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Estimating aerosol hazards from an anthrax letter

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Abstract

Events following the anthrax letter incidences in 2001 changed many previously held assumptions regarding biological aerosol hazards. As there were significant fatalities resulting from the sorting of a single anthrax letter, it suggests there is a need to reevaluate how best to measure biological aerosols and to use the information to make accurate predictions. This paper describes the biological aerosol created from manipulating envelopes containing *Bacillus subtilis globigii* spores (a simulant for anthrax). In addition, the measurements on dispersion of spores from an envelope processed by a mail sorter provide important dynamic aerosol characteristics, information previously unavailable for health risk assessment. The findings produced a number of significant aerosol dosage estimates related to spore contaminated letters. Graphic presentations show significant hazard in handling anthrax-laced letters. Aerosol particle dynamics are described and specific lethal dosage values are listed for a range of activities; these will be useful in assisting the modeling community to create more realistic predictions in hazard assessment studies.

Keywords: Anthrax letter; Biological aerosol; Lethal anthrax dosage for humans; Air transport of spores

1. Introduction

Intentionally caused lethal cases of human inhalation anthrax have been rare up till 2001. Traeger et al. (2002) reported on the first confirmed bioterrorism-related anthrax case in Florida. The Brentwood Mail Processing and Distribution Center in Washington, D.C., was extensively contaminated with *Bacillus*

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anthracis spores after two letters containing spores were processed at this facility on October 12, 2001 (Centers for Disease Control & Prevention, 2001). Borio et al. (2001) reported on two postal workers who died of inhalation anthrax at this postal station. Between 3 October and 16 November 2001, in the US, there were five cases of inhalation anthrax causing death (Bartlett, Inglesby, & Borio, 2002). By November 28, 2001, a total of 23 cases of anthrax exposure had been identified; 11 were confirmed as inhalation anthrax, and 12 were cutaneous (MMWR, 2001). Jernigan et al. (2002) summarizing the final tally from anthrax related incidences, reported that of the five fatalities, 2 had worked at the Brentwood postal station. It seems clear from the foregoing that people can become infected and have died from biological aerosol derived from anthrax spore laced letters. What is not certain is the inhaled aerosol concentrations involved in these cases.

The most recent published reference on infectious dose of anthrax spore aerosol was that for primates (Inglesby et al., 1999) taken from a US Department of Defense report that discussed biological warfare agents in what is now the former Soviet Union. The author quotes a range of 2500 to 55,000 inhaled anthrax spores as the dosage required to kill 50% of exposed people. In practice, it may never be possible to critically measure a lethal inhalation dose of anthrax under conditions of human habitation, especially involving live subjects. Yet, for a variety of reasons, there is a need to know with some precision what aerosol dosage is deemed lethal. We have reported on the airborne anthrax spore concentrations around Delivery Bar Code Sorter (DBCS) machine #17 located at the Brentwood station and have also gathered data on its surface contamination (Dull et al., 2002). In view of our recent work with aerosols, it would be of practical interest, to test if the published lethal dosage range (Inglesby et al., 1999) has any relevance for recent events pertaining to anthrax letters.

This report will demonstrate how to model aerosol hazards associated with handling spore-loaded envelopes under a variety of conditions. Also introduced will be innovative measurement techniques that were employed to estimate live aerosol particles. Finally, we will present data and an approach for estimating the aerosol concentration resulting from the processing of letters containing anthrax by DBCS #17 in the Brentwood postal station.

2. Experimental

2.1. Bacterial spores

Bacillus subtilis globigii spores (BG) were obtained from the US Department of Defense (Dugway Proving Ground, Utah). Viable spore concentration was about 1×10^{11} cfu/g. In the defence community, BG spores are routinely used as a simulant for *B. anthracis* (anthrax) spores.

2.2. Aerosol chamber and sampling equipment

The experiments were conducted in a 45 m^3 aerosol chamber (Ho, 1987) which was configured to represent a mail room or office. The chamber walls were specially coated with "mil spec" paint (MIL-P-23377) that can resist corrosive chemicals and UV exposure. This feature permitted repeated decontamination procedures between experiments. Other physical characteristics and operational functionalities have been previously described (Ho, Spence, & Hairston, 1999). When in operation, the air handling system recirculated the same air through the chamber at a rate of about 30 m^3/ min.

Located in the chamber was a variety of sampling equipment. High and low resolution slit to agar samplers were used to collect aerosolized BG spores within the aerosol chamber. The design and definition for high resolution sampling characteristics have been reported previously (Ho, Spence, & Ogston, 2001b). A table was placed at one end of the chamber to serve as a "desk". The work area in the centre of the desk was surrounded by an array of 10 high resolution (HR) slit samplers. The agar plates in the samplers turned at 1 rpm. The samplers were linked together to function sequentially to provide 10 min of continuous sampling. A similar set of samplers that ran simultaneously was located at the opposite end of the chamber.

Low resolution (LR) slit samplers (Model STA 203, New Brunswick Scientific Ltd, Edison, N.J., USA) were placed on either side of the chamber in the middle and at the opposite end. Ehrlich, Miller, and Idoine (1966) have described the use of this instrument for estimation of live biological particles in air. The agar plate in the low resolution slit samplers turned at 1 revolution per 30 min. In this study, the LR samplers were used to confirm the time of arrival of the aerosol clouds as well as to detect low aerosol concentrations. A fifth low resolution slit sampler, located in the center of the chamber, was used to obtain background aerosol samples immediately prior to the start of each trial.

2.3. *Measurement of aerosol concentrations using an optical instrument*

A Fluorescence Aerodynamic Particle Sizer version 3 (FLAPS3, Hairston, Ho, Oberreit, & Bradshaw, 2002) was used for characterizing airborne particles based on measurement of intrinsic fluorescence and particle size. The instrument was packaged in an environmental enclosure with a virtual-impactor particle concentrator (SCP, St. Paul, MN) attached. In ambient air conditions, the concentrator significantly increases the number of particles measured per unit time, which serves to improve the response time and sensitivity for detecting particles with size and fluorescence characteristics that differ from those in the ambient background. The FLAPS3 uses a 405 nm diode laser (Nichia Corporation, Tokyo, Japan) for fluorescence excitation, which permits a smaller, potentially lower cost instrument design. Principle of operation for the prototype, FLAPS 1 has been described (Hairston, Ho, & Quant, 1997). A commercial version, FLAPS 2 (Ho et al., 1999) has been field tested extensively in biological aerosol measurements. The latest version uses 2 color channels of fluorescence detection, which can improve discrimination of particles based on their fluorescence characteristics (Hairston et al., 2002). In the present application FLAPS3 was used to measure the concentration of spore particles dispersed from BG loaded letters.

