Fate of Bioterrorism-Relevant Viruses and Bacteria, Including Spores, Aerosolized into an Indoor Air Environment

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An aerosol physics test facility was used in a series of eight experiments to gather an integrated comprehensive broad base of data on the fate of surrogates of microorganisms that cause smallpox, plague, glanders, anthrax, and viral hemorrhagic fevers. The results are directly relevant to the public health issue of how to protect the occupants of buildings against bioterrorism. The test conditions were directly relevant to the indoor air environment situation, and the results can be generalized to buildings that are now occupied. The reductions in concentrations of relevant viruses and bacteria—including gram-negative fermenters and nonfermenters, gram-positive cocci and bacilli, and spores—were substantial and statistically robust. The data show that the bioterrorism-relevant aerosolized viruses and bacteria, including spores, respond like small particulates to the primary (electrical) forces that control the distribution of small particulates in a room. Further, these relevant microorganisms respond like small particulates to means designed to control airborne particulates. The results could be used to anticipate the effects of a bioterrorist attack on the public health, provide information on means that can be used to minimize such effects, and used to make decisions on how best to protect occupants of specific buildings at minimal cost and with assurance of success. Exp Biol Med 229:345–350, 2004

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The terrorist attacks of September 11, 2001, and the anthrax attacks that occurred afterward have raised questions regarding the vulnerability of building occupants to bioterrorist attack and the measures that can be taken to minimize the effects of such attacks. The concern is substantial, as can be seen in recent papers that have been published by the *Journal of the American Medical Association* (1) and the National Institute for Occupational Safety and Health (2), as well as in building engineering journals such as *Engineered Systems* (3). News reports indicate that the federal government is moving toward implementing a requirement that buildings that it leases or builds be able to minimize the effects of a bioterrorism attack.

There is a considerable amount of published data on "building-related illness" in which occupants have been sickened, for example, by mold growing under carpets and in the walls, or by bacteria and mold growing in cooling coil drain pans (4–7). This data has limited relevance to the bioterrorist situation because it involves a gradual buildup of organisms over a lengthy period, the opposite of what would happen in a bioterrorist attack. Also, the range of organisms studied has been limited and generally not the type of microorganisms that could be expected in a terrorist attack.

We have done a series of experiments in well-controlled conditions to gather a systematic body of data on bioterrorism-relevant viruses and bacteria, including spores. The organisms were aerosolized into a room that was part of a closed air handling system at a dedicated aerosol physics test facility. The organisms selected were surrogates of those that could be expected in a bioterrorist attack. For example, Langlois (8) used *Escherichia coli* MS2 bacteriophage to simulate smallpox, and Foarde *et al.* (9) used MS2 bacteriophage as a surrogate for human viruses of similar and larger sizes and shape. The test conditions were directly relevant to the indoor air environment situation in buildings, and the results can be generalized to buildings in use.

We determined whether aerosolized viruses and bacteria, including spores, of the type that might be used by a bioterrorist against the occupants of a building respond like particulate contaminants to the primary (electrical) forces that control the distribution of small particulate contaminants in indoor air. The results could be used to anticipate the effects of a bioterrorist attack, provide information

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Table 1. Characteristics of Aerosols and Particle Settling Time in Still Air

Characteristics of aerosols	
Particle size (μm)	Percentage of particles (by count)
10–30	<1
6–10	<1
3–5	<1
1–3	1
0.5–1	6
<0.5	92
Particle settling time in still air	
Particle size (μm)	Time required to settle 8 ft
100	8 secs
10	13 mins
1	19 hrs
0.1	79 days
0.01	Infinite

on means that can be used to minimize such effects, and aid in making decisions on how best to protect a building.

The first set of data collected involved five experiments using two different genera and five different strains of bacteria, including spores. *Micrococcus luteus* was used as a surrogate for gram-positive cocci because *M. luteus* is similar in size, shape, and cell wall composition to *Staphylococcus aureus*. Similarly, *Bacillus subtilis* spores and vegetative cells were used as surrogates for the gram-positive bacilli such as *Bacillus anthracis*. Culture-based measurements were made at timed intervals. The reported results indicate that the organisms respond like particulate contaminants to the primary forces that determine the distribution of particulates in a room and respond to means to decrease their concentration (10)..

