



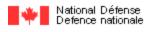
# Risk assessment of anthrax threat letters

Kournikakis, B.; Armour, S.J.; Boulet, C.A.; Spence, M.; and Parsons, B.

### DEFENCE R&D CANADA

Technical Report DRES-TR-2001-048 September 2001

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# **Risk Assessment of Anthrax Threat Letters**

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# **Defence Research Establishment Suffield**

Technical Report DRES TR-2001-048 September 2001

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# Abstract

In recent years an increasing number of letters alleging to contain anthrax have been sent to health clinics, government offices and other locations. While such "anthrax letter" incidents to date have proven to be hoaxes, all incidents must be treated as real until proven otherwise. Since no experimental studies on which to base a realistic assessment of the threat posed by these "anthrax letters" could be found, Defence Research Establishment Suffield (DRES) undertook a series of experiments to determine the extent of the hazard. In the experiments, envelopes containing *Bacillus globigii* spores (a simulant for anthrax) were opened in a mock mail room/office environment. The data measured on the dispersion of the spores were used to estimate if letters containing anthrax spores posed a significant health risk.

# Résumé

Depuis quelques années, il arrive de plus en plus souvent que des lettres censées contenir l'agent de la maladie du charbon (ou anthrax) soient envoyées à des centres de santé, à des bureaux gouvernementaux ou à d'autres endroits. Bien que, jusqu'à présent, ces incidents se soient révélés des canulars, il doivent tous être traités comme des menaces réelles, jusqu'à preuve du contraire. Étant donné que nous ne disposons pas d'études expérimentales sur lesquelles pourrait se fonder une évaluation réaliste du danger posé par les « lettres à l'anthrax », le Centre de recherches pour la défense Suffield (CRDS) a entrepris une série d'expériences pour déterminer l'importance de ce risque. Ainsi, des enveloppes contenant des spores de *Bacillus globigii* (une bactérie imitant l'agent de l'anthrax) ont été ouvertes dans un endroit simulant une salle de courrier ou un bureau. On a alors mesuré le degré de dissémination des spores, et les données obtenues ont servi à déterminer si les lettres contenant des spores d'anthrax posaient un risque important pour la santé.

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# Executive summary

In recent years an increasing number of letters alleging to contain anthrax have been sent to health clinics, government offices and other locations in the United States. While such "anthrax letter" incidents to date have proven to be hoaxes, all incidents must be treated as real until proven otherwise. The first Canadian "anthrax letter" incident occurred on 30 January 2001 at Citizenship and Immigration Canada (Ottawa, Ontario). An entire government building was shut down, illustrating how damaging and costly a single "hoax" letter can be. Since no experimental studies on which to base a realistic assessment of the threat posed by these "anthrax letters" could be found, Defence Research Establishment Suffield (DRES) undertook a series of experiments to determine the extent of the hazard. The data obtained in these experiments are now being used to provide guidance to first responders and other government departments.

In this study (non-pathogenic) *Bacillus globigii* (BG) spore contaminated envelopes, opened within the DRES aerosol test chamber (1,800 cu. ft.), were used to estimate the aerosol release from an "anthrax letter". 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 *Bacillus anthracis* (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 is a very "passive" form of dissemination, the results indicate the dispersion to be far more effective than initially suspected. Significant numbers of respirable aerosol particles (>99% in the 2.5 to 10  $\mu$ m size range) were released upon opening envelopes containing 0.1 or 1.0 grams of BG spores. Inhalational anthrax is virtually 100% fatal if left untreated. This study shows that a lethal dose could be inhaled within seconds of opening an anthrax spore filled envelope. If the person remained in the room for 10 minutes, the minimum time in which a HAZMAT team might be able to respond and effect a rescue, he could inhale 480 LD<sub>50</sub>s from a 0.1 gram filled envelope and 3,080 LD<sub>50</sub>s from a 1.0 gram filled envelope. In addition, the aerosol would quickly spread throughout the room so that other workers, depending on their exact locations and the directional air flow within the office, would likely inhale lethal doses. Envelopes with the open corners not specifically sealed could also pose a threat to individuals in the mail handling system.

Kournikakis, B.; Armour, S.J.; Boulet, C.A.; Spence, M.; Parsons, B. 2001. Risk Assessment of Anthrax Threat Letters. TR-2001-048 Defence Research Establishment Suffield.

# Sommaire

Depuis quelques années, il arrive de plus en plus souvent que des lettres censées contenir l'agent de la maladie du charbon (ou anthrax) soient envoyées à des centres de santé, à des bureaux gouvernementaux ou à d'autres endroits aux États-Unis. Bien que, jusqu'à présent, ces incidents se soient révélés des canulars, il doivent tous être traités comme des menaces réelles, jusqu'à preuve du contraire. Au Canada, le premier cas de « lettre à l'anthrax » s'est produit en janvier 2001 à Citoyenneté et Immigration Canada (Ottawa, Ontario). Un édifice gouvernemental au complet a été évacué, ce qui illustre bien les coûts et les dérangements que peut entraîner un simple canular. Étant donné que nous ne disposons pas d'études expérimentales sur lesquelles pourrait se fonder une évaluation réaliste du danger posé par les « lettres à l'anthrax », le Centre de recherches pour la défense Suffield (CRDS) a entrepris une série d'expériences pour déterminer l'importance de ce risque. Les données tirées de ces essais sont maintenant utilisées par d'autres ministères et des personnes travaillant comme premiers intervenant.

