INHALATION ANTHRAX: DOSE RESPONSE AND RISK ANALYSIS

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The notion that inhalation of a single *Bacillus anthracis* spore is fatal has become entrenched nearly to the point of urban legend, in part because of incomplete articulation of the scientific basis for microbial risk assessment, particularly dose-response assessment. Risk analysis (ie, risk assessment, risk communication, risk management) necessitates transparency: distinguishing scientific facts, hypotheses, judgments, biases in interpretations, and potential misinformation. The difficulty in achieving transparency for biothreat risk is magnified by misinformation and poor characterization of both dose-response relationships and the driving mechanisms that cause susceptibility or resistance to disease progression. Regrettably, this entrenchment unnecessarily restricts preparedness planning to a single response scenario: decontaminate until no spores are detectable in air, water, or on surfaces—essentially forcing a zero-tolerance policy inconsistent with the biology of anthrax. We present evidence about inhalation anthrax dose-response relationships, including reports from multiple studies documenting exposures insufficient to cause inhalation anthrax in laboratory animals and humans. The emphasis of the article is clarification about what is known from objective scientific evidence for doses of anthrax spores associated with survival and mortality. From this knowledge base, we discuss the need for future applications of more formal risk analysis processes to guide development of alternative non-zero criteria or standards based on science to inform preparedness planning and other risk management activities.

SPECULATION AND FACT ABOUT inhalation anthrax have often been indistinguishable both in the scientific literature and in public media since the 2001 anthrax attacks. Misinformation,* which has been unintentionally spread, is pervasive concerning the doses of *Bacillus anthracis* believed to be associated with inhalation anthrax. In order to increase society's knowledge of the risk associated with exposure to *B. anthracis* spores and progression to symptomatic inhalation anthrax, advances must be made in developing and communicating scientific knowledge about the relationship between doses of *B. anthracis* spores and various responses (eg, survival or mortality). Understanding of dose-response relationships and the underlying mechanisms of disease resistance are essential for effective risk management and preparedness planning. Therefore, the need to clarify what information about inhalation anthrax

*Misinformation—false or misleading information that is spread unintentionally—includes urban legends (untrue stories that are widely believed because they speak to a widespread fear, hope, or other emotion). Definitions are available from: http://usinfo.state.gov/media/Archive/2005/Jan/26-288268.html.

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is speculation and what is fact (ie, directly supported by scientific data) motivated us to write this article.

In 2001, public policy–makers and risk managers, as well as others interested in or affected by risk management policies and decisions, did not have access to the scientific evidence (ie, dose-response data) and accompanying models that have been developed for *B. anthracis* in the past 55 or more years. Further, quantitative knowledge of the mechanisms of pathogenesis and innate resistance to anthrax has not advanced greatly since the pioneering work of Robert Koch and Louis Pasteur in the late 1800s for this disease of great antiquity. The consequence of this lack of knowledge was that a single remediation strategy was considered: decontaminate until no spores are detected in air and on surfaces. This strategy can be interpreted as essentially a zero-tolerance policy for exposure to a single *B. anthracis* spore. The direct costs to implement this strategy in a few buildings (approximately $300 million[1,2]), in addition to other indirect costs associated with building closures and disruption of services for months to years following the anthrax attacks, may be considered disproportionate to an actual decrease in risk. A further complication in the absence of comprehensive risk analysis, including the analytic-deliberative process,[†,3] is that the level of protection actually achieved by alternative strategies remains unknown.

The purpose of this article is to present information about dose-response relationships for *B. anthracis*, the causal agent of inhalation anthrax, as an essential step to begin a productive analytic-deliberative process. Data that support the existence of a threshold region of a dose-response curve below which adverse effects are unlikely[4] are presented from recent experimental studies and declassified historical experimental reports on inhalation anthrax. These data support the need to develop new options for risk management, particularly alternatives to the current zero-tolerance strategy for managing contamination with *B. anthracis* spores. Acceptance of a non–zero-tolerance strategy for *B. anthracis* contamination is unlikely without exercise of a formal risk characterization, which involves cycles of the analytic-deliberative process.[3]

We believe that dose-response assessment is a fundamental evidence-based process essential for the advancement of risk management, which includes the development of risk-based exposure standards for inhalation exposure to *B. anthracis* spores. The present lack of clarity regarding what is scientific fact and what is more speculative opinion about *B. anthracis* dose-response relationships has promoted the misunderstanding that a single *B. anthracis* spore is fatal. In this article, we will attempt to clarify what is known from objective scientific evidence of doses of anthrax spores associated with survival and mortality, and what is possible, but speculative, from risk model simulations.[5]

RISK, THE ANALYTIC-DELIBERATIVE PROCESS, AND *B. ANTHRACIS*

Communicating about risk is a prerequisite for communicating about risk of inhalation anthrax, given exposure. The term "set of triplets" was defined and illustrated by Kaplan and Garrick[6] nearly 3 decades ago in the first issue of the journal *Risk Analysis*. These risk analysts assert that risk is not a number, or even a curve, but instead is best represented by a family of curves, along with information on costs and benefits. Risk is described as a set of triplets that communicate information needed for decision making: the answers to the following questions, based on the available evidence.

1. *What can go wrong?* (set of *i* scenarios)
2. *How likely is it?* (likelihoods estimated for *i* scenarios)
3. *What are the consequences?* (damage estimated for *i* scenarios)

For scenarios of interest to decision makers, the risk analyst seeks to structure the available evidence to identify the conditions necessary to cause adverse effects, estimate the likelihood of the conditions occurring, and estimate the consequences or damages. Using inhalation anthrax as an example, a possible scenario is that a person develops inhalation anthrax after exposure to aerosolized *B. anthracis* spores. Although the likelihood for developing anthrax for this scenario cannot be directly measured, the body of evidence can support estimation. First, the inhaled dose can be estimated from environmental sampling and information about the physiology of the respiratory tract, and, then, the likelihood of illness (response) can be determined for that estimated inhaled dose from a dose-response curve derived from studies that administered controlled doses of spores to animals (see Table 1). The consequences for inhalation anthrax include biological endpoints (eg, mortality), as well as economic, social, and political costs for treatment and denial of infrastructure (eg, building closure) and remediation.