2.4. *Letter preparation*

A test letter was prepared consisting of a standard business envelope (24 × 10.5 cm), a single sheet of printer paper (28 × 21.5 cm) and either 1.0 g or 100 mg of BG spores. The powder was placed within the triple folded sheet of paper, followed by placing that into an envelope and sealed by wetting the adhesive. Nothing special was done to the corners of the envelope where a letter opener would normally be inserted. The envelope was then shaken to mimic the handling and tumbling that would occur during its passage through the postal system. This "letter" was shuffled together with nine other, identical, untreated, "letters" so that the subject opening the envelopes would not know which one contained the BG spores. In a preliminary trial, when the letters were shaken and shuffled in the aerosol chamber, an increase in the background aerosol concentration was observed. Subsequently, to achieve a low background, the letters where shaken and shuffled prior to their introduction into the chamber.

2.5. Experimental protocol

The trials were conducted with the air recirculation system on. A 10 min background aerosol sample was taken immediately before each trial. The human operator was seated at a desk and used a standard letter opener to open the letter by inserting it at the corner where the flap seals and then slitting the top of the envelope. After slitting the envelope, the sheet of paper was removed, unfolded and placed on the desk. When the envelope containing the BG spores was opened and the sheet of paper containing the BG was removed, the operator placed it on the desk as he had done with the other sheets, stood up and backed up one step taking care not to disturb the contaminated letter further. He remained standing for the duration of the 10 min sampling time. Following each experiment, the chamber air was vented to the outside through a HEPA filter and outdoor air was brought in through another HEPA filter.

2.6. Sampling

Samples collected or plated onto nutrient agar plates were incubated overnight at 37 °C. Bacterial colonies were scanned using a colony counter (Model CASBA 4, Spiral Biotech Inc., Norwood, MA, USA) to determine the number of colony forming units (cfu) present. Knowing the flow rates for the slit samplers (30 L/min), the rotation rates and the colony count, it is possible to derive a unit of measure, commonly used in the military context as, "agent containing particles per liter of air" or ACPLA (Snyder, Tripathi, Maswadeh, Ho, & Spence, 2001).

2.7. Statistical analysis

The distribution of biological data is known in many instances to be well defined by a lognormal distribution (Tillett & Carpenter, 1991). After the counts have been $\log_{10}$-transformed, the resultant data become normally distributed. We have chosen to describe the distribution of the log-transformed concentration data in terms of a theoretical normal probability distribution, often referred to as a probability density function. A probability density function is a function $[f(x)]$ that is continuous over all values of x ($\log_{10}$ ACPLA) and the area under the curve is equal to unity. Fitting the normal probability density function (Knutti, Stocker, Joos, & Plattner, 2002) to a set of data requires estimating the mean and standard deviation of the measured data, in this case, the mean and standard deviation of the log-transformed biological concentration data. Mathematically, the probability density function enables one to determine the probability of some value of the concentration occurring within a specified range of the distribution. To facilitate comparison of the probability density distributions from various experiments, each data set has been normalized by making the mean log ACPLA equal to unity.

3. Results and discussions

A low resolution slit sampler was used to establish air cleanliness before each experiment and thus there was no attempts made to normalize the data in ACPLA units. As the number of viable particles collected during background sampling was expected to be small, data for discrete colonies are presented in Fig. 1. The cumulative background data from 10 trials were pooled and summarized in Fig. 1. A linear regression curve was superimposed over the scattered plot along with the 95% prediction lines. It appears

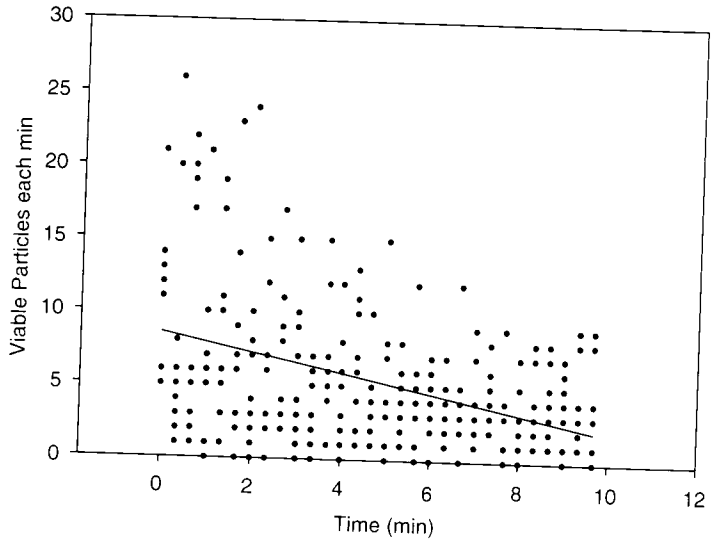

Fig. 1. Analysis of background aerosol in chamber using data taken from 10 trials. This was representative of the air cleanliness before the letter opening experiments showing the absolute number of viable particles sampled each minute at 30 L/min flow rate. It shows the cumulative data plotted as number vs time. Compared to the actual letter related aerosol levers, the background level is acceptable. A linear regression curve is superimposed over the scattered plot.

that there was a gradual decline in the concentration of aerosolized spores over time, consistent with the possibility the particles are lost to surfaces and aggregation (Ivlev, Morfill, & Konopka, 2002).

In one experiment, a BG charged envelope (1 g) was shuffled with nine untreated letters to randomize its sequence. While this was being performed, a low resolution slit sampler was monitoring possible background contamination. Fig. 2 shows the result of the contaminant spores that leaked from the envelope as a consequence of the shuffling action. There was an initial rapid concentration rise followed by decay, similar to what was observed for the background material. In the same figure, background data from the previous figure has been included to provide a contrast to the respective contamination levels. Clearly, leakage of particles from the envelope produced an increase of several hundred times the normal. When collection parameters were used to determine the concentration relative to air volume, a median of 5 ACPLA resulted.

