

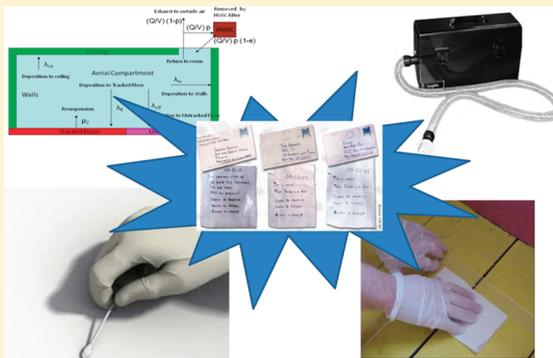
Characterizing Bioaerosol Risk from Environmental Sampling

Tao Hong^{*,†} and Patrick L. Gurian[†]

[†]Department of Civil, Architectural, and Environmental Engineering, Drexel University, 3141 Chestnut Street, Philadelphia, Pennsylvania 19104, United States

S Supporting Information

ABSTRACT: In the aftermath of a release of microbiological agents, environmental sampling must be conducted to characterize the release sufficiently so that mathematical models can then be used to predict the subsequent dispersion and human health risks. Because both the dose–response and environmental transport of aerosolized microbiological agents are functions of the effective aerodynamic diameter of the particles, environmental assessments should consider not only the total amount of agents but also the size distributions of the aerosolized particles. However, typical surface sampling cannot readily distinguish among different size particles. This study evaluates different approaches to estimating risk from measurements of microorganisms deposited on surfaces after an aerosol release. For various combinations of sampling surfaces, size fractions, HVAC operating conditions, size distributions of release spores, uncertainties in surface measurements, and the accuracy of model predictions are tested in order to assess how much detail can realistically be identified from surface sampling results. The recommended modeling and sampling scheme is one choosing 3, 5, and 10 μm diameter particles as identification targets and taking samples from untracked floor, wall, and the HVAC filter. This scheme provides reasonably accurate, but somewhat conservative, estimates of risk across a range of different scenarios. Performance of the recommended sampling scheme is tested by using data from a large-scale field test as a case study. Sample sizes of 10–25 in each homogeneously mixed environmental compartment are sufficient to develop order of magnitude estimates of risk. Larger sample sizes have little benefit unless uncertainties in sample recoveries can be reduced.



■ INTRODUCTION

After the 2001 anthrax attacks, many researchers have focused on how to effectively estimate human health risk during a bioterrorist attack, so that appropriate response actions can be taken.^{1–16} Thus, many models have been developed, including pathogen dose–response models^{17,18} and environmental transport models.^{19–23} These previous modeling efforts have recognized that the size distribution of the particulates has a substantial impact on the risk. For example, a previous study estimated that a *B. anthracis* spore concentration of 100/m² on a floor would correspond to a risk of one in a thousand if the spores were finely aerosolized 1 μm diameter particles, but one in a million if the spores were present as 10 μm diameter clumps.²⁴ Smaller particles settle more slowly and are less readily removed by HVAC system filters.¹⁹ These properties allow them to disperse over larger areas and persist longer in the air than larger particles. In addition, fine particulates are more respirable, and thus present greater risks than larger particles when inhaled.¹⁷

Previous modeling efforts have generally accounted for these aerosol size effects by modeling a number of discrete particle sizes.^{17,19,24,25} However, little previous research has examined how to conduct sampling in order to effectively parametrize these models. Although some aerosol samplers can provide information on particulate sizes, aerosol concentrations decline

rapidly after a release making it unlikely that response will be rapid enough to characterize a release based on aerosol concentrations. The material used in the attack may not be recovered in sufficient quantity for a size distribution analysis to be conducted. Surface sampling techniques generally provide information on only the total number of organisms or gene copies present, not the size fractions of particulates with which organisms are associated. Because different size particles partition into various environmental compartments at different rates, the concentrations found in different environmental compartments convey some information on the size distribution of the release. Having even limited information on the size distribution of the release may improve estimates of risk resulting from the release.

This study addresses the question of how much can be learned from simple aggregate concentration measurements, such as could reasonably be made after an actual release. To assess how well risk can realistically be identified from surface sampling results, a variety of alternative model formulations and sampling schemes are evaluated following a 7-step framework.

Received: January 23, 2012

Revised: April 24, 2012

Accepted: May 8, 2012

Published: May 8, 2012

The recommended modeling and sampling scheme is then applied to a case study using parameter estimates from a large scale field test, which aims to provide insights between a sampling scheme's reliability and its required sample size.

METHODOLOGY

The method has two components. First, forward modeling is conducted, in which characteristics of a release are assumed and a fate and transport model is used to estimate spore concentrations in different environmental compartments. Four particle sizes are used in this model, following previous forward modeling efforts.¹⁹ The *B. anthracis* spore concentrations predicted by the fate and transport model then serve as inputs for different inverse modeling approaches. Because only three environmental compartments are considered appropriate for postincident sampling (walls, floor, and HVAC filters), no more than three particle sizes can be identified for the inverse model. Different combinations of sampling locations and particle sizes are evaluated on their ability to match the forward modeling results. The overall goal of this analysis is to

identify sampling and modeling schemes, specifically as a combination of sampling locations and modeled particle sizes, which allow the release quantities for different spore sizes, their associated risks, and the amount exiting the release room to be characterized with the least error.

Fate and Transport Model (Forward Modeling). The general fate and transport model for a simple office with a HVAC system is expressed as

$$\frac{d\vec{M}(t)}{dt} = \vec{T}\vec{M}(t) \tag{1}$$

where $\vec{M}(t)$ is the quantity of spores in different compartments, and \vec{T} is a matrix of transfer coefficients. Based in Figure 1, the modeled office is divided into 7 internal compartments: air, tracked floor, untracked floor, walls, ceiling, HVAC, and the nasal passages of an occupant of the office, and the eighth compartment that consists of all areas external to the room. Thus the general fate and transport model can be written as