The complexity of risk analysis contributes to difficulty in communicating effectively about risk. Risk analysts and stakeholders may communicate about risk using the formal analytic-deliberative process described by the National Research Council.[3] The analytic-deliberative process is designed to facilitate recursive cycles of dialogue between risk analysts and stakeholders as knowledge about the risk due to a particular hazard expands. Such public discourse builds on iterative, structured analyses of the changing available body of evidence and the advantages and disadvantages as-

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[†]Definitions, descriptions, and principles available at http://www.riskworld.com/Nreports/1996/risk_rpt/html/nr6aa045.htm

Table 1. Experimental Evidence for Anthrax Lethality by Known Inhalation Exposures

Host	Infectivity Estimate (LD_{50}[a] or other as footnoted; estimated number of viable spores)	Reference	Description
Guinea pig	190,000	7	Susceptibility differences between species
Monkey (rhesus)	200,000		
Mouse	1,450,000		
Rat	7,000,000		
Dog	8,000,000		
Guinea pig	370,000[b]	8	98% single B. anthracis spores in highly reproducible clouds as dry product
Rabbit	600,000[b]		
Mouse	1,400,000[b]		
Monkey (rhesus)	45,000-640,000	9	Single spores
Guinea pig	340,000-5,300,000		
Monkey (rhesus)	1,000-9,000	10	Wet B. anthracis in open air
Sheep	3,000-9,000		Freeze-dried B. anthracis in chamber
Guinea pig	32,000-97,000		Wet B. anthracis in chamber
Monkey (rhesus)	59,000	L. Pitt, USAMRIID, personal communication, May 17, 2004	Dry B. anthracis head-only mask
Rabbit	105,000		
Chimpanzee	32,000-67,000	11	Correlates clinical behavior and tissue alterations
Monkey (cynomolgus)	4,000[c]	12	Estimates based on unpublished dataset
Monkey (cynomolgus)	1,000-6,000[d]	13	Chronic exposure to goat-hair mill air
Studies below: highest doses tested not causing adverse effects			
Human	~1,000	14	Exposure in air sampled from two goat-hair processing mills
Monkey (rhesus)	100,000,000	15	U.S. Army publication reporting administration in bronchials
Sheep	~3,000	16	Declassified UK report
Dog, guinea pig, sheep	~100,000-400,000	17	Declassified U.S. Army report

[a]LD_{50}: lethal dose for 50% of animals in dose group, also reported as LRE (lethal respiratory exposure causing 50% mortality); approximate inhaled doses generally estimated from spore concentrations per L air, exposure duration, and allometric relationships between body weight and breathing rate.

[b]Reported only as lethal doses following inhalation exposure (percentage of lethality unspecified); study also reported lethal doses by subcutaneous route (100-1,000 spores for rabbit and guinea pig; 10-100 spores for mouse).

[c]95% confidence limit for LD_{50}: 2-9,000 spores.

[d]LD_{10} to LD_{25}: lethal doses for 10-25% of animals in dose group.

sociated with alternative risk management options. For example, cycles of the analytic-deliberative process expanded knowledge of risks associated with climate change[‡] and exposures to naphthalene and influenced the development of policy options.

Several groups of scientists have modeled possible B. anthracis releases using assumptions and judgments to generate alternative hypothetical scenarios with regard to exposure and adverse effects.[18-20] The actual inhaled doses from possible exposure scenarios for B. anthracis releases are largely unverifiable, and perhaps indeterminate or unknowable. In our opinion, simulation models for inhalation anthrax releases are informative as discrete hypothetical "what if" scenarios, but the simulation results are not necessarily generalizable to applications in the development of regulatory policy.

For regulatory support and analytic-deliberative process, a cohesive mechanistic understanding of inhalation anthrax resulting from animal and human exposures is needed. Theoretically, it is possible that a single spore is sufficient to cause disease. One can assume low-dose linearity of the dose-response relationship, fit empirical models to animal

[‡]Intergovernmental Panel on Climate Change report available at http://www.ipcc.ch/ipccreports/ar4-syr.htm.

dose-response data, and extrapolate (between species and outside the range of observed doses) to predict risk estimates for humans.[10,21] We note the concern that the 2 fatalities reported in Connecticut and New York City during the 2001 anthrax attacks can be interpreted as evidence suggesting that mortality was caused by low doses of *B. anthracis* spores. However, we agree with the perspectives of Brachman[22] and Cohen and Whalen[23] that the 2001 anthrax investigations did not provide any data on infecting dose for these cases. To our knowledge, no scientific data from controlled dose-response experiments such as those listed in Table 1 or epidemiologic studies directly support the idea that a single *B. anthracis* spore is fatal. The body of evidence supports the theory that resistance to or tolerance of inhaled *B. anthracis* spores is observed at low doses, a theory offered as early as 1966 based on occupational evidence.[13,22,23] More recently, Cohen and Whalen calculated that exposures to 600 spores per day resulted in low risk (upper confidence limit of 6×10^{-8} or 6 occupational illnesses in 100 million exposures).[23] In addition, it appears to us that there are no direct scientific data that weaken the evidence of thresholds of resistance for inhalation anthrax or support the exclusion of threshold models from risk assessment. From our perspective, a key topic for inclusion in future analytic-deliberative process deliberations is the current non-threshold bias for dose-response models for inhalation anthrax.

Risk Analysis Includes Dose-Response Assessment

The established frameworks for risk analysis[3,24-27] include dose-response or consequence assessment as a major element in the risk assessment process. In risk analysis, assumptions are commonly used to bridge data gaps, and deliberation of the use and assessment of impacts of such assumptions are key components in the analytic-deliberative process. A systematic analysis of the dose-response evidence for inhalation anthrax is essential before misinformation can (or will) be deliberated and revised or rewritten.