In contrast, it can be seen that opening a 1 g letter produced significantly higher aerosol concentrations (Fig. 3). Both sets of samplers recorded a peak aerosol concentration followed by a gradual non-linear decline in concentration with time. The sampler closest to the letter opening source measured the highest ACPLA. At this point in our work, it is not clear to what extent processes like particle aggregation, gravitational settling and adhesion to surfaces play in the decay profile of BG aerosol. In aerosol physics, decay of aerosol concentration due to particle aggregation has been described before (Smoluchowski, 1917). But similar studies with biological particles in air have not been found in the literature. However, for biological cells in liquid, analogous aggregation characteristic has been studied for human blood platelets (Hantgan, 1985). The author observed that platelet aggregation was characterized by single exponential decay with a rate constant. It was noted platelets did not follow the Smoluchowski aggregation model, unlike the polystyrene microspheres that were used to validate the calculations. Ohshima and Kondo (1993) argued that particles with soft biological membrane structures (soft particle theory) follow different

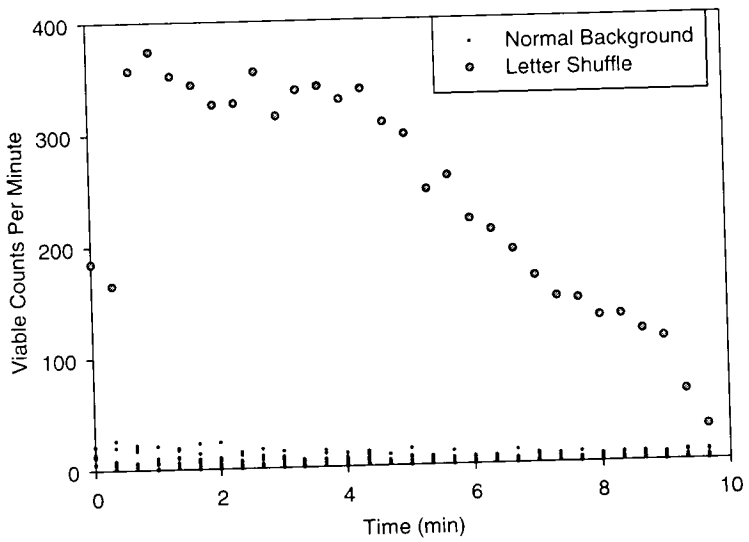

Fig. 2. Comparison between background aerosol cleanliness and a situation where a letter loaded with 1 g BG spores was shuffled among other envelopes (day 59). Viable spores were measured with a low resolution slit sampler operating at 50 L/min and rotating at 30 min/revolution. Estimated median contaminant particle concentration was 5 ACPLA, whilst the lower 25% of the population are < 3 ACPLA and that for the upper 25% is > 7 ACPLA.

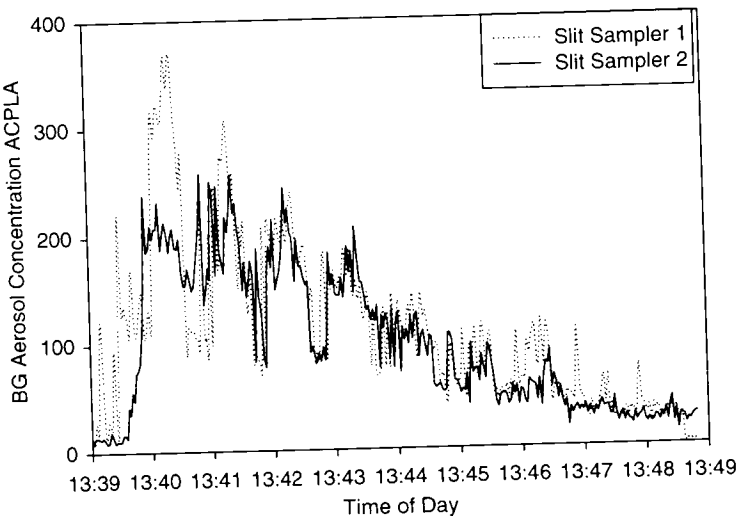

Fig. 3. High Resolution Slit Sampler data from 1.0 g release from day 59. The dotted trace shows data collected from the samplers at the desk where the envelope was opened while the solid one shows the data collected from the samplers at the opposite end of the aerosol chamber. Each curve is composed of data from 10 sampler plates.

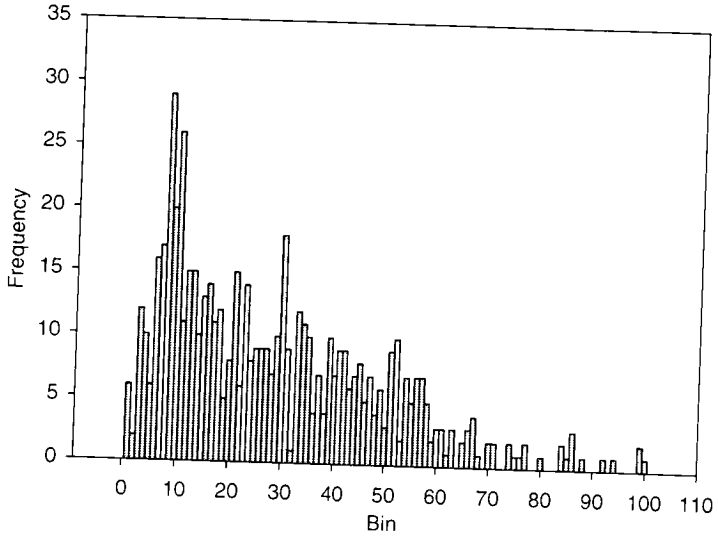

Fig. 4. Aerosol generated from opening a letter containing 1 g of BG spores. Data is presented as frequency distribution of the captured viable spores from a typical experiment (day 59). The data came from pooling the ACPLA measurements from both slit sampler sets. Normality Test (Kolmogorov–Smirnov) failure at K–S Dist. $= 0.103$ $P < 0.001$ providing a median of 85 ACPLA, whilst the lower 25% of the population are < 38 ACPLA and that for the upper 25% is > 150 respectively.

aggregation kinetics. They concluded that the surface potential of a biological cell exhibits different behavior, in contrast to the case of rigid particles described by the mobility formula of Smoluchowski. Hayashi, Tsuneda, Hirata, and Sasaki (2001), who measured the surface potential of a number of bacterial cells, concluded that cell surface potentials based on the soft particle theory were lower than those estimated by the conventional Smoluchowski formula. All these reports serve to provide evidence to conclude that the decay in BG particle concentrations is probably due to aggregation under a regime between the characteristics described by Ohshima and Smoluchowski. Although it is beyond the scope of this paper to define the physics of spore aggregation, it is correct to assume that the decay characteristics seen in Fig. 3 are well explained by the theories mentioned above. Hence, an aerosol cloud is expected to behave dynamically with respect to time in some fundamental way. Fig. 4 shows a frequency distribution plot of the data presented in Fig. 3. The data fail a normal distribution test; hence log transform is applied in subsequent analyses.

Because the aerosol concentration derived from opening an envelope containing 1 g BG spores approached the upper limit of resolution for the HR slit samplers, a series of experiments were performed using only 100 mg. A typical example of the results is shown in Fig. 5. Here it can be seen that the peak aerosol concentrations are correspondingly lower than the 1 g sample while similar decay characteristics can be observed. In this case, the two sampling arrays showed very similar results, suggesting that the aerosol distribution within the small confines of the chamber was fairly even.