$$\frac{d\vec{M}}{dt} = \begin{pmatrix} \dot{M}_{air} \\ \dot{M}_{tf} \\ \dot{M}_{utf} \\ \dot{M}_w \\ \dot{M}_f \\ \dot{M}_{ec} \\ \dot{M}_{ce} \\ \dot{M}_n \end{pmatrix} = \begin{pmatrix} [(1 - e_f)p - 1]\frac{Q}{V} - \left(\lambda_{tf} + \lambda_{utf} + \lambda_w + \lambda_{ce} + \frac{Ie_n}{V}\right) & \mu_2 & 0 & 0 & 0 & 0 & 0 & 0 \\ & \lambda_{tf} & -\mu_2 & 0 & 0 & 0 & 0 & 0 \\ & \lambda_{utf} & 0 & 0 & 0 & 0 & 0 & 0 \\ & \lambda_w & 0 & 0 & 0 & 0 & 0 & 0 \\ & e_f p \frac{Q}{V} & 0 & 0 & 0 & 0 & 0 & 0 \\ & (1 - p)\frac{Q}{V} & 0 & 0 & 0 & 0 & 0 & 0 \\ & \lambda_{ce} & 0 & 0 & 0 & 0 & 0 & 0 \\ & \frac{Ie_n}{V} & 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix} \begin{pmatrix} M_{air} \\ M_{tf} \\ M_{utf} \\ M_w \\ M_f \\ M_{ec} \\ M_{ce} \\ M_n \end{pmatrix} \tag{2}$$

where $M_{air}(t)$ is the number of spores in the air compartment, $M_{tf}(t)$ is the number of spores on the tracked surface of the floor, $M_{utf}(t)$ is the number of spores on the untracked surface of the floor, $M_w(t)$ is the number of spores on the walls, $M_f(t)$ is the number of spores on the filter, $M_{ec}(t)$ is the number of spores in the external compartment, $M_{ce}(t)$ is the number of spores on the ceiling, $M_n(t)$ is the number of spores in the occupant's nasal passages. M_{air} , M_{tf} , M_{utf} , M_w , M_f , M_{ec} , M_{ce} , and M_n are all given in units of spores (number of organisms). Next we define the following parameters: Q is the discharge from the air compartment (m^3/s), μ_2 is the resuspension rate from the tracked surface into the air compartment (s^{-1}), p is the fraction of air recirculated into the building by the HVAC system, e_f is the efficiency of the filter at removing particles, e_n is the efficiency of the nasal passages at removing particles, I is the inhalation rate of the occupant (m^3/s), and V is the volume of the room (m^3). λ_{tf} , λ_{utf} , λ_w , and λ_{ce} are the deposition rates for aerosolized pathogens onto the tracked surface, the untracked surface and the floors, walls, and ceiling respectively (s^{-1}),

which can be expressed by parameters representing the indoor air flow conditions:

$$\lambda_{tf(utf)} = \frac{A_{tf(utf)}}{V} \times \frac{v}{1 - e^{-\pi v/2\sqrt{Dk_e}}} \tag{3}$$

$$\lambda_w = \frac{A_w}{V} \times \frac{2}{\pi} \sqrt{Dk_e} \tag{4}$$

$$\lambda_c = \frac{A_c}{V} \times \frac{v}{e^{\pi v/2\sqrt{Dk_e}} - 1} \tag{5}$$

where D (m^2/s) is the particle diffusivity, k_e (s^{-1}) is turbulence intensity, and v (m/s) is particle settling velocity, which is given in eq 6 as a function of the particle's diameter (d , unit of m), the viscosity of air (μ_{air} , unit of $kg/(m \times s)$), the density of the particle (ρ_p , unit of kg/m^3), and the density of air (ρ_{air} , unit of kg/m^3):

$$V_t = \frac{gd^2(\rho_p - \rho_{air})}{18\mu_{air}} \tag{6}$$

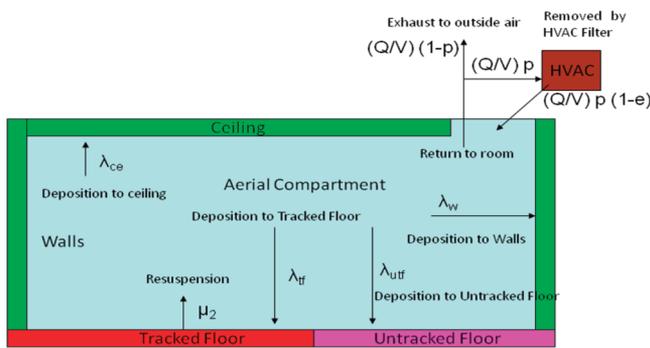


Figure 1. Schematic of single room office suite.

For the details of the solution to eq 2, please refer to Hong et al.²⁴ Parameter values are provided as Supporting Information (Table S1).

Such compartment modeling approaches are widely used, although their limitations must be acknowledged. This modeling approach assumes that each compartment is completely mixed, which is not accurate at the immediate time of a release but becomes much more accurate as the release disperses over time. Thus, the methodology presented here is intended for use in areas somewhat removed from the initial release such that concentrations on different surfaces (walls, untracked floor, and HVAC filter) can be considered reflective of more or less the same time-averaged air concentration. The model used here is based on literature studies of how particulates behave in the indoor environment.^{1,19,26,31} In the case of *B. anthracis* spores, a verification of the above-mentioned fate and transport model has been undertaken³² by comparing the results of a completely mixed compartment model with observations of *B. anthracis* spore concentrations from the Hart Senate Office Building reported by Weis et al.¹⁰ The observed behavior of the spores was generally consistent with model predictions, allowing the model to be used to generate order of magnitude estimates of risk.³² This verification analysis used a Bayesian Monte Carlo approach to identify release parameters. Although the Bayesian Monte Carlo approach has promise, it does require the identification of prior distributions for parameters and thousands of model runs to characterize posterior distributions, which is complicated and computationally intensive. In the following section, the potential of a more straightforward and rapid classical approach to identifying release characteristics is evaluated.

Particle Identification (Inverse Modeling). Because all of the processes in eq 2 are first order, the release quantity (\vec{M}_r) can be expressed as the product of a matrix, commonly termed the inverse transfer matrix (T^{-1}) and the mass of deposited *B. anthracis* spores in each environmental compartment (\vec{M}_s).³³

$$\vec{M}_r = T^{-1} \vec{M}_s \quad (7)$$

The inverse transfer matrix (T^{-1}) in eq 7 is a function of time, as specified by the fate and transport model. This matrix goes through an initial period of rapid change, when deposition from the air compartment is the dominant process. Then resuspension, a much slower process, becomes the rate controlling process. This is illustrated in Figure S1 which shows the mass in different compartments over time for the four different particle sizes considered. The mass in each compartment varies initially and then stabilizes within several hours of the release. This stabilization is not a true steady state, as eventually resuspension

will deplete concentrations on tracked surfaces (and in fact a gradual decline in tracked floor concentration can be seen over several hours for the 10 μm fraction, as this fraction is most readily resuspended). However, this period after the initial deposition phase is termed “quasi-steady state” because surface concentrations are roughly stable over the time period during which initial release characterization would be expected to occur (days). The T^{-1} matrix can be considered roughly constant in this period.

