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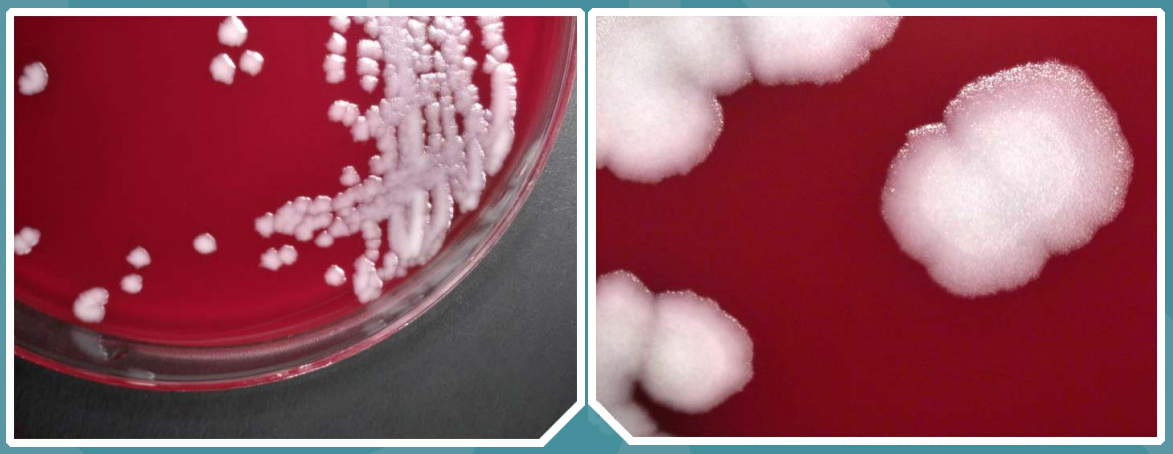
# Review of *Bacillus anthracis* Dose-Response Data for Human Health Risk Assessment



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# Review of *Bacillus anthracis* Dose-Response Data for Human Health Risk Assessment

United States Environmental Protection Agency
Cincinnati, Ohio, 45268

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## Disclaimer

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## Acronyms

| | |
|---|---|
| ADME | adsorption, distribution, metabolism, and excretion |
| AIC | Akaike Information Criterion |
| AMWG | Anthrax Modeling Working Group |
| BBDR | biologically based dose-response |
| BMD | benchmark dose |
| $BMD_x$ | benchmark dose for response in x% of individuals |
| $BMDL_x$ | the 95% lower statistical confidence limit of the BMD when the 95% lower confidence limit is applied to the estimated slope parameter value |
| BMR | benchmark response |
| BslA | *Bacillus anthracis* S-layer protein A |
| CBRN | chemical, biological, radiological, and nuclear |
| CDC | U.S. Centers for Disease Control and Prevention |
| CFD | computational fluid dynamics |
| CSM | conceptual site model |
| CFU | colony forming unit(s) |
| CI | confidence interval |
| DAF | dosimetric adjustment factor |
| DDEF | data-derived extrapolation factor(s) |
| DHHS | U.S. Department of Health and Human Services |
| EISD | Exposure - Infection - Symptomatic illness–Death |
| EPA | U.S. Environmental Protection Agency |
| ET | edema toxin |
| $F_r$ | regional fraction deposition |

| | |
|---|---|
| GSD | geometric standard deviation |
| HED | human equivalent dose |
| HHRA | human health risk assessment |
| $H_o$ | null hypothesis |
| ID | infectious dose |
| $ID_x$ | infectious dose for x% of individuals |
| LD | lethal dose |
| $LD_{50}$ | median lethal dose |
| LOAEL | lowest observable adverse effect level |
| LT | lethal toxin |
| MAPKK | mitogen-activated protein kinase kinase |
| MMAD | mass median aerodynamic diameter |
| NHP | nonhuman primate |
| NOAEL | no observable adverse effect level |
| PBBK | physiologically based biokinetic |
| PBPK | physiologically based pharmacokinetic |
| PCR | polymerase chain reaction |
| POD | point of departure(s) |
| $RDD_A$ | Regional Deposited Dose for the Animal |
| $RDD_H$ | Regional Deposited Dose for the Human |
| RDDR | Regional Deposited Dose Ratio |
| $SA_r$ | regional surface area |
| SD | standard deviation |

| | |
|---|---|
| USAMRIID | U.S. Army Medical Research Institute of Infectious Diseases |
| UF | uncertainty factor |
| µm | micrometer |

## Executive Summary

As one of the lead federal agencies supporting decontamination activities after a biological incident, the U.S. Environmental Protection Agency (EPA) has been systematically evaluating microbial dose-response data and their application for decision-making to support emergency management and decontamination activities. Risk-based approaches are desirable because they provide a formalized process to evaluate the hazard posed by these agents. The hazard posed by a release of *Bacillus anthracis* spores has made this agent a focus of considerable research by the EPA and others to identify and evaluate available data for microbial risk assessment. Given advances in the body of knowledge, a systematic review of *B. anthracis* data that can be used to support the development of a dose-response relationship or the use of *B. anthracis* dose-response data in a human health risk assessment (HHRA) is now warranted.

Given the breadth of available microbial dose-response data, science questions were generated to focus review on data necessary to perform a HHRA for *B. anthracis*. The following science questions are considered in the evaluation:

- What natural history data are available to inform development of a site-specific conceptual site model (CSM) for the generic exposure scenario?
- What data are available to support the development of the hazard identification, including disease pathogenesis data?
- What data support the use of the rabbit and nonhuman primate animal models for development of dose-response relationships?
- What dose-response data are available for inhalation and oral exposure in the rabbit, nonhuman primate, and human that may be appropriate for development of a microbial dose-response relationship?

- What are available approaches to model a microbial dose-response relationship?
- How might an animal-to-human extrapolation be conducted with *B. anthracis* dose-response data and what data are available?

Results were presented using the *EPA Framework for Human Health Risk Assessment to Inform Decision Making* (U.S. Environmental Protection Agency, 2014a) (hereinafter: the framework) as an organizing structure to report results from evaluation of the science questions.

A considerable body of knowledge is now available for the development of a site-specific HHRA for *B. anthracis*. There are sufficient data to develop the CSM and generate the hazard identification, as well as data and methods to generate a dose-response relationship for *B. anthracis* and conduct a partial interspecies extrapolation. While there are sufficient data to generate a quantitative HHRA, data quality and the presence of data gaps may contribute to potentially high levels of uncertainty in the risk assessment outputs. Depending on the intended use of the risk assessment outputs, these data may not be acceptable for all types of risk-based decision-making. Microbial risk assessors who are assisting in the initial planning and scoping element of the HHRA should take care to communicate these potential data limitations to decision-makers early in the process.

The most significant data gap relates to the lack of high quality dose-response data, defined as possessing sufficient quality to be categorized as Key Data. This clearly affects the rigor of the risk assessment. An additional data gap is the lack of basic mechanistic data for the initiation of infection and dynamics of the early infection process. These mechanistic data would greatly assist in the confirmation of appropriate dose metrics and inform the interspecies extrapolation process. However, alternative dose metrics can be assessed to see if substantive differences in

outputs result from different choices and the interspecies extrapolation process can be conducted in part to address kinetic elements.

This effort also revealed science policy gaps related to generation of a site-specific HHRA for B. anthracis inhalation exposure. Science policy gaps also affect current readiness to generate a site-specific HHRA for *B. anthracis* inhalation exposure. The selection of appropriate benchmark response (BMR) targets for reporting and risk-based decision-making for microbial pathogens is a current policy gap. While technical knowledge may inform BMR selection relative to known data set characteristics for benchmark dose (BMD) modeling, selection of values for reporting and risk-based decision-making may incorporate numerous policy considerations. An additional science policy gap is the management of uncertainty in the interspecies extrapolation given the current inability to address dynamic differences between the animal model and the human. In addition to a statement of this uncertainty in the risk characterization, a default adjustment factor could be considered for use until further data or methodologies are available.

# 1 Introduction

As one of the lead federal agencies supporting decontamination activities after a biological incident, the U.S. Environmental Protection Agency (EPA) has been systematically evaluating microbial dose-response data and their application for decision-making to support emergency management and decontamination activities. Risk-based approaches are desirable because they provide a formalized process to evaluate the hazard posed by these agents. The potential hazard resulting from exposure to residual biological contamination after buildings or other areas are cleared for re-entry is a significant concern for decision-makers. The hazard posed by residual contamination is greatest for biological agents that are highly persistent, resistant to decontamination, and with potential to cause serious or lethal illness at relatively low doses.

Interest in low-dose dose-response relationships for *Bacillus anthracis* exposure can be traced to data gaps made apparent during the civilian response to the 2001 anthrax letter event. The importance of the assessment of low-dose *B. anthracis* exposures, such as those potentially resulting from bioterrorism, was identified in publications shortly after the 2001 anthrax letter event (Dull et al., 2002; Haas, 2002; Peters and Hartley, 2002; Gutting et al., 2008). Ongoing preparedness activities have continued to identify the need for the assessment of low-dose exposures (Coleman et al., 2008; Taft and Hines, 2012; Gutting et al., 2013).

Potential health effects from a release of *B. anthracis* spores have made this agent a focus of considerable research by the EPA to identify and evaluate available data for microbial risk assessment. Although *B. anthracis* is the most highly studied of the currently known biothreat agents, significant data gaps have been identified for the microbial dose-response analysis of human exposure to low-dose exposures (Wilkening, 2006).

There is no technical or regulatory consensus for a *B. anthracis* dose-response relationship suitable for risk-based decisions (Taft and Hines, 2012). The lack of a dose-response relationship for *B. anthracis* is one significant impediment to the use of risk-based management approaches. However, there are multiple steps in the risk assessment process that incorporate microbial dose-response data. There has been considerable research performed since 2001 to better understand inhalation anthrax and its potential transmission after a biological incident. However, a systematic review is needed to evaluate currently available open source *B. anthracis* data to assess its suitability for use in a human health risk assessment (HHRA) microbial dose-response analysis. This report conducts a systematic review of *B. anthracis* dose-response data that can be used to inform development of a dose-response relationship or to support the use of *B. anthracis* dose-response data in a HHRA.

## 2 Purpose and Scope

The primary purpose of this report is to provide open source data and analysis approaches that can be used to develop a site-specific HHRA for *B. anthracis*. The report presents the results of an agent-specific planning activity for *B. anthracis* that evaluated published dose-response data, identified data and process gaps for microbial dose-response analysis of the agent, and identified science policy gaps that may be filled to conduct a site-specific HHRA for this agent. The data are organized following guidelines in the *EPA Framework for Human Health Risk Assessment to Inform Decision Making* (U.S. Environmental Protection Agency, 2014a).

Given the breadth of available microbial dose-response data, science questions were generated to focus review on data necessary to perform a HHRA for *B. anthracis*. The following science questions are considered:

- What natural history data for *B. anthracis* are available to inform development of a site-specific conceptual site model (CSM) for the identified exposure scenario?
- What data are available to support the development of the hazard identification, including disease pathogenesis data?
- What data support the use of the rabbit and nonhuman primate animal models for development of dose-response modeling of *B. anthracis*?
- What dose-response data are available for inhalation and oral exposure in the rabbit, nonhuman primate, and human that may be appropriate for development of a microbial dose-response relationship for *B. anthracis*?
- What are available approaches to model a microbial dose-response relationship for *B. anthracis*?

- How might an animal-to-human extrapolation be conducted with *B. anthracis* dose-response data and what data are available?

The intended audience is the human health risk assessor who is familiar with EPA HHRA guidance and has experience conducting microbial risk assessment. However, individuals with a research interest in microbial dose-response analysis of *B. anthracis* may find utility in the report for planning research to address data gaps or developing methodology for assessment purposes.

# 3 Framework for Microbial Human Health Risk Assessment

The U.S. Environmental Protection Agency (2014a) framework (hereinafter: the framework) for HHRA is designed for use with physical, chemical, or biological stressors. Stressors in this context are agents with the potential to cause harm. According to the framework, risk assessment is the iterative evaluation of the following elements: (1) planning, scoping, and problem formulation elements prior to the actual risk assessment; and (2) exposure assessment, effects assessment, and risk characterization steps of the risk assessment (Figure 3-1).

