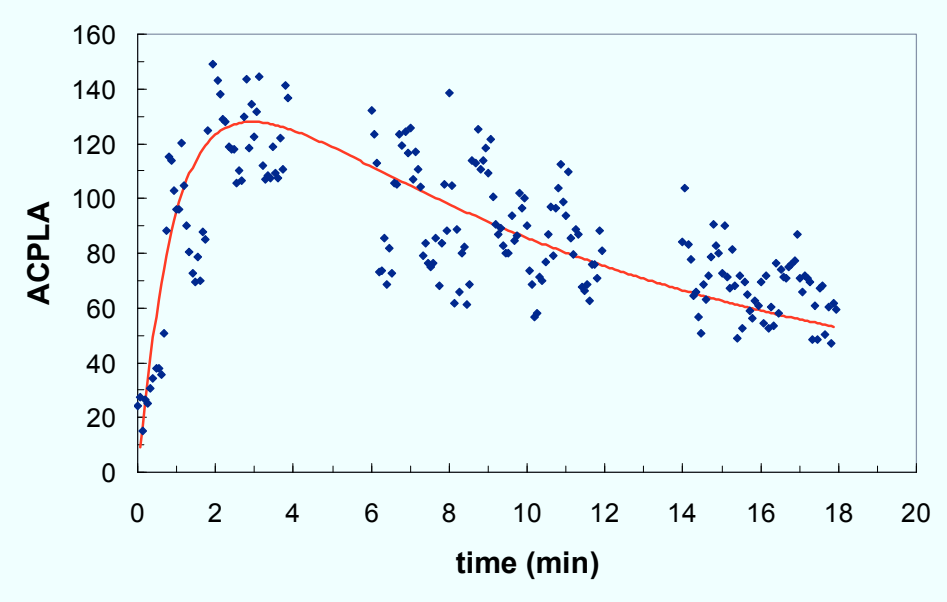

Figure 1: The number of agent containing particles per litre of air (ACPLA) measured by the High Resolution slit samplers beginning at time = 0 minutes when a letter contaminated with 1.0 g dry BG powder was opened. Total number of replicate BG letter dispersions was n=4. The exponential decay of the data is illustrated by the fitted solid curve (see text for more details).

Figure 2 shows the total number of CFUs determined to have been deposited on the microfibre filter located at the inhalation port of the canister. Also shown are background levels measured over a 30 min period prior to the letter opening experiments. The average number of CFUs for the five letter trials was 1,136,600 with a standard error of 195,200 (17%). The discrepancy between the total number of viable bioaerosol particles measured by the slit samplers and the number of viable individual organisms collected at the inhalation port of the filter canister was substantial, differing by a factor of 40. Accordingly, this may be accounted for by particles that were comprised of aggregates of BG spores over the entire particle size distribution of the bioaerosol.

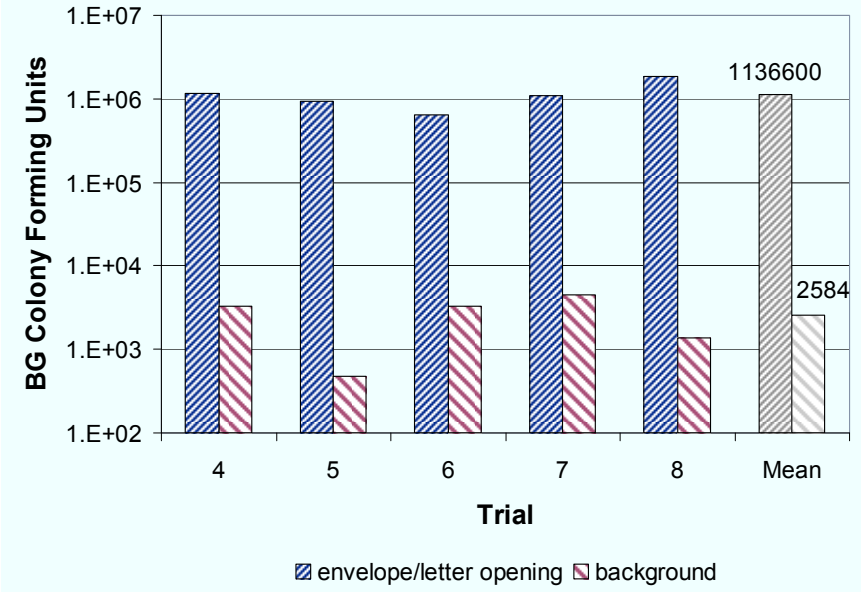

Figure 2: Total number of CFUs from viable BG spores deposited on the microfibre filter at the inhalation port of the canister worn by the individual who opened the letter contaminated with 1.0 g dry BG powder. Background values are also shown. The mean for each series of trials is presented on the right side of the graph.

