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Secondary Aerosolization of Viable *Bacillus anthracis* Spores in a Contaminated US Senate Office

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ON OCTOBER 15, 2001, A LETTER containing threatening language and a light tan powdery substance was opened in the mail handling area of a Senate office suite in the Hart Senate Office Building, Washington, DC. Federal officials removed the letter and shut down the local air handling systems. The letter was transported to the US Army Medical Research Institute of Infectious Disease and was subsequently confirmed to contain viable *Bacillus anthracis* (anthrax) spores that were dispersible in air.¹ Scanning electron microscopy of the spores used in the Senate office attack showed that they ranged from individual particles to aggregates of 100 μm or more. Spores were uniform in size and appearance and the aggregates had a propensity to pulverize¹ (ie, disperse into smaller particles when disturbed).

Following the attack, nasal swabs were collected by other investigators from more than 7000 building occupants and cultured for *B anthracis*. Twenty of 38 individuals in the office suite where the envelope was opened had positive nasal swab tests including 13 individuals present in the vicin-

Context Bioterrorist attacks involving letters and mail-handling systems in Washington, DC, resulted in *Bacillus anthracis* (anthrax) spore contamination in the Hart Senate Office Building and other facilities in the US Capitol's vicinity.

Objective To provide information about the nature and extent of indoor secondary aerosolization of *B anthracis* spores.

Design Stationary and personal air samples, surface dust, and swab samples were collected under semiquiescent (minimal activities) and then simulated active office conditions to estimate secondary aerosolization of *B anthracis* spores. Nominal size characteristics, airborne concentrations, and surface contamination of *B anthracis* particles (colony-forming units) were evaluated.

Results Viable *B anthracis* spores reaerosolized under semiquiescent conditions, with a marked increase in reaerosolization during simulated active office conditions. Increases were observed for *B anthracis* collected on open sheep blood agar plates ($P < .001$) and personal air monitors ($P = .01$) during active office conditions. More than 80% of the *B anthracis* particles collected on stationary monitors were within an alveolar respirable size range of 0.95 to 3.5 μm .

Conclusions *Bacillus anthracis* spores used in a recent terrorist incident reaerosolized under common office activities. These findings have important implications for appropriate respiratory protection, remediation, and reoccupancy of contaminated office environments.

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ity of the mail area and 7 workers on an interconnected lower floor. Additionally, 2 workers from an adjacent office suite that entered an adjoining contaminated hallway and 6 emergency responders who entered the office or hallway had positive nasal swab tests.

The building was officially closed to the public on October 17, 2001, with access to the contaminated suite limited to forensic investigators only. This study was completed after forensic investigation and prior to remediation of the Hart Senate Office Building.

Information regarding primary aerosolization of *B anthracis* spores has been reported,²⁻⁵ but few data are available regarding secondary aerosolization in-

doors. The purpose of this investigation was to evaluate secondary aerosolization of viable *B anthracis* spores under both quiescent and active office conditions. Understanding secondary aerosolization (reaerosolization) of

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B anthracis spores in building environments is essential for exposure assessment and risk evaluation following bioterrorism attacks. Such understanding will also guide cleanup strategies for readily dispersible bioaerosols.

METHODS

Environmental samples were collected in the affected Senate office suite

(total area approximately 1200 sq ft) beginning 25 days after the initial incident. Stationary and personal air samples and surface samples were collected during 3 separate building entries (TABLE 1). Initial semiquiescent sampling was followed by second and third rounds of sampling under simulated active office conditions. All analyses were conducted such that only vi-

able spores or spore aggregates were recorded.

During semiquiescent sampling, movement was minimized in the suite while air and surface samples were collected from various locations. During the semiquiescent sampling, the sample team (wearing sterile gloves, boots, hooded protective suits, and powered air purifying respirators with P.100 cartridges) placed sampling devices in the locations indicated in the FIGURE and left the suite to reduce air turbulence for the duration of the sample collection period. Following semiquiescent sampling, active office conditions were simulated to reflect routine behaviors in a busy office environment (ie, paper handling, active foot traffic, simulated mail sorting, moving trash containers, patting chairs). There was no activity in the office suite several days prior to or between sampling periods.

There are no validated environmental sampling or risk assessment methods for *B anthracis* contamination. Questions regarding collection techniques, laboratory extraction efficiency from environmental media, and appropriate methods for air monitoring remain unanswered. Accordingly, in this investigation a variety of environmental sampling methods were used to assess their usefulness for estimating environmental exposure and risk from *B anthracis* spores. Samples and sample locations were based on plausible exposure pathways (both inhalation and dermal) and were selected based on proximity to the original release, pedestrian traffic patterns within the suite, representative exposures to the staff in the work area, and areas of interest for spore transport within the office suite (eg, computer monitors).

