



Implications of Low Level Human Exposure to Respirable *B. anthracis*

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Abstract

A comprehensive literature review and associated new analysis of human exposure to different doses of respirable spores of Bacillus anthracis, and subsequent development, or not, of inhalational anthrax were conducted. The objective of the review was to evaluate actual human data collected at low doses (~600 respirable particles under 5 microns in diameter per day).

The key findings of the paper are: (a) Exposure of individuals to 600 or more spores was common in U.S. textile mills before 1960; (b) If 600 spores were sufficient to induce disease, inhalational anthrax would have been common; (c) Inhalational anthrax was very rarely observed; and (d) Therefore, 600 spores may not be sufficient to induce disease in a healthy individual who is not egregiously predisposed to anthrax, lung disease, or is immuno-compromised. The upper confidence limit for the probability that disease would result from exposure to 600 spores or less is 0.000 000 06. Furthermore, it is not appropriate to characterize 600 or fewer spores as LD_x for any value of x, however small.

Only nine “adventitious” cases of inhalational anthrax in the U.S. have been documented in the past 107 years for which there are no reasonable prospects of identifying evidenced-based explanations. For risk management, occupational safety, and related public-health concerns, the rarity, not the mystery, of these cases is the key point. The presence of such a small number of unexplained cases is a moot point for risk assessments and planning studies.

Literature Review

A literature review and associated analysis of pathogenic and non-pathogenic inhalational exposure to spores of *Bacillus anthracis*, and subsequent development, or not, of inhalational anthrax was conducted. Other manifestations of the disease, gastrointestinal and cutaneous, were not reviewed. The literature search was not limited to a computer-based algorithm, such as that contained in PubMed™ or Google Scholar™, although it did include such searches as part of the effort. The “old-fashioned” ap-

proach of reviewing well-known papers, such as Brachman, Henderson, Dahlgren, Brookmeher, and Meselson was followed by a screening and possible review of each of the citations in each of those papers. A similar follow-up was made, as appropriate, of the citations in the referenced papers. This search was conducted manually. In addition, a screening and/or review were conducted of bibliographies prepared by Phillips and Jemski for Fort Detrick; these contained thousands of papers on infectious agents, including anthrax spores. The conclusions of this paper result from a new analysis of the primary data published in some of those other papers.

1. Direct Evidence from Textile Mills

a. The 1957 Epidemic in Manchester, New Hampshire

During a 10-week period in 1957, 5 cases of inhalational anthrax occurred among the employees of a factory in Manchester, New Hampshire that processed imported goat hair into cloth. During the same period, 4 cases of cutaneous anthrax were reported (Brachman et al., 1960; Plotkin et al., 1960).

The history of cutaneous anthrax in the Manchester mill provided significant evidence for a clustering of cases. The clustering theory supports the idea that those clusters, like the 1957 cluster of inhalational and cutaneous cases, resulted from specific batches of egregiously contaminated hair, not an upper percentile of the “normal” distribution of concentrations. The 1957 outbreak was preceded by a cluster of complaints about excessive dust from the current batch of goat hair:

“In the mill under study, workers who are affected by dust will usually complain to the company nurse, who is on duty every working day and who is required to keep accurate records. Since 1946 there have been 51 complaints of dust made to the nurse, all by card-comb workers. ... 28 (55 percent) of the 51 complaints were made during 1956 and 1957...the complaints have tended to come in clusters. The last cluster occurred from August 14 to 21, six days before the onset of the first anthrax case. Although no

more complaints were recorded after August 21, several observers noted what seemed to them to be an unusual level of dust during the rest of August and September.”