To compare the aerosol populations from the 1 g and 100 mg samples, we have presented the data in terms of a probability density function (PDF) plot as introduced by Wadsworth (1990) and applied by Knutti et al. (2002) where they discussed the effect of aerosols on climate change. Fig. 6a shows the PDF for the two aerosol populations (data $\log_{10}$ transformed) generated by the envelopes containing 100 mg and 1 g BG. Executing this data transform makes it possible to use conventional statistical descriptors

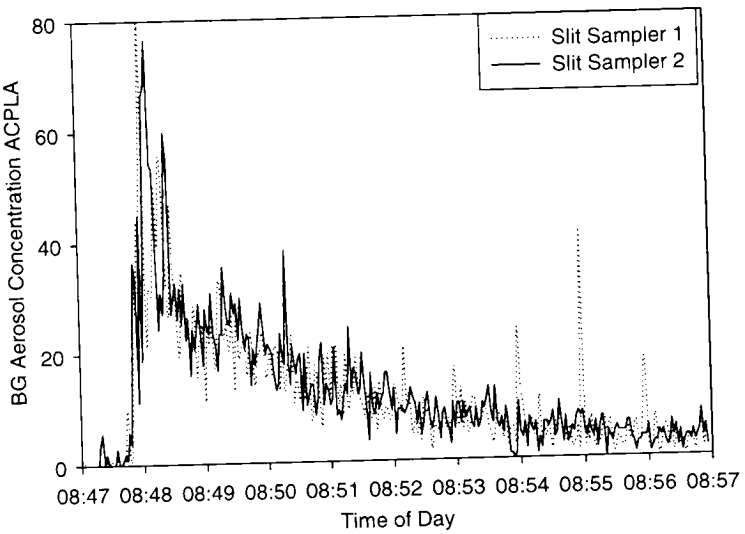

Fig. 5. High Resolution Slit Sampler data from 100 mg release, trial 092. The dotted trace shows the data collected from the samplers at the desk where the envelope was opened while the solid one shows that from the samplers at the opposite end of the aerosol chamber. Each curve is composed of data from 10 sampler plates.

such as average and standard deviation, conforming to recommended treatment for bacterial data (Tillett & Carpenter, 1991). Each plot represents the pooling of six slit sampler data sets from three experimental runs. The left-hand curve (100 mg samples) shows symmetric upper and lower boundaries where the peak represents the average concentration of the aerosol over the measurement period. In contrast, the 1 g sample curve has a truncated upper limit, probably a reflection of sampler saturation. Chang et al. (1995) have previously discussed potential sampling errors due to particle overlap, partly as a result of high aerosol concentrations impacting on agar surfaces. Fig. 6a also illustrates that the mean concentrations of the two populations are almost one log unit apart, roughly corresponding to the difference in the respective BG load used in the envelopes.

It would be instructive to be able to compare these PDFs with other measurements, for example with those from background studies. However, due to their vastly different concentration ranges, putting all the native PDFs in one graph would cause severe visual disparity making direct comparison impractical. One solution to overcoming this problem is to normalize the Y axis to unity and this is shown in Fig. 6b. After executing this procedure, it can be seen that the respective means are still the same, as are the upper and lower boundaries. The only change is seen in the alteration of the slopes, each being slightly distorted by steeper incline.

With this caveat established, it is now possible to examine the PDFs from the earlier measurements. In Fig. 7, the PDFs from the 1 g and 100 mg letters are reproduced alongside those from shuffling the unopened letter (replot of Fig. 2) and background contamination (replot of Fig. 1). It can be seen that the four sample means span a wide concentration range from about log −1 to 2. Of particular interest is the continuous overlap between the concentrations, first, between shuffling an envelope and opening the 100 mg loaded letter and second, between the 100 mg and 1 g envelopes. It illustrates a continuum of aerosol concentrations relating to different potential aerosol threat activities. By inference, it also suggests

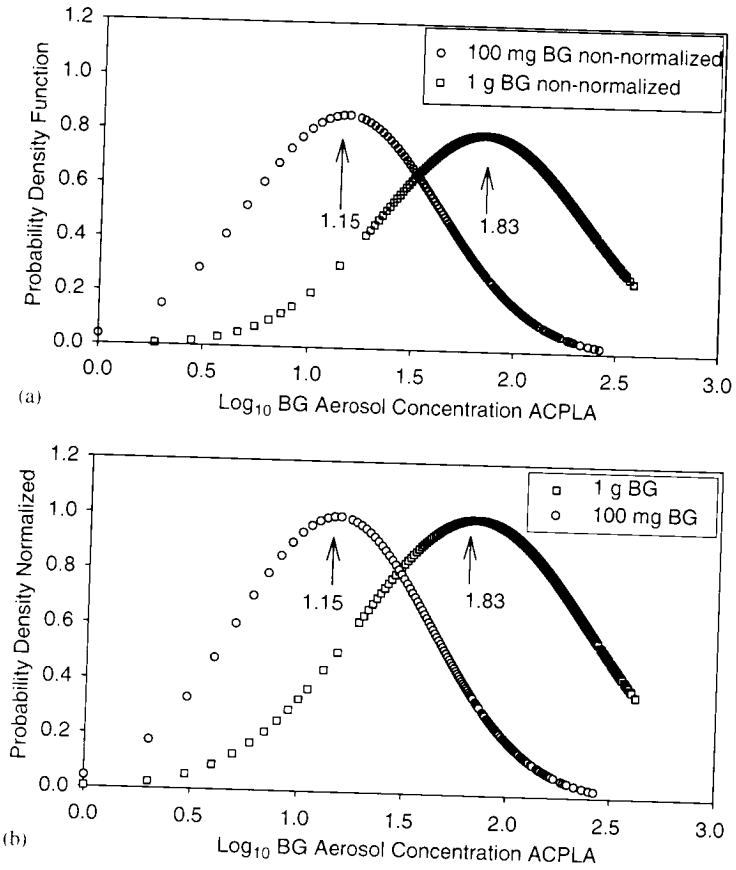

Fig. 6. (a) Native probability density function plot. Each curve was derived from pooled data of 3 replicates representing that from 1 g and 100 mg samples. Their respective mean was 1.83 and 1.15, standard deviation was 0.5 and 0.46. (b) Normalized probability density function plot of pooled data from 3 replicates representing that from 1 g and 100 mg material.

that in real threat scenarios, the assumption of discrete measurements (single numbers often found in the literature) may be an incorrect if not misleading way of presenting lethal dosages.