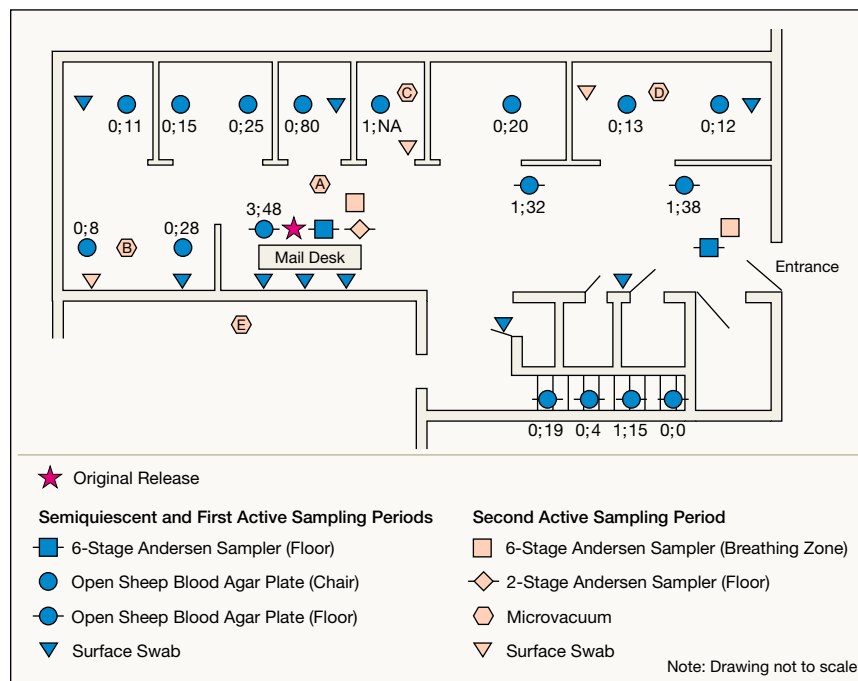
Environmental sampling methods included air monitoring with stationary and personal sampling devices (devices worn by the sample team to characterize colony-forming unit [CFU] levels in their breathing zone) that actively collected spores from a known volume of air as well as open blood agar plates that passively collected spores deposited from the Hart Senate Office Building aerosol.

Table 1. Chronology of Secondary Aerosolization Sampling in the Hart Senate Office Building After the Primary Aerosolization Event on October 15, 2001*

| | November 10, 2001 AM (Semiquiescent Sampling) | November 10, 2001 PM (1st Active Sampling Period) | November 15, 2001 (2nd Active Sampling Period) |
|---|---|---|--|
| Personal breathing zone samples | 3 | 3 | 4 |
| Passive SBA plates | 17 | 17 | NA |
| Microvacuum | NA | NA | 5 |
| Andersen samples (floor position) | 2 | 2 | 1 |
| Andersen samples (breathing zone level) | NA | NA | 2 |
| Surface swab samples | 9 | 9 | 3 |

*SBA indicates sheep blood agar (5%); NA, not applicable. Environmental samples for *Bacillus anthracis* were collected in the Senate suite following the initial forensic investigation and prior to remediation. The types of samples collected, the number of samples in each category, and the collection schedule are presented.

Figure. Active and Passive Environmental Sample Locations in the Hart Senate Office Building



NA indicates not available. Numerical values of viable *Bacillus anthracis* colonies represent passive samples collected by placing open sheep blood agar plates during semiquiescent and first active sampling periods (semiquiescent; first active). Surface swab samples in the second active sampling period had no corresponding control samples.

Surface samples were collected to help characterize the presence of *B anthracis* contamination on a variety of surface types using both microvacuum devices and sterile swabs. These environmental samples were collected under both quiescent and active office conditions to assess the influence of human movement within the suite on environmental spore concentrations.