During the latter part of the outbreak, the mill began to use a detergent to scour the hair; this was likely in response to the extreme dustiness of the batch, but it had the effect of “enhancing” the spores, or increasing the number of respirable particles in the air. While use of the detergent began too late to account for the first case, it may well have prolonged, or exacerbated the outbreak, because the number of respirable particles would have been much greater than if conditions had resembled those in the South Carolina mill in Brachman’s subsequent study (Brachman, 1980), which did not use detergent.

b. Air Sampling in Manchester and Philadelphia

Air sampling was performed at the Manchester mill where the 1957 outbreak occurred, and in a control mill in Philadelphia (Dahlgren et al., 1960), at a time period believed to be representative of normal operations. No inhalational anthrax cases had been reported at the Philadelphia mill, and the outbreak at Manchester had ceased. Thus, these air measures represent “typical” variations in spore concentrations, rather than the egregious concentrations from a bad batch of hair used in Manchester. Two full 8-hour days were sampled in each mill and the number of respirable particles (under 5 microns in size) inhaled per employee during 8 hours was calculated. In Manchester, one of the days yielded 150 respirable particles; the other yielded 700 respirable particles. In Philadelphia, the 2 days of sampling yielded 400 and 500 respirable particles per day per employee, respectively.

Watson and Keir (1994) quote a sentence from Brachman: “A worker in the card room of the Manchester mill was calculated to have inhaled approximately 600-

2150 anthrax-bearing particles per 8-hour shift, of which 150-700 were less than 5 microns in diameter.” The context of this sentence in the Brachman paper (Brachman et al., 1960), based on research reported by Dahlgren, (Dahlgren et al., 1960) makes it clear that this measurement was taken during a time frame (February 1958) in which there were no cases of anthrax. Unfortunately this is not entirely clear from Watson and Keir’s presentation, especially in their Table 1, which is misleading in its association of this measured level of inhaled particles with the earlier outbreak. A similarly misleading association of low spore dose with disease appears in Fennelly’s (2004) extrapolation of data.

c. Brachman’s Air Sampling Study

The most important study for assessing a typical industrial level of exposure to anthrax spores is Brachman’s study, “Industrial Inhalation Anthrax” (Brachman et al., 1966). The main focus of the study was the response of Asian cynomolgus macaques’ exposure to air piped in from a goat hair mill. The study demonstrated that these animals have much higher susceptibility to inhalational anthrax compared to humans. We cannot draw any conclusions from the macaque exposures, in part because of the usual difficulties of generalizing or extrapolating from animal data to human responses, and also because the macaques in Brachman’s study were in poor health before the experiment started, which may have increased their susceptibility to inhaled pathogens.

Of more concern for the present analysis, Brachman also tracked the concentration of respirable spores of *B. anthracis* in a working goat hair mill in South Carolina for 47 consecutive days. This mill had never had a case of inhalational anthrax in the 2.5 years of operation before the workers were vaccinated.

These air sampling records provide us with invaluable data about typical exposures in such mills. The distribu-

Table 1
32 U.S. Cases of Inhalational Anthrax. (Adapted from Holty, 2006.)

Number of Cases	Case IDs	Exposure
5	30-34	1957 Manchester mill outbreak
1	23	Across alley from the same mill as 1957 outbreak, 9 years later
4	28, 44, 45, 51	Workers at other hide or hair mills
3	17, 52, 53	Craftsman using hide or hair products
1	29	Electrician in laboratory that studied anthrax
9	1-9	Direct exposure to 2001 anthrax letters
23	Subtotal	
2	10, 11	Adventitious cases in late 2001
3	35, 40, 41	Adventitious cases near the same tannery, 1948-1957
4	42, 43, 47, 49	Adventitious cases 1923-1947, unknown exposure

tion of exposures observed during this period, in which no humans suffered any ill effects, provides an example of a distribution that is “safe” for humans. (The Manchester outbreak was clearly the result of abnormal conditions rather than the upper tails of the “safe” distribution; and most likely, the 4 other cases in U.S. textile and leather mills in the 20th century were as well.)

Brachman reports over a period of 47 consecutive days of normal operations of the South Carolina mill; air from the hood over the picking machine was piped to the macaques during working hours, and fresh air from outside was piped to them when the mill was not in operation. The picking section of the mill was in operation (indicated by nonzero spore counts) on 31 of the 47 days.