At quasi-steady state, if the diameters of spores are divided into four groups: 1 μm, 3 μm, 5 μm, and 10 μm,¹⁶ eq 7 can be expanded to give

$$\begin{bmatrix} M_{r1} \\ M_{r3} \\ M_{r5} \\ M_{r10} \end{bmatrix} = \begin{bmatrix} K_{surfA_1} & K_{surfA_3} & K_{surfA_5} & K_{surfA_{10}} \\ K_{surfB_1} & K_{surfB_3} & K_{surfB_5} & K_{surfB_{10}} \\ K_{surfC_1} & K_{surfC_3} & K_{surfC_5} & K_{surfC_{10}} \\ K_{surfD_1} & K_{surfD_3} & K_{surfD_5} & K_{surfD_{10}} \end{bmatrix}^{-1} \begin{bmatrix} M_{s_surfA} \\ M_{s_surfB} \\ M_{s_surfC} \\ M_{s_surfD} \end{bmatrix} \quad (8)$$

here $K_{surf i_j}$ is the distribution coefficient for spores with diameter of j on surface i , $M_{s_surf i}$ is the unbiased mass measurement of the total spores on surface i , and M_{rj} is the initial release quantity for spores whose diameters are j μm. It is the M_{rj} values which need to be identified from sampling so that the impacts of the release can be modeled. Each size fraction that is considered introduces another row to the left-hand side of eq 8 which requires an additional distribution coefficient and surface to be measured to identify the release quantity (that is there must be an additional row on the right-hand side).

Because of the difficulties associated with recovering samples from surfaces,^{34–36} leading to potential errors in the sampling and analysis steps, measurements from the surfaces vary from their true values. Thus, it is necessary to evaluate the impacts of such errors in measurements of \vec{M}_s on the characterized release quantity, \vec{M}_r . Simulated measurement errors are constructed by Hadamard multiplying (element by element product, symbol \circ) \vec{M}_s , the environmental compartment concentration values from the fate and transport model, by a coefficient (\vec{Z}):

$$\vec{M}_r = T^{-1} (\vec{M}_s \circ \vec{Z}) \quad (9)$$

For the initial evaluation the errors in the \vec{Z} matrix are assumed to be normally distributed with mean of 1 and standard deviation of 0.3. The value of 0.3 corresponds to fairly large errors (the 95% interval extends from a 60% underestimate to a 60% overestimate) reflecting the desire to identify an approach robust to even large measurement errors. After the initial evaluation using these assumed errors, the framework is subsequently applied using errors calibrated to data from a large-scale field sampling exercise.

Modeled Scenarios. Three sets of size distributions of released *B. anthracis* spores are employed to test the robustness of candidate modeling and sampling schemes. The nominal size distribution is based on the lab analysis of the 2001 anthrax letter attack: the fractions of 1, 3, 5, and 10 μm are 0.14%, 1.46%, 8.40%, and 90%, respectively.³⁷ The second set doubles the quantity of spores with diameters of 1, 3, and 5 μm, and reduces the amount of 10 μm; thus the new fractions are 0.28%, 2.92%, 16.80%, and 80%, respectively. This size fraction represents the situation where more fine particles are released, which is termed “light”. The third size fraction, termed “heavy”, has half the number of 1, 3, and 5 μm spores (size fractions are

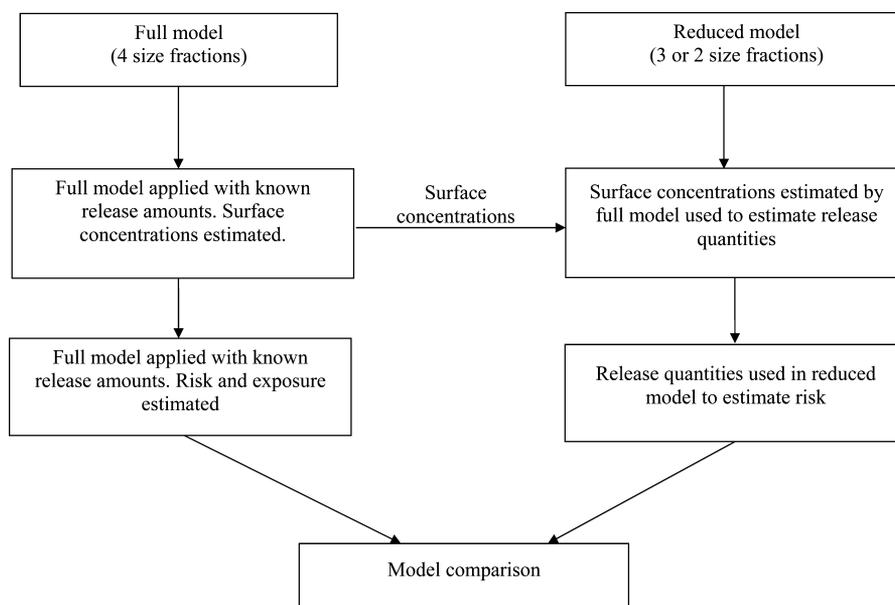


Figure 2. Evaluation framework used in this study.

0.07%, 0.73%, 4.20%, and 95%) as compared to the nominal situation. Because different HVAC operation situations might change the fate and transport properties of released spores and impact human exposure, three HVAC operating conditions are considered, representing low, medium, and high air recirculation rates ($p = 0.5, 0.75, 0.95$) for each set of size distributions considered. In addition, surface concentration measurements with errors (model predictions multiplied by a coefficient matrix \bar{Z}) and without errors are considered. Since the impacts of measurement error are modeled by a matrix \bar{Z} whose elements are random numbers, it is necessary to propagate this uncertainty. Thus medians and 90% confidence intervals from Monte Carlo simulations with 1000 iterations are included. In total each candidate scheme is evaluated under 18 conditions (3 HVAC operational conditions \times 3 size fraction distributions \times with and without measurement errors).

Evaluation Framework. Only 3 out of 8 modeled locations (wall, untracked floor, and HVAC filter) are considered feasible for sampling in this study. The indoor air, external air, ceiling, and human nasal passage compartments in the fate and transport model (eq 2) are excluded due to the relatively low concentration values that would be expected in these compartments several hours after a release, which could not be measured accurately. The tracked floor is excluded because it provides essentially the same information as the untracked floor but with less reliability as it is less stable over time. This indicates that no more than three size fractions can be identified from the release quantity (eq 7 will be underdetermined if one tries to identify four size fractions), while the full model includes four size fractions.