What is shown in Fig. 7 probably represents a reasonable facsimile of the probable low end of the concentration range arising from activities related to handling an anthrax letter in a variety of ways. To make this exercise complete and thus useful for predicting a probable dose that caused the lethal effects at the Brentwood postal station, we would require similar data generated from a letter sorting machine. Fortunately we have surveyed the area surrounding the Brentwood Delivery Bar Code Sorter (DBCS) machine #17 which had processed the anthrax letter(s) and came away with impressions on the airflow patterns in this area (Dull et al., 2002). We noted the operation of this machine with respect to how envelopes were routed through a series or rollers and pulleys. More importantly, in a separate venue, we were able to measure BG aerosol generated from processing letters with 1 g loads using a similar DBCS enclosed in a chamber. However, due to logistical expediency, aerosol measurement was preformed with a UV fluorescence optical instrument (FLAPS3) described in methods.

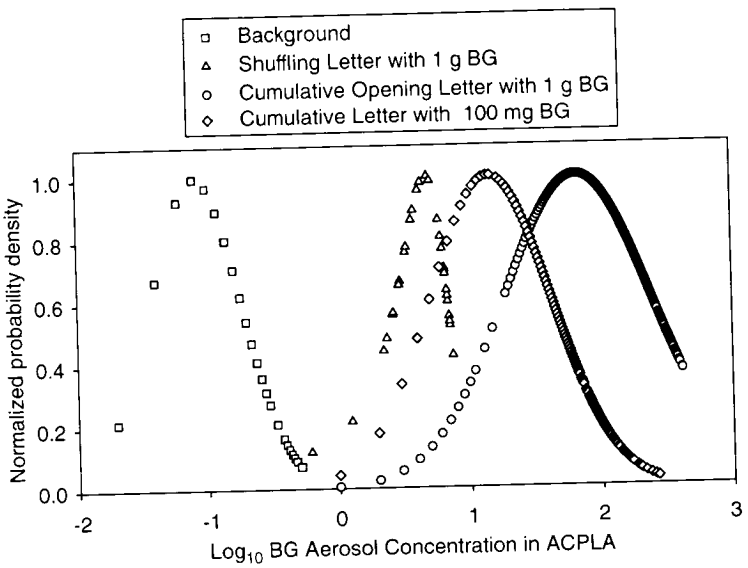

Fig. 7. Comparison of normalized probability density function plots for aerosol content measurement during background (mean = −1.1; SD = 0.35), letter shuffling (mean = 0.67; SD = 0.2) and opening of letters.

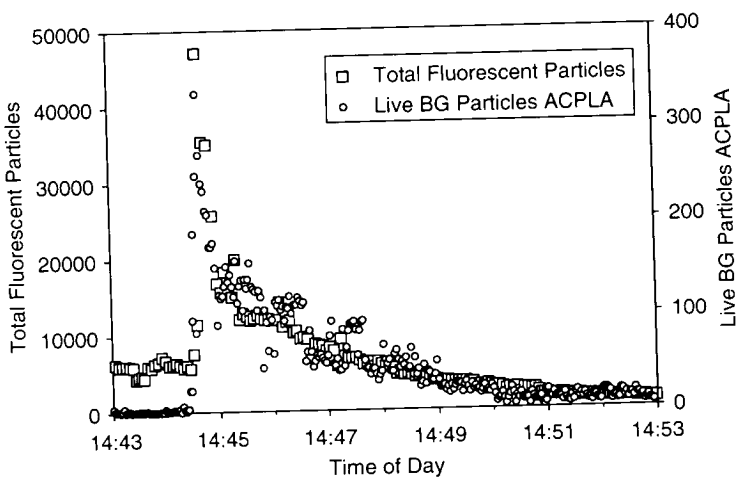

Fig. 8. Fluorescent aerosol particles associated with opening an envelope containing 100 mg BG spores. Data derived from a trial (day 9) where a FLAPS instrument was used to monitor fluorescent particles to be correlated with the live particles samplers by high resolution slit samplers.

The fluorescence data obtained with a FLAPS type instrument has been shown to correlate well with live biological particles (Ho, 2002). A typical optical measurement of the biological aerosol dispersed from opening a 100 mg loaded letter is shown in Fig. 8. Sampling time for FLAPS was 3 s, very similar to that for the high resolution slit samplers (2 s). This performance characteristic permits resolving the concentration dynamics of a cloud with good fidelity, as illustrated in the rapid rise time peak seen at 14:45.

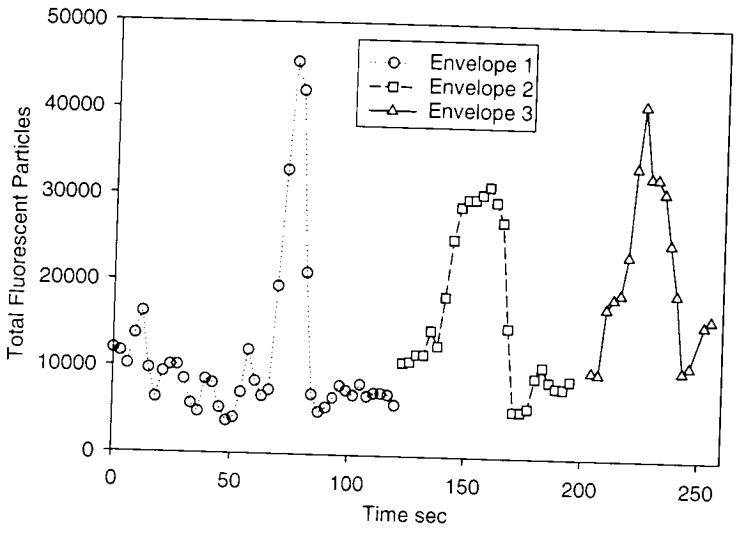

Fig. 9. Aerosol generated from a mail sorter. Fluorescent aerosol particles associated with passage of envelopes each containing 1 g BG spores. Three separate runs are summarized in this plot.

In this figure, the initial fluorescent noise was due to unspecified background, probably attributed to human activities. At the end of the measurement period, the air cleared itself of most particles, biological as well as contaminants. It is not unusual for background air to contain fluorescent particles. Humans produce significant numbers just by walking past the FLAPS instrument. We have attributed this phenomenon to shedding of biological material off the human skin, hair and other sources. Again, we would like to emphasize that even though the fluorescent background may be there, BG levels were low as established in Fig. 1.

By replotting the live particle concentration versus the fluorescent particle numbers (Fig. 9), the resultant calibration curve provides the value for the slope of the linear regression that is useful for predicting ACPLA equivalents from optical measurements. From Fig. 10, the equation becomes ACPLA = optical data/slope and applying it to data in Fig. 9 provides the results shown in Fig. 11.