The second set of data collected involved three experiments using the gram-negative glucose-fermenting and nonfermenting bacteria as well as bacteriophage. *Serratia marcescens* was used as the surrogate for the gram-negative glucose-fermenting bacilli. Examples of organisms in this group are *Yersinia pestis*, *Salmonella typhi*, and *Shigella* that cause plague, typhoid fever, and shigellosis, respectively (11).

Pseudomonas fluorescens was used as the surrogate for the gram-negative, glucose nonfermenting bacilli. Examples of organisms in this group are *Burkholderia mallei*, *Burkholderia pseudomallei*, and *Francisella tularensis*, which cause glanders, melioidosis, and tularemia, respectively (11).

The *E. coli* MS2 bacteriophage was used as the surrogate for viruses. Examples of organisms in this group are varicola; filoviruses such as Ebola; and alphaviruses such as Venezuelan, Eastern, and Western equine encephalitis, which causes smallpox, viral hemorrhagic fevers, and viral encephalitis, respectively (11).

We report here on the second set of data collected that, when considered with the first set, gives us a directly relevant comprehensive broad base of data from which decisions can be made. We also discuss the implications and application of this broad base of data in the protection of occupants of buildings against bioterrorist attack.

Primary Determiners of Particulate Distribution in Indoor Spaces

The earth has a natural background electrical field, which varies with time and place, but is generally about 150–200 V/m (12). In indoor spaces, the natural background field is distorted by the electrical characteristics of the materials in equipment, furniture, walls, clothing, and carpets. The electrical fields generated by computers and other electrical and electronic equipment and the electric fields generated by the power lines in the walls also distort the ever-present background field. In a room, these factors create what could be envisioned as a complex three-dimensional web of voltage gradients often referred to as electrical field lines. Adding to the complexity is that humans frequently carry a substantial electric charge that also influences the background electrical field. Several thousand volts on a man wearing wool trousers in dry air are not unusual (13). This is what sometimes causes an electric shock when one touches a doorknob.

Aerosols vary in size, concentration, and settling time. More than 98% of particulates in room air are small, less than 2 μm in size, and essentially do not settle out of the air by gravity, as shown in Table 1 (12). Air currents in a room entrain and move large particulates, carrying them into the ducts and then into the filters. Most small particulates are not entrained and moved by air currents because their cross-sectional area is so small. The motion of small particulates is primarily determined by the typical electrical fields that exist in all rooms. The small particulates tend to move along the electrical field gradients and deposit in and on people and objects. Thus, relatively few small particulates are returned to the ducts and, as a consequence, relatively few reach the filters.

As small particulates in the air age, many collide, forming larger particulates. This natural process is called coagulation. The coagulated particulates are more readily moved by air currents because they have a large enough cross-sectional area to be entrained by air currents. The coagulated particulates thus tend to be returned to the ducts. Once they are in the duct system, they are carried to and trapped in the filters as a function of factors such as filter efficiency or static pressure drop (13).

In summary, the typical electrical fields in indoor spaces; the electrical characteristics of particulates; and the electrical characteristics of people, objects, and surfaces in the space are some of the primary determiners of the fate of small particulates (those smaller than about 2 μm). These interactions, in large part, determine the deposition of particulates in and on people, objects, and walls in a space.

There is a large body of information on the electrical interactions involving airborne particulates in indoor spaces (12).

From a bioterrorism standpoint, if relevant airborne viruses and bacteria, including spores, in a room act as small particulates act, then we can apply the available knowledge touched on above to better understand the distribution, inhalation, and body deposition of them in a space. We will also have means available to minimize their concentrations in spaces such as office buildings and other facilities if they are injected into the air system.