Dans cette étude, on a utilisé des enveloppes contaminées par des spores de *Bacillus globigii* (non pathogène), ouvertes à l'intérieur de l'enceinte d'essais du CRDS pour les aérosols (1 800 pi cu), pour évaluer la quantité de spores libérées dans l'air lors de l'ouverture d'une enveloppe contenant l'agent de l'anthrax. On a élaboré un plan et un protocole expérimental de manière à simuler ce qui pourrait se passer dans un bureau, une salle du courrier ou un secrétariat central si une enveloppe contenant des spores de *Bacillus anthracis* (agent de l'anthrax) y était reçue et ouverte. On s'est servi d'échantillonneurs à fente et de filtres pour mesurer et suivre la dissémination des aérosols à la suite de l'ouverture de l'enveloppe.

Bien que l'ouverture d'une telle enveloppe constitue une forme de dissémination très « passive », les résultats indiquent que la dissémination est beaucoup plus grande que ce qu'on avait prévu à l'origine. Des quantités importantes de particules aérosols inhalables (>99 % dans l'intervalle de grandeur de 2,5 à 10  $\mu$ m) ont été libérées lors de l'ouverture d'enveloppes contenant 0,1 ou 1,0 grammes de spores de *Bacillus globigii*. Lorsque l'anthrax est contracté par inhalation , il est fatal dans pratiquement 100 % des cas s'il n'est pas traité. L'étude indique qu'une dose mortelle serait inhalée dans les secondes suivant l'ouverture d'une enveloppe remplie de spores d'anthrax. Si la personne demeure dans la pièce pendant 10 minutes, délai minimal avant l'intervention d'une équipe HAZMAT, elle pourrait inhaler 480 LD<sub>50</sub> à partir d'une enveloppe contenant 0,1 gramme et 3 080 LD<sub>50</sub> à partir d'une enveloppe contenant 1,0 gramme de spores. En outre, l'aérosol se répandrait rapidement dans la pièce et d'autres employés pourraient vraisemblablement inhaler des doses mortelles, selon l'endroit où ils se trouvent et la circulation de l'air dans le bureau. Les enveloppes dont les coins ne sont pas entièrement scellés peuvent aussi présenter des risques aux individus qui les manipulent.

Kournikakis, B.; Armour, S.J.; Boulet, C.A.; Spence, M.; Parsons, B. 2001. Risk Assessment of Anthrax Threat Letters. TR-2001-048 Defence Research Establishment Suffield.

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# Introduction

In recent years an increasing number of letters alleging to contain anthrax have been sent to health clinics, government offices and other locations in the United States [1]. While such "anthrax letter" incidents to date have proven to be hoaxes, all incidents must be treated as real until proven otherwise. The first Canadian "anthrax letter" incident occurred on 30 January 2001 at Citizenship and Immigration Canada (Ottawa, Ontario) [2]. An entire government building was shut down, illustrating how damaging and costly a single "hoax" letter can be.

DRES has had many inquiries about the true risks from "anthrax letters". Since no experimental studies on which to base a realistic threat assessment of these "anthrax" letters could be found, DRES undertook preliminary experiments from 26 February to 11 April 2001 to obtain sufficient data to provide realistic and informed answers. In this study *Bacillus globigii* (BG) spore contaminated letters were opened within the DRES aerosol test chamber (1,800 cu ft) to measure the actual aerosol release resulting from the passive dissemination of the BG spores contained within the envelope.

This work is considered to be an important first step in providing a realistic threat assessment of "anthrax letters". These findings will be of value to the intelligence and counterterrorism communities, and to numerous other government departments as well as to the first responders, both military and civilian, who must deal with "real" incidents.

# **Bacterial spores**

*Bacillus globigii* spores (in dry powder form) were donated by the US Department of Defense (Dugway Proving Ground, Utah). Stock concentration powder was  $\sim 1 \times 10^{11}$  cfu/gm. *Bacillus globigii* (BG) spores are routinely used as a simulant for *Bacillus anthracis* (anthrax) spores.

# Aerosol chamber and sampling equipment

Experiments were conducted in the DRES Aerosol Chamber which was configured to represent a mail room, central registry or office. The chamber is  $18 \times 10 \times 10$  ft and has a volume of 1,800 cu ft. When operating, the air handling system recirculates the same air through the chamber at 1,050 cu ft/min.

Figure 1 illustrates the chamber and the sampling equipment. High and low resolution slit to agar samplers were used to collect aerosolized BG spores within the aerosol chamber.

A 6 x 4 x 3 ft table was placed at one end of the chamber to serve as a "desk". The work area in the center of the desk was surrounded by 10 high resolution (HR) slit samplers (locally made). The agar plate in samplers turned at 1 revolution per minute (rpm). The samplers were linked together to function sequentially, in the order given in the figure, to provide 10 minutes of continuous sampling. A second set of 10 high resolution samplers, also linked together to function sequentially, was placed on a table at the opposite end of the chamber.

Low resolution (LR) slit samplers (Model STA 203, New Brunswick Scientific Ltd, Edison, N.J., USA) were placed on either side of the chamber in middle and at the opposite end (see Figure 1). The agar plate in the low resolution slit samplers turned at 0.1 rpm. A fifth low resolution slit sampler, located in the center of the chamber, was used to take a background aerosol sample immediately prior to the start of each trial.

A Dichotomous sampler (virtual impacter, Model WSA 245, Grasby Anderson Inc., Atlanta, GA, USA) was used to collect samples onto 37 mm, glass fibre filters. The sampler is designed to collect particles in two size ranges, coarse (2.5 to 10  $\mu$ m) and fine (<2.5  $\mu$ m).