Sorting letters with a DBCS is a relatively violent process compared to just opening a letter. The BG aerosol dispersed from the DBCS can be seen by the naked eye so it is not surprising that FLAPS could measure the aerosol as fluorescent particles (Fig. 9). In this experiment, blank envelopes were processed through the sorter at its operational speed and this generated non-specific fluorescent particles. Fluorescent dyes are known to be used in the paper industry to enhance visual whiteness (http://www.leathernet.com/chromtrip/frames.htm). Dye particles are dispersed when envelopes go through the sorter, producing a constant level of background fluorescent "noise", seen in Fig. 9 as part of the region below the 10,000 particle baseline. When a loaded envelope came by, BG aerosol was detected as a signal about 3 times higher than background for a period of about 15 s. A second envelope was processed and the signal measured exhibited a broader persistence of almost 30 s. Finally a third envelope came by, producing a particle profile more complex than the previous two. The observations from this experiment show that dry spore material within sealed envelopes does produce dispersed contaminants during normal processing by a DBCS. In the presence of non-specific dye particles, it is possible that part of the aerosol, especially

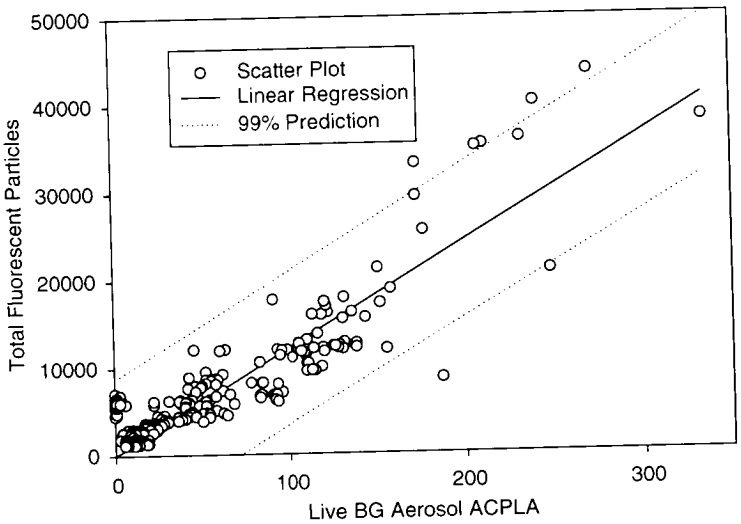

Fig. 10. Relationship between live BG particles and fluorescent particles. Regression fit $R^2 = 0.74$; slope = 121.84.

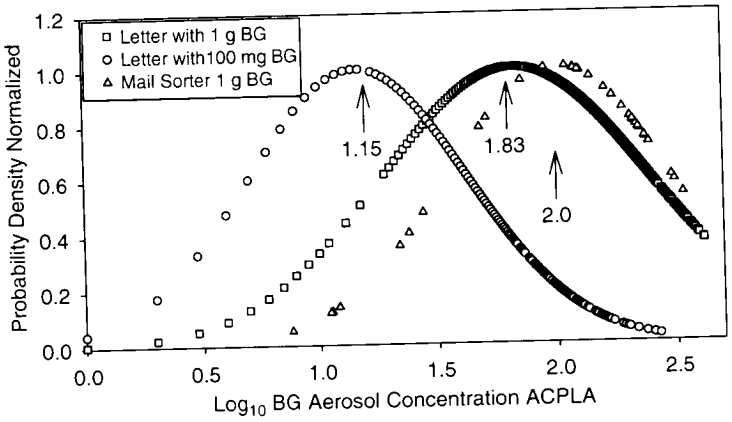

Fig. 11. Comparison of mail sorter associated aerosol contamination from 1 g spore in an envelope. Mail sorter data were obtained from pooling three separated runs (mean = 2.0; SD = 0.3). Aerosol concentrations from opening BG loaded envelopes are reproduced from Fig. 6 for comparison.

the lower concentration fraction, may not have been correctly measured by the optical method used here but the bulk of the signals are on the whole detectable.

To convert optical data attributed to sorting letters containing BG to equivalent viable spore ACPLA data, we elected to subtract the "noise" from the signals and then pooled the derived values for presentation as a PDF curve (Fig. 11). The mean aerosol concentration from sorting 1 g loaded envelopes is about log 2 ACPLA with a standard deviation of 0.3. The fact that this is higher than what was measured when an envelope containing a similar amount of BG was opened by hand is not unexpected because the sorting process is more violent.

In order to predict the aerosol concentration that might have caused mortality in Brentwood, a few assumptions have to be made. Using this 1 g BG mail sorter letter as a starting point, a number of corrections can be assumed to make the mean concentration more realistic. The scaling corrections are listed below:

1. Assume 3 g of actual anthrax load in the original letters; Inglesby et al. (2002) suggested there might have been 2 g anthrax material in the original letter that passed through the DBCS, so factoring to 3 g appears reasonable. Any more than this amount will make the letter bulge out slightly, giving it an unnatural appearance.
2. +10% for less than perfect sampling efficiencies in various instruments; there has been criticism that the slit sampler inlet nozzle design is not 100% efficient (Upton, Mark, Douglass, Hall, & Griffiths, 1994).
3. Model aerosol decay after the initial puff; Fig. 9 illustrates that the initial aerosol peak lasted less than 30 s so it may be more representative of the real conditions to examine the contents after that.

Taking all these factors into consideration and with rounding, the data transform steps can be summarized as follows. A scaling factor of 3 (plus sampler efficiency correction) was applied to the pooled triplicate data from the mechanically sorted letter to obtain a calculated mean of log 2.7 ACPLA. The probability density profile thus obtained is an assumed representation of the hazard for the anthrax aerosol that might have been produced by the Brentwood DBCS (Fig. 12). Unlike historical anthrax studies where animals were given regulated dosages of freshly generated aerosol via elaborate plumbing (Druett, Henderson, Packman, & Peacock, 1953), at Brentwood, the human exposures most likely came from a cloud with a peak concentration lasting no more than 15–30 s, similar to the dynamics shown in Fig. 9. In addition, with the passage of time, the cloud would have gone through decay characteristics not unlike what we have described in Figs. 3 and 5 for the letter opening trials.

It is important to correctly describe what happens to this decaying cloud over an extended period of time. Again from anecdotal information, it is known that none of the 2 fatalities were operators of the DBCS machine. So for them to acquire a lethal dose, the element of time, especially after the initial puff had occurred, must be a factor to be considered. So we need to map the decaying aerosol concentrations and define a value that is close to the realistic scenario. To achieve this, we pooled replicate data from the decay profile obtained from opening the 1 gm letter (Fig. 3). This was followed by applying a scaling factor of 7.2 to the data set so that the mean log ACPLA concentration equaled 2.7. Recall that this value is similar to the 3 gm mail sorter modeling exercise. We had to do this because the original 1 gm mail sorter data set did not have an accurate decay profile, this being obscured by the high fluorescent dye background. As a surrogate, the 1 gm open letter model was adopted (Fig. 3). Finally, with reference to the decay profile shown in Fig. 3, the last 7 min of the transformed decay curve was excised and reprocessed to obtain the probability density function plot with a mean of log 2.55 ACPLA (Fig. 12). To justify the necessity of this exercise, we have used information based on the floor plan around DBCS #17 and also the estimated location of the fatalities to project a bubble of space where this cloud can exert its influence (Dull et al., 2002). Two factors were considered critical if hazard prediction were to be meaningful; firstly, there was a significant airflow crossing DBCS #17 towards a series of opened doors at the truck loading bays. Second, all fatalities had assigned work stations in the path of this air flow. What is not known is the actual flow velocity but this can be assumed to be moderate. Thus this 7 min assumption was considered the most likely time bracket for people not immediately adjacent to the DBCS to be exposed.