Because coagulation is one of the most important phenomena in the interactions of aerosols, an assessment of whether these microorganisms respond as small particulates in a well-established coagulation acceleration procedure can give us fundamental information on their behavior in indoor air and on means to control them. Theory and much laboratory data indicate that accelerating coagulation can enhance the effectiveness of filters in removing small particulates (13, 14). Thus, this is a clear-cut experimental mechanism and procedure that can be used to determine whether these microorganisms relevant to bioterrorism can be influenced and controlled in the same manner as particulates in room air.

One way to accelerate coagulation is to use equipment to generate a specific nonhomogeneous electrical field within a section of duct, downstream of the filter, as shown in Figure 1. What happens is that within this nonhomogeneous electrical field within the duct, coagulation is accelerated. The coagulated particulates then enter the room where, somewhat like snowballs rolling down a hill, they coagulate or sweep up additional small particulates. The air currents then carry the now large particles from the room into the duct and are then trapped in the filters (15).

In this article, we report data indicating that surrogates of relevant airborne pathogenic bacteria, including spores, and viruses respond in a manner similar to airborne particulates.

Materials and Methods

Test Facility. All testing was done in a dedicated aerosol physics facility, which includes a room 2.75 × 4.25 × 2.50-m high (9 × 14 × 8 ft) with a floor of vinyl tile. The walls and ceiling are constructed of drywall that is coated with three applications of polyurethane. The room has its own closed-circuit air handling system. The volume of the room itself is 62.6 m^3 (1008 ft^3), and the duct volume (inside and outside the room) is 4.7 m^3 (76 ft^3).

Air entered the room through supply diffusers on one side (as shown in Fig. 1), passed across the room, and exited through return grills into a duct at a rate of 21 room air changes per hour at a velocity of 225 ft/min. Similar results with this system were obtained at air change rates of 5–21 per hour; 21 was used in accordance with standard engineering practice to minimize test time. In the duct, the air passed sequentially through a 30% filter, two electrical field screens, and a blower, and then re-entered the room through the supply diffusers. The 30% efficient filters are 30% American Society of Heating, Refrigerating, and Air-Conditioning Engineers (ASHRAE) dust spot per standard 52.1 and carry a fractional size efficiency Minimum Efficiency Reporting Value (MERV) value of 8 per Std 52.2. This is a 2-in. thick, pleated panel filter with a glass media. The relative humidity in the room, measured with a sling psychrometer, was approximately 76%, which minimized the possible organism desiccation that the relatively high air change rate could have caused. Between test runs, the air in the room was UV irradiated by a ceiling-mounted model xx-40s UV lamp (UVP Co., Upland, CA) and purged to the outside for 30 mins and replaced by air-conditioned office air. Separate tests were done to determine if viable organisms remained in the room at the end of the purge period. These tests were done using impingers to sample, which was also done in the experiments and with the settling plates. The results indicated that no viable bacteria or viruses remained at the end of the purge.

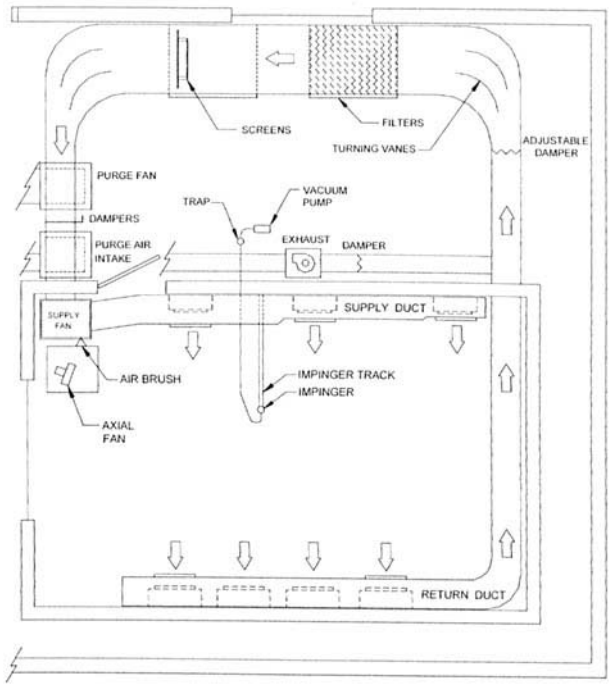

Figure 1. View from above of aerosol test facility: room and ductwork inside and outside of the room.