The canister on the respirator worn by the experimental subject was fitted with a special collection filter (37mm, glass fibre) to collect the bacterial spores that would have normally been inhaled by the clerk opening the envelopes.

Rodac plates were used to collect surface BG contamination from the protective clothing (Tyvek coveralls) worn by the subject.

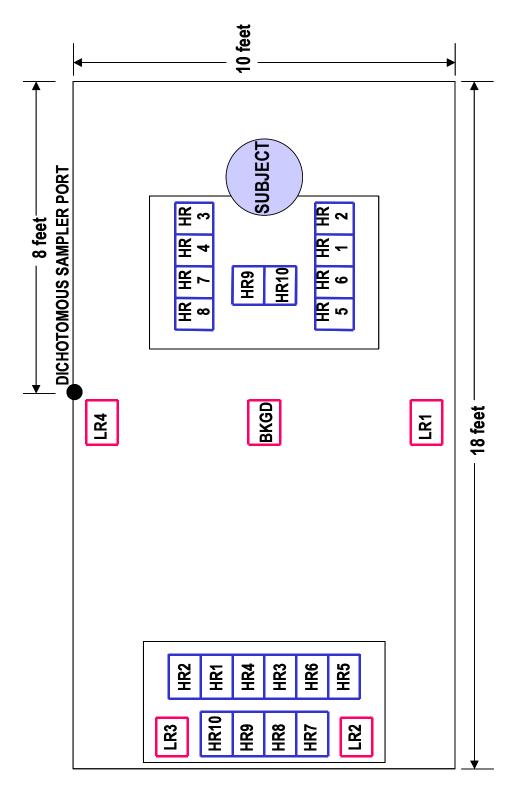


Figure 1. Layout of instrumentation in DRES aerosol chamber.

### "Letter" preparation

Since the authors were able to obtain little information on actual "anthrax letters", a standard letter was developed for the trials. It consisted of a standard business envelope ( $4.125 \times 9.25$  in), a single sheet of copier paper ( $8.5 \times 11$  in) and either 1.0 gram or 0.1 gram of BG spores. Initially, a fill of 1.0 gram was chosen as it appeared to be a "reasonable" amount and could not be detected by feeling the envelope. The fill was subsequently reduced to 0.1 grams because the very heavily contaminated slit sampler plates from the 1.0 gram release could not be accurately scanned.

A "letter" was prepared by putting BG spores in the center of a sheet of paper, folding it over into thirds, placing the folded sheet into the envelope and sealing using the adhesive present on the envelope. Nothing was done to seal the corners of the envelope where a letter opener would normally be inserted. The envelope was then shaken to mimic the handling and tumbling that would occur during its passage through the postal system. This "letter" was shuffled together with nine other, identical, untreated, "letters" so that the subject opening the envelopes would not know which one contained the BG spores and would, consequently, treat all the envelopes the same. In a preliminary trial, when the letters were shaken and shuffled in the aerosol chamber, an increase in the background aerosol concentration was observed. In the trials reported here, the letters where shaken and shuffled prior to their introduction into the chamber.

## **Experimental protocol**

Data in this report are from six trials conducted with the air recirculating system on, three with a 1.0 gram fill and three with a 0.1 gram fill.

Immediately before each trial, a 10 minute background aerosol sample was taken.

A subject wearing protective clothing and a Canadian C4 respirator played the role of a mail clerk. He was seated at the "desk" and used a metal letter opener to open the envelopes. At the start of the trial he pushed a switch to activate the samplers and then commenced opening the envelopes. He slit each envelope open, removed the sheet of paper, unfolded it and laid it on the desk. When he opened the contaminated envelope containing the BG spores, he laid the sheet of paper on the desk as he had done with the other sheets, stood up and backed up one step taking care not to disturb the contaminated letter further. He remained standing for the duration of the 10 minute sampling time. At the end of the sampling period, Rodac plates were used to collect surface BG contamination at several positions (back and front) on the protective clothing worn by the subject.

Following each experiment, the chamber air was vented to the outside through a HEPA filter and outdoor air was brought in through another HEPA filter.

## Sampling

Samples collected or plated onto nutrient agar plates were incubated overnight at 37°C. Bacterial colonies were scanned using a colony counter (Model CASBA 4, Spiral Biotech Inc., Norwood, MA, USA) to determine the number of colony forming units (cfu) present.

## Results

Data from the six trials with the recirculating air system on are illustrated in Figures 2 to 6 and tabulated in Table 1. In the first three trials 1.0 grams of BG spores were released (Figures 2, 3, 4,) and in the second series 0.1 grams was released (Figures 3, 5, 6). The trial number associated with each figure represents the day the experiment was done (e.g., Jan 1 = 001, Jan 2 = 002, etc). Figures 2 and 4 show data from a trial carried out with a 1 gram release on day 059. Figures 5 and 6 show data from a trial carried out with a 0.1 gram release on Day 092. Figure 3 shows photographs from trials carried out on Days 057 (1 gram release) and 064 (0.1 gram release).