3.1 Model Development

The proposed model was developed to address the considerable discrepancy observed between the number of viable BG-containing aerosol particles measured by the slit samplers and the number of viable organisms (CFUs) measured by the microfibre filters located at the inhalation port of the respirator canister. The former was calculated to be 30,975 BG-containing particles and the latter was determined to be 1,136,600 CFUs, assuming similar air flow sampling rates and a 30 min sampling time. At the simplest level, this suggests that each particle may contain approximately 37 viable BG spores. Measurements of the aerosolized BG dispersed from the contaminated letter showed that the bioaerosol consisted of a distribution of particles of various sizes. The smallest particle in fact would be a single spore, ellipsoidal in shape with the long axis approximately 0.75-1.0 μm in length. Particles larger than this would be due to the aggregation of spores to form multi-spore particle-aggregates and the relative fraction of these were observed to decrease with increasing particle diameter. A typical probability density function of the aerosol particle size distribution measured by the aerosol particle sizer is presented in Figure 3. The data have been fit with a function $f(\phi)$ to describe the distribution of particles with diameters ϕ continuous over the range shown. The form of $f(\phi)$ is assumed to be

$$f(\phi) = k_1 \exp(-k_2 \phi) \qquad (2)$$

where $f(\phi)$ is an exponential probability density function, ϕ is the particle diameter and k_1 is a normalization constant and k_2 is the exponential reduction constant, which define the shape of the distribution (see Figure 3). From Figure 3 it is evident that particles with diameters above 9.0 µm make up a very small proportion of the distribution. Accordingly, the particle diameter size distribution was truncated between 0.75 µm (single spore) and 9.0 µm. Therefore in the context of this model, the distribution of particles with diameters ϕ was considered continuous over the interval $0.75 \leq \phi \leq 9$ µm. Figure 4 is a bar graph showing the percent fraction of particles of a specific range in diameter for the probability density distribution $f(\phi)$. For example, approximately 70% of the number of BG-containing particles have diameters between 0.75 and 2 µm whilst only 0.1% of the particles have diameters between 8 and 9 µm.

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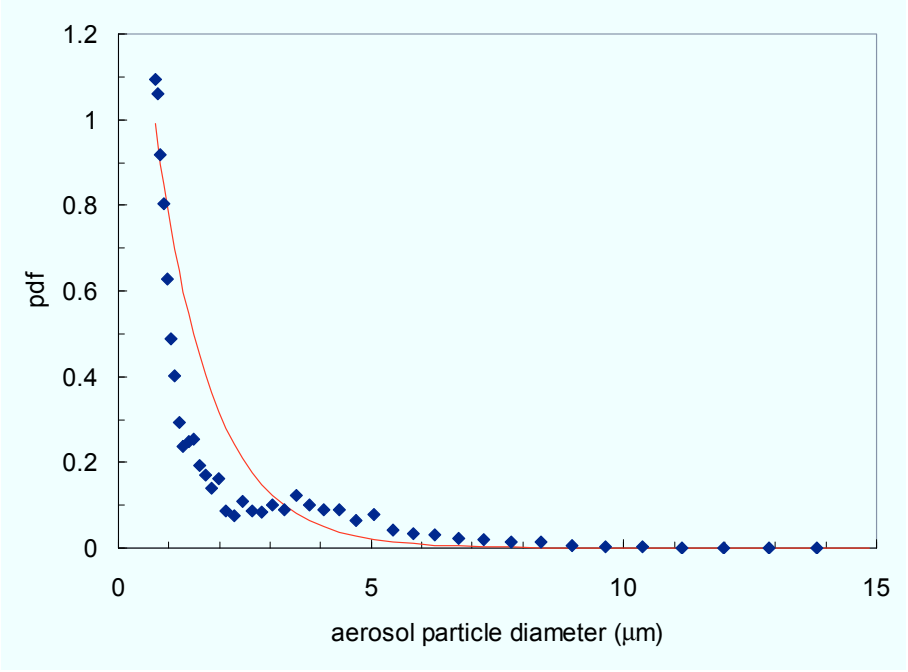

Figure 3: Probability density plot of BG aerosol data measured by aerosol particle sizer. Solid curve is a fit of the data to Equation 2. Constants k_1 and k_2 have values of 1.77 and 0.9 respectively.