Extensive dose-response data for the mortality endpoint available from selected controlled experiments using different strains and diverse animal hosts (Table 1) are presented as the primary evidence available for dose-response assessment. Select mortality data from experiments with nonhuman primates and guinea pigs were fitted to simple empirical models for use in predicting the likelihood of adverse effects in humans.[10,21] However, these models may not account for observed biological complexities such as mechanisms of innate and acquired resistance that likely cause nonlinearities in the low-dose region. We are unaware of any existing comprehensive systematic analysis of this body

of evidence to account for variability imposed by experimental protocols or biological variability in dose-response models across species. In our view, including model uncertainty (nonthreshold [linear] and threshold [nonlinear] models) and physiologic models for extrapolations between species and to the unobserved low-dose region of the dose-response curve would advance dialogue about inherent biases that perpetuate the misinformation about the infectivity of *B. anthracis*.

We present here evidence from recent studies[28] (L. Pitt, USAMRIID, personal communication, May 17, 2004; B. Leffel, USAMRIID, personal communication, February 13, 2008) and historical information that support the concept of threshold regions for resistance to inhalation anthrax. For example, recent data generated at USAMRIID illustrate survivability below a threshold region or inflection point of 10,000 inhaled *B. anthracis* spores for rhesus monkeys and rabbits (Figure 1) (L. Pitt, USAMRIID, personal communication, May 17, 2004) and 1,600 spores in an additional nonhuman primate host (B. Leffel, USAMRIID, personal communication, February 13, 2008).

Although some argue that thresholds do not exist for pathogens,[29] sufficient statistical evidence of a threshold region or inflection point where the likelihood of adverse effects increases has been demonstrated for *Salmonella pullorum*.[4] Ideally, quantitative data on dose-response and mode of action need to be integrated into predictive models used in risk analysis so that biologically sound inferences,[30] based in science, can be made from the available models. We anticipate that results from ongoing mechanistic research and physiological modeling efforts in multiple institutions will advance risk analysis by strengthening the scientific basis of risk assessment models and the rigor of extrapolation and inferencing to predict human health risks at low doses of *B. anthracis*.

Anthrax: A Global Disease in Animals and Humans

Although most people in the U.S. were unfamiliar with anthrax until 2001, it is an ancient disease with a global distribution in animals and humans (Figure 2). Despite declining incidence of anthrax in many countries, *B. anthracis* continues to cause disease in animal populations of most countries around the world.[31] The number of countries reporting human cases is smaller, but worldwide human cases are related to animal disease rates and behavioral factors such as contact of ungloved hands and arms during butchering of infected animal carcasses.[32,33]

In the past, the major risk factor for human inhalation anthrax in the U.S. and Europe was exposure to imported contaminated goat hair from Iran, Iraq, India, and Pak-
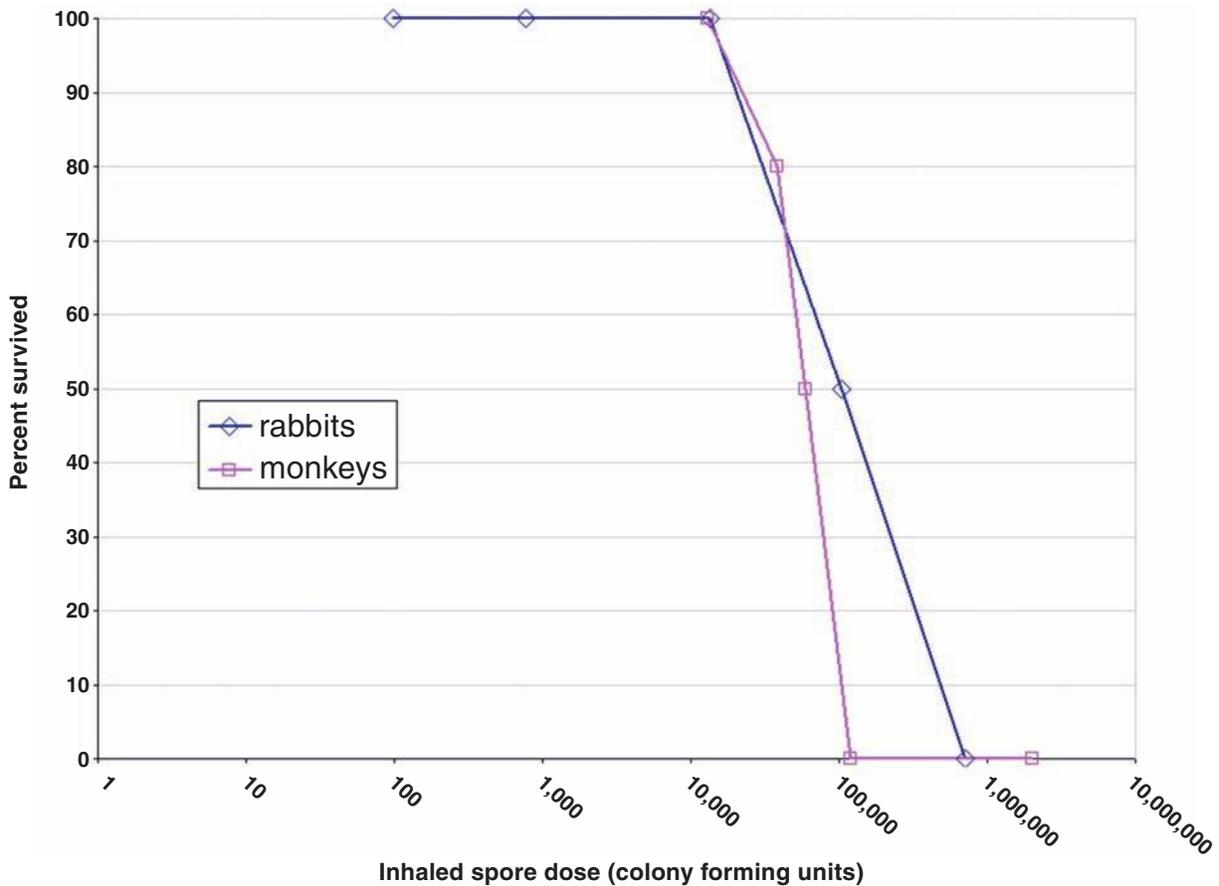
Figure. 1.   Survival curves for inhalation anthrax in rhesus monkey and rabbit.[11]