Figure 3-1 also identifies the risk assessment elements in the framework that incorporate microbial dose-response data and the report sections where the available data for *B. anthracis* are summarized and evaluated. Report content addresses two elements of the framework: problem formulation and effects assessment. In the problem formulation element (Section 4), there is a systematic identification of the factors (e.g., stressor(s), receptors, regulatory considerations) that will be evaluated in the risk assessment process (U.S. Environmental Protection Agency, 2014a). The CSM (Section 4.1) is a primary output of the problem formulation step. This CSM defines the hazard to be assessed relative to the relationships between the type and source of stressors, exposure pathways and completeness of these pathways, receptors, and types of endpoints or effects (U.S. Environmental Protection Agency, 2014a). It is presented as text, with a graphic showing the movement of the agent from the source to potential points of

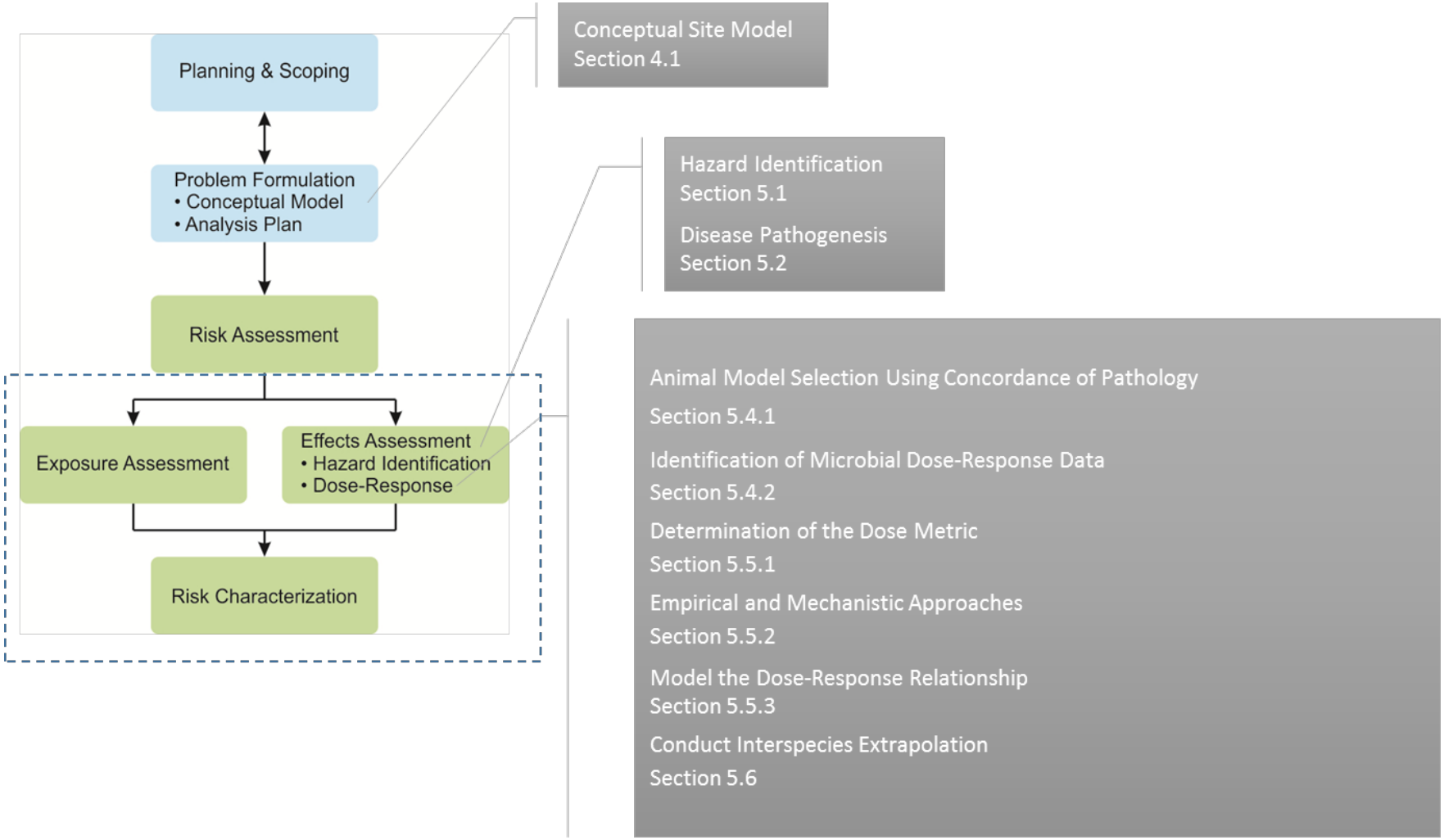


**Figure 3-1. Elements of the U.S. Environmental Protection Agency (2014a) human health risk assessment framework and associated report content.**

human exposure (U.S. Environmental Protection Agency, 2014a). The model may also include other considerations depending on the site, hazard, or other assessment-specific factors.

In the effects assessment element (Section 5), the hazard identification and dose-response assessment characterize the potential effects of exposure from the hazard being assessed (U.S. Environmental Protection Agency, 2014a). Overall, the effects assessment process considers data on the types of health effects, exposure pathways and routes of exposure associated with health effects, and associated dose-response relationship data for those effects. Specifically, the hazard identification (Section 5.1) identifies the type of hazard posed in the context of an identified exposure scenario (U.S. Environmental Protection Agency, 2014a). As part of the hazard identification for microbial hazards, data are presented on the likelihood of disease transmission and disease severity associated with exposure pathways, potentially sensitive subpopulations, and possible long-term sequelae. An evaluation of the microbial dose-response data (Section 5.4) considers both available data for animal model selection and the assessment of dose-response data. The mathematical modeling of dose-response relationship (Section 5.5) incorporates decisions regarding the dose metric used for analysis, empirical and mechanistic modeling approaches, and empirical curve-fitting within a benchmark dose analysis framework. As part of microbial dose-response analysis, approaches to conduct an interspecies extrapolation (Section 5.6) are also considered.

# 4 Problem Formulation

The risk assessment problem is the determination of the human health hazard posed by contact with low-levels of residual *B. anthracis* spore contamination in the air and on surfaces. An example of an exposure scenario consistent with this problem formulation is exposure to low levels of *B. anthracis* spores, such as might be present following application of remedial technologies after an intentional or unintentional release of spores in an indoor environment. Exposure to *B. anthracis* spores from other scenarios that are substantively similar in the route(s) and associated magnitude(s) of exposure may also be assessed using these data.

***Summary of Findings for Problem Formulation***

- Published reports support the potential for released *B. anthracis* spores to result in inhalation, ingestion, and dermal exposure with disease transmission.
- A quantitative HHRA could be developed with existing data.
- There is the potential for high levels of uncertainty associated with the quantitative HHRA outputs from limitations in dose-response data.
- The ingestion and dermal pathways are also likely to be complete but there are insufficient data to conduct a quantitative HHRA.
- The available natural history data are sufficient to generate a site-specific conceptual site model.

The problem formulation for this data evaluation is representative of a simplified, generic site. However, this does not preclude the potential presence of other exposure pathways when site-specific conditions are evaluated in an actual HHRA. The data evaluation is not inclusive of all fate and transport processes leading to potentially complete exposure pathways following an outdoor release or natural disease outbreak. For example, fate and transport pathways related to potential contamination of agricultural products and/or the food supply are not explicitly evaluated. Natural disease transmission from infected animals or associated fomites (i.e., objects or surfaces) is also not considered.

For this assessment, low dose was defined as the Rickmeier et al. (2001) value of less than $10^5$ colony-forming-unit(s) (CFU) inhaled dose. The original source for the low dose value in Rickmeier et al. (2001) was not identified, though it is presumed to be a consensus expert opinion identified by project participants. The primary reason for selection of the value of less than $10^5$ CFU inhaled dose is that it is less than the commonly cited median lethality value of $1.05 \times 10^5$ of Zaucha et al. (1998) for the rabbit. Few microbial dose-response and associated health studies are conducted with doses below the Zaucha et al. (1998) median lethality value. While it would be desirable that the defined low-dose level was reflective of a lower response level, it would not have been practical.

The majority of microbial dose-response and associated hazard data evaluated in this report are derived from spores manufactured for laboratory use, with the noted exception of the data from exposure to *B. anthracis*-contaminated mill aerosols. It is hypothesized that intentionally released manufactured spores might include some material modification (e.g., dispersants, detergents) to increase the hazard posed. However, this assessment will assume that no special processing techniques are used beyond typical laboratory practices to manufacture the spores with a consistent, highly respirable size for animal challenge studies.

### 4.1 Conceptual Site Model

A CSM can be a graphical or text description that concisely conveys the source of exposure, potential fate and transport mechanisms, completed or potentially completed exposure pathways to receptors, and associated routes of exposure. A generic CSM was generated using the problem statement description of the human health hazard posed by contact with low-levels of *B. anthracis* spore contamination (Figure 4-1). However, the presentation of this generic model

does not preclude the presence of other exposure pathways when site-specific conditions are evaluated. A site-specific evaluation must be conducted prior to the direct use of the generalized CSM in a site-specific risk assessment for *B. anthracis*.

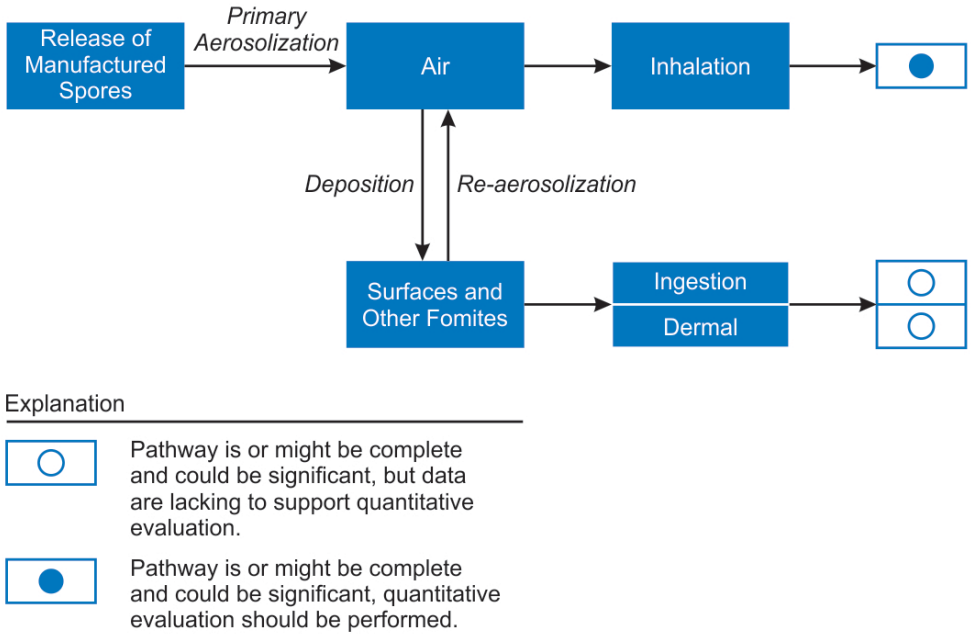


**Figure 4-1. Generic conceptual site model.**

The generic exposure scenario assessed is the release of manufactured spores. Spores of *B. anthracis* are hardy and persist for extended periods when released in indoor or outdoor environments (Inglesby et al., 2002). The exact mechanism of release is not defined (e.g., envelope, spray), but the spores are aerosolized as they are released to the air. Primary aerosolization at the point of release is the fate and transport mechanism that transports spores through the air medium to allow inhalation by the receptor. Spores may be deposited on surfaces (e.g., tables, computer screens, carpets) where they may be re-aerosolized into the air medium and remain aerosolized for extended periods. In addition to surfaces, spores may deposit on other

fomites (e.g., clothing), which may allow direct contact with the receptor, or the spores may re-aerosolize or be transported away from the initial release location with the fomite.

Re-aerosolization of *B. anthracis* spores from indoor surfaces was described after simulated office activities approximately one month after the primary aerosolization from the 2001 anthrax letter event in the Hart Senate building (Weis et al., 2002). Measurements of airborne CFU concentrations varied based on activity levels in the office area (Weis et al., 2002). Re-aerosolization of spore-containing particles in outdoor environments was also described in experimental studies using surrogates of *B. anthracis* spores (Layshock et al., 2012).

Physical transport within and between indoor and outdoor locations may lead to potentially complete exposure pathways for receptors in areas away from the initial release point. Transfer between indoor and outdoor environments (and vice versa) through building air intake and removal structures (e.g., heating, ventilation and cooling equipment), tracking from individuals, and movement via fomites during sample collection were described in studies using surrogate *B. anthracis* spores (i.e., *Bacillus thuringiensis* var. *kurstaki*) (Van Cuyk et al., 2011; Van Cuyk et al., 2012). Secondary contamination of *B. anthracis* spores in an individual's home and vehicle were reported after drums were made from contaminated African hides in a location separate from their home (U.S. Centers for Disease Control and Prevention, 2006). During an investigation of an anthrax outbreak at a textile mill in 1978, one of four sampled vacuum bags from workers' homes tested positive for *B. anthracis*, providing evidence for distant transport of spores via fomites (Bales et al., 2002). Transmission of cutaneous anthrax to children in the households of mill workers (presumably through contaminated fomites) was involved in 4% of

cases assessed in the Gold (1955) review of 117 anthrax cases in the United States between 1933 and 1955.

Oral exposure of spores is most likely to result from the transfer of spores from fomites (i.e., contaminated surfaces, clothing) to the receptor's hand and ultimately their mouth (i.e., hand-to-mouth exposure pathway). Oral exposure may also occur after inhalation of spores and subsequent mucociliary clearance from the respiratory tract to the esophagus (U.S. Centers for Disease Control and Prevention, 2010). Dermal exposure may occur through contact with deposited spores. However, this exposure pathway will not be assessed further due to the lack of available dose-response data that more closely match the exposure scenario of interest and do not involve subcutaneous inoculation.

Exposure duration of receptors may be acute, short-term, or possibly subchronic given the potential persistence of spores. For example, the exposure duration may be acute from a one-time visit (e.g., 24-hour or less exposure duration) or may be in the form of recurring daily exposure as could be anticipated after remediation in a residential or occupational land use. However, there may be peak exposures resulting in relatively high doses acutely or intermittently over time depending on receptor activities, environmental conditions, and spore particle characteristics.