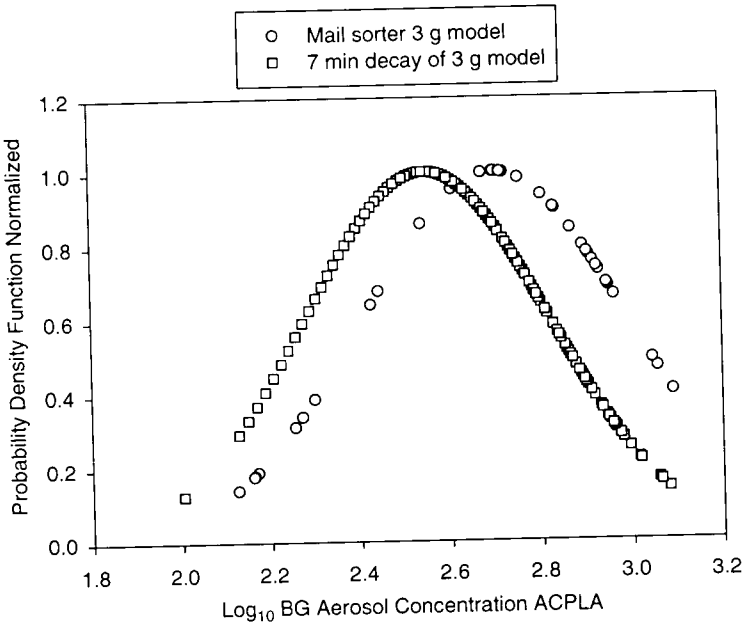

Fig. 12. Modeling the aerosol concentration from a letter with a 3 g BG load. To achieve a probability density profile where the average log BG concentration is equal log 2.7, several assumptions were made. Pooled (3 replicates) data from the 1 g letter that had been through the mail sorter was scaled up by a factor of 3. To correct for sampler inefficiencies, a 10% adjustment was added to give a final value of log 2.7 (circles). For modeling the decay characteristics of this curve, a segment (7 min) of data from the time base plot was used to generate the probability density function (squares). The mean of this threat aerosol was log 2.55 ACPLA.

The most probable aerosol cloud concentration and its decay profile can best be depicted as a time versus concentration plot (Fig. 13). It can be seen that the log of cloud concentration decays linearly and intersects the time line at about 33 min from the time the alleged anthrax letter was sorted. During this time period, the people were exposed with a fatal dose of spores. An attempt will be made to identify the possible range of exposure dose.

To perform this task, hazard predictions using measurements from this study are summarized in Table 1 along with other published information on lethal doses. In item g (Table 1), the mean decaying ACPLA concentration (log 2.55) from Fig. 12 is used to estimate a 5 min exposure lethal dose for man whose breathing rate is 20 L/min (Altman & Dittmer, 1971). The dose thus obtained is 1.6×10^5 spores, a value amazingly similar to the others listed in this table for animal studies. Item h illustrates the hazardous dose range from opening a 1 g spore loaded envelope. Again, the calculated dose range (3×10^4 to 1.7×10^5) appears to fit well within the reported animal data. If the Inglesby et al. (1999) estimate for man can be critically considered, then opening an anthrax letter can be lethal. On the lower end of the hazard scale, by using the same estimation methodology, the dosages for a man shuffling letters and opening a 100 mg letter turn out to be 2.1×10^3 and 6.3×10^3, respectively. We can now revisit the Brentwood DBCS environment to determine if it is possible for a worker to encounter a hazardous dose at a time soon after sorting an anthrax letter. With reference to Fig. 13, this exercise can be done by extrapolating the decay regression. As an example, by inspection, it can be seen that at 23 min, the aerosol level is 1 log ACPLA

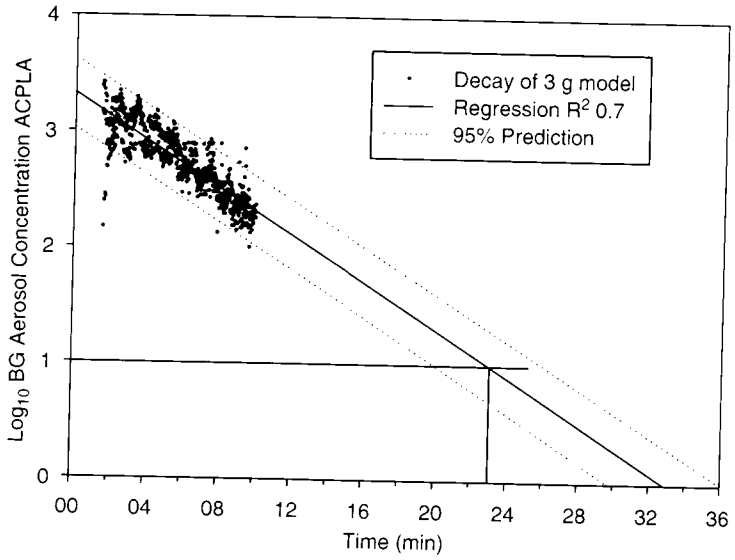

Fig. 13. Reconstruction of aerosol cloud concentration from a modeled 3 gm BG letter. The letter has been through the mail sorter at zero time. It is assumed the initial puff would decay rapidly followed by a more predictable population represented here by about 7 min of data from the series. This cloud probably has the most significant effect on the surrounding environment of the DBCS, perhaps causing fatal effects from inhalation. Also modeled is the regression curve that predicts the time when aerosol hazard becomes minimal (over 32 min). At 23 min the aerosol concentration has decayed to an estimated 10 ACPLA which works out to be equal to dose of 4.5×10^3 spores.

and this is equivalent to a dose of 4.5×10^3 spores. This dose range may be significant especially for susceptible individuals with poor respiratory fitness.