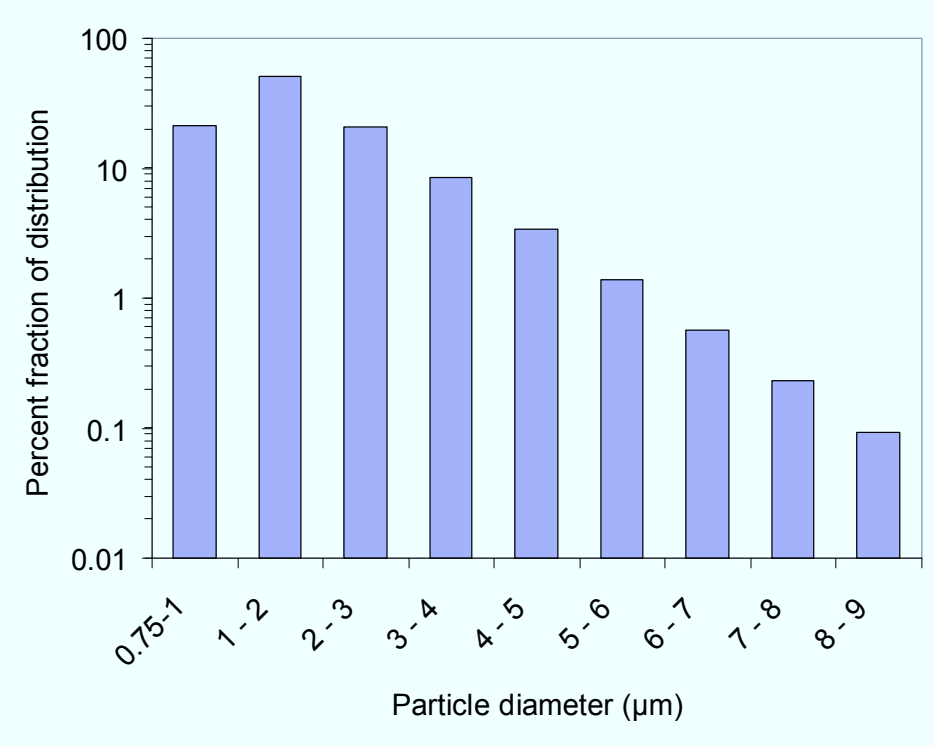

Figure 4: The percent fraction of particles of a specific range in diameter for the probability density distribution defined by Equation 2.

All aerosol particles in $f(\phi)$ were assumed to be spherical, with a volume $V_p(\phi)$. We also assume that aggregation of spores, whether it occurred pre- or post-aerosolisation, resulted in particles with a minimum spore packing density defined by η. Donev *et al.* [24] has shown by computer simulations that random ellipsoid packing densities may approach that of the densest sphere packings, ie, filling nearly 74% of space. Dividing V_p by the volume V_s of a single spore and multiplying by the packing density η, here defined as the fraction of total particle volume occupied by spores, gives the number of spores in a particle of diameter ϕ

$$N_s(\phi,\eta) = \frac{V_p(\phi)}{V_s}\eta \qquad (3)$$

An example is provided for clarity: given a particle with $\phi = 2$ μm, $V_p(\phi) = 4.189 \cdot 10^{-18}$ m^3, V_s = 9.82□10^{-20} m^3 (based on an ellipsoid shape with a long axis of 0.75 μm and the two short perpendicular axes 0.5 μm in length), and η = 0.60, the number of spores $N_s(\phi,\eta)$ in an aggregate comprising this particle was determined to be $\approx$ 26.