Exposure via inhalation or ingestion of spores can result in lethal systemic anthrax illness, with inhalation anthrax having a significantly higher degree of lethality, even with aggressive medical treatment. Lethal inhalation anthrax has been associated with both low- and high-dose inhalation exposures, though this exposure scenario is focused on the assessment of low-dose exposure. Adult and child receptors are susceptible to inhalation anthrax after inhalation exposure to spores or to gastrointestinal or oro-pharyngeal anthrax (also termed oral-pharyngeal anthrax) after oral

exposure. There is also anecdotal evidence and limited *in vitro* evidence for the presence of sensitive subpopulations (e.g., elderly, immune-compromised, toxin-sensitive) that may be more susceptible to anthrax illness than the general population (Inglesby et al., 2002; Canter, 2005; Martchenko et al., 2012). Complicated by the low number of published reports on anthrax illness in pregnant, postpartum, or lactating women, Meaney-Delman et al. (2012) noted preliminary, though not statistically significant, evidence that cutaneous anthrax may pose the potential for greater lethality than might be expected in the general non-pregnant population. However, potential confounding factors were also identified that might explain the observed higher death rates including lack of timely treatment, type of medical treatment, and location of the cutaneous lesion (Meaney-Delman et al., 2012).

Direct person-to-person transmission of *B. anthracis* illness was not identified during a review of 49 anthrax investigations conducted by the U.S. Centers for Disease Control and Prevention (CDC) between January 1950 and August 2001 (Bales et al., 2002). Anthrax retransmission was also not described during the 2001 anthrax letter event (Inglesby et al., 2002). Though extremely rare, transmission of cutaneous anthrax infection has resulted from direct contact with infectious lesions, contaminated dressings, and contact with a bath item contaminated by an infected individual (Weber and Rutala, 2001). Published evidence for maternal-to-fetal transmission was described in case reports of neonatal anthrax illness and was accompanied by anthrax bacilli identified in organs from fetal and neonatal autopsies (Meaney-Delman et al., 2012).

# 5 Effects Assessment

In the effects assessment element of the risk assessment process, the potential health effects and endpoints of microbial exposure are identified in conjunction with known relationships between the exposures as described by the exposure assessment and the likelihood of health effects for those exposures. Section 5.1 identifies and evaluates available data to conduct a hazard identification to appropriately inform a site-specific effects analysis for *B. anthracis*. Section 5.2 then builds upon the hazard identification to provide further detail on the inhalation anthrax disease pathogenesis. Section 5.3 describes and evaluates available processes to perform a microbial risk assessment.

***Summary of Findings for Hazard Identification***

- The hazard posed by exposure to *B. anthracis* spores is well documented.
- Inhalation anthrax poses the greatest threat of lethality because it is difficult to diagnose during early stages of illness and becomes rapidly lethal.
- There is considerable uncertainty in the mechanistic details of the disease process.
- There is not a clear link between mechanistic pathway(s) or tissue dose(s) associated with the lethality endpoint.
- There is uncertainty regarding the mechanistic process for the initiation of the infection.

## 5.1 Hazard Identification

The hazard identification identifies the type of health hazard posed by the potentially complete exposure pathways identified in the CSM. As further detail to accompany the hazard identification, a key event identification and description of the disease pathogenesis of inhalation anthrax is provided in Section 5.2.

The microbial pathogen *B. anthracis* exists in two forms: vegetative bacterium and spore. For *B. anthracis*, inhalation exposure of the spore form and associated pathogenic illness is the human health hazard of greatest concern. Historically, the spore form has been of greatest human health concern due to its

persistence in indoor or outdoor environments, demonstrated lethality if infection results from human inhalation exposure, and prior use in biological terrorism. Vegetative bacteria released to the environment are generally less of a threat due to their limited persistence and low likelihood of infection unless directly introduced to the bloodstream (Fisher et al., 2011). There are very limited published data on the infectious dose (ID) associated with inhalation anthrax illness and the majority of collected data are for the lethality endpoint.

Complete human exposure pathways with *B. anthracis* spores associated with anthrax illness include agricultural contact with livestock, recreational contact with wildlife, associated fomites from livestock or wildlife (e.g., soil, meat, leather, wool or hair, bone meal) (Shadomy and Smith, 2008), and occupational contact with contaminated animal products (e.g., woolen textile mill) (Brachman et al., 1960). Prior to the 2001 anthrax letter event, approximately 80% of anthrax illness in the United States was associated with industrial contact with contaminated materials and 20% was associated with agricultural exposure (Brachman, 1984). For those exposed occupationally, the primary risk factor for anthrax illness was contact with contaminated goat hair from Iran, Iraq, India, or Pakistan (Coleman et al., 2008). Incidental contact with contaminated animal products (e.g., shaving brush bristles, yarn, animal hide drums, bone meal) is associated with anthrax illness but tends to be extremely rare (Vaswami, 1955; Suffin et al., 1978; U.S. Centers for Disease Control and Prevention, 2010; Marston et al., 2011). Two releases of manufactured *B. anthracis* spores have resulted in human anthrax disease outbreaks: the accidental release of spores manufactured by a former Soviet Union bioweapons facility in Sverdlovsk in 1979, and the anthrax letter event in the United States in 2001.

The four types of anthrax illness are differentiated based on the route of exposure associated with the initiation of infection: inhalation exposure (i.e., inhalation anthrax), oral exposure (i.e., gastrointestinal anthrax or intestinal anthrax, oro-pharyngeal anthrax), dermal exposure (i.e., cutaneous anthrax), and injection exposure (i.e., injection anthrax) from subcutaneous, intramuscular, or intravenous injection of drugs contaminated with *B. anthracis* spores (Inglesby et al., 2002; Grunow et al., 2013). With the exception of the deliberate release of manufactured spores, anthrax illness is relatively rare in developed countries and most often results from contact with infected animals or contaminated animal products (Passalacqua and Bergman, 2006).

Anthrax illness has been described as having three phases: asymptomatic or incubation, prodromal or latent with nonspecific flulike symptoms, and fulminant with "severe symptomatic disease" (Bravata et al., 2006). Fulminant anthrax infection is characterized by the development of overt clinical symptoms resulting from bacteremia and subsequent systemic dissemination of bacteria and associated toxins. These symptoms can include respiratory distress (i.e., dyspnea, stridor, cyanosis leading to mechanical ventilation after respiratory failure) and shock (Holty et al., 2006). Meningoencephalitis is present in up to 50% of human fulminant inhalation anthrax cases reviewed in Holty et al. (2006). Though each type of anthrax illness can progress to a fulminant infection, inhalation anthrax poses the greatest threat of lethality because it is difficult to diagnose during early stages of illness and becomes rapidly lethal after development of severe symptoms (Inglesby et al., 2002). Even with modern medical treatment and early diagnosis, the case fatality rate of those with inhalation anthrax during the 2001 anthrax letter event was 45% (Inglesby et al., 2002). However, the fatality rate is generally estimated to be almost twice as high without antibiotics or intensive medical treatment (Inglesby et al., 2002; Hilmas et al.,

2009). In the United States, 32 cases of inhalation anthrax were reported from 1900 through 2005 (Holty et al., 2006). Slightly more than half of the cases resulted from sources of manufactured spores or contaminated animal products. Eleven cases were associated with the 2001 anthrax letter event, five occupational cases were associated with the Manchester goat hair processing plant outbreak in 1957, and one case in 1966 from a man working across the street from the Manchester plant almost a decade after the 1957 outbreak (Holty et al., 2006). From 2006 through 2013, two additional cases of inhalation anthrax were reported in the United States (U.S. Centers for Disease Control and Prevention, 2006; Griffith et al., 2014).

There are two forms of anthrax illness associated with oral exposure: gastrointestinal and oro-pharyngeal. The fatality rate for identified cases of gastrointestinal anthrax ranges from 25% to 60% (U.S. Centers for Disease Control and Prevention, 2000), though it is unknown to what extent the estimate may be biased high from overrepresentation of more clinically apparent and/or more severe cases. In a similar fashion to inhalation anthrax, early diagnosis of gastrointestinal anthrax can be difficult due to non-specific disease symptoms (Cote et al., 2011). Oro-pharyngeal anthrax generally presents in a milder form and is associated with lower fatality levels than gastrointestinal anthrax (Hilmas et al., 2009). Case fatality rate estimates for gastrointestinal and oro-pharyngeal anthrax have high uncertainty as these forms of illness are likely to be both underreported and present as a "spectrum" of severity levels ranging from subclinical to lethal illness (Sirisanthana and Brown, 2002). Anthrax infection following oral exposure is most typically associated with less developed countries (Weiner and Glomski, 2012); this may be related to increased exposure opportunities due to differing cultural norms and routine food safety practices in less developed countries. Historically, a large-scale gastrointestinal anthrax epidemic of approximately 15,000 people in Saint-Domingue (Haiti)

during the 1700s was hypothesized to result from ingestion of uncooked beef, highlighting its potential for significant foodborne outbreaks (Morens, 2002).

In the United States, gastrointestinal anthrax in an occupational setting has been reported co-incident with cutaneous anthrax, with hand-to-mouth contact of spore-contaminated materials identified as a potential route of exposure (MacDonald, 1942). Gastrointestinal anthrax was suspected after ingestion of contaminated meat, though anthrax was not clinically confirmed in the Minnesota family event in 2000 (U.S. Centers for Disease Control and Prevention, 2000). Gastrointestinal anthrax in one individual in the United States was also reported after use of a contaminated animal hide drum (U.S. Centers for Disease Control and Prevention, 2010). Hypothesized pathways of exposure of the drum user included inhalation and subsequent ingestion of airborne spores, ingestion of food that had been contaminated by individuals that previously contacted spores, ingestion of food contaminated by direct deposition of aerosol, and incidental hand-to-mouth contact after spore contact (U.S. Centers for Disease Control and Prevention, 2010). However, the absence of gastrointestinal anthrax in laboratory animals after oral challenge with very large doses of *B. anthracis* spores has led to the hypothesis that infection from the oral route may require exposure to significant amounts of vegetative bacteria (e.g., ingestion of undercooked contaminated meat) (Inglesby et al., 2002; Xie et al., 2013). Host conditions may predispose individuals to infection even at lower doses where others may be unaffected (U.S. Centers for Disease Control and Prevention, 2010).

Cutaneous anthrax currently accounts for approximately 95% to 99% of all reported human cases of anthrax illness worldwide (Shadomy and Smith, 2008), with reported lethality rates of approximately 1% with antibiotic treatment and 10 to 20% without treatment (Beatty et al.,

2003). Eleven of the 22 cases of anthrax illness during the 2001 anthrax letter event were suspected or confirmed to be cutaneous (Inglesby et al., 2002). A 7-month old infant developed cutaneous anthrax after contact with *B. anthracis* contamination during the 2001 anthrax letter event that later resulted in severe systemic illness with hemolytic anemia, renal involvement, and persistent hyponatremia (Freedman et al., 2002). This constellation of symptoms appears to be unique relative to other descriptions of cutaneous anthrax in children, as well as the development of severe systemic symptoms after timely treatment with antibiotics and corticosteroids (Freedman et al., 2002). Children who develop cutaneous anthrax typically respond very well with appropriate treatment, but the severity of presentation in this case is atypical (Freedman et al., 2002). However, it is unknown how much of the literature describing cutaneous anthrax includes consideration of cases in children less than one year of age.

First described in 2000, injection anthrax is a relatively new phenomenon for human exposure and subsequent anthrax infection (Grunow et al., 2013). This form has only been identified in European countries to date and it has been hypothesized that all cases over the past decade may have resulted from a common contamination source in heroin (Grunow et al., 2013). The case fatality rate for injection anthrax is estimated to be 30% (Grunow et al., 2013).

Long-term health impacts, also termed sequelae, have been associated with infectious disease for a number of pathogens. For example, the toxins produced by some bacteria can cause serious organ damage in those infected (e.g., kidney damage from *Escherichia coli* infection) (Food and Agriculture Organization and World Health Organization (FAO and WHO), 2003). Alternatively, post-infection response to infectious disease can include the development of auto-

immune diseases such as reactive arthritis and Guillain-Barré syndrome (Food and Agriculture Organization and World Health Organization (FAO and WHO), 2003).

The potential for long-term sequelae from inhalation or gastrointestinal anthrax infection is unknown. Opportunities to conduct studies on the potential long-term health effects associated with surviving inhalation anthrax have been extremely limited due to the rarity of cases and survival after the illness. Reissman et al. (2004) assessed the presence of long-term health effects from bioterrorism-related *B. anthracis* infection in an adult study population that survived either inhalation anthrax or cutaneous anthrax. The study took place one year after illness from the 2001 anthrax letter event in the United States. Survivors reported somatic symptoms associated with multiple body systems, psychological distress, poor life adjustment, and reduced functioning (Reissman et al., 2004). However, the confounding of bioterrorism-related exposure with anthrax illness limits the ability to draw conclusions solely attributable to anthrax illness. Reissman et al. (2004) noted that their results were supportive of other studies with the United States population that identified both physical and mental health problems associated with surviving a terrorism event.