On the 31 days the picking section was in operation, the mean number of respirable particles inhaled by a caged cynomolgus macaque was 571, with a standard deviation of 519. The respiratory rate of the macaques was estimated at 1 liter per minute, or 0.48 cubic meters in 8 hours. Taking the standard figure of 7 cubic meters in 8 hours for humans, a human exposed to the same air would inhale on average 8,331 respirable particles with a standard deviation of 7,568. However, the air to which the macaques were exposed came from the vent hood over the picking machine, not from the air in the room. Assuming a typical 90% efficiency for a 1950s ventilation system would lead to a human working in the picking room inhaling an average of 833, and a standard deviation of 757 respirable particles, Figure 1 shows the day-by-day number of spores that would be inhaled by a human working 8 hours in the mill, based on the amounts measured in the trailer. Figure 2 shows the cumulative frequency distribution of this quantity over the 31 days that the picking machine was in operation. In particular, every worker in the room inhaled 602 or more respirable particles on 17 separate days out of the 31 days.

Despite this level of exposure, the mill operated for 2.5 years before workers were initially vaccinated, with no documented cases of inhalational anthrax. During the entire test period, including weekends and holidays, 17 out of 47 days (or 36%) were days in which every worker in the most contaminated areas would inhale over 600 respirable particles containing anthrax spores, with the remainder of the days being either non-working days, or working days on which the picking machine was not used.

Thirty-six percent of 365 days equals over 120 days per year. Over the 2.5 years this particular mill had been in operation, this translates to over 300 working days of exposure by each worker to over 600 respirable particles in 8 hours. If 50 of the 250 employees in this mill were in the high exposure areas of the mill, then this means there were over 15,000 person-days of exposure to over 600 respirable spores without resulting in a single infection in the mill.

2. Other Direct Exposure Cases

Holty reports 32 cases of inhalational anthrax in the U.S. between 1900 and 2005 (Holty et al., 2006). Five of these cases were infected in the 1957 outbreak in Manchester. Four cases of inhalational anthrax, as well as a larger number of cases of cutaneous anthrax, were reported among employees of textile mills or tanneries. The last reported case of inhalational anthrax in a textile mill worker was in 1961; since then, all at-risk employees in the U.S. have been vaccinated (Brachman, 1980; Friedlander, 1999).

Case #23 (Holty et al., 2006) was a new employee in 1966 at a sheet metal shop across the alley from the same Manchester goat hair processing mill where the 1957 outbreak occurred. The goat hair mill employees were vaccinated by 1966, but the sources of the goat hair were unchanged, and the mill air was vented to the alley (Brachman, 1980).

Three of the 32 total American cases worked with animal hide or hair, and one was an electrician in a laboratory where anthrax was studied.

Finally, 9 of the 11 cases of inhalational anthrax in 2001 summarized by Holty were people in direct contact with contaminated mail, and thus, almost certainly exposed to thousands of respirable spores (Kournikakis et al., 2004).

This gives a total of 23 of the 32 cases where there was a known source of contact that could have exposed affected persons to doses of respirable spores well in excess of 600 per day. These cases are summarized in Table 1.

3. Adventitious Cases

The remaining 9 cases must be considered adventitious cases since they arose in the absence of any known direct source of exposure to large numbers of respirable spores. These cases fall into the following three groups:

A. Holty’s cases 10 and 11 were indirectly associated with the 2001 terror attacks: the elderly woman in Connecticut and the hospital worker in New York. Mail received by these two individuals may have been exposed to contact with contaminated mail, but no evidence of spores in their environments was found.

B. Holty’s cases 35, 40, and 41 all lived within 1.5 miles of, or regularly walked past the same leather factory that experienced cases during the period from 1948 to 1957.

C. Holty’s cases 42, 46, 47, and 48, all from 1947 or before, have no known connection with any source of contamination.

The scientific ramifications of the existence of recorded cases for which there is no reasonable prospect of evidence based explanations are interesting, and may prompt research efforts in years to come. The practical implications are quite different; for purposes of risk man-

Figure 1

Daily calculated intake of spores by each mill worker. (Adapted from Brachman et al., 1960.)