A noncommercial system to accelerate coagulation was installed with its screens in the duct. The upstream (HV) electrical field screen was 50 × 50 cm (20 × 20 in.), and the downstream (HF) was 60 × 60 cm (24 × 24 in.). They were spaced 7.6 cm (3 in.) apart, as shown in Figure 2. The HF screen was constructed of 0.041-in. wire mesh (Wire Cloth Mfg., Largo, FL) with 1.3-cm (0.5-in.) openings mounted on a steel frame. The HV screen was constructed of 4.7-mm (3/16 inch) flat-tinned copper braid straps (Alpha Wire Co., Elizabeth, NJ) mounted 7 cm (2.75 in.) on centers vertically on a steel frame. A model 600 electrical source (Cosatron, Tampa, FL) supplied a 25 kV DC signal to the HV screen and a 700-V rms 177 kHz signal to the HF screen to create an electrical excitation field. The current was trivial at less

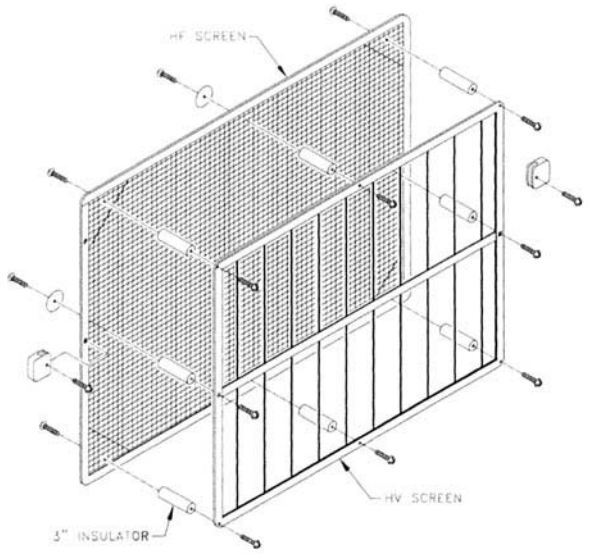

Figure 2. Construction of the in-duct electrical field screens.

than 3 milliamps. Separate tests with a Sensidyne ozone detector system (Sensidyne, Clearwater, FL) before and after the screens show that no ozone was produced by this system.

The experimentation was done in a dedicated aerosol physics test facility, which has yielded highly reliable data with particulates and chemicals (15, 16). Extensive pilot studies were carried out with viruses and bacteria, including spores, to determine the parameters that were used in the data reported below and to determine if we would have reliability with microorganisms comparable with the reliability obtained in particulate and chemical research. The reliability was comparable as can be seen in the statistical significance of the data obtained in the main experimentation carried out later and reported previously and below.

Experiment 1. *S. marcescens*, supplied by American Type Culture Collection, ATCC 13880 (Manassas, VA), was used. The bacteria were streaked for isolation on trypticase soy agar plates (BD Biosciences, Sparks, MD) and incubated at 30°C for 24 hrs. Isolated colonies were suspended in 0.1% peptone water to a turbidity equivalent to a 0.5 McFarland standard, approximately 10^8 colony-forming units (CFUs) per milliliter. The inoculum was used for all four runs done each day and was refrigerated between runs. Ten milliliters of inoculum was put into a 1-oz airbrush bottle. The bottle was then inserted through a wall access panel and mounted to a Paasche single action, external mix airbrush (Paasche Co., Harwood Heights, IL). The airbrush was located in the test room 3 ft above the floor and pointed toward the center of the room (Fig. 1). A 100-cu ft/min axial fan was mounted 1 ft below the airbrush's air stream and was pointed toward the center of the room to aid the dispersion of the airbrush spray.

A set of four runs was completed each day. Run 1 was done with the in-duct electric field (EF) system off (control). Runs 2 and 3 were done with the EF system on (test), and Run 4 was done with the EF system off (control).