Andersen 6-stage viable (microbial) particle-sizing samplers (Thermo-Andersen, Smyrna, Ga) were used to collect airborne spores to evaluate concentrations and size ranges of spores or spore aggregates. Andersen samplers were operated for 10 minutes at an air flow rate of 28.3 L/min during each sample collection period. The Andersen sampler collects spores according to nominal aerodynamic diameters on each of 6 vertically stacked agar plates. Andersen samplers use petri dishes filled with 42 mL of agar to control aerodynamics of particle impact on plates according to manufacturer-specified cutoffs of 7.0, 4.7, 3.3, 2.1, 1.1, and 0.65 μm . For this investigation, 18 mL of 5% sheep blood agar (SBA) plates (Remel Inc, Lenexa, Kan) were used for collection media. Use of reduced media volume resulted in an increase in the specified jet-to-plate distance of 0.3 cm with a corresponding increase of 0.3 μm in the particle size cutpoints.⁶ Thus, the smallest particle impacting the number 6 plate in the cascade would have a nominal diameter of 0.95 μm (ie, 0.65 μm + 0.3 μm).

For the semiquiescent and the first active testing period, 2 viable Andersen impact samplers (6-stage) were used; 1 was placed on the floor in the vicinity of the original contamination and 1 was placed on the floor 20 feet away near the common entrance to the suite (Figure). During the second active sampling period, the 2 Andersen samplers were placed at the breathing zone level in the same locations, and a specially configured 2-stage Andersen sampler was placed at a floor location near the original source zone. The final stage of this sampler was fitted with a glass fiber filter to trap any remaining viable spores smaller than the final impact stage (approximately 0.9

Box. Positive Hole Correction Method

The positive hole correction method determines a statistical probability count of colony-forming units. It represents a count of the jets that delivered the spores to the agar plates and the conversion of the jet number to a particle count by using the "positive hole" conversion formula⁷:

$$\text{Pr} = N \times \sum_{x=0}^{r+1} \left(\frac{1}{N-x} \right)$$

where Pr is the expected number of viable particulates to produce r positive holes and N is the total number of holes per stage (400). This formula is based on the principle that as the number of viable particles being impinged on a given plate increases, the probability of the next particle going into an unpenetrated hole decreases. Thus, when 9 of 10 of the holes have each received 1 or more particles, the next particle has but 1 chance in 10 of going into an unpenetrated hole. Therefore, on average, 10 additional particles would be required to increase the number of positive holes by 1.

Table 2. Stationary Air Samples of Viable *Bacillus anthracis* Particles*

| Nominal Particle Size, μm | No. of CFUs (No. Based on Positive Hole Correction Method) | | | | | |
|---|--|-------------------|----------------|--------------------------|-------------------|----------------|
| | Mail Handling Area | | | Entrance Area | | |
| | Semi-quiescent Condition | Active Conditions | | Semi-quiescent Condition | Active Conditions | |
| | Floor | Floor | Breathing Zone | Floor | Floor | Breathing Zone |
| >7.1 | 1 | 65 (71) | 3 | 0 | 13 | 0 |
| 4.8-7.1 | 0 | 19 | 13 | 2 | 9 | 0 |
| 3.2-4.7 | 2 | 57 (61) | 13 | 5 | 12 | 1 |
| 2.1-3.1 | 3 | 98 (112) | 20 | 9 | 30 (31) | 3 |
| 1.1-2.0 | 30 (31) | 400 (>2628) | 80 (90) | 40 (42) | 100 (115) | 24 |
| 0.65-1.0 | 11 | 100 (115) | 56 (61) | 13 | 23 (24) | 2 |
| Total CFUs | 47 (48) | 739 (>3006) | 185 (200) | 69 (71) | 187 (204) | 30 |
| Estimated air concentrations (CFUs/m ³) | 170 (171) | 2611 (>11 000) | 653 (707) | 244 (251) | 244 (721) | 106 |

*CFUs indicate colony-forming units. Samples were collected on 6-stage Andersen sampling devices designed to register nominal spore size for viable particles.

μm). At the end of each sample collection period, Andersen samplers were disinfected to avoid cross-contamination.

Direct colony counts on SBA plates in the Andersen samplers were obtained and the positive hole correction method (BOX) was used to acquire a statistical probability count of CFUs (TABLE 2).

In addition to stationary air samples, personal air samples were collected from the breathing zone of sample team members during all 3 rounds of sampling. Sample pumps were calibrated to operate at a flow rate of 4 L/min. The

flow rate was not intended to simulate respiratory minute ventilation but to provide efficient deposition of spores on the collection media. Collection media consisted of gelatin filters placed in 37-mm open-faced filter cassettes and located in breathing zones of team members for each sampling period. These cassettes are commonly used for personal air monitoring applications and were available with corresponding gelatin inserts conducive to the collection and direct incubation of microbial samples. Sample cassettes were placed on the front of the team mem-

bers' suits just below the shoulder and connected to a sampling pump worn at the waist by a length of Tygon tubing (Saint-Gobain Performance Plastics Corporation, Akron, Ohio).