### 5.2 Disease Pathogenesis in the Context of Key Events

A key events analysis provides the analytical framework and structure to evaluate host-pathogen interactions from exposure through response (Buchanan et al., 2009). The base assumption of the key events approach is that a series of "causally linked biochemical or biological key events" can describe the process from initial exposure through the endpoint of interest (Meek et al., 2014). Though originally developed for chemical dose-response analysis, a key events framework for the food-borne pathogen *Listeria monocytogenes* was generated to assist in the development of a

dose-response relationship (Buchanan et al., 2009). Using the general approach described by Julien et al. (2009) and Buchanan et al. (2009), a preliminary key events process for inhaled *B. anthracis* spores was generated for discussion purposes by Hines and Comer (2012). While the motivation for the *B. anthracis* key events description was to facilitate identification of data gaps in the disease process, it provides a useful framework to organize the presentation of anthrax pathogenesis data (Figure 5-1). Appendix A provides background on transmission and pathogenesis elements for biological threat agents with relevance to microbial dose-response analysis.

*Key Event 1: Inhalation and Deposition of Respirable* B. anthracis *Spore Particles*

The first key event in *B. anthracis* pathogenesis is inhalation and deposition of respirable *B. anthracis* spore particles. For the development of inhalation anthrax, spores must be inhalable, deposit in the respiratory tract, and remain viable to initiate infection. It is traditionally accepted that the transmission of inhalation anthrax infection is optimized when inhalation exposure occurs to respirable spore particles that are less than 10 µm, which have a higher deposition potential in the deeper regions of the lung than larger particles. However, Thomas (2013) notes that deposition should be evaluated as a "continuum" through the entire respiratory tract, with the potential for infection recognized along the different tissue types present.

Consistent with other inhaled microbial pathogens, larger particle size doses are generally associated with presumed infection in the upper versus lower portions of the respiratory tract (Thomas, 2013). Higher doses for lethality are hypothesized to result from higher levels of clearance in the upper respiratory tract and tissue-specific colonization features (Thomas, 2013). Particle clearance capabilities in the upper respiratory tract also favor movement of particles to

the gastrointestinal tract (Thomas, 2013). In the murine model, gastrointestinal involvement was only identified in mice challenged with 12 µm particles, but not with 1 µm particles (Thomas et al., 2010). In this same murine model, the inhalation challenge with 12 µm particles was also

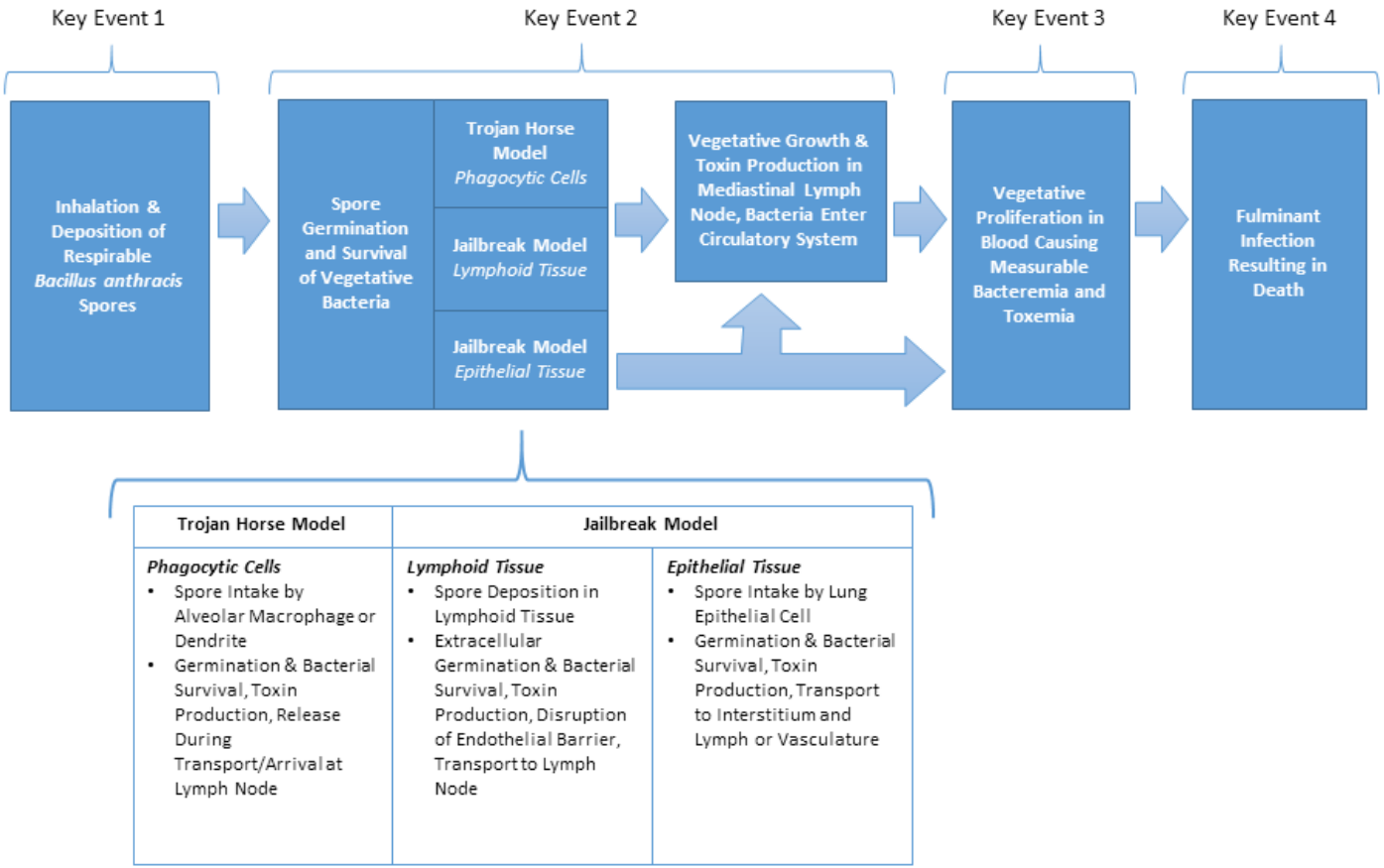


**Figure 5-1. Key events determination for inhalation anthrax modified from Hines and Comer (2012).**

associated challenge with 12 µm particles was also associated with longer average time-to-death measures than 1 µm particles (i.e., 161 ± 16.1 h versus 101.6 ± 10.4 h, respectively) (Thomas et al., 2010).

Consistent with other inhaled microbial pathogens, larger particle size doses are generally associated with presumed infection in the upper versus lower portions of the respiratory tract (Thomas, 2013). Higher doses for lethality are hypothesized to result from higher levels of clearance in the upper respiratory tract and tissue-specific colonization features (Thomas, 2013). Particle clearance capabilities in the upper respiratory tract also favor movement of particles to the gastrointestinal tract (Thomas, 2013).

Anthrax infection from inhalation exposure to larger particle sizes is associated with larger reported median lethal dose ($LD_{50}$) values and differences in disease presentation for nonhuman primate and guinea pig challenge studies (Druett et al., 1953; Goodlow and Leonard, 1961). The exposure of nonhuman primates to particle sizes greater than 10 µm has been associated with disease initiation in the upper respiratory tract, as evidenced by edema of the face and head for days prior to death from anthrax illness (Druett et al., 1953). Similar presentations of human anthrax infection were reported where infection was identified in the upper, but not lower, respiratory tract (Thomas, 2013). In the human, a limited number of case reports have been made of anthrax infection with clear indications of upper respiratory tract infection, but without any typical manifestations in the lower respiratory tract (Thomas, 2013).

*Key Event 2: Spore Germination, Proliferation, and Movement to Bloodstream*

The second key event for pathogenesis is spore germination and vegetative proliferation, ultimately leading to the release of vegetative bacteria to the bloodstream. Spore germination

leading to vegetative proliferation is indicative of infection. Two models have been developed to conceptualize the initiation of *B. anthracis* infection from inhalation exposure: the Trojan horse model (Guidi-Rontani, 2002) and the jailbreak model (Weiner and Glomski, 2012). The Trojan horse model is the first and most frequently cited model for initiation of inhalation anthrax since its publication in 2002 (Weiner and Glomski, 2012). Most of the early *in vitro* mechanistic work cited in the initial description of the Trojan horse model used the murine animal model or murine-derived cell lines (Hanna et al., 1993; Guidi-Rontani et al., 1999; Dixon et al., 2000), though Shafa et al. (1966) evaluated macrophages from the rabbit. The Trojan horse model hypothesizes the establishment of inhalation anthrax infection as an intracellular competition between the *B. anthracis* spore, host macrophage, and toxins expressed by vegetative *B. anthracis* (Guidi-Rontani, 2002). The Trojan horse model implicates both lethal toxin (LT) and edema toxin (ET) in the initiation of infection.

In the Trojan horse model, infection is initiated through engulfment of the spore by an alveolar macrophage and subsequent spore germination either during transport to, or upon arrival in, the lymph node (Guidi-Rontani, 2002). Consistent with the Trojan horse model, lung-associated lymph nodes were identified as the primary location of germination in rabbits after bronchoscopic administration[1] of spores (Lovchik et al., 2012). However, the murine (Glomski et al., 2007; Sanz et al., 2008; Dumetz et al., 2011) and guinea pig models (Twenhafel, 2010) provide preliminary evidence that transport to or through a regional lymph node may not be necessary for spore germination and anthrax illness after inhalation exposure.

[1] Bronchoscopic administration likely precludes initiation of infection in the upper respiratory tract and nasal-associated lymphoid tissue (NALT).

After the Trojan horse model was published, additional phagocytic cell types capable of transporting *B. anthracis* spores to lymph nodes were identified through in-vitro studies of human dendritic cells[2] (Brittingham et al., 2005) and murine B cells (Rayamajhi et al., 2012). Spore germination outside of phagocytic cells in a murine animal model after inhalation and oral exposure was reported in the lymphoid tissue of the respiratory tract and Peyer's patch tissues of the intestine, respectively (Glomski et al., 2007; Lowe et al., 2013). Spore translocation into lung epithelial cells was also reported from an *in vivo* murine study, providing a hypothetical direct intracellular route for spores to the lymphatic system (Russell et al., 2008).

To accommodate these new data, the jailbreak model expanded the Trojan horse model for initiation of infection in three important ways: (1) increased emphasis on the host-pathogen interactions in lymphoid and epithelial tissues, (2) broadened the role of alveolar macrophages to include important elements of host defense, and (3) expanded the number of potential cellular carriers to initiate infection (Weiner and Glomski, 2012). The jailbreak model is unique because it provides a conceptually consistent approach to model the early stages of infection across the three natural routes of exposure:  inhalation, gastrointestinal, and cutaneous anthrax (Weiner and Glomski, 2012). Multiple pathways by which inhalation anthrax may be initiated from the same route of exposure were identified (Weiner and Glomski, 2012). The use of multiple distinct pathways for infection would not be unique to *B. anthracis* as multiple pathways have been identified for other microbial pathogens (e.g., salmonellae, shigellae, *Listeria monocytogenes*) (Weiner and Glomski, 2012). Lowe et al. (2013) has also clarified that the identification of multiple pathways does not imply that mediastinal lymph node-initiated infections are not

[2] Dendritic cells were identified in the original description of the Trojan horse model as possibly providing an additional vehicle for transport to the lymphatic system and subsequent germination location (Guidi-Rontani, 2002).

occurring, but that alternative or additional pathways may not be recognized without study approaches designed to capture the data.

New concepts introduced in the jailbreak model include the potential for extracellular germination of spores that does not require an intracellular phagocytic location for germination, while still allowing for subsequent transport to the lymph system (Weiner and Glomski, 2012). The differing role for toxins in early infection is also notable. In the jailbreak model, spores germinate in an extracellular environment and toxins are necessary to damage the integrity of cellular barriers to facilitate access to the lymph system (Weiner and Glomski, 2012). In contrast, toxins in the Trojan horse model facilitate successful intracellular germination through modulation of host defenses in the phagocytic cell (i.e., the oxidative burst process) (Weiner and Glomski, 2012).

*Key Event 3: Vegetative Proliferation Leads to Measurable Bacteremia and Toxemia*

The establishment of anthrax infection requires the successful germination of spores in a host environment that is conducive for proliferation and dissemination of vegetative bacteria to the bloodstream (Guidi-Rontani, 2002). Systemic infection then allows for continued bacterial proliferation in blood and tissue, toxin production, and other virulence factors that are necessary for potential development of fulminant anthrax.

Lowe et al. (2013) hypothesized that the host environment for germination and growth may have downstream effects on the dissemination pattern of systemic infection. Similarities in the terminal bacterial burden in organs, but varying numbers of bacteria and differing kinetics of release based on the initial site of spore germination (e.g., lymphoid tissue versus phagocytes in

draining lymph node) were identified in murine studies (Lowe et al., 2013). Equivalent studies have yet to be conducted in the rabbit and nonhuman primate.

Dissemination allows for the vegetative bacteria to be presented to new host environments relative to the initial environment(s) associated with germination and initial proliferation. An *in vitro* evaluation of the germination of *B. anthracis* Sterne spores and proliferation of vegetative bacteria in rabbit, nonhuman primate, and human sera found the rabbit sera to be the most hospitable proliferation medium relative to the nonhuman primate and human (Bensman et al., 2012). Interestingly, the same *in vitro* study reported differences in the species sera most hospitable to germination, with spore germination highest in nonhuman primate sera, moderate in human sera, and only limited in rabbit sera (Bensman et al., 2012).