The impinger used, XX100-3750 (Millipore, Bedford, MA), is comparable in shape, configuration, and size to the AGI-30 (ACE Glass, Vineland, NJ) according to the manufacturer's published specifications. Both draw air at approximately 12.5 L/min at the same pressure drop. Three glass impingers were each filled with 30 ml of brain heart infusion broth (BD Biosciences). The impingers sampled the air at 5, 25, and 45 mins after the bacteria spray stopped. Each impinger was clamped onto the end of a wooden bar and wrapped with 20-gauge grounded copper wire. This assembly could be slid in and out of the room on a wooden track. Vacuum tubing was fitted onto the outlet on the side of the impinger. The impinger was then inserted through an access panel into the center of the room 3 ft above the floor. The distal end of the impinger tubing was attached outside the room to an Erlenmeyer flask, which was used as a trap. Another piece of vacuum tubing connected the flask to a 3.5-cfm vacuum pump.

The procedure for a run was to turn on the room air system, then the axial fan, and then the airbrush compressor set at 20 psi. The airbrush was operated until the bacterial suspension was sprayed into the room, which took about 1 min. The axial fan optimized the dispersion of the bacteria. The bacteria were allowed to circulate for 5 mins so that they were distributed throughout the room and duct system. The vacuum pump was turned on, and room air was drawn through the impinger for 5 mins. Then the room air system was briefly turned off while the impinger was pulled back through the wall access panel. One milliliter of liquid from the impinger was evenly spread onto a 150 × 15-mm trypticase soy agar plate (BD Biosciences). At the 25-min and 45-min points, the procedure was repeated. Duplicate and/or triplicate plating was performed on all of the bacteria, including spores, as well as the bacteriophage. At the end of the run, the room air was UV irradiated and purged to the outside to complete the run.

The EF was then turned on, and Runs 2 and 3 were completed in the same way. The EF was then turned off, and Run 4 was completed.

All sample plates were incubated at 30°C for 24 hrs. The number of CFUs was counted, and the data were recorded as CFUs per plate.

Experiment 2. All conditions were the same as above except *P. fluorescens*, which was supplied by American Type Culture Collection, ATCC 13525 (Manassas, VA), was used. The airflow in the room was made turbulent by having two floor fans in operation in the room.

Experiment 3. All conditions were the same as Experiment 1 except for the following: the MS2 *E. coli* bacteriophage and *E. coli* bacteria were used. Serial dilutions of a stock MS2 *E. coli* bacteriophage solution, containing 10^{12} PFUs/ml, were made in 0.1% peptone water. Ten milliliters of the resulting 10^9 PFUs/ml dilution was put into a 1-oz airbrush bottle and sprayed into the test room. The impingers sampled the air at 5, 25, and 45 mins after the MS2 bacteriophage spray stopped. Next, 0.2 ml of liquid

Table 2. Percent Reduction in Bacteria and Viruses in Test Groups Compared with Control Groups for Each Microorganism (Statistical Significances of the Differences Are also Given)

	Time (min)	Percent reduction	Significance
S. marcescens	25	45	0.004
	45	69	0.004
P. fluorescens	25	43	0.004
	45	51	0.004
MS2 *E. coli* bacteriophage	25	28	0.004
	45	48	0.004

from the impinger was placed into an empty 150 × 15 mm petri dish, and 0.2 ml of the *E. coli* suspension, with a turbidity equivalent to a 0.5 McFarland standard, was also placed into the petri dish. Twenty-five milliliters of agar overlay media (L.B. Miller broth with 0.75% agar, BD Biosciences ref 292780) that was melted and cooled in a 46°C water bath was added to the petri dish. The MS2 bacteriophage, the *E. coli*, and the agar overlay media were mixed and allowed to harden. The sample plates were incubated at 36°C for 24 hrs. The number of PFUs was counted, and the data were recorded as PFUs per plate.

Results

The comparisons between test and control groups for the gram-negative glucose-fermenting and nonfermenting bacilli and the viruses are summarized in Table 2 and described in detail below.