The steps of the model evaluation framework used in this study are illustrated in Figure 2: (1) the full model, consisting of all 4 size fractions, is run and concentrations in the different environmental compartments at quasi-steady state, as well as the risk to the occupants of the room and the amount of spores exiting the room, are simulated; (2) a set of compartments is chosen for measuring *B. anthracis* spore concentrations, where the selected compartments should provide concentrations that are homogeneous and greater than the analytical detection limit; (3) particle sizes are selected to be modeled; given that

only 3 environmental compartments are considered suitable for measurement and that the full model has 4 particle sizes, the number of size fractions to be modeled based on available measurements must be reduced from the number in the full model, hence this is termed the reduced model; (4) the inverse transfer matrix at quasi-steady for the reduced model is computed based on the modeled particle sizes and surfaces to be measured (i.e., particle sizes and environmental compartments not selected in Steps 2 and 3 are excluded from the model), and the matrix (\bar{Z}), representing measurement error, is generated; (5) Monte Carlo simulations are conducted, and the release quantities of the particle sizes for the reduced model are estimated: when surface measurements are error free, the release quantities are calculated with eq 7. When simulated measurement errors are included in the environmental concentrations, eq 9 is used. When measurement errors are present, it is possible to obtain negative estimates of mass. Zeros are substituted for any negative estimates; (6) using the estimated release quantities and the reduced model (i.e., the model formulated in Step 4), the risk to occupants of the room and the number of spores exiting this room are calculated; and (7) the ratios of the reduced model and full model are determined for the risk to occupants and amount of spores exiting the room.

For an identification approach to be effective, the ratios of quantities such as human risk and the amount of spores exiting the room between the reduced form and the full model (based on four size fractions) should be close to one but should not have much risk of falling substantially below one. Ratios greater than one represent conservative models, meaning that a model that overestimates risk to the occupants of the room, which in most cases would be preferred to an approach that underestimates risk. Likewise an approach that overestimates the number of spores leaving the room (which would inform estimates of risk to those downwind of the release) would generally be preferred over an approach that underestimates the number of spores leaving the room. However, in order to provide a reasonable prediction, these ratios should not be too far away from one.

Table 1. Results for Approaches to Identify Three Size Fractions^a

selected compartments	size fractions to be identified (μm)	set of size distribution	HVAC operation condition	ratio of occupants' risk (compared to full model)			ratio of spores exiting the room (compared to full model)				
				no measurement error	measurement error			no measurement error	measurement error		
					50%	5%	95%		50%	5%	95%
untracked floor, walls, HVAC filter	1,3,10	nominal	0.50	1.15	1.83	0.68	5.02	1.06	1.34	0.88	2.12
			0.75	1.14	1.92	0.73	4.90	1.06	1.38	0.90	2.14
			0.95	1.11	1.68	0.69	4.61	1.05	1.31	0.89	2.10
		light	0.50	1.20	1.37	0.59	3.48	1.12	1.26	0.85	1.99
			0.75	1.18	1.31	0.57	3.38	1.11	1.25	0.86	1.94
			0.95	1.14	1.27	0.55	3.18	1.09	1.21	0.85	1.91
		heavy	0.50	1.11	2.47	0.88	6.56	1.03	1.41	0.91	2.32
			0.75	1.10	2.56	0.88	6.63	1.03	1.45	0.94	2.35
			0.95	1.08	2.32	0.89	6.45	1.03	1.42	0.91	2.30
	1,5,10	nominal	0.50	1.00	1.40	0.61	3.70	1.00	1.19	0.85	1.70
			0.75	1.00	1.44	0.65	3.58	1.00	1.20	0.87	1.70
			0.95	1.00	1.31	0.63	3.35	1.00	1.16	0.86	1.68
		light	0.50	1.00	1.12	0.55	2.53	0.99	1.10	0.81	1.53
			0.75	1.00	1.08	0.53	2.47	0.99	1.10	0.83	1.51
			0.95	1.00	1.07	0.54	2.33	0.99	1.07	0.82	1.46
		heavy	0.50	1.00	1.82	0.80	4.76	1.00	1.24	0.87	1.91
			0.75	1.00	1.88	0.81	4.80	1.00	1.28	0.89	1.91
			0.95	1.00	1.77	0.77	4.69	1.00	1.25	0.86	1.88
	3,5,10	nominal	0.50	1.00	2.53	0.79	6.05	1.00	1.52	0.96	2.69
			0.75	1.00	2.58	0.89	6.05	1.00	1.54	0.95	2.71
			0.95	1.00	2.35	0.92	5.61	1.01	1.48	0.96	2.66
		light	0.50	1.00	1.80	0.75	3.99	1.00	1.36	0.92	2.39
			0.75	1.00	1.71	0.78	3.75	1.01	1.35	0.91	2.37
			0.95	1.00	1.57	0.77	3.53	1.01	1.28	0.90	2.25
heavy		0.50	1.00	3.43	1.03	8.13	1.00	1.61	0.97	2.98	
		0.75	1.00	3.48	1.02	8.47	1.00	1.65	1.00	3.02	
		0.95	1.00	3.21	1.00	8.24	1.00	1.60	0.97	2.93	
overall					0.75	8.47		0.90	3.02		

^aThe size fractions of 1, 3, 5, and 10 μm for the nominal scenario are 0.14%, 1.46%, 8.40%, and 90%. The size fractions of 1, 3, 5, and 10 μm for the light scenario are 0.28%, 2.92%, 16.80%, and 80%. The size fractions of 1, 3, 5, and 10 μm for the heavy scenario are 0.07%, 0.73%, 4.20%, and 95%. If a negative release quantity is identified, it will be assumed 0. Bold shows the recommended approach. Values in the "overall" row come from the lowest 5% and the highest 95% ratios.

RESULTS

The first modeling and sampling schemes considered are those with three different size fractions. The fraction of spores with the diameter of 10 μm is always identified because it constitutes the vast majority of the release, while the other two identification targets are selected from the remaining three candidate particle sizes. Table 1 presents results for identification approaches for three particle sizes. If the surface sampling results are perfect (no errors), ratios for the occupants' risk, and the amount of spores exiting the room are very close to 1, indicating that these approaches closely match the full model. However, once sampling inefficiency and potential errors are considered, the median ratios from some sampling schemes overestimate risk by a factor of 2 or 3. This indicates that predictions for the release quantity and ratios are sensitive to measurement errors. Across the different size fraction identification schemes, the ones using 3, 5, and 10 μm s as the identification targets outperform others, because the fifth percentiles for the ratios of human health risk and the amount of spores exiting the room are closer to 1, which means

adopting this sampling scheme reduces the potential extent of underestimation due to sampling error.