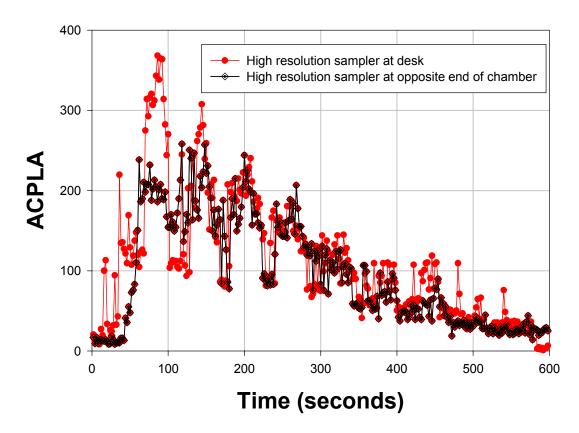
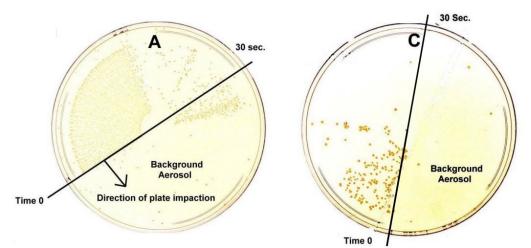


Figure 2. High Resolution Slit Sampler data from 1.0 gram release, Trial 059. The gray circles show the data collected from the samplers at the desk where the envelope was opened while the black diamonds show the data collected from the samplers at the opposite end of the aerosol chamber. Each curve is composed of data from 10 samplers which functioned in sequence.

The experimental protocol was designed so that the subject would not know which of the 10 envelopes placed at the "desk" in the aerosol chamber actually contained the BG spores. Consequently, zero time on each of the figures corresponds only to the opening of the first envelope, not necessarily the opening of the BG spore containing envelope. In all cases, however, the BG spore containing envelope was opened within the first 60 seconds of sampling. As illustrated in Figure 2, opening an envelope containing 1 gram of BG spores resulted in a sharp rise in the aerosol particle concentration (number of "Agent Containing Particles Per Litre of Air" (ACPLA)) that slowly declined over the course of the 10 minute sampling period.



A) 1st minute 1 gram release C) 1st minute 0.1 gram release



B) 2nd minute 1 gram release D) 2nd minute 0.1 gram release

Figure 3. Examples of High Resolution Slit sampler plates from 0.1 gram (Trial 064) and 1.0 gram (Trial 057) release of *Bacillus globigii* spores. The other trials showed similar results with the only difference being in how far the first plate of the series had rotated before the spore containing envelope was opened.

The number of ACPLA's is determined by scanning bacterial colonies growing on the slit plate. Ideally, the sampled aerosol concentration should be such that slit sampler collects only a modest number of viable particles on the surface of the slowly rotating agar plate. These particles will grow into distinct well defined bacterial colonies which can be accurately scanned. Figure 3A illustrates the first minute of sampling from a 1.0 gram release for a sampler located on the desk. Samples of the background aerosol concentration, represented on the bottom portion of the plate, resulted in the growth of very few colonies. By the time the 45 second mark was reached, the aerosol concentration was very high, resulting in heavy bacterial growth which interfered with the accuracy of scanning. The pattern of heavy growth continued through the sampling period as represented by the plate from the second minute of sampling (Figure 3B).

The aerosol, produced by opening the BG spore containing envelope, was not confined to the area of the desk but spread throughout the chamber. ACPLA values were almost as high at the opposite end of the chamber, shortly after opening the envelopes (Figures 2, 5).

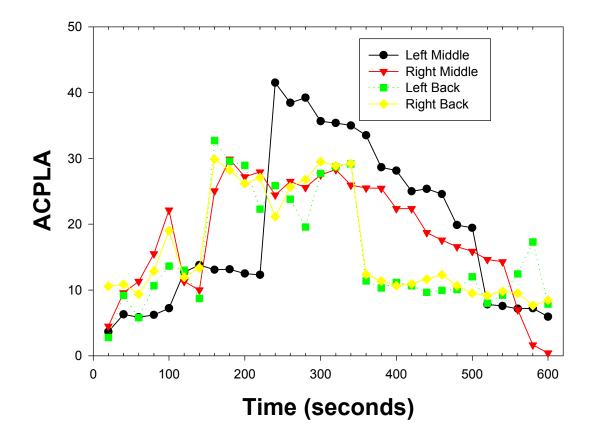


Figure 4. Low Resolution Slit Sampler data from 1 gram release, Trial 059 (4 sampler locations).

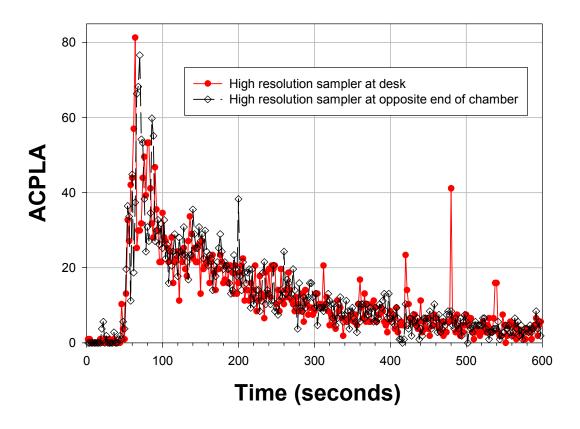


Figure 5. High Resolution Slit Sampler data from 0.1 gram release, trial 092. The gray circles show the data collected from the samplers at the desk where the envelope was opened while the black diamonds show the data collected from the samplers at the opposite end of the aerosol chamber. Each curve is composed of data from 10 samplers which functioned in sequence.