Open plates were placed in workstations, on the floor, and within the stairway to estimate spore settling during and following various levels of human activity in the suite. Seventeen SBA plates were placed in various locations and at various heights throughout the office during the semiquiescent and the first active sampling period. Ten plates were placed on office chairs, 3 at various floor locations, and 4 on the steps of an internal office stairway (Figure). Plates were opened for 45 minutes to collect viable spores then closed and wrapped with parafilm.

A total of 17 surface samples were collected on fabric office dividers, carpets, paper files, and near the source of the original contamination. A microvacuum sampler was used to quantify the surface loading of *B anthracis* on a variety of surface types. Microvacuum samples were collected using personal air monitoring pumps operated at a calibrated flow rate of 4 L/min. Filter cowl containing gelatin filters with a nominal pore size of 3 μm (having submicron retention efficiencies) were connected to the pump with tubing to form a microvacuum device. Sampled areas were defined by a 100-cm² template, then vacuumed using a slow back and forth motion first in one direction, and then perpendicular to the original direction. Microvacuum samples were collected at workstations in 5 different office areas during the second active sampling period.

Swab samples were used to assess the presence of *B anthracis* contamination on an additional 12 surfaces. Sterile nylon swabs moistened with sterile water were used to sample both vertical and horizontal surfaces as defined by 100-cm² templates. Areas were swabbed in perpendicular directions using a slowly progressing S-shaped motion and then placed in sterile 15-mL tubes. Nine swab samples were collected for both the semiquiescent and first active sampling periods: 3 vertical semigloss latex painted

surfaces (2 doors and 1 wall), 3 computer monitors, and 3 individual mailboxes.

Aseptic handling techniques were used throughout the sampling and analytical process. All samples were labeled immediately following collection using predetermined sample codes. Samples were placed in individual resealable bags and immediately shipped to the analytical laboratory with blind identification codes and under chain-of-custody. Field blank samples (quality-control samples used to ensure adherence to sterile microbiologic technique) were included at a frequency of 10%.

Samples were evaluated for the presence of viable *B anthracis* at the Naval Medical Research Center in Silver Spring, Md. Gelatin filters were removed from the filter cassettes and placed directly on SBA plates. Swabs and glass fiber filters were macerated in 3.0 and 7.5 mL, respectively, of sterile phosphate-buffered saline for approximately 1 minute to free viable spores. Following maceration, a 1.0-mL aliquot of each sample was removed and heat shocked at 65°C for 15 minutes to reduce viable vegetative bacteria in the sample. A 200- μL aliquot of each heated sample was spread on an SBA plate and plates were incubated at 37°C for 14 hours. Following incubation, bacterial colonies morphologically consistent with *B anthracis* were counted and recorded. Rapid real-time polymerase chain reaction assays were used to confirm the identity of suspect *B anthracis* colonies.^{8,9} At least 1 suspect colony from each plate was tested for the presence of the genetic markers *pag* and *cyaB*, specific to the virulence plasmids pXO1 and pXO2, respectively. Following polymerase chain reaction confirmation of selected suspect colonies, the number of *B anthracis* colonies on each plate was reported. Analyse-It Software version 1.64 (Analyse-It Software Ltd, Leeds, England) was used for statistical analyses and $P < .05$ was considered significant. All sample team members were specially trained in response to extremely hazardous environments and all participation was voluntary. The US Federal

Incident Command System reviewed and approved the study. Incident Command System is a system used to organize and manage participating groups during emergency response situations.

RESULTS

Results for the 6-stage Andersen air samples are presented in Table 2. Positive hole correction results are presented below where applicable. Comparison of floor samples between semiquiescent and active conditions showed an increase in viable spore collection across all sampler stages at both the mail area (48 vs >3006 total CFUs) and entrance area (71 vs 204 total CFUs) locations. In the mail area, stationary Andersen breathing zone samples showed an increase compared with semiquiescent sampling taken previously at floor level (200 vs 48 total CFUs). Estimated airborne spore concentrations collected near the floor over a 10-minute period ranged from 171 to 251 CFUs/m³ during the semiquiescent period. For the active period, airborne CFU concentrations ranged from 721 to more than 11 000 and 106 to 707 CFUs/m³ for floor and breathing zone samples, respectively. This represents as much as a 65-fold increase in CFUs under active conditions compared with semiquiescent conditions. Approximately half of the CFUs had corrected nominal diameters ranging from 1.4 to 2.4 μm , with more than 80% ranging from 0.95 to 3.5 μm . Results from the 2-stage Andersen sampler indicated no viable spores less than a corrected nominal diameter of 0.95 μm .