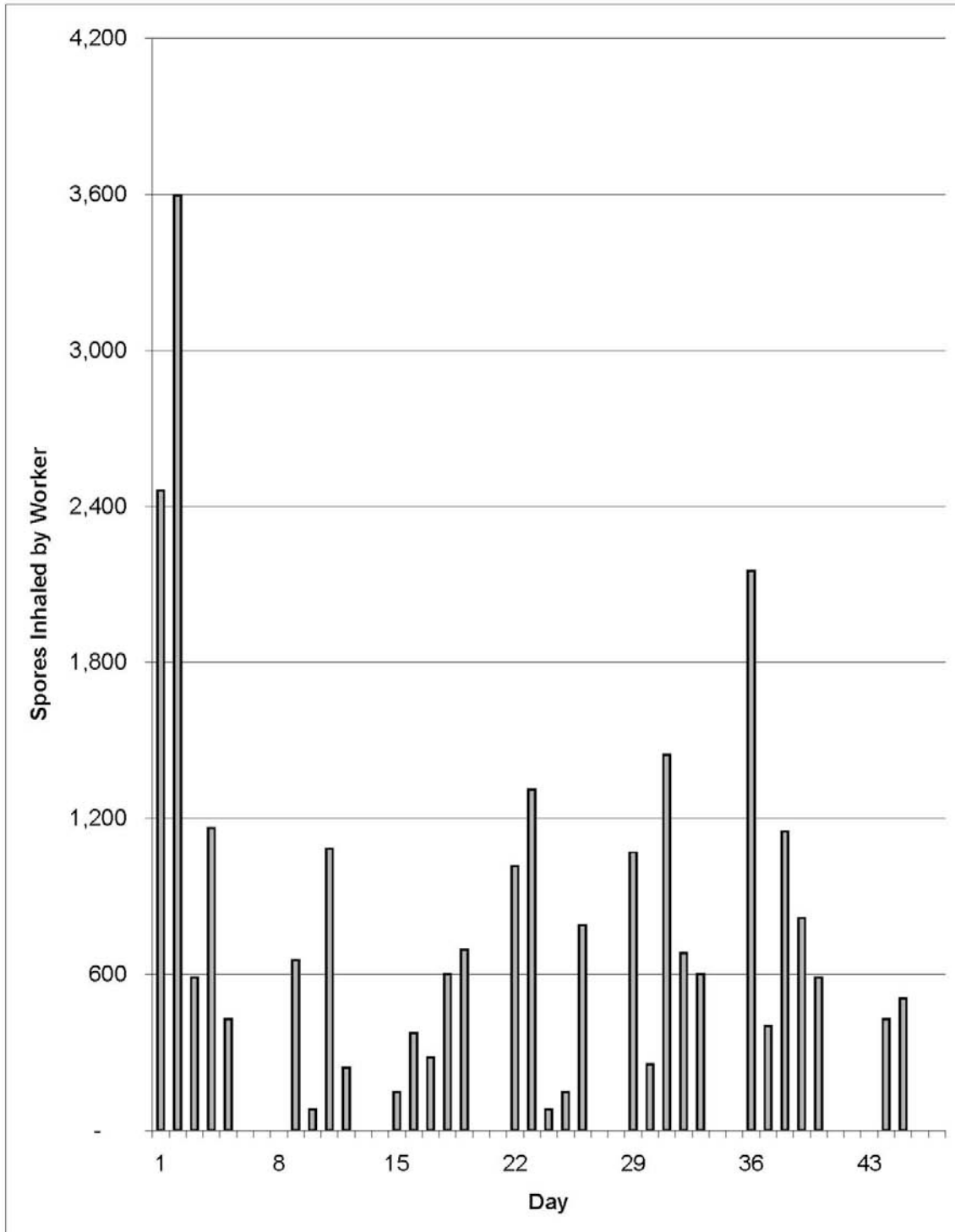