Few inhalation anthrax datasets for the rabbit report survival after measurable bacteremia. Survival without medical treatment after development of anthrax bacteremia was reported in two unvaccinated animals in the multiple-dose, low-dose rabbit study (U.S. Environmental Protection Agency, 2012b). Fellows et al. (2001) also reported survival after anthrax bacteremia when vaccinated rabbits were challenged with isolated strains from diverse geographic locations. Incidence of bacteremia for two isolates were reported as 70% and 80%, with accompanying survival rates of 90% and 100%, respectively. However, bacteremia levels were relatively low (i.e., <100 CFU/mL) (Fellows et al., 2001).

In contrast, survival after measurable low-level bacteremia was reported more often for unvaccinated nonhuman primates (Albrink and Goodlow, 1959; Saile et al., 2011; Henning et al., 2012) and vaccinated nonhuman primates (Ivins et al., 1996; Ivins et al., 1998; Fellows et al., 2001). Consistent with reports for the vaccinated rabbit, the levels of bacteremia were low (i.e.,

<100 to 200 CFU/mL) in the vaccinated nonhuman primate. From a key events perspective, the presence of measurable bacteremia appears to be strongly correlated with development of lethal anthrax infection, but in itself is not 100% predictive.

Bacteremia provides for a significant toxin loading to develop due to the upregulation of toxin production by vegetative bacteria (Cote et al., 2011). The LT and ET anthrax toxins may be released through extracellular vesicles containing toxin or in association with the capsule (Ezzell et al., 2009). Host cell proteins are receptors for the toxins, with differential expression of these proteins in cell lines associated with varying levels of cellular lethality when exposed to anthrax toxin (Martchenko et al., 2012). Each toxin affects cell signaling pathways that are present throughout the body in almost every cell type (Moayeri and Leppla, 2009). As a result, the response to the toxin is varied and dependent on the exposure and dose of exposed cells and tissues.

The LT is a zinc metalloproteinase that affects the mitogen-activated protein kinase kinases (MAPKKs) that are critical to many diverse cellular functions (Moayeri and Leppla, 2009). The ET is a calmodulin-dependent adenylate cyclase that produces cyclic 3',5'-adenosine monophosphate (cAMP), a compound also capable of affecting cellular signaling pathways (Moayeri and Leppla, 2009). The level of cooperative action of the toxins is a current area of uncertainty. The anthrax toxins have been described to work in an “additive or synergistic” fashion when both toxins are present (Lovchik et al., 2012), with the potential for “cooperative” action of the two toxins also reported for *in vitro* cellular studies using murine dendritic cells (Tournier et al., 2005). Recent reviews should be consulted for more detailed information on

toxins and toxin action (Tournier et al., 2007; Moayeri and Leppla, 2009; Guichard et al., 2012; Lowe and Glomski, 2012).

*Key Event 4: Development of Fulminant Infection*

Fulminant anthrax is associated with a presentation of "severe symptomatic disease" that can rapidly progress to severe respiratory distress, shock, and death (Bravata et al., 2006). Terminal bacteremia (i.e., vegetative bacteria in bloodstream) can be extremely high relative to other microbial pathogens, with levels of $10^9$ CFU/mL reported in the nonhuman primate (Friedlander et al., 1993). More typical reported values for the nonhuman primate range from $10^6$ to $10^8$ CFU/mL, with published examples including Ivins et al. (1996) and Ivins et al. (1998). In the rabbit animal model, terminal bacteremia concentrations were identified in the range of $10^5$ to $10^7$ CFU/mL in the single-dose study and $10^1$ to $10^5$ CFU/mL in the multiple-dose study (U.S. Environmental Protection Agency, 2011a, 2012b). However, there were also animals in each study that died with anthrax-illness related symptoms but no measureable bacteremia concentrations (U.S. Environmental Protection Agency, 2011a, 2012b). In contrast, toxemia can be more variable in its presentation from the appearance of symptoms to death, with non-detection even in animals that die with symptomatic disease.

While the action of toxins in the early stages of anthrax illness is thought to affect the functioning of phagocytic cells, the systemic accessibility of toxins in the later illness stages provides for the expression of widespread and tissue-specific toxicity. However, there is considerable uncertainty in known connections between the cell type and the pathway(s) associated with the toxicity (Moayeri and Leppla, 2009). To date, there are no mechanistic pathway(s) or tissue dose(s) that can be definitively associated with the lethality endpoint.

There is also in-vitro evidence for non-toxin mediated virulence factors that may be associated with lethality. Lethality in the rabbit resulted from intravenous challenge with *B. anthracis* Vollum strain vegetative bacteria mutants that lost production of toxins (Levy et al., 2014). However, additional non-toxin virulence factors that are hypothesized to contribute to anthrax lethality include sepsis from high bacteremia levels, proteases, *B. anthracis* S-layer protein A (BslA), and other factors yet to be identified (Friedlander, 2001; Guichard et al., 2012; Weiner and Glomski, 2012; Coggeshall et al., 2013; Remy et al., 2013). The sepsis hypothesis has received the most attention to date. The hypothesis acknowledges the role of toxins in reducing immune system effectiveness, but associates lethality with the extremely high bacteremia levels of fulminant illness (Stearns-Kurosawa et al., 2006; Coggeshall et al., 2013). Alternately, Cote et al. (2011) recognized the high terminal bacteremia concentration and hypothesized that host death resulted from a combination of toxemia and additional virulence factors.

### 5.3 Overview of Microbial Dose-Response Analysis

Dose-response analysis evaluates the relationship between exposure and the likelihood of identified health effects or outcomes (U.S. Environmental Protection Agency, 2014a). The resulting dose-response relationship is then compared to the results of the exposure assessment to determine the likelihood of adverse effects. There are three main steps in the development of a microbial dose-response relationship: (1) evaluation of microbial dose-response data, (2) modeling the dose-response relationship, and (3) conducting interspecies extrapolation to a human equivalent dose (HED) (Table 5-1). Table 5-1 identifies the key questions associated with each main step and the report section where data to evaluate the key questions are presented. The evaluation of data to answer the key questions is guided by current microbial dose-response analysis practice and data describing *B. anthracis* pathogenesis. As additional information to

supplement Section 5.1, Appendix B provides a review of historical themes in modeling *B. anthracis* dose-response relationships.

**Table 5-1. Development of Microbial Dose-Response Relationships**

| Steps in Microbial Dose-Response Analysis | Key Questions | Report Section |
|---|---|---|
| Evaluate the microbial dose-response data (Section 5.4) | What animal models are appropriate to generate dose-response data for *B. anthracis*? | Section 5.4.1 Animal Model Selection Using Concordance of Pathology |
| | What dose-response data are available and of sufficient quality to generate a dose-response relationship for *B. anthracis*?<br><br>What endpoints can be evaluated with available dose-response data? | Section 5.4.2 Identification of Microbial Dose-Response Data |
| Model the dose-response relationship (Section 5.5) | What dose metrics can be supported based on available disease pathogenesis and other dose-response data?<br><br>What assumptions are associated with a given dose metric? | Section 5.5.1 Determination of Dose Metric |
| | What types of empirical and mechanistic models may be suitable for *B. anthracis*?<br><br>Can mechanistic models be supported by available dose-response data for *B. anthracis*? | Section 5.5.2 Empirical and Mechanistic Modeling Approaches |
| | What approaches can be used to mathematically model the dose-response relationship and estimate the POD? | Section 5.5.3 Mathematically Modeling the Microbial Dose-Response Relationship |
| Conduct interspecies extrapolation to a HED (Section 5.6) | What is a general framework that can be used for interspecies extrapolation of *B. anthracis*? | Section 5.6.3. Proposed Framework for Interspecies Extrapolation for *B. anthracis* |
| | What data for the rabbit, nonhuman primate, and human are available to evaluate the kinetics and dynamics of *B. anthracis* pathogenesis? | Section 5.6.4 Available Kinetic Data<br><br>Section 5.6.5 Available Dynamic Data |
| | How can available data be incorporated in the extrapolation process? | Section 5.6.6 Summary of Extrapolation Framework for *B. anthracis* |

POD -- point of departure
HED -- human equivalent dose

## 5.4 Evaluate the Microbial Dose-Response Data

The evaluation of microbial dose-response data in this section will consider determination of appropriate animal models to generate a dose-response relationship relevant for the human and evaluation of available dose-response data for the appropriate animal models and the human (Table 5-2).

**Table 5-2. Evaluation of Microbial Dose-Response Data**

| Step in Microbial Dose-Response Analysis | Key Questions | Report Section |
|---|---|---|
| Evaluate the microbial dose-response data (Section 5.4) | What animal models are appropriate to generate dose-response data for *B. anthracis*? | Section 5.4.1 Animal Model Selection Using Concordance of Pathology |
| | What dose-response data are available and of sufficient quality to generate a dose-response relationship for *B. anthracis*?<br><br>What endpoints can be evaluated with available dose-response data? | Section 5.4.2 Identification of Microbial Dose-Response Data |

### *5.4.1 Animal Model Selection Using Concordance of Pathology*

This section will evaluate suitability of the rabbit and nonhuman primate animal models for the development of human dose-response relationships for inhalation anthrax. Based on general similarity in the pathology of the human and the animal models, the rabbit and nonhuman primate are identified as suitable for inhalation anthrax studies of pathogenesis (Zaucha et al., 1998; Leffel and Pitt, 2006; U.S. Food and Drug Administration, 2007; Goossens, 2009; Twenhafel, 2010). While rodent species (e.g., mouse) have been used for studying various elements of anthrax pathogenesis, potential variation in response to fully virulent strains and differences in immune system activity may limit the utility of these animal models for broader applications (U.S. Food and Drug Administration, 2007). Given the relative scarcity of oral

dosing studies reporting pathology, an animal model assessment was not conducted for this route of exposure. Animal model selection should be based on the utility of an animal model to answer the specific research question(s) being considered (Goossens, 2009). However, a process to assess animal model suitability for extrapolation to a human *B. anthracis* dose-response relationship has not been proposed (Pitt and LeClaire, 2005; Leffel and Pitt, 2006; Coleman et al., 2008).

***Summary of Findings for Animal Model Selection***

- The rabbit and nonhuman primate exhibit many commonalities in the type of lesions and tissues identified for inhalation anthrax in the human.
- Differences were not identified between the rabbit and the nonhuman primate for anthrax pathology that do not have a time-dependency for incidence or severity in presentation.
- The rabbit and nonhuman primate are suitable animal models for development of dose-response relationships for the human.

For this evaluation, the suitability of animal models for extrapolation to human *B. anthracis* dose-response relationships was determined by an assessment of general concordance in published anthrax pathology between the human and the animal models. The key human histologic findings for the assessment of animal models identified by Twenhafel (2010) were used to assess published anthrax pathology of the rabbit and nonhuman primate relative to that of the human. The ultimate use (e.g., basic pathogenesis research, medical countermeasures) of the selected animal models was not specified in Twenhafel (2010). Twenhafel (2010) evaluated human pathology data from Sverdlovsk (Abramova et al., 1993; Grinberg et al., 2001) and the 2001 anthrax letter event (Jernigan et al., 2001) to generate the following list of key human pathological findings: pneumonia; splenic lymphoid depletion; meningitis; hepatic, gastrointestinal, and urogenital hemorrhage and/or inflammation; anthrax bacteremia; and anthrax toxemia.

#### *5.4.1.1 End-stage Pathology*

The vast majority of published pathology data for the evaluated animal models are representative of end-stage illness. However, exceptions include a nonhuman primate serial sacrifice study that evaluated a subset of tissues associated with early infection events (Berdjis et al., 1962) and a serial pathology study in the rabbit at 30, 60, and 72 hours post-challenge for selected tissues (Peterson et al., 2007). The pathology reported from scheduled sacrifice studies may also include animals that have inhalation anthrax in earlier stages of the disease (i.e., not end-stage) and may therefore introduce early or intermediate disease stages in the described pathology. However, these occurrences are not specifically identified in reports and therefore cannot be systematically evaluated.

Comparisons of anthrax pathology provided in published reports can be challenging for many reasons. Vasconcelos et al. (2003) noted the inherent difficulty in comparisons of pathology reported in nonhuman primate studies due to fundamental differences in study design and quality controls (e.g., animal age, *B. anthracis* strain, dose, particle size, pre-existing lung lesions from mites). Differences in pathology descriptions and disease definitions also complicate comparisons of the presence, absence, or severity of identified pathological conditions (Fritz et al., 1995; Vasconcelos et al., 2003). Additionally, distinguishing between gross versus histopathologic observations can be challenging based on the limited data reported for some studies (Fritz et al., 1995). Likewise, the existence of anthrax pathology can be missed for animals lacking gross lesions typically associated with inhalation anthrax (i.e., atypical disease presentations) if microscopic examination of tissues is not conducted (Vasconcelos et al., 2003). The lack of these data could bias the reported data set of inhalation anthrax pathology toward only the histopathology associated with gross pathological features.

Characteristics of inhalation anthrax pathology also affect the comparison of reported study results. One key consideration is the potential role of time-dependency in lesion development, whereby lesion progression and anatomical location for specified tissue locations are associated with survival time post-challenge. For example, defined pathological outcomes (e.g., meningeal hemorrhage, adrenal inflammation and necrosis, hepatic necrosis) were reported more commonly in nonhuman primates that survived four or more days post-challenge relative to those that survived shorter time periods (Vasconcelos et al., 2003). Similarly, the rapidity of death from inhalation anthrax in the rabbit has been attributed to a decreased incidence and severity of mediastinal lesions relative to the human, who typically exhibits a longer survival time (Zaucha et al., 1998). The extension of human survival afforded by medical treatments (e.g., antibiotics, aggressive medical care) may confound comparisons with animal pathology unless similar medical treatments are employed to extend the illness duration in the animal model. The prolongation of survival through the use of antibiotics in later stages of illness without prevention of death was reported during early studies of nonhuman primates by Gleiser (1967). As an additional complicating factor, dose-dependency has also been hypothesized to affect formation of specific lesions (Gleiser et al., 1963). As these factors have relevance for the comparison of animal model data, they will be considered further in Section 5.4.1.5.