As was noted above, only a certain proportion of spores in a population are considered to be viable, ie, will germinate into culturable organisms. We correct for this inherent property by multiplying $N_s(\phi,\eta)$ by a spore viability coefficient, σ, to give the number of viable spores in a particle of diameter ϕ

$$N_{sv}(\phi,\eta) = N_s(\phi,\eta)\sigma \tag{4}$$

In the example just given above for $N_s(\phi,\eta) \approx 26$, assuming a spore viability coefficient of $\sigma = 0.35$ [14], the number of viable spores in a particle of diameter $\phi = 2$ μm becomes $N_{sv}(\phi,\eta) \approx 9$. Figure 5 shows the number of viable spores as a function of particle diameter ϕ calculated from Equation 3 and 4 over the range $0.75 \leq \phi \leq 9$ μm. As expected, this increases with a higher spore viability coefficient. It follows that to determine the total number of viable spores in a population of aerosol particles, Equation 4 must be applied over the entire particle size distribution $f(\phi)$. The expression for this is given by

$$N_{T_{sv}} = N_p \int_{\phi_l}^{\phi_u} N_{sv}(\phi,\eta,\sigma) f(\phi) d\phi \tag{5}$$

where N_p is the total number of BG aerosol particles measured by the slit samplers at a given air sampling flow rate and duration of time, ϕ_l is the lower limit of the aerosol particle diameter, ϕ_u is the upper limit of the aerosol particle diameter, $f(\phi)$ is given by Equation 2 and $N_{sv}(\phi,\eta,\sigma)$ is defined as the number of spores, adjusted for packing density and viability, in a particle of diameter ϕ (Equation 4). Equation 5 has an exact closed form solution

$$N_{T_{sv}} = \frac{N_p k_1 \eta \sigma}{ab^2}\left[\Psi(\phi_u) - \Psi(\phi_l)\right] \tag{6}$$

where

$$\Psi(\phi) = \left(-\frac{6}{k_2^4} - \frac{6\phi}{k_2^3} - \frac{3\phi^2}{k_2^2} - \frac{\phi^3}{k_2}\right)\exp(-k_2\phi)$$

and N_p is the total number of BG aerosol particles, η is the packing density, σ is the spore variability coefficient, a and b are the long and short axis lengths of the ellipsoid representing a single BG spore, ϕ_l is the lower limit of the aerosol particle diameter, ϕ_u is the upper limit of the aerosol particle diameter, k_1 and k_2 are constants that define the probability density exponential function given by Equation 2.