THE BIOLOGY OF INHALATION ANTHRAX AND ITS IMPACT ON DOSE-RESPONSE MODELS

istan. These regions were, and may still be, epizootic or hyperendemic for *B. anthracis*. Contamination of approximately 50% of imported goat hair lots was documented prior to 1960.[34] Attack rates paralleled environmental contamination of air and surfaces in goat hair mills, with the highest contamination detected in the early stages of processing (picking, carding, and combing), and generally declining rates with progression to the final processing step, weaving goat hair cloth.[35] Eighteen cases of inhalation anthrax were reported in the U.S. in the 1900s, and 10 of these cases were associated with wool and hides.[34] The remaining cases were associated with indirect exposures (3 cases), exposures to a home craftsman (1 case), laboratory workers (2 cases), and unknown sources (2 cases). Human inhalation cases in millworkers were dependent on the level of gross contamination, proximity to the source, and host factors.[36] When these mill industries shut down and moved abroad, associated human cases in the U.S. ceased, as did the related outbreaks in livestock downstream of the mills. Occupational cases of inhalation anthrax among wool sorters in the UK ceased by 1939, largely due to the Anthrax Prevention Act of 1919, which required decontamination of mohair, raw wool, and alpaca bales imported from specific risk areas prior to processing.

The biological basis of the clinical disease associated with inhaled *B. anthracis* spores (inhalation anthrax) is poorly understood, particularly with regard to host-pathogen interactions related to pathogen dose. Although inhaled and retained doses are unknown, and indeterminate, for all recorded human anthrax cases, significant observations of human pathology include occurrence of hemorrhagic mediastinal lymphadenitis, with the most severe lesions in the mediastinal lymph nodes rather than in respiratory tract tissues.[37-39] From infection of the lung associated lymphatics, bacteremia and septicemia can develop, thus resulting in secondary damage to other tissues during systemic infection. In our view, the major uncertainty in prediction of adverse effects from inhalation anthrax in humans is incomplete knowledge of the relationship between external exposures to *B. anthracis* spores and their deposition and clearance by innate and adaptive host defenses.

Some differences in pathology and dose-response relationships for inhalation anthrax in animals[7-10] may be at-

Global Anthrax Outbreak Status, 2004

Outbreak Status

- ■ Hyperendemic
- ■ Endemic
- ■ Sporadic
- ■ Probably Free
- □ Free
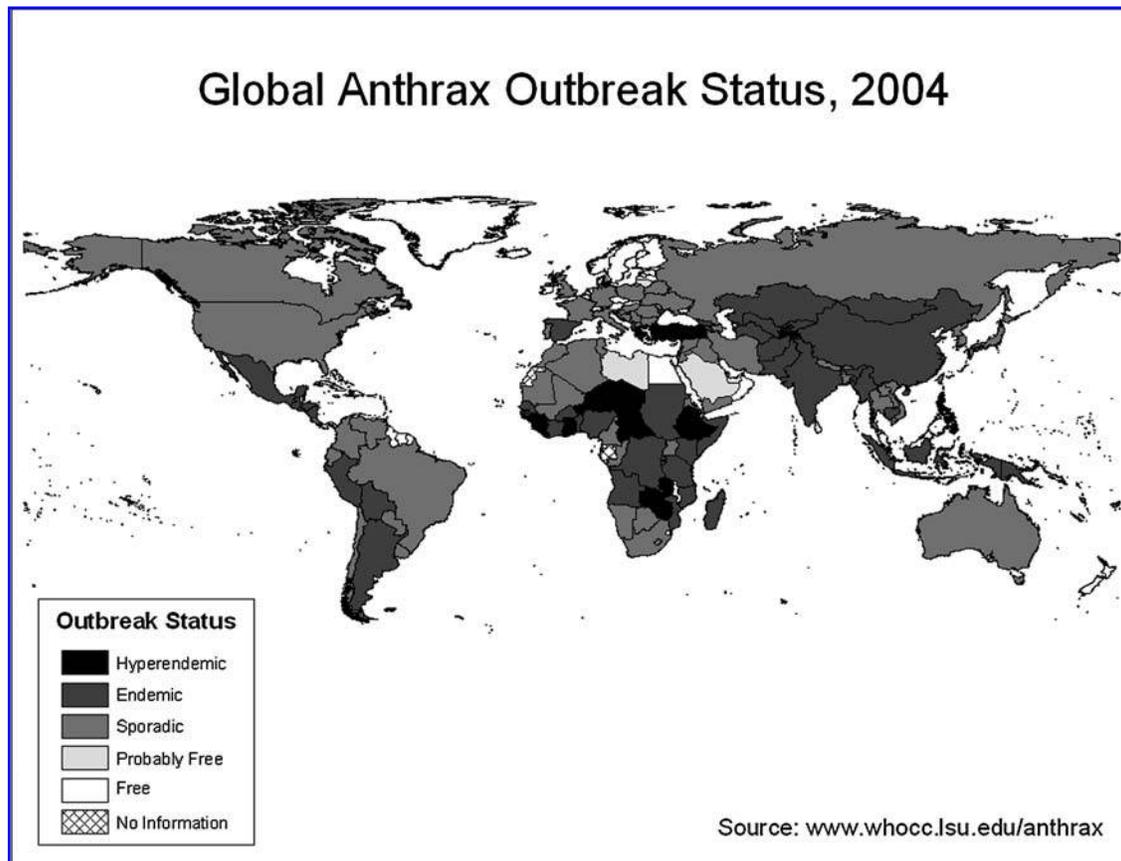- ▨ No Information