In the second series of experiments, 0.1 grams of BG spores were used. Aerosol distributions, similar to those observed for the 1.0 gram releases, were observed for the 0.1 gram releases. The high resolution slit sampler at the desk showed a sharp rise in aerosol particle concentration after opening the envelope followed by a decline over the 10 minute sampling period (Figure 5). Similar data were observed from the high resolution slit sampler at the other end of the chamber (Figure 5) and the low resolution slit samplers (Figure 6). The bacterial colonies growing on the slit sampler plates (Figures 3C, 3D) were clearly visible as well defined distinct colonies which could be accurately scanned.

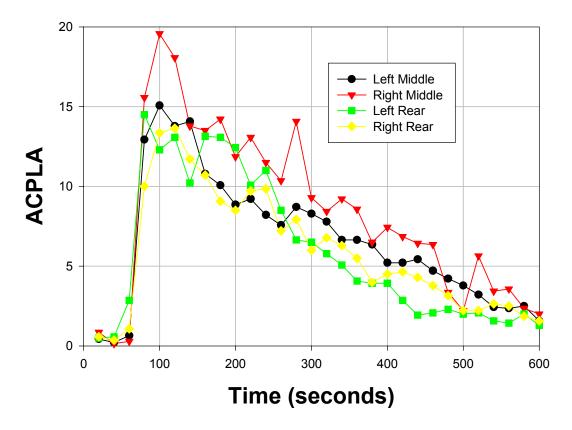


Figure 6. Low Resolution Slit Sampler data from 0.1 gram release, Trial 092 (4 sampler locations).

During each trial, particles were collected by a Dichotomous Sampler and on the filter on the canister worn by the subject. Data (Table 1) was reasonably consistent from trial to trial and, as expected, the mean concentration from the 1.0 gram releases was about 10 times that of the 0.1 gram releases.

In all cases, greater than 99% of the total particles collected by the dichotomous filter were collected by the coarse filter indicating that the particles were in the 2.5 to 10  $\mu$ m size range.

The Rodac plates from the first few trials were so heavily contaminated that they could not be accurately counted. Consequently, Rodac plates were not used for the remainder of these experiments.

Table 1. Filter Collection Data					
Trial Day	Canister Filter (total cfu; 10 min sample)	Dichotomous Filter (cfu/L air)			
057	1.0 x 10 <sup>7</sup>	9.5 x 10 <sup>4</sup>			
059	4.8 x 10 <sup>6</sup>	6.4 x 10 <sup>4</sup>			
060	5.3 x 10 <sup>6</sup>	7.3 x 10 <sup>4</sup>			
(Mean <u>+</u> S.E.)*	$(6.7 \pm 1.7) \ge 10^6$	$(7.7 \pm 0.9) \ge 10^4$			
067	6.6 x 10 <sup>5</sup>	1.8 x 10 <sup>4</sup>			
074	8.9 x 10 <sup>5</sup>	8.6 x 10 <sup>3</sup>			
092	8.6 x 10 <sup>5</sup>	8.0 x 10 <sup>3</sup>			
(Mean <u>+</u> S.E.)*	$(8.0 \pm 0.7) \ge 10^5$	$(1.2 \pm 0.3) \ge 10^4$			
Note: First 3 trials reported here used envelopes with a 1 gram BG spore fill while the second set of 3 trials used a 0.1 gram BG spore fill.					
* (mean total cfu ± standard error.)					

# Discussion

Although "anthrax letters" have been sent to many locations in the United States over the past few years [1], the first incident in Canada occurred on 30 January 2001 [2, 3]. This incident in which a letter claiming to contain anthrax was sent to the Office of the Minister of Citizenship and Immigration [2, 3] was rapidly followed by hoax letters sent to Ontario government offices in Toronto and to a Wal-Mart store in Victoria [4].

When requested to assess the risk from "anthrax" letters, DRES staff was not able to locate any experimental data on which to base a realistic threat assessment. The experiments described in this report were designed to produce quantitative information on the amount of material aerosolized when an envelope containing BG spores (to simulate anthrax spores) was opened under conditions representative of that in a mail room, central registry or office environment so that a preliminary assessment of the threat posed by opening an "anthrax letter" could be estimated.

Before starting the experiments, it was assumed the opening of an envelope constituted a very "passive" form of dissemination that would produce minimum aerosolization of the BG spores unless additional energy was added to aid dissemination (e.g., panic behavior, strong air flows). This assumption proved incorrect. Almost immediately upon opening the envelope a significant aerosol concentration was observed in the area of the "desk" (Figure 2) which declined slowly over the 10 minute sampling period. The high resolution slit sampler plates themselves (Figure 3) were so densely packed with bacterial colonies that the concentrations (ACPLA values) for the 1 gram trials are believed to be artificially low due to the difficulty in scanning such densely packed plates. The high concentration, and rapidity with which the aerosol spread to the other end of the chamber (Figures 2, 3) was also unexpected. The very heavy contamination on the back and front of the clothing worn by the subject was also unexpected.

To increase the accuracy of the high resolution slit sampler data, it was necessary to reduce the density of the bacterial colonies so that the plates could be more accurately scanned. This was accomplished by decreasing the BG spore fill to 0.1 gram. As is shown in Figure 5, the initial concentration upon opening an envelope containing 0.1 grams of BG spores exceeded 80 APCLA. This can be compared with the 350 APCLA observed upon opening the envelope containing the 1.0 gram fill (Figure 2). Thus, the suspicion that the densely packed slit plates were underestimated was confirmed.

As observed with the 1 gram letter, the aerosol produced on opening the 0.1 gram envelopes quickly dispersed throughout the chamber and decayed slowly over the 10 minute sampling period (Figure 5).