If two size fractions are to be estimated, again the 10 μm size fraction is always included in the model because it accounts for the majority of spores. The other identification target is selected from the 1, 3, and 5 μm diameter size fractions, while two sampling surfaces are selected from the untracked floor, wall, and HVAC filter. Table S2 provides the results for the identification approaches based on two sampling surfaces. If measurement error is not considered, three candidate sampling schemes satisfy the evaluation criteria: (1) identifying 5 and 10 μm particle sizes by sampling from the untracked floor, and the walls; (2) identifying 5 and 10 μm particle sizes by sampling from the walls and the HVAC filter; and (3) identifying 3 and 10 μm particle sizes by sampling the untracked floor and the HVAC filter. However, if the effect of measurement error is considered, the fifth percentile ratios for the human risk are less than 0.5 for the selected combinations, indicating serious underestimations of risk are possible due to sampling error. As a result, none of the two particle size approaches are recommended.

The results of single particle sampling approaches are summarized in Tables S3 and S4. As might be expected, single particle approaches cannot capture the diversity of behavior of different particulate sizes and perform poorly.

APPLICATION OF THE SAMPLING SCHEME

Based on the above results, identifying 3, 5, and 10 μm particle size fractions based on samples from the untracked floor, walls, and the HVAC filter is recommended as a modeling and sampling scheme. To examine how such an approach might be applied, and what degree of uncertainty would be present in results, concentration measurements from a large scale field test are analyzed below.

In September 2008, Battelle Energy Alliance conducted five release events of *Bacillus atrophaeus*, a surrogate for *B. anthracis*, at Idaho National Laboratory (INL) in a typical two-story commercial building (Building PBF632) in order to support the Department of Homeland Security (DHS), the Environmental Protection Agency (EPA), and the Joint Program Executive Office Chemical and Biological Defense's (JPEOCBD) Sample Collection Operation Test Plan. Among the five tested release events, Events 1, 2, and 4 were performed on the first floor of the building, while Events 3 and 5 were performed on the second floor. For each release event, *Bacillus atrophaeus* spores were aerosolized through a battery-powered generator, and surface concentrations were measured using collection methods: vacuum, wipe, and swab. Between release events, the building was decontaminated and the effectiveness of decontamination was verified by clearance samples. For details of this field test, please refer to the official report.³⁸

To create the matrix \bar{Z} , it is necessary to estimate sample recovery variability and uncertainty associated with different collection methods. This was done using settling plate samples as a reference by a multivariate regression model. Recovery efficiencies (γ_j) are estimated as

$$Y_{i,j,k} = \sum_{i=1}^{n_i} \beta_i I_{i,k} + \sum_{j=1}^{n_j} \gamma_j J_{j,k} + \varepsilon_{i,j,k} \quad (10)$$

where the dependent variable is the log transformed surface concentration $Y_{i,j,k}$, i indexes the combination of room and sampling event where the sample was taken (thus rooms are indexed separately for each of the sampling events), j indexes collection method, and k indexes the measurements within each room–event and sampling method combination. The model has two classes of parameters: (1) β_i the nuisance parameters, which account for the effects of $I_{i,k}$, the indicator of location–event combination ($I_{i,k}$ is 1 when the k th sample is from the i th location–event combination and 0 otherwise), and (2) γ_j the collection method recovery fractions, which account for the effects of $J_{j,k}$, the collection method indicator ($J_{j,k}$ is 1 when the k th sample is sampled by the j th collection method and 0 otherwise). The error terms of this regression ($\varepsilon_{i,j,k}$) are collection method specific, following a normal distribution with a mean of zero and a standard deviation of σ_j ($\varepsilon_{i,j,k} \sim N(0, \sigma_j)$). Parameters are estimated by maximum likelihood estimation (MLE) using data from the 3 release events with more than 55% detectable concentrations (Events 1, 4, and 5) for a total of 550 observations, which consists of 146 swabs samples, 76 vacuum samples, 227 wipes samples, and 101 settling plate samples. The inverse of the information matrix is used to estimate standard errors of model parameters. The standard error of γ_j , denoted by σ_j , is of particular interest since this is the

uncertainty in mean recovery (compared to the settling plate data that were used as a reference). The normality of the residual errors from the best fitted model has been verified, and values of σ_j and σ_p are present in Table 2 by sample collection method.

Table 2. Standard Deviation and Its Uncertainty for the Error Term (Log Scale)^a

collection method	uncertainty σ_j	variability σ_j	total error for indicated sample size		
			$(\sigma_j^2 + (\sigma_j^2/n))^{1/2}$		
			$n = 1$	$n = 4$	$n = 25$
swab	0.32	0.94	0.99	0.57	0.38
vacuum	0.18	0.67	0.69	0.38	0.22
wipe	0.25	0.97	1.00	0.54	0.31

^a σ_j is uncertainty in mean recovery for different collection method, while σ_j is variability in the recovery from sample to sample.

For samples taken from an unknown surface, the overall uncertainty has two sources, uncertainty in mean recovery (σ_j) and variability in the recovery from sample to sample (σ_j). The sampling variability can be reduced by increasing sample size. However, unless a reference method is available, increasing the sample size will not reduce uncertainty in the recovery fraction. Although a reference method (i.e., settling plate data) was available for this field study, one would not be available in the aftermath of an actual biological attack. Thus for a given collection method j , its residual error, (i.e., the elements of matrix \bar{Z}), is generated by the following distribution:

$$\ln \varepsilon_j \sim N\left(0, \sqrt{\sigma_j^2 + \frac{\sigma_j^2}{n}}\right) \quad (11)$$

where n is the sample size.

Based on errors calculated in this manner and shown in Table 2, nine different \bar{Z} matrices are developed, one for each of three sample collection methods (swabs, vacuum, and wipes) and three sample sizes (1, 9, and 25). These \bar{Z} matrices estimated from the field data are tested on the previously recommended sampling and modeling scheme, taking samples from untracked floor, wall, and the HVAC filter to identify 3, 5, and 10 μm . A Monte Carlo simulation of 1000 iterations is used for each sampling scheme, and the ratios of human risk and amount of spores exiting the room are computed. The impacts of imperfect sample recovery and sample size are investigated by checking the quotients between the 95th percentile and fifth percentile of these two ratios, which represents the uncertainty factor of the results. The closer this quotient is to 1, the less uncertainty in the sampling scheme. Figure 3 illustrates this quotient as a function of sample size for nine sampling schemes. Uncertainties in number of spores exiting the room are smaller than those in health risks to occupants of the release room. Thus health risk to occupants of the room drives the sample size requirement. Based on these inputs, it is suggested to take 25 samples from each environmental compartment (walls, floor, HVAC filter in a homogeneously mixed zone), which results in roughly 1 order of magnitude difference between the 95th percentile and fifth percentile estimations for risk estimates in the release room. Sample sizes larger than 25 from each environmental compartment within the same homogeneously mixed zone provide little benefit as the