Source: www.whocc.lsu.edu/anthrax

Figure. 2.   Global distribution of anthrax in animals and humans from 2004 data. Map key: black, hyperendemic/epizootic >99 livestock cases/10^6; dark gray, enzootic, 30-99 livestock cases/10^6; medium gray, sporadic, <30 livestock cases/10^6; light gray, white, free or probably free; cross-hatched, unknown. Courtesy of World Anthrax Data Site; available at http://www.vetmed.lsu.edu/whocc/.

tributed to experimental factors in addition to host species differences. Risk assessors should consider the available body of evidence as comprehensively as possible, including in vivo and in vitro studies, to resolve apparent inconsistencies within and between species and draw inferences appropriate for predicting risk in humans. Although many details of host-pathogen interactions for inhalation anthrax are partially or qualitatively characterized in various experimental systems in vitro, the rates and capacities of spore clearance in vivo in human and animal models are unknown. Thus, gaps in knowledge of pathophysiology, including dose-dependency of innate and active host defenses, merit deliberation with researchers who could address the needs for verification and validation of risk assessment model simulations.

Dose-dependent survival is documented for inhalation anthrax in many animal systems. For example, in murine studies in vivo and in vitro, Pickering and colleagues reported 100% survival of mice that retained ~1,000 spores in their lungs and decreasing frequency of survival at higher doses.[40] Mice retaining ~100,000 and ~3,000,000 spores in their lungs had 20-50% and 80-100% mortality, respectively. The animals retaining the highest doses induced cy-

tokine responses, evidence for differential immune responses at high spore doses. These results support the concept that innate defenses (eg, alveolar macrophages) are sufficient to clear low-dose challenges, while enhanced immune defenses are triggered by higher doses.

Other studies document additional evidence for physiological factors that likely contribute to host survival of aerosol exposures to *B. anthracis* spores. Greater than 70% clearance of inhaled spore doses was observed in multiple animal species immediately after exposure,[7,8] likely via filtering in the upper respiratory tract and the mucociliary escalator that transfers spores and other particles from the ciliated airways of the respiratory tract to the expired air and gastrointestinal tract. Impaired clearance of spores deposited in the lungs of mice with depleted populations of alveolar macrophages was associated with significantly lower survival than mice with normal macrophage populations.[41] These innate host defenses likely constrained further distribution of retained doses in guinea pig lungs (exceeding a million spores immediately after exposure); by 24 hours postexposure, spore counts were 5×10^5 in lungs and 70 (range 0-254) in lung-associated lymph nodes in these mice.[8] In addition to host factors that constrain infec-

tion, a conserved pathogen regulatory mechanism may also constrain disease progression for low doses of *B. anthracis.* Disruption of quorum sensing, coordinated control of arrays of bacterial genes in response to a quorum or minimal density of bacterial populations, was recently demonstrated to inhibit *B. anthracis* growth and virulence gene expression in vitro.[42] These results suggest that the presence of a single *B. anthracis* spore in the lung, a macrophage, or a lymph node may not result in growth, disease progression, or mortality.

In summary, we conclude that dose-response models for inhalation anthrax should incorporate key nonlinear or sublinear effects of physiological factors influential in host-pathogen interactions that likely constrain progression of infection. Development and testing of physiological models of deposition and clearance for inhalation anthrax in animals and humans or Physiologically Based BioKinetic/Bio-Dynamic (PBBK/BD) modeling[43-45] may soon expand the knowledge base for drawing valid inferences from the body of evidence in animal systems to reliably predict the likelihood and magnitude of inhalation anthrax risk in humans. At present, both threshold and nonthreshold dose-response models should be introduced in the analytic-deliberative process for anthrax.

PHYSIOLOGICALLY BASED DOSE-RESPONSE ASSESSMENT

Little discussion of the risk of inhalation anthrax to date focuses on interpretation of the most relevant body of direct evidence for inhalation anthrax dose-response relationships from controlled experimental studies (Table 1). In addition to documenting an adverse effect (mortality) and susceptibility to inhalation anthrax after exposures to thousands or millions of *B. anthracis* spores, Table 1 also documents resistance to high doses of spores in humans, sheep, dogs, and guinea pigs from multiple investigators.[7,14,16,17] Additional evidence merits further consideration:

1. Data presented in Figure 1 were generated from ongoing vaccine and treatment efficacy research for *B. anthracis* (L. Pitt, USAMRIID, personal communication, May 17, 2004). The survival curves for monkeys and rabbits exposed to *B. anthracis* spores illustrated in this figure document 100% survival for both animal species challenged with up to 10,000 spores.
2. A recently compiled dataset from declassified U.S. Army studies (B. Thran, USACHPPM, personal communication, December 3, 2006) includes observations from *B. anthracis* exposure of 11,601 guinea pigs and 694 rhesus monkeys. Collectively in the guinea pig studies, 100% survival was observed in 128 dose groups (totaling 1,325 survivors) exposed to aerosolized *B. anthracis* spores, at

estimated doses ranging from 20 to 443,243 spores. Of 5,758 guinea pigs exposed at estimated doses in what may be considered the high-dose region (>10,000 spores), 3,716 survived challenge. Collectively in the rhesus monkey studies, 100% survival was observed in 15 dose groups (totaling 132 survivors) exposed to 10 to 320 *B. anthracis* spores. Of 254 rhesus monkeys exposed at estimated doses in the high-dose region (>10,000 spores), 130 survived challenge. The United States Army Center for Health Promotion and Preventive Medicine (USACHPPM) and others are exploring the low-dose behavior of an array of empirical dose-response model forms for this dataset in addition to the traditional probit and exponential models applied previously.[10,21] Ongoing and future analysis of this large body of evidence includes nonlinear mixed effects modeling, Bayesian analysis, artificial neural network analysis, benchmark dose modeling, and physiological extrapolation modeling.