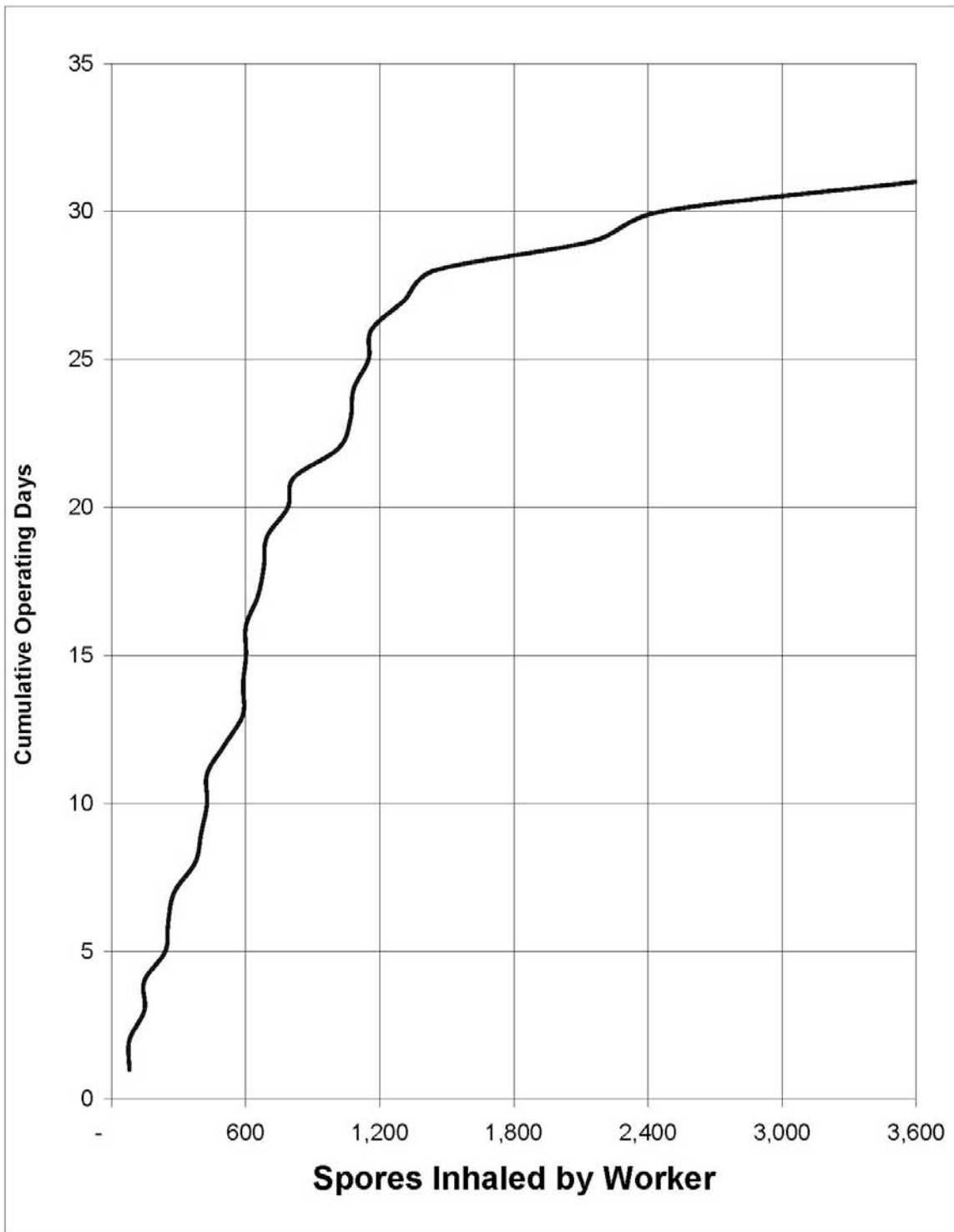