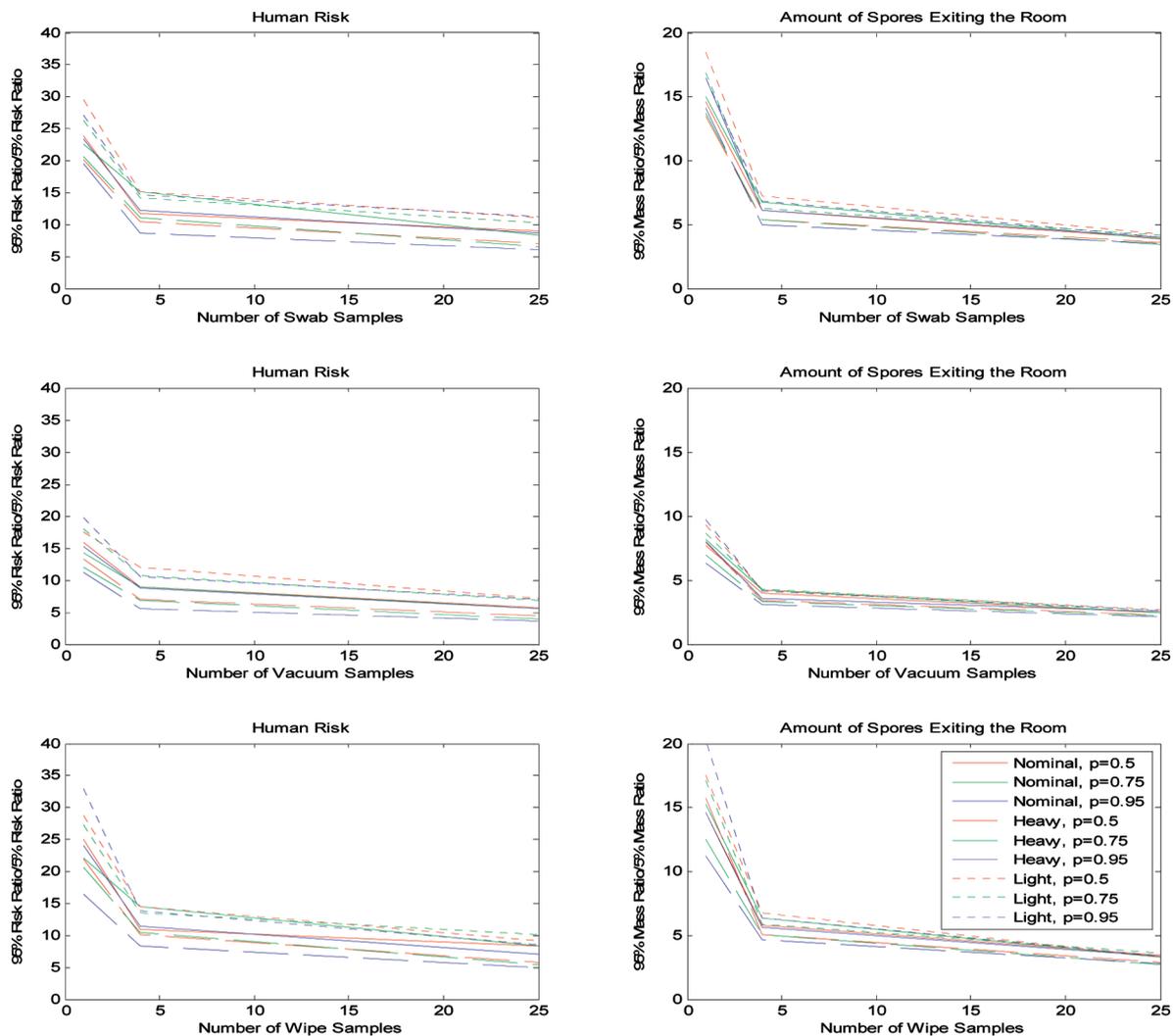


Figure 3. Relationship between a sample scheme's amount of uncertainty (ratio of 95th to 5th percentiles of estimated risk) and its sample size for different ventilation recirculation rates ($p = 0.5$, $p = 0.75$, and $p = 0.95$) and different particle size distribution (light, heavy, and nominal, see text for definitions) (Identification targets are 3, 5, and 10 μm , and samples are taken from untracked floor, wall, and the HVAC filter)O.

remaining uncertainty is dominated by uncertainty in mean recovery (σ_r) rather than sampling variability (σ_j). Given that a reference method (i.e., settling plates) would not be available in a real release, additional samples will not reduce uncertainty in recovery rates. Accordingly once the sample size is sufficient to reduce the effect of σ_j on overall uncertainty, then there is little benefit to further sampling. One potential option to reduce σ_r would be to conduct positive control studies.³⁹ Although this option is not explored further here, the expected reduction in σ_r could be used with the approach described here to estimate the benefit of such positive control studies. Among the three sample collection methods, samples taken by wipes have the most sampling variability and hence require the largest sample size, followed by swab and vacuum. One should be aware that results may be sensitive to specifics of sampling techniques (material used for sampling, sample extraction method used, see for example Solon et al.⁴⁰) and thus conclusions might change as improved sampling methods are developed (see Griffith⁴¹ for a summary of the performance of a variety of sampling methods).

DISCUSSION

This analysis provides a framework for determining how much detail can feasibly be included in models and provides guidance on appropriate sampling schemes. This analysis suggests that (1) a 3 size fraction model (3, 5, and 10 μm), which can be estimated based on aggregate surface sampling of the untracked floor, wall, and the HVAC filter, provides reasonable and conservative estimates of risk and number of spores leaving the release room; and 2) in an example application, a sample size of about 25 (in each environmental compartment of a homogeneously mixed zone) provided order of magnitude estimates of risk, but unless a reference is available to enable uncertainties in recovery to be reduced, there is little benefit to taking more than 25 samples from each surface within a homogeneously mixed zone.