3. The data in Table 1 document significant mortality observed in multiple animal species at doses from thousands to millions of *B. anthracis* spores. However, Table 1 also includes multiple studies documenting survival without treatment for multiple animal species dosed at hundreds to hundreds of thousands of spores. Application of previously mentioned PBBK/BD modeling for risk analysis integrates knowledge of the underlying mechanisms of deposition and clearance of spores from the respiratory tract and associated lymphatics in animals and humans. Such PBBK/BD modeling can be used to estimate the actual dose of spores to regions of the respiratory tract for a given exposure and resolve apparent inconsistencies in dose-response data for various animal species. PBBK/BD modeling also can serve as a heuristic tool for probing our understanding of mechanisms of disease and for identifying and prioritizing data needs. With advances in development and validation of human and animal PBBK/BD models, risk analysts will be able to apply principles of dosimetry to predict equivalent internal and external human doses associated with survival or mortality, and perhaps other adverse endpoints, from animal data.

Currently, physiologic or PBBK/BD models are in development for *B. anthracis* spore deposition and clearance in guinea pigs, rabbits, nonhuman primates, and humans.[43-46] Future research should be directed at bridging the gaps in the existing datasets, including extrapolation from the mortality endpoint to morbidity endpoints and early events of disease progression; adjustment for intra- and interspecies host effects; and extrapolation from high-dose to low-dose regions of dose-response curves. As new studies are designed, PBBK models can be updated and modified to strengthen the scientific basis of future dose-response as-

sessments and contribute to subsequent cycles of the analytic-deliberative processes for inhalation anthrax.

Credible dose-response assessment is problematic when objective scientific data or verifiable *facts* are confused with *expert opinions*, such as the widely cited infectivity estimates for *B. anthracis* spores (8,000-10,000; 8,000-50,000; 2,500-55,000). Neither source often cited for these infectivity values[47,48] actually includes any experimental details from primary research or data tables or figures to support these values. Despite extensive searching, to date we have identified no source of experimental data for these values. In addition, the datasets available before 2000 that measured inhaled or retained doses did not measure responses other than mortality;[28,36] however, subclinical (asymptomatic) infections were noted. Thus, these values likely reflect lethality estimates (LDs, perhaps LD_{50}s), not doses causing infection (IDs) or morbidity. To our knowledge, IDs have not been systematically measured for inhalation anthrax. ID and LD are not interchangeable, from our perspective of medical microbiology. Not all researchers and analysts share our interpretations. However, unbiased communication of the states of knowledge and disagreement is critically important in the analytic-deliberative process.

EPIDEMIOLOGIC DATA ON INHALATION ANTHRAX

Evidence gathered during epidemiologic investigations generally does not provide the explicit dose and response data needed for dose-response modeling. For example, the number of spores inhaled or retained, the total number of individuals in the exposed populations, and the doses that actually caused disease or no disease are not typically measured in epidemiologic investigations. Cohen and Whalen provide an analysis of the 32 inhalation anthrax cases reported between 1900 and 2005 as a result of exposure in U.S. textile mills, other direct exposures (ie, the 2001 anthrax event), and unknown exposures (9 "adventitious" cases).[23] We agree with Cohen and Whalen that frequent low-dose occupational exposures on the order of hundreds of spores per day to mill workers were within a threshold region for human resistance, and that infrequent high-dose exposures caused rare cases of inhalation anthrax in mill environments. Further evidence that the mill worker scenario is relevant for biothreat assessment is the observation that the virulence of strains isolated from mill workers was comparable to strains used in U.S. Army testing.[34] Additional epidemiologic studies that also provide meaningful insights into the potential dose-response relationships are noted below.

Temporal and spatial patterns for epidemiologic evidence,[49-51] as well as experimental evidence in Table 1 and other published studies,[52,53] is consistent with increasing likelihood and severity of adverse effects with increasing doses of many different pathogens in susceptible hosts. Unexpected clusters of inhalation (and cutaneous) anthrax cases, including fatalities in 1979 and 2001, led to epidemiologic investigations of these incidents. Spatial and temporal patterns of human and animal cases from the 1979 epidemic (Table 2) suggest distribution of high doses of *B. anthracis* spores downwind of the release site. However, in-

Table 2. Indirect Evidence for Livestock and Human Exposures Following Sverdlovsk Release in 1979

Exposure Sites	Distance from Release Site (km)	Number of Farms Reporting Animal Fatalities	Species	Number of Reported Fatalities[a] by Date in Early April										Totals
				4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	
Sverdlovsk, Rudnyi	2-7	4	Sheep			1	1					1		3
			Cattle		1		2							3
			Pigs				1		1					2
Bolshoye S, Malye S, Pervomayskyi	16-23	25	Sheep	6	15	5	2		2	1		1		32
Kashino, Sysert	32-34	12	Sheep	3	3		1	1		1		1	1	11
			Cattle									1		1
Abramovo, Averino, Novoipatovo	52-67	14	Sheep		1	3	3	2					1	10
			Cattle					1	1		1	1		4
Total animal fatalities		55		9	20	9	10	4	2	3	2	5	2	66
Total fatalities by animal species			Sheep	9	19	9	7	3		3	1	3	2	56
			Cattle		1		2	1	1		1	2		8
			Pigs				1		1					2
Reported human fatalities	<4.5	Not applicable	Humans					1	4	5	4	4	5	23

[a]Livestock fatality data translated from Dzhupina;[54] human fatality data from Guillemin.[55]

complete and conflicting accounts of the details of the release[55,56] limit the utility of these data for dose-response assessment. In our judgment, for the epidemiologic investigations from 1979 to the present, the actual doses of spores inhaled, the actual number of people (and livestock) exposed, and the actual doses causing and not causing disease are indeterminate. Simulation results for possible scenarios for the releases suggest interesting opportunities for future verification testing of modeling assumptions that may or may not be true.