In all cases, greater than 99% of the total particles collected by the dichotomous sampler were collected by the coarse filter, indicating that the particles were in the 2.5 to 10  $\mu$ m size range. Particles in this size range do not consist of single spores but of agglomerations of several spores [5] and constitute a definite respiratory hazard [6].

While the slit sampler data provided clear evidence of the rapid creation of the aerosol, the time course of its dispersion through the chamber, and its slow decline, the data from the canister filter and dichotomous filter (Table 1) can be used to assess the potential threat from the aerosol.

In their consensus statement on "Anthrax as a Biological Weapon", Inglesby *et al* [5] state that, "Based on primate data, it has been estimated that for humans the  $LD_{50}$  (lethal dose sufficient to kill 50% of the persons exposed to it) is 2,500 to 55,000 inhaled anthrax spores."

The data from the canister filter collection indicates the number of aerosol particles the subject in the chamber could have inhaled had he been an unprotected office worker exposed to a real "anthrax letter". Each viable aerosol particle contains one or more viable spores and, when cultured, will produce a colony (one cfu). In the following calculation it is assumed that one particle is equivalent to one cfu whereas a single particle may contain several viable spores. Consequently, an exposure based on cfu may underestimate the actual number of viable spores to which the person was exposed. If the worst case scenario is assumed and the lower figure of 2,500 spores is used as the LD<sub>50</sub>, the subject could have inhaled 320 LD<sub>50</sub>s upon opening the 0.1 gram envelope and 2,680 LD<sub>50</sub>s from the 1 gram envelope. If the higher figure of 55,000 spores is used as the LD<sub>50</sub>, he could have inhaled 15 LD<sub>50</sub>s from the 0.1 gram envelope.

The data from the dichotomous filter collection provides a measure of the particle concentration in the chamber air. Using this value and the minute volume, a calculation can be made of the number of particles the subject in the chamber could inhale during a 10 minute exposure. The minute volume depends on activity, sex, age, weight and physical condition. The Radiological Health Handbook [7] gives minute volumes of 7.5 L/min at rest and 20 L/min doing light work for a 70 kg adult male. For moderate exercise, a range of 22.5-49 L/min is given for adult males and 22-25 L/min for adult females [8]. Under stress the minute volume is expected to increase and values of up to 50 L/min were observed for novice parachutists before the jump and after landing [9].

To calculate the exposure an unprotected person could receive if he remained in the chamber for the 10 minute exposure period the following assumptions were made: (a) uniform mixing is assumed for the chamber (e.g., the concentration measured by the dichotomous filter is truly representative of the concentration throughout the chamber) (b) a minute volume of 10 L/min is representative for a person doing office work. If the worst case scenario is assumed and the lower figure of 2,500 spores is used as the LD<sub>50</sub>, persons in the chamber could have inhaled 480 LD<sub>50</sub>s when the 0.1 gram envelope was opened and 3,080 LD50s from the 1 gram envelope. If the higher figure of 55,000 spores is used as the LD<sub>50</sub>s from the 1 gram envelope. If the breathing rate of the person were to increase due to the stress of the situation (e.g., 20-30 L/min) these exposures would double or triple (e.g., 9,240 LD<sub>50</sub> exposure from a 1 gram release and a 30 L/min breathing rate).

All the experiments described in this study were carried out with the HVAC air recirculation system in the chamber operating at its normal rate (1,050 cfm). Follow-on experiments in still air (with the HVAC system in the chamber turned off) have been initiated. Results to date (data not shown) show that the aerosol takes longer to diffuse through the chamber and that

higher concentrations persist in the area where the envelope is opened. This suggests that the airflow in the chamber is not the major cause of the aerosolization.

In summary, so called "passive" dissemination of anthrax spores from an envelope presents a far more serious threat than had been previously assumed. Not only is the person opening the envelope at risk, but so are other people in the same room. The quantity of spores needed to cause significant injury is so small it cannot be casually detected by feeling the unopened envelope. This small quantity needed could be mailed, undetected, anywhere in the world. The dramatic results, obtained in this study of a very basic scenario, clearly indicate the need to fully understand the risks and devise improved methods of consequence management. It is only a matter of time until a real "anthrax letter" arrives in some mail room.

During the course of these experiments, DRES staff became aware of similar experiments being carried out by the Ottawa-Carleton First Responder Group [10]. The experiments, which were conducted in the Ottawa-Carleton Regional Police Service Forensic Identification Laboratory, used 3.5 grams of Redwop Finger Print Powder (Brilliant Red) as the anthrax simulant. Two different sized envelopes (8.5 x 11 in and 9.5 x 4 in) were opened by hand or with a letter opener and contamination was measured using an UV light source. When some letters were handled prior to opening, sufficient powder leaked out through the unsealed corners of the envelope to contaminate the subject's hands and other files and papers on the desk. All trials showed that contaminant could be dispersed in the immediate and surrounding area, on the person opening the envelope, on fomites (letter openers, pens, papers, files) and into the exhaust HVAC grilles. As in the case of the DRES experiments, these experiments show that a real "anthrax letter" would pose a serious threat to not only the person opening the envelope but to others in the room. If the envelope was not completely sealed (e.g., specifically sealing the open corners), it could also pose a threat to individuals in the mail handling system.

# Follow-on

The information obtained in these experiments was used in the preparation of the brochure "Anthrax Letter – Background Information and Response Guidelines", prepared by DRES at the request of the Provost Marshall (see Annex A).

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# References

1. Bioterrorism Alleging Use of Anthrax and Interim Guidelines for Management – United States, 1998, *MMWR*, February 05 **1999**, 48, 69-74.