Implicit in this approach is the assumption that the mean concentration is to be used to estimate risk. This is appropriate for estimating exposure within a homogeneously mixed zone when risk estimates are based on either the exponential or beta-Poisson dose response function, as both these functions are based on population average exposures and allow for Poisson variability in exposure across individuals.⁴² Exponential and

beta-Poisson models have both been used successfully to describe *B. anthracis* dose response.^{17,43}

Calculations involved in this paper depend on a wide variety of parameter inputs and modeling assumptions. Model sensitivity analyses indicate that resuspension rate³² and dose response¹⁶ are key parametric uncertainties in estimating risk. Structural uncertainties are also important. In particular, the applicability of the complete mixing assumption is of great importance and should be carefully assessed for each application. While uniform mixing key is generally true over time for fine particles as deposition rates are slow relative to mixing rates, the immediate vicinity of a release may contain large amounts of coarse particles due to rapid deposition of these particles before mixing can occur. Thus, the complete mixing assumption could underestimate risk to the people who were exposed at close range and right after a release, but would provide more accurate risk estimates as exposures became more removed in space and time from the initial release. More detailed work, such as computational fluid dynamics modeling could provide guidance as to how to interpret surface concentrations in the immediate vicinity of a release. Whether this framework is applicable can be readily ascertained from sampling data. Areas of a room showing spatial variability in sampling results would be areas for which the complete mixing assumption is not valid. Results from areas with consistent and uniform concentrations would be appropriate for use with this method.

■ ASSOCIATED CONTENT

📄 Supporting Information

Figure of the number of spores in different environmental compartments over time for a single room model; figure of the distribution of *B. anthracis* with different diameters after 8 h; tables containing parameter inputs and results for different identification approaches. This material is available free of charge via the Internet at <http://pubs.acs.org>.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: hongtao510@gmail.com. Address: Office of Research and Development, U.S. Environmental Protection Agency, 960 College Station Road, Athens, Georgia 30605, United States.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This research was funded through the Center for Advancing Microbial Risk Assessment, supported by the U.S. Environmental Protection Agency and U.S. Department of Homeland Security, under the Science to Achieve Results (STAR) grant program (grant R83236201). We appreciate valuable discussion and inputs from Ken Fan and Nicholas Dudley Ward.

■ REFERENCES

- (1) Price, P. N.; Sohn, M. D.; Lacommare, K. S. H.; McWilliams, J. A. Framework for evaluating anthrax risk in buildings. *Environ. Sci. Technol.* **2009**, *43* (6), 1783–1787.
- (2) Wein, L. M.; Craft, D. L.; Kaplan, E. H. Emergency response to an anthrax attack. *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100* (7), 4346–4351.
- (3) Brookmeyer, R.; Johnson, E.; Bollinger, R. Public health vaccination policies for containing an anthrax outbreak. *Nature* **2004**, *432* (7019), 901–904.

- (4) Inglesby, T. V. Anthrax as a biological weapon, 2002: Updated recommendations for management (vol 287, pg 2236, 2002). *JAMA-J. Am. Med. Assoc.* **2002**, *288* (15), 1849–1849.

- (5) Dewan, P. K.; Fry, A. M.; Laserson, K.; Tierney, B. C.; Quinn, C. P.; Hayslett, J. A.; Broyles, L. N.; Shane, A.; Winthrop, K. L.; Walks, I.; Siegel, L.; Hales, T.; Semenova, V. A.; Romero-Steiner, S.; Elie, C.; Khabbaz, R.; Khan, A. S.; Hajjeh, R. A.; Schuchat, A.; Washington, D. C. A. R. T. Inhalational anthrax outbreak among postal workers, Washington, DC, 2001. *Emerg. Infect. Dis.* **2002**, *8* (10), 1066–1072.

- (6) Brachman, P. S., Inhalation Anthrax. *Ann. N.Y. Acad. Sci.* **1980**, *353*.

- (7) Bradley, A. P.; Tanja, P.; Yeskey, K. Public Health in the Time of Bioterrorism. *Emerg. Infect. Dis.* **2002**, *8*, 10.

- (8) Dull, P. M.; Wilson, K. E.; Kournikakis, B.; Whitney, E. A. S.; Boulet, C. A.; Ho, J. Y. W.; et al. *Bacillus anthracis* aerosolization associated with a contaminated mail sorting machine. *Emerg. Infect. Dis.* **2002**, *1044–1047*.

- (9) Hughes, J. M.; Gerberding, J. L. Anthrax bioterrorism: lessons learned and future directions. *Emerg. Infect. Dis.* **2002**, *8* (10), 1013–4.

- (10) Weis, C. P.; Intrepido, A. J.; Miller, A. K.; Cowin, P. G.; Durno, M. A.; Gebhardt, J. S.; Bull, R. Secondary aerosolization of viable *Bacillus anthracis* spores in a contaminated US Senate Office. *JAMA* **2002**, *288* (22), 2853–8.

- (11) Brookmeyer, R.; Johnson, E.; Barry, S. Modelling the incubation period of anthrax. *Stat. Med.* **2005**, *24* (4), 531–542.

- (12) Doolan, D. L.; Freilich, D. A.; Brice, G. T.; Burgess, T. H.; Berzins, M. P.; Bull, R. L.; Graber, N. L.; Dabbs, J. L.; Shatney, L. L.; Blazes, D. L.; Bebris, L. M.; Malone, M. F.; Eisold, J. F.; Mateczun, A. J.; Martin, G. J. The US capitol bioterrorism anthrax exposures: Clinical epidemiological and immunological characteristics. *J. Infect. Dis.* **2007**, *195* (2), 174–84.

- (13) Sanderson, W. T.; Stoddard, R. R.; Echt, A. S.; Piacitelli, C. A.; Kim, D.; Horan, J.; Davies, M. M.; McCleery, R. E.; Muller, P.; Schnorr, T. M.; Ward, E. M.; Hales, T. R. *Bacillus anthracis* contamination and inhalational anthrax in a mail processing and distribution center. *J. Appl. Microbiol.* **2004**, *96* (5), 1048–1056.

- (14) Coleman, M. E.; Thran, B.; Morse, S. S.; Hugh-Jones, M.; Massulik, S. Inhalation anthrax: Dose response and risk analysis. *Bio Secur. Bioterror.* **2008**, *6* (2), 147–159.

- (15) Mitchell-Blackwood, J.; Gurian, P. L.; O'Donnell, C. Finding Risk-Based Switchover Points for Response Decisions for Environmental Exposure to *Bacillus anthracis*. *Hum. Ecol. Risk Assess.* **2011**, *17* (2), 489–509.

- (16) Hong, T.; Gurian, P. L.; Huang, Y.; Haas, C. N. Prioritizing Risks and Uncertainties from Intentional Release of Selected Category A Pathogens. *PLoS One* **2012**, *7* (3), e32732.

- (17) Bartrand, T. A.; Weir, M. H.; Haas, C. N. Dose-response models for inhalation of *Bacillus anthracis* spores: Interspecies comparisons. *Risk Anal.* **2008**, *28* (4), 1115–1124.