The events following the Sverdlovsk release are uncertain in part because disinformation was supplied in deliberate attempts to conceal illicit operation of the Sverdlovsk facility. Mortality was reported by a scientific source[54] in sheep, cattle, and pigs predominantly from sites 16 to 67 km from the Sverdlovsk release site nearly a week before the first human fatalities were recorded, within 4.5 km of the release (Table 2). It is unclear if penned livestock were exposed to higher doses or longer durations of exposure, or if livestock were innately more susceptible than humans. Hypothetical simulation models developed to retrospectively reconstruct release, dispersion, and inhalation doses for humans and animals exposed in the 1979 release represent only *possible* scenarios and are, therefore, speculative.[5] In our view, although the magnitude of exposure (dose) to individuals downwind of the release is unknowable, we speculate that large numbers of humans and animals were exposed and relatively few developed anthrax.

For the 2001 anthrax attacks,[§] investigations began only after inhalation anthrax was diagnosed. Therefore, delays in the identification of the event may have resulted in human exposures over extended periods in contaminated environments. The first 2 inhalation anthrax cases in Florida were eventually linked to exposure via a contaminated letter opened at the American Media Incorporated (AMI) building in Boca Raton, Florida.[59] Despite distribution of spores throughout all 4 floors of the building, none of the remaining 98 AMI workers developed anthrax (inhalation or cutaneous). In the New York City area, at the NBC studios and the *New York Post* offices, other contaminated letters caused cutaneous anthrax cases but no inhalation anthrax cases. Two additional fatal inhalation anthrax cases (a 61-year-old hospital stockroom worker in New York City[60,61] and a 94-year-old Connecticut woman[38]) have fueled speculation that low doses were responsible because *B. anthracis* spores were not detected in environmental sampling. This scenario of low-dose fatalities is possible, but scientific evidence supporting this possibility is lacking.[22,23] Another scenario that is possible is that these women inhaled thousands or millions of spores from cross-contaminated mail, as reported by Beecher.[62] In addition, these fatalities may also reflect high-susceptibility scenarios. The total numbers of exposed people in Connecticut, Florida, and New York, as well as the doses potentially received, are unknown; however, based on the few cases of inhalation anthrax observed, we speculate that large numbers of people were exposed and few developed disease, consistent with the existence of a threshold region below which innate and adaptive protective mechanisms are likely to confer resistance to progression of inhalation anthrax.

The second batch of *B. anthracis* contaminated letters was mailed from New Jersey to Senators Tom Daschle and Patrick Leahy in Washington, DC. The principal victims of these letters were Washington-area postal workers who handled the letters or were present when they were being sorted. Most of the mail sorter infections were apparently due to *B. anthracis* spores being forced through the envelopes' fiber matrix during high-speed sorting. In addition to contaminating adjacent letters, the spores contaminated the high-speed sorting equipment and became aerosolized when the equipment was cleaned with compressed air. Postal workers accounted for 20 of the patients, including 7 inhalation anthrax victims. These letters, one of which was actually opened and contaminated the Hart Senate Office Building, became the subject of intense concern and investigation. Detection of 200 to 7,900,000 spores was reported for cross-contaminated envelopes postmarked the same day as the Daschle and Leahy letters.[46] Few to hundreds, thousands, or millions of spores could have been inhaled by any of the victims, in addition to others potentially exposed in the vicinity of the letters in the workplace or post office or via cross-contaminated mail. People handling the approximately 85 million pieces of mail processed before closure of the contaminated facilities[63] may have been exposed to the range of spores reported by Beecher.[62] Therefore, due to the large number of people expected to have been exposed and the few cases of illness observed, we again speculate that a threshold region exists below which disease is unlikely and innate and adaptive resistance is possible.

Additional epidemiologic evidence from the 2001 attacks that supports the concept of a threshold region for human resistance to inhalation anthrax was recently reported by Doolan and colleagues.[28] Researchers classified 124 subjects into groups based on their proximity when the contaminated letter was opened in Senator Daschle's office as a proxy for exposure to aerosolized *B. anthracis* spores. The exposure groups ranged from definitive exposure (subjects within the exposure zone with positive nasopharyngeal swabs) to unexposed negative controls. Although no attempt was made to reconstruct individual doses for each exposure classification, dose-dependency was demonstrated as relative increases in priming of cell-mediated immunity ob-

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[§]In summary, 22 cases of anthrax (11 inhalational, 11 cutaneous) were identified; 5 of the inhalational cases were fatal from October 4 to November 20, 2001.[57,58]

served in the individuals more likely to have inhaled greater numbers of spores in closer proximity to the release. Another finding was that 4 of 20 individuals in the negative control group (outside the Hart Senate Office Building during letter opening) developed cell-mediated immune responses but remained asymptomatic for inhalation anthrax. Although the inhaled doses are known only qualitatively for this event, this study provides indirect evidence suggesting that innate clearance for expected doses of relatively low numbers is an effective clearance mechanism, consistent with a threshold region of resistance.

Our position that epidemiologic data are consistent with threshold regions of resistance for inhalation anthrax is supported by multiple investigations in addition to the 2001 anthrax attacks and the 1979 Sverdlovsk release. Investigations for 2 other inhalation anthrax cases, unrelated to the 2001 event, were also initiated after diagnosis of cases of fulminant septicemia.[64,65] Again, delays in diagnosis may have resulted in human exposures over extended periods in contaminated environments. Both cases involve drum makers who were presumably exposed while working closely on contaminated hides without ventilation or respiratory protection as part of the drum-making process. We speculate that each of the drum makers inhaled higher levels of *B. anthracis* spores than exposed co-workers and family members visiting them or their working environments. During the investigations of one of the cases, 7 people who were exposed in contaminated spaces, but who had not worked on the unprocessed hides, were provided postexposure prophylaxis long after the drum maker's disease progressed to near-fatal sepsis. The lack of infection in the co-workers and family members is consistent with the innate host defenses as an effective mechanism conferring resistance to inhalation anthrax.

Similarly, a human inhalation anthrax case among 3 fatalities reported between 1961 and 1980 from England and Wales was associated with handling of second-hand sacks contaminated by bone meal,[66] another exposure scenario consistent with high-dose exposures to the handler and low-dose exposures to others in the same environment. Accordingly, we advocate and stress the importance of introducing a synthesis of the direct and indirect scientific evidence for inhalation anthrax dose-response assessment in a more formal cycle of analytic-deliberative process than this manuscript to support policy development and selection of detection, remediation, and reentry standards for *B. anthracis* and other biothreat agents.