Figure 2

Cumulative frequency distribution of spores over 31 days of mill operations in 1966. (Adapted from Brachman et al., 1960.)



agement, emergency planning, occupational safety, and related public-health or societal-level concerns, the rarity rather than the mystery of these cases is the key point.

These adventitious cases account for only 9 individuals in 107 years, and each represents reported clinical anthrax with no evident exposure to large numbers of spores. An obvious question is how to reconcile these cases with the evident safety, after exposure to hundreds of spores, in an estimated 150,000 person-years of textile worker exposures (60 years x 50 mills in any given year x 50 workers in most contaminated part of each mill).

Some of these individuals are known to have had predisposing health conditions that would place them in the upper tail of a susceptibility distribution, or, equivalently, at the lower tail of a distribution of individual pathogenic dose. However, the presence of a sufficient number of respirable spores to overcome a (perhaps compromised) immune system, and lead to an inhalational anthrax infection, still invites speculation. Perhaps a small number of spores might cause disease in the most susceptible people, or these cases might represent a particularly virulent strain that has never been isolated or identified, or perhaps each of these unlucky individuals was exposed to one mega-particle (but still dust-sized) containing a very large number of spores. Despite these, or other possible explanations, no generally-accepted clinical explanation for these cases is available. Science will likely never know precisely what caused these cases.

Without confirmed clinical explanations, the presence of such a small number of adventitious cases is a moot point for risk assessments and planning studies. There is no particular reason to assume they share exposure explanation, or even etiology. Furthermore, since the underlying explanations remain unknown, the appropriate preventive, or remedial actions are equally unknown.

4. Discussion

The objective of this review was to evaluate actual human data collected at low doses (~600 respirable particles per day). Exposure to naturally-occurring spores, as well as to enhanced spores was reviewed. Strains were reported as virulent; there is no reported evidence of a significant variation in virulence across these studies. Particle size is a relevant factor; only respirable particles under 5 microns in diameter can penetrate the alveolar surface and cause disease. These particles are referred to as "spores" although they may contain one or more spores.

The argument that a small number of spores do not produce disease (with the possible exception of a few individuals per ten million who are extraordinarily predisposed to anthrax) is a statistical argument, supported by the biological fact of non-specific immune response (Guidi-Rontani, 2002), but fundamentally an empirical observation based on the following points:

a. Exposure of Individuals to 600 or More Spores per Day Was Common Among U.S. Textile Workers

The environmental testing in the textile mills clearly demonstrated up to 3,000 spores per day were inhaled by workers on one day, and 600 or more respirable spores were inhaled on each of 15 separate days. In addition to the direct collection of spore-particles from the circulating air, the pre-vaccine prevalence of cutaneous anthrax is additional strong evidence of anthrax spores in the mill environment. The appropriate conclusion from these observed facts is that 600 spores is not any LD, but rather, is a lower boundary of the maximum non-infectious dose for inhalation anthrax in humans.

b. If 600 Spores were Sufficient to Cause Disease, Inhalational Anthrax Would Have Been Common

Every worker in the most contaminated areas of the South Carolina mill received daily exposure to 600, or more respirable spores 17 times during the 47-day test period. Over the 60 years of this type of operation in many mills, hundreds or thousands of cases would have been a statistical certainty.

c. Inhalational Anthrax Was Very Rarely Observed

Neither the workers in these mills, nor the far greater number of workers in similar mills throughout the developed world in the 6 decades between the introduction of 20th century ventilation standards and the widespread use of vaccination, developed inhalational anthrax. The main exception is the New Hampshire outbreak for which there is strong evidence of egregiously contaminated materials.

d. Therefore, 600 Spores May Not Be Sufficient to Induce Disease

There are no studies with known, low, pathogenic exposures that include follow-up histopathology, or clinical confirmation of disease. There are no low-dose studies with associated probability of disease based on exposure. Models purporting to emulate low-dose studies by extrapolating from studies in the vicinity of LD50, or higher (Meselson et al., 1994; Wilkening, 2006) are not the same as actual experimental data using low doses. The mill evidence clearly implies that the infections at Sverdlovsk must have been caused by more than 600 respirable particles per person.

Based on overwhelming evidence from reported scientific studies and observations of this reportable disease, an exposure of 600 spores per day would not be expected to cause disease in a healthy individual who is not egregiously predisposed to anthrax or lung disease, or is immuno-compromised.