A detailed summary table of end-stage pathology for the rabbit, nonhuman primate, and human is provided in Appendix C, Data Summary Table for End-stage Inhalation Anthrax Pathology of the Human, Nonhuman Primate, and Rabbit.

#### *5.4.1.2 Human*

Available human anthrax pathology data originate from the 1957 anthrax occupational outbreak, the 1979 Sverdlovsk outbreak, the 2001 anthrax letter event, and anecdotal published case reports (Table 5-2). There are varying levels of comprehensiveness and detail in the reported pathology, ranging from complete pathological descriptions to highlights or generalized findings. Table 5-2 identifies primary sources describing human inhalation anthrax cases. However, there may be potential for some overlap in descriptions of an individual case. Interpretation of the published data on the pathology of human inhalation anthrax is complicated by: (1) application of varying types of medical treatment and (2) cases resulting from exposure to different strains or spore products (e.g., Ames strain manufactured spore products versus mill aerosol strains).

**Table 5-3. Reported Human Autopsy or Pathology Data by Outbreak or Event**

| Outbreak/Event (Strain) | Reported Data |
|---|---|
| 1957 Occupational Outbreak (Unknown mill aerosol strain[s]) | Albrink et al. (1960)<br>Plotkin et al. (2002) |
| 1979 Sverdlovsk Outbreak (Unknown multiple strains)* | Abramova et al. (1993)<br>Grinberg et al. (2001) |
| 2001 Anthrax Letter Event (Ames strain) | Barakat et al. (2002)<br>Borio et al. (2001)<br>Bush et al. (2001)<br>Gill and Melinek (2002)<br>Guarner et al. (2003)<br>Jernigan et al. (2001)<br>Mina et al. (2002) |
| Anecdotal Events (Unknown strains) | Albrink (1961) - Electrician who worked in microbiology laboratory in an unspecified year (Unknown strain)<br>Brachman et al. (1961) – Male with sarcoidosis in 1958 and woman in 1948† (Unknown mill aerosol strain[s])<br>Gold (1955) – Handyman in carding room of mill in 1942 (Unknown mill aerosol strain[s])<br>LaForce et al. (1969) – Worker across alleyway from goat hair processing plant in 1966 (Unknown mill aerosol strain[s])<br>Suffin et al. (1978) – Weaver exposed to yarn in 1976 (Unknown multiple strains associated with animal-origin yarn)<br>U.S. Communicable Disease Center (1961) – Secretary in goat hair and wool plant outside Philadelphia in 1961 (Unknown mill aerosol strain[s]) |

* See Jackson et al. (1998) for more information on Sverdlovsk strains

† The 1951 case described as the "housewife" in Brachman et al. (1961) did not include autopsy or pathology data

Data on human inhalation anthrax pathology without any medical treatment are not available, as most individuals receive medical treatment when the severity of illness associated with fulminant anthrax is exhibited. For example, inhalation anthrax cases in the 1957 occupational outbreak were given antibiotics at some point prior to final diagnosis or death. This makes it difficult to fully determine the human pathology without medical treatment. To obtain comparable animal model pathology data, animal studies would need to incorporate the same types of medical treatments. The effectiveness of medical treatment for inhalation anthrax has increased substantially between the earlier outbreaks (e.g., 1957 occupational outbreak, Sverdlovsk) and the 2001 anthrax letter event outbreak; this has led to higher survival rates and possibly longer times to death for those that do not survive. However, strain-specific differences in inhalation anthrax pathology have also contributed to the identified differences. Many of the pre-Sverdlovsk cases resulted from exposure to unknown strains of animal mill aerosol or finished product (e.g., yarn) of animal origin, whereas the Sverdlovsk outbreak, the 2001 anthrax letter event outbreak, and the case in the electrician described by Albrink (1961) resulted from exposure to Ames or an unidentified manufactured spore product strain(s) (Table 5-3).

Table 5-4 provides a summary of reported human pathology relative to the Twenhafel (2010) list of key histopathological findings. The two most pronounced gross autopsy findings of human inhalation anthrax victims were pleural effusions and mediastinal lymph nodes with edema and hemorrhage (Guarner and del Rio, 2011). Serosanguinous pleural effusions were identified in five of the eight patients who died during the 2001 anthrax letter event, with the confirmed presence of *B. anthracis* antigens in the pleurae thought to explain the reported severity of these lesions (Guarner et al., 2003). “Massive hemorrhagic mediastinitis” was identified in two of

three of the fatal inhalation anthrax cases in the 1957 occupational outbreak reviewed by Plotkin et al. (2002), with mediastinal lymph nodes described as enlarged and edema-filled.

Notable differences in pathology were described between the victims of Sverdlovsk and the 2001 anthrax letter event, with greater progression of disease reported in Sverdlovsk victims (Guarner and del Rio, 2011). The first point of difference between the Sverdlovsk and the 2001 anthrax letter event victims was the relative presence of high- and low-pressure hemorrhages. In the 2001 anthrax letter event, higher pressure hemorrhages were less prominent than in the Sverdlovsk cases. The second main difference was that the Sverdlovsk victims exhibited extensive

**Table 5-4. Summary of Human Pathology Relative to Twenhafel (2010) Key Findings**

| Pathology | Human |
|---|---|
| Pneumonia | Pleural effusions (at autopsy or drained prior to death) (LaForce et al., 1969; Jernigan et al., 2001; Barakat et al., 2002; Mina et al., 2002; Guarner et al., 2003)<br>Pulmonary edema (Abramova et al., 1993; Mina et al., 2002), including intra-alveolar and interstitial edema with focal hemorrhage and fibrin deposition (Barakat et al., 2002)<br>Necrotizing, hemorrhagic pneumonia with primary foci present (Abramova et al., 1993)<br>Perihilar interstitial pneumonia (Grinberg et al., 2001) and acute bronchial pneumonia (Grinberg et al., 2001) |
| Splenic lymphoid depletion | Splenomegaly with hemorrhage (Albrink et al., 1960), congestion (Suffin et al., 1978), and necrosis (Barakat et al., 2002; Guarner et al., 2003)<br>Moderate to marked lymphocytolysis, minimal atrophy of follicles, and thickening of Bilroth cords (Grinberg et al., 2001) |
| Meningitis | Meningitis (Inglesby et al., 2002), including hemorrhagic meningitis (Plotkin et al., 2002)<br>Cardinal's Cap from hemorrhage of leptomeninges (Inglesby et al., 2002); more frequently identified from Sverdlovsk than 2001 anthrax letter event victims (Guarner and del Rio, 2011) |
| Hepatic hemorrhage or inflammation | Intrasinusoidal inflammation present (Grinberg et al., 2001)<br>Kupffer cells mildly to moderately hypertrophic and hyperplastic, minimal to mild centrilobular, and coagulation necrosis noted infrequently (Grinberg et al., 2001) |
| Gastrointestinal hemorrhage or inflammation | Gastrointestinal submucosal lesions (Abramova et al., 1993; Inglesby et al., 2002)<br>Necrosis, hemorrhage, and edema of the ileum (Albrink et al., 1960) |
| Urogenital hemorrhage or inflammation | *None reported for human in identified sources* |

hemorrhage in the meninges (i.e., Cardinal's Cap) and higher burdens of *B. anthracis* in the brain and intestines (Guarner and del Rio, 2011). In contrast to the identification of meningeal spread in approximately 80% of the Sverdlovsk cases as reported by Grinberg et al. (2001), a considerably lower case rate of meningitis or post-mortem evidence of meningeal spread was identified in the 2001 anthrax letter event cases (Guarner et al., 2003). Hypothesized reasons for these differences included differing *B. anthracis* strains, earlier case recognition, and more effective treatment protocols in the 2001 anthrax letter event (Guarner et al., 2003; Guarner and del Rio, 2011).

Splenomegaly was reported during the 1957 occupational outbreak (Albrink et al., 1960), anecdotal case reports (Suffin et al., 1978), and the 2001 anthrax letter event (Barakat et al., 2002; Guarner et al., 2003). Splenic congestion, a condition that can contribute to presentation of splenomegaly, was also identified in 86% of the 41 cases for which microscopic data were evaluated in the Sverdlovsk outbreak (Grinberg et al., 2001).

#### *5.4.1.3 Rabbit*

Inhalation anthrax pathology for *B. anthracis* Ames strain exposure has been described for two rabbit breeds (Table 5-5). Table 5-5 identifies studies reporting pathology of end-stage inhalation anthrax, with the exception of Peterson et al. (2007). The New Zealand white rabbit is the most commonly used breed of domesticated rabbit (*Oryctolagus cuniculus*) for anthrax pathology studies. Peterson et al. (2007) reported that the pathology in the Dutch-belted dwarf rabbit resulting from intranasal *B. anthracis* administration was generally consistent with that identified for New Zealand white rabbits by Zaucha et al. (1998) and Yee et al. (2010) after aerosol challenge. An absence of sex-related differences in the development of antigenemia or

bacteremia after aerosol challenge was described in the New Zealand white rabbit (Yee et al., 2010). In a comparison of the pathology resulting from bronchoscopic versus aerosol challenge, Lovchik et al. (2012) reported that the "typical" histopathology lesions identified were consistent with those described by Zaucha et al. (1998) and Yee et al. (2010).

**Table 5-5. Studies Reporting Inhalation Anthrax Pathology by Rabbit Breed**

| Rabbit Breed | Study Citation (Strain) |
|---|---|
| New Zealand White Rabbit | Lovchik et al. (2012) (Ames)<br>Peterson et al. (2007)* (Ames)<br>U.S. Environmental Protection Agency (2011a) (Ames)<br>U.S. Environmental Protection Agency (2012b) (Ames)<br>Yee et al. (2010) (Ames)<br>Zaucha et al. (1998) (Ames) |
| Dutch-belted Rabbit | Peterson et al. (2007) (Ames) |

* Reports serial sacrifice pathology for up to 72 hours post-challenge, no end-stage pathology

A detailed summary table of end-stage pathology for the rabbit, nonhuman primate, and human is provided in Appendix C, Data Summary Table for End-stage Inhalation Anthrax Pathology of the Human, Nonhuman Primate, and Rabbit. Table 5-6 summarizes the published rabbit pathology relative to the key findings of Twenhafel (2010).

A review describing gross lesions identified in New Zealand white rabbits after aerosol challenge found blood from the nose, splenomegaly, adrenal gland hemorrhage, hemorrhage in the mandibular lymph node, and lung edema (Twenhafel, 2010). In the same review, reported histopathology included interstitial pneumonia, splenitis, and lymphadenitis with destruction of lymphoid tissues noted in the spleen; mediastinal, mandibular, and mesenteric lymph nodes; and Peyer patches in the small intestine and sacculus rotundus (Twenhafel, 2010).

**Table 5-6. Summary of Rabbit Pathology Relative to Twenhafel (2010) Key Findings**

| Pathology | Key Findings |
|---|---|
| Pneumonia | No reported pneumonia, but suppurative inflammation in lung (U.S. Environmental Protection Agency, 2011a, 2012b) |
| Splenic lymphoid depletion | Splenomegaly, with acute fibrinous splenitis (Zaucha et al., 1998; Yee et al., 2010; Lovchik et al., 2012); necrosis (Zaucha et al., 1998; Yee et al., 2010; Lovchik et al., 2012); hemorrhage (Zaucha et al., 1998; Lovchik et al., 2012); lesions (Lovchik et al., 2012)<br>Lymphocyte depletion (Lovchik et al., 2012) |
| Meningitis | Meningitis with suppurative inflammation (U.S. Environmental Protection Agency, 2011a); bacilli in meninges (Peterson et al., 2007)<br>Brain and/or meningeal lesions with no leukocytic infiltrate (Zaucha et al., 1998) |
| Hepatic hemorrhage or inflammation | Pathology not reported after inhalation exposure, one identification after intravenous dosing in Nordberg et al. (1961) |
| Gastrointestinal hemorrhage or inflammation | Hemorrhage, necrosis, and lymphoid depletion in appendix (U.S. Environmental Protection Agency, 2012b)<br>Edema, hemorrhage, and necrosis in cecum (U.S. Environmental Protection Agency, 2012b) |
| Urogenital hemorrhage or inflammation | Ovarian hemorrhage (Zaucha et al., 1998) |

Two high-dose studies reported the pathology for New Zealand white rabbits challenged with single inhaled doses of approximately $10^7$ inhaled CFU of *B. anthracis* Ames spores (Zaucha et al., 1998; Yee et al., 2010). Zaucha et al. (1998) is the classic anthrax pathology rabbit study. The most "prominent" pathology findings reported for the 22 New Zealand white rabbits were hemorrhage and edema in the spleen, lymph nodes, lungs, gastrointestinal tract, and adrenal glands (Zaucha et al., 1998). Lesions were typically hemorrhagic, necrotic, and exhibited minimal localized leukocytic response (Zaucha et al., 1998). Necrosis was reported in the mediastinal lymph nodes of 100% of the challenged rabbits, in the mandibular lymph nodes of 89% of the challenged rabbits, and in the mesenteric lymph nodes of 59% of the challenged rabbits (Zaucha et al., 1998). Zaucha et al. (1998) hypothesized that the increased incidence and severity of lesions in the submandibular node may have been associated with direct

oropharyngeal deposition or mucociliary clearance of previously deposited spores lower in the respiratory tract. Acute mediastinitis was infrequently identified, with lesions noted to be less severe than in the human (Zaucha et al., 1998). The spleen exhibited necrosis, inflammation, hemorrhage, and significant lesions (Zaucha et al., 1998). Pathology was also reported for a single high-dose control group that was identified as generally consistent with that identified by Zaucha et al. (1998) (U.S. Environmental Protection Agency, 2011a).