- (18) Huang, Y.; Haas, C. N. Time-dose-response models for microbial risk assessment. *Risk Anal.* **2009**, *29* (5), 648–661.

- (19) Sextro, R. G.; Lorenzetti, D. M.; Sohn, M. D.; Thatcher, T. L. Modeling the spread of anthrax in buildings. *Indoor air 2002*, Monterey, CA, 2002; Monterey, CA, 2002; pp 506–511.

- (20) Riley, W. J.; McKone, T. E.; Lai, A. C. K.; Nazaroff, W. W. Indoor particulate matter of outdoor origin: Importance of size-dependent removal mechanisms. *Environ. Sci. Technol.* **2002**, *36* (2), 200–207.

- (21) Waring, M. S.; Siegel, J. A. Particle loading rates for HVAC filters, heat exchangers, and ducts. *Indoor Air* **2008**, *18* (3), 209–224.

- (22) Sreedharan, P.; Sohn, M. D.; Nazaroff, W. W.; Gadgil, A. J. Influence of indoor transport and mixing time scales on the performance of sensor systems for characterizing contaminant releases. *Atmos. Environ.* **2007**, *41* (40), 9530–9542.

- (23) Sreedharan, P.; Sohn, M. D.; Gadgil, A. J.; Nazaroff, W. W. Systems approach to evaluating sensor characteristics for real-time monitoring of high-risk indoor contaminant releases. *Atmos. Environ.* **2006**, *40* (19), 3490–3502.

(24) Hong, T.; Gurian, P. L.; Ward, N. F. D. Setting Risk-Informed Environmental Standards for *Bacillus anthracis* Spores. *Risk Anal.* **2010**, *30* (10), 1602–1622.

(25) Reshetin, V. P.; Regens, J. L. Simulation modeling of anthrax spore dispersion in a bioterrorism incident. *Risk Anal.* **2003**, *23* (6), 1135–1145.

(26) Sohn, M. D.; Apte, M. G.; Sextro, R. G.; Lai, A. C. K. Predicting size-resolved particle behavior in multizone buildings. *Atmos. Environ.* **2007**, *41* (7), 1473–1482.

(27) Thatcher, T. L.; Lunden, M. M.; Revzan, K. L.; Sextro, R. G.; Brown, N. J. A concentration rebound method for measuring particle penetration and deposition in the indoor environment. *Aerosol Sci. Technol.* **2003**, *37* (11), 847–864.

(28) Thatcher, T.; Sextro, R.; Ermak, D. *Database of Physical, Chemical and Toxicological Properties of CB Warfare Agents for Modeling Airborne Dispersion In and Around Buildings*; Lawrence Berkeley National Laboratory: Berkeley, CA, 2000.

(29) Sohn, M. D.; Sextro, R. G.; Gadgil, A. J.; Daisey, J. M. Responding to sudden pollutant releases in office buildings: 1. Framework and analysis tools. *Indoor Air* **2003**, *13* (3), 267–276.

(30) Nazaroff, W. W. Indoor particle dynamics. *Indoor Air* **2004**, *14*, 175–183.

(31) Nazaroff, W. W.; Cass, G. R. Mathematical-modeling of indoor aerosol dynamics. *Environ. Sci. Technol.* **1989**, *23* (2), 157–166.

(32) Hong, T.; Gurian, P. L. A Bayesian Approach to Model Calibration for Weaponized *B. Anthracis* Risk Assessment. *Environ. Sci. Technol.* **2012**, under review.

(33) Ramaswami, A.; Milford, J. B.; Small, M. J. *Integrated Environmental Modeling: Pollutant Transport, Fate, and Risk in the Environment*; Wiley: Hoboken, NJ, 2005.

(34) Frawley, D. A.; Samaan, M. N.; Bull, R. L.; Robertson, J. M.; Mateczun, A. J.; Turnbull, P. C. B. Recovery efficiencies of anthrax spores and ricin from nonporous or nonabsorbent and porous or absorbent surfaces by a variety of sampling methods. *J. Forensic Sci.* **2008**, *53* (5), 1102–1107.

(35) Estill, C. F.; Baron, P. A.; Beard, J. K.; Hein, M. J.; Larsen, L. D.; Rose, L.; Schaefer, F. W., III; Noble-Wang, J.; Hodges, L.; Lindquist, H. D. A.; Deye, G. J.; Arduino, M. J. Recovery Efficiency and Limit of Detection of Aerosolized *Bacillus anthracis* Sterne from Environmental Surface Samples. *Appl. Environ. Microbiol.* **2009**, *75* (13), 4297–4306.

(36) Brown, G. S.; Betty, R. G.; Brockmann, J. E.; Lucero, D. A.; Souza, C. A.; Walsh, K. S.; Boucher, R. M.; Tezak, M.; Wilson, M. C.; Rudolph, T. Evaluation of a wipe surface sample method for collection of *Bacillus* spores from nonporous surfaces. *Appl. Environ. Microbiol.* **2007**, *73* (3), 706–10.

(37) Kuhlman, M. R. *Preliminary SPOT Report on Particle Size Analyses*; Battelle Memorial Institute: Columbus, OH, 2001.

(38) Amidan, B. G.; Pulsipher, B. A.; Matzke, B. D. *Statistical Analyses of Second Indoor Bio-Release Field Evaluation Study at Idaho National Laboratory*; PNNL-18932; Other: 400904120; 2009; p Medium: ED; Size: PDFN.

(39) Skolnick, E. B.; Hamilton, R. G. Legacy science suggests improved surface-testing practices for detection of dispersed bioagents in bioterrorism response. *2004 National Environmental Monitoring Conference*, Washington, DC, 2004.

(40) Solon, L.; Gurian, P. L.; Perez, H. The Extraction of a *Bacillus anthracis* Surrogate from HVAC Filters. *Indoor Built Environ.* **2012**, in press.

(41) Griffith, K. Comparison of Methods for Quantification of Surface Contamination with Microbial Spores in an Office Building. Masters Thesis, Department of Civil, Architectural, and Environmental Engineering, Drexel University, Philadelphia, PA, 2011.

(42) Haas, C. N.; Rose, J. B.; Gerba, C. P. *Quantitative Microbial Risk Assessment*; Wiley: New York, 1999.

(43) Mitchell-Blackwood, J.; Gurian, P. L.; Lee, R.; Thran, B. Variance in *Bacillus anthracis* Virulence Assessed through Bayesian Hierarchical Dose-Response Modeling. *J. Appl. Microbiol.* **2012**, in press.