Taking into consideration all of the mills in developed countries between the introduction of ventilation systems and the widespread practice of vaccination, we would find a far larger number of person-days. The few cases of inhalational anthrax that exist among them are likely attributable to individual batches of egregiously contaminated materials. Even if these cases represent the high tail of a distribution of contamination, this still provides very strong evidence that doses of 600 respirable particles per day, or less, present no discernible threat of inhalational anthrax.

An upper confidence limit for the probability that disease would result from inhalation of 600 spores in a day, based on inhalation of 600 or more spores, on over 120 days per year for 60 years by each of 50 workers located in the most contaminated parts of each of 50 mills, is $1/(120 \times 60 \times 50 \times 50) = 0.000\ 000\ 06$. It is important to note that this does not mean that 600 is the number of spores associated with any numeric LD₅₀; lethal dose estimates are not typically based on this type of direct computation from observation, and are intentionally not computed here. Many scientific models for inhalational anthrax and other diseases are based on various mathematical formulas applied to high-dose estimates of lethality to estimate effects of low-dose exposures. For such models, extrapolation is often extended well beyond the domain of observed data.

This approach needs to be reconceptualized in light of our new analysis of the low-dose human exposures to *B. anthracis* spores. In the meantime, 600 spores per day is an appropriate level to use as a threshold for preparedness planning and risk analysis.

References

- Brachman, P. S. (1980). Inhalation anthrax. *Ann. NY Acad. Sci.*, 353, 83-93.
- Brachman, P. S., Plotkin, S. A., Bumford, F. H., & Atchison, M. M. (1960). An epidemic of inhalation anthrax, the first in the 20th century: II Epidemiology. *Am. J. Hygiene*, 72, 6-23.
- Brachman, P. S., Kaufmann, A. F., & Dahldorf, F. G. (1966). Industrial Inhalation Anthrax. *Bacteriological Reviews*, 30(3).
- Dahlgren, C. M., Buchanan, L. M., & Decker, H. M. (1960). Bacillus anthracis aerosols in goat hair processing mills. *American Journal of Hygiene*, 72, 24-31.
- Fennelly, K. P., Davidow, A. L., Miller, S. L., Connell, N., & Ellner, J. J. Airborne infection with Bacillus anthracis—From mills to mail. *Emerg. Infect. Dis.* (serial on the Internet), June 2004. Available at: www.cdc.gov/ncidod/EID/vol10no6/02-0738.htm
- Friedlander, A. M., Pittman, P. R., & Parker, G. W. (1999). Anthrax Vaccine: Evidence for safety and efficacy against inhalational anthrax. *Journal of the American Medical Association*, 282, 2104-2106.
- Guidi-Rontani, C. (2002). The alveolar macrophage: The Trojan horse of Bacillus anthracis. *Trends in Microbiology*, 10(9).
- Holty, J. C., et al. (2006). Systematic Review: A Century of Inhalational Anthrax Cases from 1900 to 2005. *Ann. Intern. Med.*, 144, 270-280.
- Kournikakis, W., Ho, J., & Duncan, S. (2004). Objective Assessment of the Risk from Anthrax Letter Terrorist Attacks. *Technical Report, DRDC Suffield TR 2004-304*.
- Meselson, M., Guillemin, J., Hugh-Jones, M., et al. (1994). The Sverdlovsk anthrax outbreak of 1979. *Science*, 266, 1202.
- Phillips, G. B., & Jemski, J. V. (1965). Microbiological Safety Bibliography. Miscellaneous Publication 6, U.S. Army Biological Laboratories, Fort Detrick, July 1965.
- Plotkin, S. A., Brachman, P. S., Utell, M., Bumford, F. H., & Atchison, M. M. (1960). An epidemic of inhalation anthrax, the first in the 20th century: 1. Clinical features. *Am. J. Med.*, 29, 992-1001.
- Watson, A., & Keir, D. (1994). Information on which to Base Assessment of Risk from Environments Contaminated with Anthrax Spores. *Epidemiol. Infect.*, 113, 479-490.
- Wilkening, D. A. (2006). Sverdlovsk revisited: Modeling human inhalation anthrax. *Proceedings of the National Academy of Sciences of the United States of America*, 103(20), 7589-7594.