Risk Analysis: Guide for the Future?

Decision makers responsible for reopening contaminated buildings during the 2001 anthrax attacks were subject to misinformation and the limitations of scientific knowledge.

No synthesis of the available data on inhalation anthrax dose-response relationships was prepared at that time for systematic application in site-specific risk assessment simulations. Also, risk-based criteria for decontamination and reentry had not been set for biothreat agents and continue to remain elusive. The current lack of a comprehensive knowledge-base for use in site-specific microbial risk assessment for biothreat agents continues to limit resolution of the pervasive complicated questions, "How clean is clean?" more than 6 years after the anthrax attacks, and "What is acceptable risk?" decades after publication of the milestone risk analysis contribution of Kaplan and Garrick.[6]

Advice by Sandman offered months following the anthrax attacks—"speculate responsibly"[67]—merits consideration for future risk analysis efforts regarding inhalation anthrax and other infectious diseases. The blurring of speculation and fact for inhalation anthrax is so pervasive that Sandman's advice is even more relevant for future deliberations about inhalation anthrax. For example, the 2 presumed low-dose fatalities in New York City and Connecticut that strongly influenced remediation decisions following the 2001 anthrax attack are not, in our opinion or the opinions of other scientists,[22,23] objective scientific evidence for low infecting doses causing inhalation anthrax. Stakeholders will need more transparent treatment of the primary evidence, objective scientific data from controlled studies, that supports risk analysis (risk assessment, risk communication, and risk management).[3,24,25] The need to clarify what information about inhalation anthrax is *speculation* and what is *fact* (directly supported by scientific data) motivated this article.

We were also motivated to write this article by Sandman's position that consideration of non-zero standards for inhalation anthrax has merit.[68] More than 4 decades ago, Brachman and colleagues reported that doses of 500 spores were "not necessarily or consistently dangerous" to mill workers,[34] and, more recently, Cohen and Whalen derived an estimate of 600 spores per day as an interim science-based threshold for preparedness planning and risk analysis.[23] These positions, based on related bodies of evidence, challenge the idea that a single spore is fatal. In this article, we have introduced an additional evidence-based approach as a next step to systematically derive human exposure guidelines for biothreat agents: dose-response assessment that incorporates PBBK/BD modeling. Future advances in mechanistic modeling may distinguish between existence of a threshold region associated with low probability of adverse effects and a true threshold of resistance to inhalation anthrax. While we do not advocate the position that risk is zero for exposure to a single spore, neither do we advocate anything less than a scientifically balanced analysis of the available body of evidence and commitment to the analytic-deliberative process for preparedness planning. We believe that public policy–makers and risk managers, as well as

stakeholders, will *not* accept non-zero standards for managing anthrax contamination without a systematic science-based methodology and wider exercise of more formal risk characterization, including iterative analytic-deliberative processes. Some urgency exists for facilitating public deliberation of the body of data and analyses for inhalation anthrax that integrates risk analysis information (risk assessment, documenting the scientific basis for risk assessment models; risk management, documenting the impact of alternative policies and interventions to reduce risk; and risk communication, facilitating dialogue about risk, its assessment, and its management options).

Though applications of the analytic-deliberative process for inhalation anthrax have been limited to date, ideally, the analytic-deliberative process would integrate science (via risk assessment) and other societal influences (via risk management and risk communication) to support a more complete exercise of possible scenarios and their risk management options. For example, hearings on siting biocontainment-level laboratories would likely be more productive as exercises of the analytic-deliberative process. Though difficult, proactive dialogue on risk analysis with stakeholders is essential to enhance effectiveness of our preparedness and response planning for future biothreat attacks. The subjective belief in the idea that a single spore is fatal and the use of infectivity estimates that lack scientific support limit preparedness and response planning unnecessarily. The wisdom of the current strategy of decontaminating until viable spores are nondetectable on surfaces and in air merits reconsideration.

Risk analysts continue to assess the anthrax attacks and work toward improving dose-response assessments for *B. anthracis*, as well as other pathogens. In addition to considering the traditional applications of empirical modeling with prediction of a probit slope or a point estimate, such as the LD_{50}, from a lethality curve, dose-response assessments incorporating biological mechanisms of disease resistance and susceptibility would significantly improve the biological plausibility of risk analysis models. Novel approaches[43-45] are being developed for risk analysis, beginning with inhalation anthrax to resolve the question of "acceptable risk" using dose-response assessment, to include PBBK/BD modeling to address the likelihood and consequences associated with alternative assumptions, interspecies extrapolations, and low-dose extrapolations that risk managers require. Dialogue between risk analysts and laboratory researchers has begun in support of physiological model development since knowledge of the biological events of inhalation anthrax pathogenesis across host species is necessary for interpreting the available data. This ongoing work is important to enhance the knowledge base for inhalation anthrax necessary for development of alternative non-zero clean-up and reentry standards that merit more urgent consideration.

CONCLUSIONS

The synthesis provided herein regarding inhalation anthrax dose-response relationships serves as a preliminary step in the analytic-deliberative process that is necessary to clarify what is known (*fact*) about inhalation anthrax and what is *speculation*. A more extensive exercise of the analytic-deliberative process is needed before societal acceptance of alternative non-zero reentry strategies for *B. anthracis* is feasible. Such analysis would include model uncertainty (nonthreshold and threshold models) and physiologic models for extrapolations between species and to the largely unobserved low-dose region of the dose-response curve. The analytic-deliberative process is needed to counter inherent biases that perpetuate the mistaken ideas about the infectivity of *B. anthracis* and inform risk management decisions regarding different levels of protection for different populations, development of alarm levels for biosensors for detection and reentry based on risk rather than exposure, and more effective use of limited resources for preparedness, response, and recovery options for future biothreat attacks.

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