Data were also obtained from studies where limited pathology results were reported as part of a larger study design. Yee et al. (2010) conducted a high-dose exposure study and noted general pathological concordance with the Zaucha et al. (1998) results. Peterson et al. (2007) described the pathology identified during serial sacrifices of aerosol-challenged animals at 36 hours (n=3), 60 hours (n=3), and 72 hours (n=1). Histologic lesions, by order of prominence, were present in the mediastinal lymph node, lungs, spleen, and thymus (Peterson et al., 2007). Lesions exhibited edema/fibrin, necrosis/depletion, hemorrhage, and differing levels of leukocytic infiltration (Peterson et al., 2007). Lovchik et al. (2012) reported consistency in the pathological lesions in rabbits bronchoscopically challenged with lethal doses of *B. anthracis* with that previously described in the rabbit by Zaucha et al. (1998) and Yee et al. (2010) for aerosol challenges.

Pathology from low-dose *B. anthracis* aerosol challenge studies was also reported for the New Zealand white rabbit (U.S. Environmental Protection Agency, 2011a, 2012b). An acute single low-dose study with a challenge dose of approximately $10^2$ to $10^5$ inhaled CFU was conducted (U.S. Environmental Protection Agency, 2011a). A follow-on study using a similar design that incorporated multiple doses of approximately $10^2$ to $10^4$ inhaled CFU per day for 15 days was

then performed (U.S. Environmental Protection Agency, 2012b). The challenges took place Monday through Friday; there were no weekend challenges.

Gross and microscopic pathology reported for both studies was concordant with Zaucha et al. (1998), with gross lesions correlated with histological findings of hemorrhage, necrosis, edema, and suppurative inflammation (U.S. Environmental Protection Agency, 2012b).

One pathological finding of interest was the identification of granulomas/pyrogranulomas in one individual (Rabbit 38) of the U.S. Environmental Protection Agency (2012b) multiple-dose study. In the single-dose study, multinucleated giant cells were reported as tending toward formation of granulomas, though no actual granulomas were identified. One interpretation for the presence of the granuloma or pre-granulomas was that the removal of organic debris (e.g., food particles or hair and debris from vascular access ports) (Taketoh et al., 2009) was impaired by systemic macrophage dysfunction that can be associated with high levels of bacteremia and associated sepsis (U.S. Environmental Protection Agency, 2012b). However, the pathophysiological data for the rabbit did not include signs indicative of fulminant anthrax necessary to induce sepsis (i.e., showed elevation in telemetry parameters with abnormality only in the respiratory rate, single low positive bacteremia sample). There is one other pyrogranuloma reported in the literature relating to inhalation anthrax and it was described in a vaccinated animal that survived inhalation anthrax (U.S. Food and Drug Administration, 2002). Interestingly, the pulmonary lesions reported by Gleiser et al. (1968) were consistent with the characteristics of an early granuloma and were identified in animals thought to be innately resistant to inhalation anthrax infection. In this context, the granuloma may simply be a non-specific indicator of a vigorous host response to a bacterial challenge.

However, the use of a venous access port in the U.S. Environmental Protection Agency (2012b) and U.S. Environmental Protection Agency (2011a) studies may provide an additional confounding factor to the interpretation of the granuloma in the multiple-dose study and the early stage granulomas described in the single-dose study. Granulomas were reportedly associated with the use of venous access ports in studies of rats (Taketoh et al., 2009); however, the study did not have a control group for statistical comparison. Accordingly, further study using a fully virulent low-dose *B. anthracis* spore strain without the inclusion of confounding factors (e.g., venous access port, vaccination status) will be necessary before the granuloma can be attributed to its proper cause.

#### *5.4.1.4 Nonhuman Primate*

Published pathology data from inhalation exposure to *B. anthracis* were identified by nonhuman primate species, *B. anthracis* strains, and sources (Table 5-7). With the exception of Berdjis et al. (1962), who used a serial sacrifice study design, the identified reports describe end-stage pathology from inhalation of *B. anthracis* aerosols. Reported pathology outcomes from studies or treatment groups that included medical treatments (e.g., anti-toxins, antibiotics) or other treatment protocols were not included in the summary pathology table in Appendix C. An example of data from a treatment protocol would include the pathology reported from penicillin-treated monkeys in Gochenour et al. (1962).

**Table 5-7. Studies Reporting Inhalation Anthrax Pathology by Nonhuman Primate Species and Strain**

| Nonhuman Primate (Species) | Study Citation (Strain) |
|---|---|
| Chimpanzee (*Pan troglodytes*) | Albrink and Goodlow (1959) (Vollum rB) |
| Rhesus Monkey (*Macaca mulatta*) | Berdjis et al. (1962)[*†] (Vollum-189)<br>Gochenour et al. (1962)[†] (Vollum-189)<br>Friedlander et al. (1993) (Vollum 1B strain)<br>Fritz et al. (1995) (Vollum 1B strain, Ames strain)<br>Gleiser et al. (1963)[†] (Vollum-189) |
| Cynomolgus Macaque (*Macaca fascicularis*) | Brachman et al. (1966) (Goat Hair Mill Aerosol, Unknown Strain[s])[‡]<br>Dalldorf et al. (1971) (Goat Hair Mill Aerosol, Unknown Strain[s])[‡]<br>Henning et al. (2012) (Ames)<br>Vasconcelos et al. (2003) (Ames) |
| African Green Monkey (*Chlorocebus aethiops*) | Twenhafel et al. (2007) (Ames) |
| Common Marmoset (*Callithrix jacchus*) | Lever et al. (2008) (Ames) |

[*] Serial sacrifice pathology reported Days 1 through 6, no end-stage pathology reported
[†] Originating technical report for papers is Gochenour (1961)
[‡] Papers report pathology from same study of nonhuman primate exposure to goat hair mill aerosol in South Carolina, originating technical report for papers is Dalldorf and Kaufman (1966)

In the 1950s through the 1960s, anthrax studies by the U.S. Army laboratories (predecessors of the current U.S. Army Medical Research Institute of Infectious Diseases [USAMRIID] laboratories) typically used the cynomolgus monkey (*Macaca fascicularis*) (U.S. Food and Drug Administration, 2002). There was one published study reporting pathology after exposure to goat hair mill aerosols of unknown strain(s) that used the cynomolgus monkey (Brachman et al., 1966; Dalldorf et al., 1971). The rhesus monkey (*Macaca mulatta*) was also used in the 1960s in controlled exposure laboratory studies with the Vollum-189 strain (Berdjis et al., 1962; Gochenour et al., 1962; Gleiser et al., 1963). During the resurgence period of anthrax research from 1990 through 2000, the rhesus monkey was the most commonly used species until the rhesus monkey became increasingly expensive and difficult to access (Twenhafel et al., 2007). Since that time, additional nonhuman primate species were evaluated including the African green monkey (*Chlorocebus aethiops*) and common marmoset (*Callithrix jacchus*) (Lever et al., 2008;

Twenhafel, 2010), while the cynomolgus monkey also experienced a resurgence in use (e.g., Vasconcelos et al. (2003); Henning et al. (2012)). All studies conducted in 2003 or later with these nonhuman primate species or the cynomolgus monkey used the Ames strain of *B. anthracis*.

The assessment of nonhuman primate pathology of the lung is complicated by lung mite (*Pneumonyssus simicola*) parasitism in most rhesus monkeys used for testing during the 1960s. Studies that reported lung mites in challenged monkeys include Berdjis et al. (1962) and Gleiser et al. (1963). At the time of challenge, the mites contributed to lung lesions, which became sites of superinfection with *B. anthracis* (Fritz et al., 1995). Therefore, comparisons of lung pathology between rhesus monkeys and other nonhuman primates may be difficult based on the availability of one study (Fritz et al., 1995) that reported pathology of rhesus monkeys without mite infection. Noting the similarity in pathology between the rhesus monkey and the fulminant necrotic and hemorrhagic pneumonia described by Abramova et al. (1993) during the Sverdlovsk outbreak, Fritz et al. (1995) hypothesized that the described nonhuman primate pathology resulting from infection under conditions of pre-existing lung lesions may mimic that of the human with pulmonary compromise and have utility in that context. Hemorrhagic pneumonia has been reported in the nonhuman primate (Albrink and Goodlow, 1959; Fritz et al., 1995; Lever et al., 2008), as well as hemorrhage of varying severity, absent pneumonia, in the lung (Gleiser et al., 1963; Vasconcelos et al., 2003; Twenhafel et al., 2007).

This assessment examined nonhuman primate species as one group for the evaluation of the pathology data. However, species-specific data that indicate a lack of concordance with expected human pathology or that of other nonhuman primate species were also highlighted.

The nonhuman primate species exhibited generally consistent clinical and pathological outcomes after exposure to lethal inhalation doses of *B. anthracis* (Twenhafel, 2010). Though few low-dose studies have been conducted, one study reported similar pathology across a range of low-dose (200 to $2 \times 10^4$ CFU) and high-dose ($2 \times 10^4$ CFU to $1 \times 10^7$ CFU) challenges for the African green monkey (Twenhafel et al., 2007). Similarities in response were identified for age (e.g., adult versus juvenile) and sex (e.g., male versus female) in the dose range of $2 \times 10^4$ to $5 \times 10^{10}$ CFU (Twenhafel, 2010).

A detailed summary table of end-stage pathology for the rabbit, nonhuman primate, and human is provided in Appendix C, Data Summary Table for End-stage Inhalation Anthrax Pathology of the Human, Nonhuman Primate, and Rabbit. Table 5-8 summarizes the published nonhuman primate pathology relative to the key findings of Twenhafel (2010).

**Table 5-8. Summary of Nonhuman Primate Pathology Relative to Twenhafel (2010) Key Findings**

| Pathology | Key Findings |
|---|---|
| Pneumonia | Pleural effusions (Albrink and Goodlow, 1959; Dalldorf et al., 1971; Vasconcelos et al., 2003; Twenhafel et al., 2007); though not reported in rhesus macaque (Twenhafel et al., 2007)<br>Hemorrhagic pneumonia (Albrink and Goodlow, 1959; Lever et al., 2008); low incidence of pneumonia (2/13) but presence of hemorrhages (Fritz et al., 1995);<br>Alveoli filled with edema often mixed with fibrin, hemorrhage, macrophages, and neutrophils (Twenhafel et al., 2007); acute suppurative inflammation (4/14) (Vasconcelos et al., 2003) |
| Splenic lymphoid depletion | Splenomegaly (Albrink and Goodlow, 1959; Middleton and Standen, 1961; Gleiser et al., 1963; Lever et al., 2008); low incidence identified from one study (3/13) (Fritz et al., 1995) or described as mild (Twenhafel et al., 2007);<br>Histiocytosis (Fritz et al., 1995); hemorrhage in splenic marginal zone (Fritz et al., 1995); necrosis of lymph follicles and/or necrosis of red and white pulp with hemorrhage (21/23) (Dalldorf et al., 1971) |
| Meningitis | Meningitis (9/21) (Dalldorf et al., 1971); suppurative meningitis (10/13) (Fritz et al., 1995) |
| Hepatic hemorrhage or inflammation | Liver congestion (Albrink and Goodlow, 1959; Lever et al., 2008)<br>Diffuse hepatic congestion, fibrin deposition, and expanded germinal center (Lever et al., 2008); lymphocytic depletion (Fritz et al., 1995)<br>Acute inflammation/leukocytosis (13/14) and acute necrosis (5/14) in liver (Vasconcelos et al., 2003); sinusoidal leukocytosis (9/10), necrosis (6/10) and acute inflammation (4/10) (Henning et al., 2012) |
| Gastrointestinal hemorrhage or inflammation | Hemorrhage of various severity in the small and large intestine serosa and esophagus mucosa (Fritz et al., 1995); or stomach mucosa and/or submucosal tissues (Fritz et al., 1995; Vasconcelos et al., 2003);<br>Acute colitis with necrotizing vasculitis (1/13) (Fritz et al., 1995), necrosis of villus tips in ileum or jejunum (9/14) (Vasconcelos et al., 2003), or with stomach inflammation (2/14) or ulceration (1/14) (Vasconcelos et al., 2003)<br>Edema, congestion, and hemorrhage in the gastrointestinal tract (Twenhafel et al., 2007) |
| Urogenital hemorrhage or inflammation | Periovarian or peritesticular congestion and/or hemorrhages (Twenhafel et al., 2007)<br>Ovarian hemorrhage and necrosis (1/14) (Vasconcelos et al., 2003) |

Gross pathology in the nonhuman primate from inhalation anthrax includes edema, hemorrhage, and varying levels of necrosis in the lungs, lymph nodes, and spleen (Fritz et al., 1995; Leffel and Pitt, 2006). Generally mild levels of leukocytic infiltration in the tissues were also reported, with mild levels typically indicating a highly susceptible host (Fritz et al., 1995). Gross and histological changes in lymphoid tissues are a key pathological outcome in fulminant inhalation anthrax. Lymphoid tissues exhibiting consistent pathology across nonhuman primate species are the mediastinal lymph nodes (specifically, the tracheobronchial lymph node) and the spleen, with

hallmark lesions, including the presence of necrohemorrhagic lymphadenitis and generalized lymphoid depletion (Twenhafel, 2010). However, the marmoset animal model as described by Lever et al. (2008) exhibits a relatively low incidence of "classic lesions" in the lymph nodes (1 of 6 marmoset with enlarged and hemorrhagic tracheobronchial lymph nodes) and an absence of meningitis (Twenhafel, 2010).