- 2. John Ward, Bacterial envelope mailed to minister, *Medicine Hat News*, 1 February 2001.
- **3**. Jeff Sallot, Hoax highlights vulnerability to bioterrorism, *The Globe and Mail*, 2 February 2001.
- 4. Garry Ackerman and Jeffrey Allan, Will we be ready, *The Globe and Mail*, 8 February 2001.
- 5. T.V. Inglesby, D.A. Henderson, J.G. Bartlett, M.S. Ascher, E. Eitzen, A.M. Friedlander, J. Hauer, J. McDade, M.T. Osterholm, T. O'Toole, G. Parker, T.M. Perl, P.K. Rusell, K. Tonat, Anthrax as a Biological Weapon: Medical and Public Health Management, *JAMA*, **1999**, 281, 1735-45.
- 6. E.M. Eitzen, Use of Biological Weapons, in Textbook of Military Medicine, Medical Aspects of Chemical and Biological Warfare, F.R. Sidell, E.T. Takafuji and D.R. Franz, ed. 1997, 437-50.
- 7. Radiological Health Handbook, Public Health Service, U.S. Department of Health, Education and Welfare, Rockville, MD, **1970.**
- 8. P.L. Altman and D.S. Dittmer, Respiration and Circulation, Biological Handbooks, Federation of American Societies for Experimental Biology, Bethesda, MD, **1971**.
- 9. W.D. Fenz and S. Epstein, Gradients of physiological arousal in parachutists as a function of an approaching jump, *Psychosomatic Medicine*, **1967**, 29, 33-51.
- 10. M. Callaghan, D. Raymond, S. McLennan and M. Best, Investigation of Dispersal Patterns of Contamination in Letters, private communication, 9 April 2000.

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# List of symbols/abbreviations/acronyms/initialisms

DND	Department of National Defence
ACPLA	Agent containing particles per litre of air
CFU	Colony forming unit
LD <sub>50</sub>	Lethal Dose 50%

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#### ANNEX A

Brochure "Anthrax Letters - Background Information and Response Guidelines"

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#### **References:**

Approval of Cipro® for Use After Exposure to Inhalational Anthrax (<u>http://www.fda.gov/bbs/topics/ANSWERS/ANSO1030.html</u>)

B. Kournikakis, S. J. Armour, C. A. Boulet, M. Spence and B. Parsons, Risk Assessment of Anthrax Threat Letters, Technical Report 2001-048, Defence Research Establishment Suffield, in press.

Bioterrorism Alleging Use of Anthrax and Interim Guidelines for Management – United States, 1998, *MMWR*, 48 (**1999**) 69-74. (http://www.cdc.gov/mmwr/preview/mmwrhtml/00056353.htm

How to Handle Anthrax Threats in the Office, Indianapolis-Marion County, Emergency Management Agency, Safety Bulletin (<u>http://www.indygov.org/ema/anthrax.safety-Anthrax</u>)

WMD Threats: Sample Guidelines, National Domestic Preparedness Office, 15 March 1999.

Anthrax Facts, Special Bulletin, National Domestic Preparedness Office, 6 April 1999.

Anthrax Fact Sheets, Centers for Disease Control and Prevention, National Center for Infectious Diseases, Division of Bacterial and Mycotic Diseases, November 2000. (http://www.cdc.gov/ncidod/dbmd/diseaseinfo/anthrax\_g.htm)

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Anthrax Letters Background Informatic

DEFENCE

#### Background

In recent years there have been an increasing number of incidents in which letters alleging to contain anthrax have been sent to health clinics, government offices and other locations. While all such incidents to date have proven to be hoaxes, the threat is real. All incidents must be treated as real until proven otherwise! This will minimize the risk in the event of a real incident and protect the crime scene.

The guidelines provided here are related to **anthrax threats only** and are not all inclusive but provide a reasonable variety of potential situations.

Local management should consider having an in-house Emergency Response Plan to deal with biological threats. If a threat letter is received, **remain calm!** Even if the letter or package really does contain anthrax spores, you are not in immediate danger. Anthrax is not transmissible from person to person.

Common features of previous anthrax threat letters included: no return address, excess postage, hand written or poorly typed address, misspelling of common words, restrictive marking such as "personal", "confidential", excessive weight or a feeling of a powdery substance.





# **DRES Defence Research Establishment Suffield**

# Background Information and Response Guidelines



However, it cannot be assumed that future letters will appear at all out of the ordinary.

n If a suspicious letter or package is unopened and no material is leaking out, or if a letter or package is opened and no powder is contained within (but there is a claim of anthrax), the risk of contracting anthrax is extremely low.



# RESPONSE GUIDELINES

#### For the Person(s) Immediately Exposed

You have opened a letter or package that claims to contain anthrax and has powder inside the envelope. The powder may or may not have spilled out onto your desk, the floor, your hands or your clothing.

#### The key to controlling the situation is not to panic and to limit the exposed area and people.

Your first goals are to relax, remain calm and contain the contamination as follows:

- Do not disturb the letter any further. Do not pass it around. Do not try to clean up the powder or brush it off your clothing.
- Stay in your office or immediate work area. This also applies to co-workers in the same room. Lock the door, if applicable, to prevent others from entering the area and becoming contaminated. Remember you are not in immediate danger.
- Call for help. This may be to your supervisor, an in-house emergency response or to 911 depending on your own situation.
- Keep your hands away from your face to avoid contaminating your eyes, nose or mouth.
- If possible (without leaving your workspace), wash your hands.
- If possible have the building ventilation system shut down and turn off any fans or equipment that is circulating air around your workspace.
- Wait for help to arrive.