Experiment 1. *S. marcescens*, supplied by American Type Culture Collection (ATCC 13880), was used. To eliminate possible trend effects, the following run pattern was used: Runs 1 and 4 were EF-off (controls), and Runs 2 and 3 were EF-on. In the beginning of the analysis, each run was considered individually. The 5-min data point was considered the start point. Because it would vary slightly from run to run, the data for each run was normalized by taking 25- and 45-min data points as percent of bacteria remaining compared with the 5-min data point for each run. Thus, the start point for each of the runs was made equivalent.

The normalized data from Runs 1 and 4 were averaged, and the data from Runs 2 and 3 were averaged. Then a percent reduction or increase in CFUs was calculated for the EF-on plate averages as compared with the EF-off plate averages. The derivative eight data points for the 25-min samplings and for the 45-min samplings were analyzed individually by use of the binomial test. The differences between EF-on versus EF-off at the 25-minute sampling were significant at the 0.004 level, and there was a median reduction in CFUs with EF-on of 45%. The differences between EF-on versus EF-off at the 45-min sampling were significant at the 0.004 level, and there was a median reduction in CFUs with EF-on of 69%.

Experiment 2. *Pseudomonas fluorescens*, supplied by American Type Culture Collection (ATCC 13525), was used. The run pattern used was as above, and the data were normalized. A percent reduction or increase in CFUs was calculated and analyzed as above. The derivative eight data points for the 25-min samplings and for the 45-min samplings were analyzed individually by use of the binomial test. The differences between EF-on versus EF-off at the 25-min sampling were significant at the 0.004 level, and there was a median reduction in CFUs with EF-on of 43%. The differences between EF-on versus EF-off at the 45-min sampling were significant at the 0.004 level, and there was a median reduction in CFUs with EF-on of 51%.

Experiment 3. MS2 *E. coli* bacteriophage was used. The run pattern used was as above, and the data were normalized. A percent reduction or increase in PFUs was calculated for the EF-on plate averages as compared with the EF-off plates. The derivative eight data points for the 25-min samplings and for the 45-min samplings were analyzed individually by use of the binomial test. The differences between EF-on versus EF-off at the 25-min sampling were significant at the 0.004 level, and there was a median reduction in PFUs with EF-on of 28%. The differences between EF-on versus EF-off at the 45-min sampling were significant at the 0.004 level, and there was a median reduction in PFUs with EF-on of 48%.

In Table 3 are the results that were obtained in the earlier set of experiments with the gram-positive cocci and bacilli, including spores (10).

Table 3. Percent Reduction in Bacteria, Including Spores, in Test Groups Compared with Control Groups for Each Microorganism (Statistical Significances of the Differences Are also Given)

	Time (min)	Carolina strain		ATCC strain	
		Percent reduction	Significance	Percent reduction	Significance
M. luteus	25	28	0.03	29	0.003
	45	29	0.006	35	0.003
B. subtilis vegetative cells	25	57	0.03	46	0.008
	45	76	0.03	78	0.008
B. subtilis spores	25	—	—	46	0.004
	45	—	—	52	0.004

Discussion

The results provide the first integrated comprehensive broad base of data bearing on the fate of viruses and bacteria, including spores, which may be used by a bioterrorist against building occupants. The test conditions are directly relevant to the indoor air environment situation in buildings, and the results can be generalized to buildings that are now occupied. The reductions in concentrations of relevant viruses and bacteria, including gram-negative glucose fermenters and nonfermenters and gram-positive cocci, bacilli, and spores were substantial and statistically robust.

The data show that bioterrorism-relevant aerosolized viruses and bacteria, including spores, respond like small particulates to the primary (electrical) forces that control the distribution of small particulates in a room. Further, these relevant microorganisms respond like small particulates to means designed to control airborne particulates.

The results could be used to anticipate the effects of a bioterrorist attack, provide information on means that can be used to minimize such effects, and aid in decision making regarding the best way to protect specific buildings. For example, the results indicate that much of the body of knowledge developed for particulate control in an indoor environment can be used to control bioterrorism-relevant viruses and bacteria, including spores, in such an environment. Thus, there is now a body of directly relevant data available to the public that provides guidance as to which occupant protection measures may reduce morbidity and mortality during a bioterrorist attack.

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