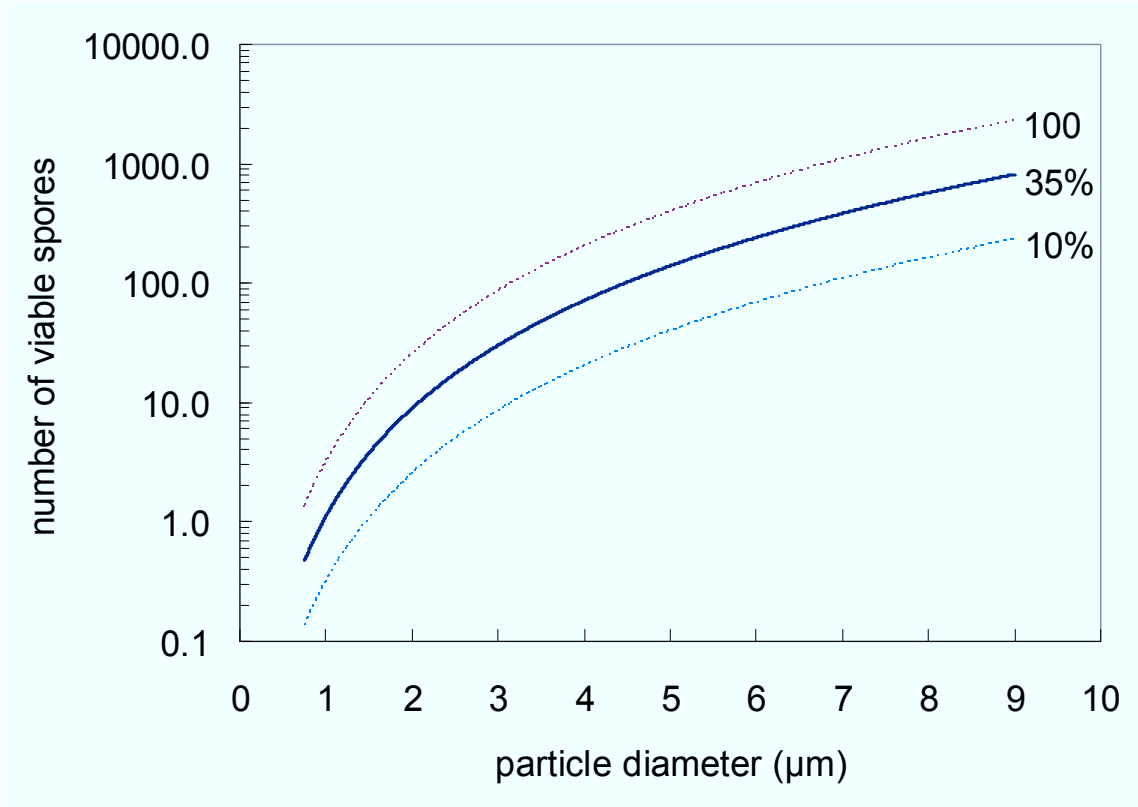

Figure 5: Calculated number of viable spores as a function of aerosol particle diameter. Packing density was kept constant at 0.6. Labeled curves are for spore viability. For example, middle curve, 35% of the spores in the aerosol particles were viable; these data were used in the model. Lower curve only 10% of the spores in each aerosol particle were viable, and upper curve, all the spores comprising the aerosol particle were viable.

Inputting N_p = 30975 into Equation 5 and calculating the expression inside the brackets using k_1 = 1.77, k_2 =0.9, η = 0.60, σ = 0.35, and ϕ_l = 0.75, ϕ_u = 9.0, the value for $N_{T_{sv}}$ was calculated to be ≈ 536,057 viable spores. Adjusting this value to take into consideration the sampling efficiency of the slit samplers gives an estimated 1,072,113 viable spores. This is within ~10% of the total CFUs that we measured by the microfibre filter sampling technique at the inhalation port of the respirator canister when similarly adjusted for sampling efficiency. Varying the packing density of spores in an aggregate particle has a significant impact on the total number of spores available within the particle distribution. Table 1 provides the effect of varying the packing coefficient on the predicted number of viable spores $N_{T_{sv}}$. The model shows that as the packing coefficient increases, which corresponds to a decrease in the void space within the particle-aggregate, the total number of viable spores for a given N_p is seen to increase.

Table 1: Effect of changing packing coefficient on total viable spores ($N_{T_{sv}}$) calculated by model. Spore viability coefficient was kept constant at 0.35. Assumed slit sampler efficiency of 50%. Shaded row shows model output values for $N_{T_{sv}}$ not adjusted for sampling inefficiency and adjusted for sampling inefficiency.

Packing Coefficient	$N_{T_{sv}}$	
	(not adjusted for sampling inefficiency)	(adjusted for sampling inefficiency)
0.1	89,343	178,686
0.2	178,686	357,371
0.3	268,028	536,057
0.4	357,371	714,742
0.5	446,714	893,428
0.6	536,057	1,072,113
0.7	625,399	1,250,799

In a study by Dahlgren et al. [25] examining anthrax exposure in workers at a goat hair mill, they determined that workers inhaled up to 500 anthrax particles of at least 5 µm in diameter in an 8 h shift. No workers contracted respiratory anthrax. Similar to BG, particle-aggregates of anthrax are expected to be comprised of viable and non-viable spores, numbers of which will depend on the size of the particle and packing density. Using the same input parameters as above, the model proposed here calculates that the workers might have been exposed to approximately 5910 individual viable spores, assuming a particle size distribution between 0.75 and 5 µm (no correction made for sampling efficiency). Clearly, within the context of the exposure scenario studied by Dahlgren *et al*. [25], a dosage of 500 particles over 8 h remained below the infectious limit. The model estimate of 5910 infectious organisms is at the low end of the LD_{50} based on primate data, and is therefore not inconsistent with the absence of respiratory anthrax observed by Dahlgren et al.