Locations and results of viable colony counts on the 17 open SBA plates (10 on chairs; 7 on the floor) collected during semiquiescent and active periods are shown in the Figure. During the semiquiescent period, 5 of the 17 plates were positive for *B anthracis* (median, 0 CFU; range, 1-3 CFUs; 95% confidence interval [CI], 0-1). In comparison, 14 of 15 plates (1 plate was left in the suite and was desiccated beyond use) during the first active sampling period were positive for *B anthracis* (median, 15 CFUs; range, 4-80 CFUs; 95% CI, 11-28) illustrating a significant increase in colony

Table 3. Personal Air Monitoring Results*

| Team Member | Semiquiescent | | Active Period 1 | | | Active Period 2 | | |
|-------------|---------------|--|-----------------|--|---------|-----------------|--|---------|
| | No. of CFUs | Estimated Air Concentration, CFUs/m ³ | No. of CFUs | Estimated Air Concentration, CFUs/m ³ | P Value | No. of CFUs | Estimated Air Concentration, CFUs/m ³ | P Value |
| 1 | 7 | 14.1 | 36 | 85.6 | .17 | 20 | 49.1 | .01 |
| 2 | 1 | 2.0 | 7 | 16.7 | | 12 | 26.9 | |
| 3 | 3 | 6.0 | 1 | 3.3 | | 22 | 49.1 | |

*CFUs indicate colony-forming units. Results for the personal air monitors represent exposures integrated over the total time spent in the Hart Senate Office Building. During quiescent sampling the team spent more than half of the time in less contaminated hallway areas; therefore, results may underestimate exposure in more contaminated areas of the suite. P value comparisons with semiquiescent sample measurements.

counts ($P < .001$; using a 2-tailed non-parametric Wilcoxon signed rank test).

Results of personal air monitor samples collected from team members during each of the sampling periods are presented in TABLE 3. Filters from all 10 of the samples were positive for *B anthracis*. Results were positive for *B anthracis* during semiquiescent office conditions (mean, 4 CFUs; range, 1-7 CFUs) and increased during active office conditions (mean, 14 CFUs; range, 1-36 CFUs). There was a significant increase in the number of CFUs collected on personal air samples during the second active test period ($P = .01$; 1-tailed paired *t* test with 2 *df*) but not the first active test period ($P = .17$) when compared with the semiquiescent sampling period. A 1-tailed statistical test was used with the expectation that the number of airborne viable CFUs would increase (rather than decrease) when activity increased in the suite.

Six of the 9 surface swab samples taken during the semiquiescent and first active period were positive; 3 vertical mailbox surfaces (range, 3-43 CFUs) and 3 computer screens (range, 2-150 CFUs), with little change in viable spore counts in response to increased activity. Three swab samples collected from vertical wall surfaces during each sampling period were negative. During the second active sampling period, sequential swab samples of a computer monitor screen sampled in the off, then on position, resulted in a 25-fold increase in viable colony counts on the charged screen. Deposition of spores on the charged monitor may indicate influence of electrostatic effects on spore behavior.

Additionally, 5 microvacuum samples were taken in different office

Table 4. Microvacuum Samples Collected in the Hart Senate Office Suite During Second Active Period*

| Location | Samples, CFUs/m ² | | | |
|-----------------|------------------------------|------------------------|----------------------------|---------------|
| | Carpet | Fabric Office Dividers | Smooth Horizontal Surfaces | Tops of Files |
| A (Mail area) | ... | NA | NA | NA |
| B (Workstation) | 1500 | 0 | 2000 | 0 |
| C (Workstation) | 4000 | 200 | 6800 | NA |
| D (Workstation) | 1100 | 100 | 100 | 100 |
| E (Workstation) | 4600 | 0 | 2500 | 0 |

*Ellipses indicate too numerous to count; NA, not applicable; and CFUs, colony-forming units. Microvacuum samples of selected 100-cm² surfaces within the office suite. Homogeneity of spore distribution across surface areas sampled should not be assumed.

areas during the second period of activity to evaluate contamination of different types of surfaces (TABLE 4). Although microvacuum samples showed substantial viable spore contamination of carpeted and smooth horizontal surfaces, very little contamination of vertical fabric workstation dividers or the tops of paper files was found. No CFUs were found on the field blanks collected from any of the sample types during the course of the investigation.