#### For the First Responder

You are responding to an incident involving the alleged use of anthrax. Most incidents are hoaxes, however, all must be treated as if real. Whether or not anthrax is present a criminal event has occurred. You must arrive on the scene with appropriate protective gear (to avoid becoming affected yourself) and decontamination supplies (including dilute bleach (1 part household bleach plus 9 parts water)), water, plastic bags, coveralls (for affected persons who need fresh clothing, etc).

Your first goals are to secure the area to minimize the spread of contamination and protect the crime scene (for later evidence collection), to decontaminate those persons affected and to remove them for medical treatment.

Decontamination procedures for affected persons will vary depending on the degree of exposure. All persons in the room are at risk. The person who opened the envelope and any others who came into direct contact with the envelope after it was opened are at greatest risk. Assist affected persons by:

- Wetting their clothing down with water or dilute bleach (e.g., with a garden sprayer) to reduce the risk of secondary aerosols.
- Removing their clothing and personal items (e.g., watches, rings, keys) and placing them in marked plastic bags.
- Having them wash their hands.
- Having them change into fresh clothing.
- If a shower is available in the building, have them take a thorough shower with soap and water and change into a second set of fresh clothing.

If a shower is not available, you must transport the affected persons to hospital for decontamination and treatment. (Affected persons properly decontaminated on scene do not need to be decontaminated again at the hospital.)

Once at the hospital, affected persons must be seen by a physician. Antibiotic treatment should be provided immediately. The US Food and Drug administration approved the use of Ciprofloxacin for the treatment of inhalational anthrax and made the following recommendation on administration of the drug:

"The recommended adult dose of Cipro® for postexposure inhalational anthrax is 500 milligrams given orally twice a day. The recommended pediatric dose of Cipro® for post-exposure inhalational anthrax is 15mg/kg given orally twice a day. The adult intravenous dose is 400 mg twice a day; the pediatric intravenous dose is 10 mg/kg twice a day. Treatment with Ciprofloxacin should begin as soon as possible. The drug should be administered for a total of 60 days."

Some personal items that may have been contaminated may be decontaminated using dilute bleach (e.g. keys).

Bacillus anthracis



#### What is anthrax?

Anthrax is an acute infectious disease caused by the spore-forming bacterium Bacillus anthracis. Anthrax most commonly occurs in wild and domestic animals (cattle, sheep, goats, antelopes, bison and other herbivores). Anthrax can also occur in humans when they are exposed to infected animals or tissue from infected animals. Anthrax is found globally.

#### How is anthrax transmitted?

Anthrax infection can occur in three forms: cutaneous (skin), the most common form, inhalation, and gastrointestinal. Bacillus anthracis spores can live in the soil for many years. Humans can become infected with anthrax by handling products from infected animals, by inhaling anthrax spores from contaminated animal products or by eating undercooked meat from infected animals. Anthrax is not transmissible from person to person.

Intestinal: Gastrointestinal anthrax, characterized by an acute inflammation of the intestinal tract, may follow the consumption of contaminated meat. Initial signs of nausea, loss of appetite, vomiting, fever are followed by abdominal pain, vomiting of blood, and severe diarrhea. Intestinal anthrax results in death in 25% to 60% of cases.

### How is anthrax diagnosed?



#### What are the symptoms of anthrax?

Symptoms usually occur within 7 days and vary depending on how the disease was contracted.

Cutaneous: Most (about 95%) anthrax infections occur when the bacterium enters a cut or abrasion on the skin. Skin infection begins as a raised itchy bump resembling an insect bite that develops into a vesicle within 1-2 days and then a painless ulcer, usually 1-3 cm in diameter, with a characteristic black necrotic center. Lymph glands in the adjacent area may swell. Deaths are rare with appropriate antibiotic treatment but occur in about 20% of untreated cases.

Inhalation: Initial symptoms may resemble a common cold. After several days, the symptoms may progress to severe breathing problems and shock. Inhalation anthrax results in death in greater than 90% of cases, unless promptly treated with appropriate antibiotics.

Anthrax is diagnosed by isolating Bacillus anthracis from the blood, skin lesions, or respiratory secretions or by measuring specific antibodies in the blood of persons with suspected cases.

#### Is there a treatment for anthrax?

Yes, antibiotic treatment should be initiated as soon as possible.

If left untreated, the disease can be fatal.

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#### 14. ABSTRACT

(U) In recent years an increasing number of letters alleging to contain anthrax have been sent to health clinics, government offices and other locations. While such "anthrax letter" incidents to date have proven to be hoaxes, all incidents must be treated as real until proven otherwise. Since no experimental studies on which to base a realistic assessment of the threat posed by these "anthrax letters" could be found, Defence Research Establishment Suffield (DRES) undertook a series of experiments to determine the extent of the hazard. In the experiments, envelopes containing Bacillus globigii spores (a simulant for anthrax) were opened in a mock mail room/office environment. The data measured on the dispersion of the spores were used to estimate if letters containing anthrax spores posed a significant health risk.

15. KEYWORDS, DESCRIPTORS or IDENTIFIERS

(U) Descriptors: Terrorism; Anthrax; Biological agents; Unconventional warfare; Aerosols; Antipersonnel agents; Procedures; Safety; Threat evaluation; Sampling; Biological agent detection; Simulation; Biological agent simulants

Identifiers: Biological warfare; Hoax letter; Air circulation; Mail processing centres; Mailrooms



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