4 Summary

A model was developed to define a relationship between numbers of BG spore containing particle-aggregates measured by the slit samplers and numbers of individual viable spores comprising the particles measured by entrapment on a microfibre filter medium. The exponential probability density distribution in the model proposed here describes a particle size distribution wherein most of the particles in the aerosol would have diameters less than 3 µm. Using actual aerosol particle size distribution data collected by a aerodynamic particle sizer, an estimate was derived of the total number of individual viable spores in a population of particles measured by

slit samplers. The value calculated by the model, when corrected for aerosol sampling inefficiencies, differs by ~10% from the actual number of viable organisms measured by the microfibre filter at the inhalation port of the respirator filter canister. Our assumed value for packing density, while not based on an actual measurement, is a physically realistic number; it is less than the optimum packing density of spheroids (~75%) and similar to the minimum solid volume in a cubic-centred packing arrangement of spheres of equal size (~52%). The value for spore viability comes from a detailed study by Ho and Fisher [14] and should be highly representative of the BG used in this study. The authors acknowledge that near the source of an aerosol release the spatial aerosol concentration gradient can be very large. At the time of release one would not expect the aerosol concentrations at the slit sampler location and at the inlet to the respirator to be exactly the same. However, BG was measured by the slit samplers within seconds of opening the contaminated letter. Clearly, the aerosolised BG disperses rapidly outward from the location where the letter was opened. Given that aerosol sampling occurred for tens of minutes, it is not unreasonable to expect that over time, the transient differences in the aerosol concentrations between the two sampling locations would average out. The intention of the proposed model is not to show exactitude between the two sampling methods but rather to illustrate that only a relatively simple model using aerosol particle size distributions and first principle packing density and spore viability is necessary to satisfactorily explain the difference between the aerosol particle results obtained by slit sampling and viable organisms obtained by direct inhalation sampling.

The issue of viable particles versus viable organisms has wide implications, particularly in terms of estimated lethal dose. In this study, the slit sampler data, which is a measurement of numbers of viable particles only, falls within the estimated LD_{50} range for anthrax. However, the data obtained from the microfibre filter at the inhalation port of the respirator canister shows that the number of discrete individual viable organisms that may comprise such a distribution of particles, exceeds the upper end of the LD_{50} by over an order of magnitude. Clearly this is cause for concern. Particle-aggregates of multiple spores may be far more resilient and aggressive point-sources of acute infection, and far fewer particle-aggregates will be necessary to push respiratory exposures into the lethal range. The model demonstrates that it becomes extremely important to factor in particle size, spore aggregation and spore viability into aerosol particle measurements.

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List of symbols/abbreviations/acronyms/initialisms

DND	Department of National Defence
DRDC	Defence Research & Development Canada
BG	*Bacillus atrophaeus* (also called *Bacillus subtilis var niger*)
LD_{50}	lethal dose sufficient to kill 50% of persons exposed
ACPLA	agent containing particles per litre of air
CFU	colony forming units
HR	high resolution

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Two different sampling methods were employed to measure a biological aerosol disseminated from a contaminated letter: slit samplers and a microfibre filter medium located at the inhalation port of a respirator canister worn by the individual who opened the letter. Measurements obtained from slit samplers may only be interpreted in terms of numbers of viable particles. Particles may be comprised of single or multiple organisms. Microfibre filter data are presented in terms of the number of colony forming units, which refer to individual viable organisms. We observed a significant difference between the number of viable particles and the number of colony forming units measured by these two techniques. The former was calculated to be 30975 *Bacillus atrophaeus* (BG) containing particles and the latter was determined to be 1136600 CFUs, assuming similar air flow sampling rates. At the simplest level, this suggests that each particle may contain 37 viable BG spores. It is far more likely however, that aerosolised BG consists of a distribution of particles of various sizes which, depending on the volume of the particle, would contain different numbers of spores. A model was developed to define a relationship between numbers of BG spore containing particle-aggregates measured by the slit samplers and numbers of individual viable spores comprising particles measured by entrapment on the microfibre filter medium. From this an estimate was derived of the total number of individual viable spores in a population of aerosol particles measured by slit samplers. The model assumes a relatively simple probability density function distribution for particle size and incorporates realistic assumptions regarding particle diameters, packing density and spore viability. The value predicted by the model, when corrected for aerosol sampling inefficiencies, differs by only ~10% from the actual number of viable organisms measured by the microfibre filter at the inhalation port of the respirator filter canister.

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