COMMENT

The importance of secondary aerosolization of *B anthracis* spores associated with a bioterrorism attack has been discussed by a number of researchers.¹⁰⁻¹⁴ However, few empirical data existed to allow for scientifically based public health conclusions or recommendations. Although research conducted by the military has shown that *Bacillus subtilis* spores, used as a surrogate for *B anthracis*, can reaerosolize with varying activities in outdoor environments,^{13,15} until now, no published data have been available concerning secondary aerosolization of *B anthracis* spores indoors. Prior to the attacks in the fall of 2001, con-

sensus recommendations from the Working Group on Civilian Biodefense¹¹ suggested only a slight risk of acquiring inhalational anthrax by secondary reaerosolization from heavily contaminated surfaces. These recommendations were based on an incident involving accidental release of *B anthracis* in Sverdlovsk, Russia,⁵ occupational studies of workers in goat hair processing mills,¹⁶ and modeling analyses by the US Army.¹² The Working Group on Civilian Biodefense recognized that its recommendations were based on interpretation and extrapolation from an incomplete knowledge base and needed to be regularly reassessed as new information becomes available.¹¹ A recent reassessment by the consensus group includes a precautionary note regarding reaerosolization of *B anthracis* spores based, in part, on work presented here.¹⁷

This investigation presents empirical findings concerning secondary aerosolization of viable *B anthracis* spores following a bioterrorism incident indoors. Among the limitations of the work are the severe schedule constraints, limited availability of equipment, and the extreme conditions un-

der which the investigation was planned and implemented. Both empirically observed and substantially increased spore concentrations were recorded on open SBA plates during active conditions in the office suite. Elevations of CFUs recorded on personal air monitoring devices during active vs semiquiescent office conditions are consistent with military investigations showing activity-related increases in airborne spore exposures outdoors.¹³ However, the personal air monitor data reported in this study are limited due to high variability and small sample size.

During simulated activities, airborne concentrations of viable *B anthracis* spores within the office ranged from 2 to 86 CFUs/m³ for personal air monitors and 100 to more than 11 000 CFUs/m³ for stationary Andersen samples, with more than 80% of the spores falling into the respirable range (<5 µm). Relatively higher collection efficiencies on stationary monitors may be due to sample locations within the contaminated suite, higher air flow rates through the stationary sampling devices, or the sample team personal monitors integrating exposure over both contaminated and noncontaminated areas of the Hart Senate Office Building (personal monitors were activated on entry to the building 6 floors below the contaminated suite).

Using a mean (SD) respiratory rate of 1.38 m³/h (0.66) reported for office workers,¹⁸ estimated inhalation exposures to *B anthracis* in the breathing zone were 119 and 250 CFUs/h for personal air monitors and breathing zone Andersen samplers, respectively. Based on CFU concentrations recorded by floor level Andersen samplers, estimated exposures were as high as 15 000 CFUs/h. Additionally, findings of airborne *B anthracis* spores during the initial semiquiescent sampling period suggest that even minimal movements may result in resuspension of viable spores. These findings were recorded almost a month following the original incident, despite the removal of the contaminated letter from the suite.

Determining the magnitude of inhalational risks from reaerosolized *B an-*

thraxis spores is uncertain. Reliable human data on the minimum infective dose for inhalational *B anthracis* is lacking. Individual susceptibility, virulence of the strain, and spore physical characteristics may all have profound impacts on the dose necessary to cause inhalational anthrax.^{3,4} Primate model extrapolations suggest an estimated human median lethal dose between 2500 and 55 000 spores,¹⁰ with the highest infectivity associated with clouds of single spores, vs multispore aggregates.⁴ Recent primate studies have demonstrated inhalational infectivity of *B anthracis* following exposure to only a few spores.¹⁹ Human cases of inhalational anthrax have also been reported involving minimal exposures.¹⁶ Risk predictions indicate that infective doses may be as low as 1 to 3 spores¹⁴ and these predictions may be reflected in the 2 cases of inhalational anthrax in New York and Connecticut still under investigation.²⁰

This work clearly demonstrates a potential for secondary aerosolization of viable *B anthracis* spores originating from contaminated surfaces in an indoor environment. As a result, precautions to protect exposed decontamination workers and area occupants are indicated.

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Analysis and interpretation of data: Weis, Intrepido, Miller, Cowin, Durno, Gebhardt, Bull.

Drafting of the manuscript: Weis, Intrepido, Miller, Cowin.

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