There is evidence to suggest that lethal doses vary for different individuals. For example mice have been shown to be distinctly susceptible or resistant to lethal infection by the toxigenic, nonencapsulated Sterne vaccine strain. The LD_{50} for the susceptible mice was approximately 10^3 spores of the Sterne strain, whereas the resistant mice were killed by 10^6 or more spores (Welkos, Keener, & Gibbs, 1986). Brachman (1980), in his review of historical anthrax studies, mentioned that certain chronic health conditions such as alcoholism might render an individual more susceptible to infection. Marcy and Merrill (1987) cited some evidence to suggest that cigarette smoking alters the respiratory tract's ability to defend itself from infection. Some subjects with chronic bronchitis have colonization of the lower respiratory tract with bacteria. Both smokers and patients with chronic respiratory disease appear to have a higher frequency of respiratory infections and an increased severity of symptoms when infected. Although there is no substantiating medical evidence as proof, these findings may partially help explain why the operator of DBCS #17 did not get infected while others caught lethal doses of anthrax.

4. Conclusions

A carefully designed set of measurements has been presented on spore aerosol dispersal from letters containing BG and the raw data has been used for hazard prediction. By using a high resolution slit sampler array, it is possible to demonstrate variation in the live spore particle concentrations with respect

Table 1
Summary of anthrax aerosol dosage for animal studies and estimates for man

Item	*B. anthracis* lethal dose	ACPLA[a] ($\log_{10}$)	References	Comments GP = guinea pig, M = monkey
a	2.57×10^5 spore	7.6×10^4 (4.9)	Broster and Hibbs (1990)	Gp, Vollum strain, in lungs
b	3.2×10^5 spore	9.5×10^4 (4.98)	Broster and Hibbs (1990)	Gp, Ames strain, in lungs
c	5.1×10^6 spore	1.5×10^6 (6.2)	Broster and Hibbs (1990)	Gp, New Hampshire strain
d	10^4 to 10^6 spore	$\sim 3 \times 10^3$ to 10^5 (4.5–5.5)	Jones, Beedham, Turnbull, Fitzgeorge, and Manchee (1996)	Gp, 3 strains, in lungs
e	5.5×10^4 spore LD_{50}		Ivins et al. (1996)	M, Ames, inhaled dose
f	4.1×10^5 4.5 μm spore LD_{50}	$\sim 6 \times 10^5$ (5.8)	Druett et al. (1953)	Gp, Margin of error = 4x, Harper and Morton 1953
g	1.6×10^5 spore	355 (2.55)	This paper calculated for man	Assume 5 min exposure; 3 gm BGmail sorter model
h	3×10^4 to 1.7×10^5	68–375 (1.83–2.57)	This paper calculated for man; mean to peak values	Assume 5 min exposure; 1 gm BG open letter
i	2.5×10^3 to 5.5×10^4 LD_{50}		Inglesby et al. (1999), indirect reference	Estimated from primate data and applied to man

Notes: in a, b, c and d, exposure time was 5 min; e did not report breathing rate. Breathing rate for moderate human exercise falls in a range of 22.5–49 L/min for adult males and 22–25 L/min for adult females (Altman & Dittmer, 1971); 20 L/min is used for postal workers doing mild to moderate work (items g and h).

Multipliers (ACPLA × 4.5 × 20 × 5) have been used to convert ACPLA units to lethal dosages.

[a]Agent containing particles per liter of air (ACPLA), calculated data assuming each 4–4.5 μm particle contains 4.5 viable spore (Ho, Spence, & Duncan, 2001a) and GP breathing rate = 0.15 L/ min and exposure time of 1 min (Druett et al., 1953).

to time. It can be seen that aerosol clouds decay over time and the decay profiles have been converted into probability density function plots so that different scenarios can be compared. By this procedure, we have shown the progressive increase in mean aerosol concentrations in a number of dispersal conditions. Such increments paralleled changes in the amount of BG spore available for dispersal. For logistical reasons, we could not use the slit sampler while testing a mail sorter and an optical detector was used instead. However it was possible to transpose the optical measurements from a fluorescent analyzer into ACPLA units. Mail sorter aerosol dispersal data were crucial to meaningful prediction of the lethal dosage responsible for the fatalities at the Brentwood postal station. The resultant estimated aerosol dosages were comparable to those used in animals summarized in Fig. 14. This exercise gave us the opportunity to state, for the first time, a dosage that might have proved lethal for the postal workers. It also allowed prediction of the hazards from opening or shuffling spore loaded letters. It would appear that handling anthrax letters in all the conventional ways practiced in an office could be a significant hazard with potential lethal consequences. The data do not support Peter and Hartleys' (2002) suggestion that as few as 1–3 spores may cause infection in humans. We have also introduced the concept of biological aerosol decay and attempted to offer explanations for the phenomenon. Apart from the conventional electrostatic forces that have been defined for inanimate particles, biological adhesion, a recent discovery that can be quantified by atomic force microscopy (Bowen, Fenton, Lovitt, & Wright, 2002) may exert significant influence on spore interaction with each other. If this were proven to be correct, biological decay may have a major

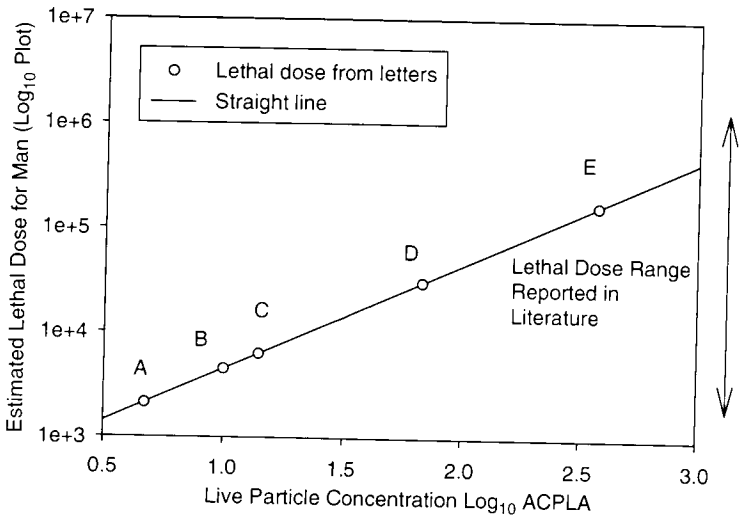

Fig. 14. Summary of mean lethal dose estimates from handling letters with different spore loads. Lethal doses are calculated as the product of ACPLA values and a multiplying factor defined in Table 1. The straight line shows a continuum of measured doses from letter handling in comparison to that found in the public domain. A = shuffling 1 g BG letter, B = lower limit of lethal hazard prediction at Brentwood postal station, C = opening 100 mg BG letter, D = opening 1 g BG letter and E = estimated lethal dose for postal workers. Shown on the right-hand axis is the lethal dose range for animals cited in open literature.

impact on biological hazard modeling. Current models are mostly based on gas molecule behavior and when applied to biological hazard prediction, may suffer unpredictable distortions and inaccuracies (Yee, 1999).

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