Splenomegaly (i.e., enlargement of the spleen) is a gross pathology outcome commonly identified in the nonhuman primate (Albrink and Goodlow, 1959; Gleiser et al., 1963; Lever et al., 2008). Splenomegaly was also identified in the pathology reported when fulminant anthrax developed from intracutaneous dosing of rhesus monkeys with *B. anthracis* (Vollum 1B) spores (Middleton and Standen, 1961). Gross splenic changes have been described as enlargement with rounded edges, dark red color, and an appearance to similar to "blackberry jam" (Twenhafel, 2010).

Though there is some variation in the frequency with which splenomegaly was reported across nonhuman primate species, there may not be a meaningful splenic pathology difference across the nonhuman primate species when considering the general consistency in reported histopathological data. Fritz et al. (1995) reported a lower incidence of splenomegaly (3 of 13) in the rhesus monkey relative to that reported as "frequently seen" by Gleiser et al. (1963). However, Fritz et al. (1995) also reported characteristic microscopic lymphoid changes (e.g., splenic histiocytosis [12/13], lymphoid depletion [13/13], and hemorrhage in spleen marginal zone [7/13]) present in the majority of monkeys without regard to the presence of gross splenomegaly. Gleiser et al. (1963) identified histopathological changes in the spleen as similar to the lymph node (i.e., necrosis, hemorrhage, "depopulated" state), though a quantitative

description was not provided. Vasconcelos et al. (2003) reported mild splenomegaly (enlarged 1.5- to 2-fold) in 13 of 14 cynomolgus monkeys challenged with high doses of *B. anthracis*. However, the reported histopathological data provided did not extend beyond a general description of lymphocytolysis and general congruence in the pathology with the intrathoracic lymph nodes. Dalldorf et al. (1971) reported splenic changes of necrosis in the red and white pulp of the spleen with hemorrhage in 14 of 23 cynomolgus monkeys, but did not identify gross pathology relating to general spleen enlargement. Lever et al. (2008) noted gross pathology indicative of splenomegaly in 2 of 6 marmoset, yet described microscopic findings in 6 of 6 marmoset of lymphoid depletion, necrosis, fibrin, and hemorrhage, as well as acute inflammation.

There were also species-specific differences in the reporting of pleural effusions across the nonhuman primate species. Pleural effusions were identified in the chimpanzee, cynomolgus macaque, and African green monkey (Albrink and Goodlow, 1959; Dalldorf et al., 1971; Vasconcelos et al., 2003; Twenhafel et al., 2007). However, pleural effusions were not reported in the rhesus monkey (Gleiser et al., 1963; Twenhafel et al., 2007) and marmoset (Lever et al., 2008). The relevance of this difference is not currently known.

Cardiac tissue lesions or the associated myocardium were identified more frequently in the cynomolgus monkey than in the rhesus monkey when Vasconcelos et al. (2003) compared their cynomolgus study results with those for the rhesus monkey reported by Fritz et al. (1995) and Gleiser et al. (1963). These lesions have not been reported in the human (Vasconcelos et al., 2003), though pericardial effusions were identified in 2001 anthrax letter event cases (Jernigan et al., 2001). The presence of differing pathology may be an area of true difference in tissue or

organ-specific susceptibility among the nonhuman primate species (Vasconcelos et al., 2003) and the human.

#### *5.4.1.5 Results of Concordance Analysis for Similarity between Rabbit, Nonhuman Primate, and Human Pathology*

The rabbit and nonhuman primate exhibit many commonalities in the type of lesions and tissues associated with inhalation anthrax pathology in the human. For example, Zaucha et al. (1998) identified that the end-stage pathology of anthrax in the rabbit as being "remarkably similar" to the human. Vasconcelos et al. (2003) reported that the "pattern of inhalation anthrax lesions" was similar among the cynomolgus monkey, rhesus monkey, and the human.

The principal anthrax lesions of edema, hemorrhage, and necrosis are present in a variety of common tissues in the rabbit, nonhuman primate, and human. However, this constellation of pathology is generally consistent with descriptions of animal models susceptible to fulminant inhalation anthrax infection (Gleiser et al., 1963) and is not unique to the rabbit and nonhuman primate animal models. Lesion differences among susceptible animals are manifested by differing levels of inflammation and infiltration of leukocytic elements into existing lesions (U.S. Food and Drug Administration, 2002), whereby less susceptible animals exhibit greater inflammation and leukocytic infiltration than more susceptible animals, which rapidly succumb to illness.

The lymphoid tissues are the primary target for anthrax lesion development in susceptible animals (U.S. Food and Drug Administration, 2002), specifically the lymph nodes draining the lungs, pharynx, or gastrointestinal tract, the spleen, and lymphoid tissues associated with the gastrointestinal tract (e.g., Peyer's patches, sacculus rotundus, appendix). The most commonly affected lymph nodes are the thoracic lymph nodes, including the mediastinal lymph nodes

(human, rabbit, and nonhuman primate), the submandibular (rabbit), and the cervical lymph nodes (nonhuman primate). Anthrax pathology of the affected lymph nodes includes necrosis, hemorrhage, and depletion and/or destruction of lymphocytes (lymphocytolysis), with these characteristics identified in the overall pathology of the rabbit, nonhuman primate, and human (Dalldorf et al., 1971; Abramova et al., 1993; Zaucha et al., 1998; Guarner et al., 2003). Movement to and through the lymph node or other more direct routes to the bloodstream allow for systemic accessibility of the pathogen. This allows for infection and associated pathology to be exhibited in distant nonlymphoid tissues in the rabbit and nonhuman primate, including adrenal glands, ovarian or testicular tissues, and myocardial tissue (Gleiser et al., 1963; Fritz et al., 1995; Zaucha et al., 1998; Vasconcelos et al., 2003; Twenhafel et al., 2007).

There are two areas of difference between the anthrax presentation in the human and the nonhuman primate. The first is the presentation of splenomegaly or splenic histopathology. As described in Section 5.4.1.4, variation among the nonhuman primates in the presence or absence of splenomegaly has been reported. There are also conflicting reports regarding the presence or absence of splenomegaly in the human. Based on reports from Sverdlovsk from Abramova et al. (1993) and Grinberg et al. (2001), Vasconcelos et al. (2003) determined that humans do not typically exhibit splenomegaly. Fritz et al. (1995) also identified a limited occurrence of splenomegaly in the human. However, splenomegaly was described in earlier human inhalation anthrax case reports by Albrink et al. (1960) and Suffin et al. (1978). Histopathology conducted on four of the 2001 anthrax letter event cases described splenic histopathology to include congestion (3 of 4 individuals) and necrosis (1 of 4 individuals) (Guarner et al., 2003). Available gross pathology is very limited from the Sverdlovsk outbreak and frequency of splenomegaly is unknown. However, histopathology on stored tissues from Sverdlovsk reported splenic

pathology to include lymphocytolysis, splenic congestion, and presence of neutrophils (Grinberg et al., 2001), which are not inconsistent with presentation of splenomegaly. Alternately, *B. anthracis* strain-specific effects may be contributing to apparent differences in the presentation of splenomegaly in the human, as the historic human data were reflective of exposure to mill aerosol strains, whereas later data reflected exposure to the Ames strain in the 2001 anthrax letter event or the mixture of strains in Sverdlovsk.

Meningitis has been reported as a second differentiator in anthrax pathology between the rabbit and the nonhuman primate animal model due to identified absence of meningitis in the rabbit (Twenhafel, 2010). Zaucha et al. (1998) described a low incidence of hemorrhage associated with *B. anthracis* bacilli in the rabbit brain or meninges and noted the lack of leukocytic infiltration in these lesions. Since that report, one study reported meningitis with suppurative inflammation in a high-dose (c. $10^6$ CFU) control group rabbit (1 of 25 rabbits) (U.S. Environmental Protection Agency, 2011a). The absence of "full blown" meningitis is hypothesized to result from the rapidity of disease progression in the rabbit, which limits the opportunity for inflammation and leukocytic response (Zaucha et al., 1998; Leffel and Pitt, 2006). Meningitis lesions in the rabbit were typically noninflammatory when compared to the suppurative, inflammatory lesions described in the nonhuman primate and human (U.S. Food and Drug Administration, 2002). Interestingly, the rabbit that exhibited meningitis in the U.S. Environmental Protection Agency (2011a) study had a time-to-death of four days, which was at the high end of the range for time-to-death values (i.e., 2 to 3 days, mean of 2.4 days) reported by Zaucha et al. (1998). Alternately, Zaucha et al. (1998) hypothesized that strain differences could be contributing to variation in the incidence of meningitis in the rabbit versus nonhuman primate as earlier nonhuman primate studies used Vollum strains as reported in Fritz et al. (1995) and

Gleiser et al. (1963). However, studies conducted since that time with the Ames strain in nonhuman primate species have reported meningitis in the African green monkey with a similar incidence as prior nonhuman primate studies (Twenhafel et al., 2007), as well as suppurative meningitis with hemorrhage in the cynomolgus monkey (Vasconcelos et al., 2003).

Time-dependency in anthrax pathology also contributes to differences in lesion tissue location and presentation among the rabbit, nonhuman primate, and human (U.S. Food and Drug Administration, 2002; Leffel and Pitt, 2006). However, this poses a challenge for the systematic evaluation of anthrax pathology of animal models and the human because of recognized differences in the time-to-death values typically associated with each group. As described earlier, the rabbit typically exhibits the shortest time-to-death values as evidenced by the commonly cited value of 2 to 3 days of Zaucha et al. (1998). The nonhuman primate exhibits a wider range of values for time-to-death, with 3 to 8 days reported in Fritz et al. (1995). The human with a slightly longer time-to-death values as evidenced by the reported range of value of 5 to 8 days in Jernigan et al. (2001). As identified earlier, a complicating factor for interpretation of human pathology data is the unknown contribution that magnitude of dose or initiation of medical treatment may play in resulting time-to-death and/or pathology.

The relationship between survival time and lesion development was first recognized over 50 years ago in the nonhuman primate (Albrink et al., 1960; Berdjis et al., 1962). When evaluating a possible connection between the use of antibiotics and the presence of meningitis in study animals, Albrink et al. (1960) hypothesized that antibiotics may reduce damage in non-central nervous system tissues and prolong life, such that individual bacteria that travel to the meninges have sufficient time to multiply and develop into meningitis. Time-dependent development of

lesions was also described in the nonhuman primate without medical or antibiotic treatment post-challenge. Nonhuman primates with an extended survival time post-challenge relative to shorter-lived animals in the same study were more commonly found to exhibit disease progression in specific tissues (e.g., adrenal inflammation and necrosis, hepatic necrotic lesions, meningeal hemorrhage, cerebral vasculitis) (Vasconcelos et al., 2003). As would be expected, the severity of lesions may also be affected by the length of survival time for disease progression. Lesions and associated inflammation in the mediastinal area (mediastinitis) were described in the nonhuman primate and the human, though a lesser severity of mediastinitis was noted for the rabbit relative to the human (Zaucha et al., 1998). Zaucha et al. (1998) hypothesized that a longer disease progression would provide necessary time for expansion of the infection from the lymph nodes to the surrounding mediastinal tissues.

Overall, the human exhibits less susceptibility than the rabbit and nonhuman primate, with the result being a longer period of disease progression (i.e., longer time-to-death after challenge) (U.S. Food and Drug Administration, 2002; Leffel and Pitt, 2006). The increased time of length of disease allows for development of more inflammatory elements of the pathology (U.S. Food and Drug Administration, 2002). As an example, the rabbit typically exhibits less severe mediastinal lesions, reduced incidence of pneumonia, and a lack of leukocyte invasion in the meninges and brain than species less susceptible to anthrax (Leffel and Pitt, 2006) and generally has the shortest time-to-death after challenge.

The purpose of the concordance review was to evaluate available pathology data for the nonhuman primate and to select appropriate dose-response data for lethality to extrapolate to the human. However, this review should not be directly applied to other endpoints (e.g., infection)

without additional analysis. As noted previously, the key histopathology findings in the human identified by Twenhafel (2010) were used as a starting point. These findings included hepatic, gastrointestinal, and urogenital hemorrhage and inflammation; pneumonia; splenic lymphoid depletion; and meningitis. In the evaluation of animal models for the testing of medical countermeasures, a close replication of the human disease state is desired to ensure the treatment being assessed is protective of a full range of adverse anthrax illness outcomes in addition to lethality (e.g., meningitis, organ, or tissue damage). In contrast, animal model selection for dose-response analysis focuses identification on key events associated with disease progression relative to the identified endpoint of interest (i.e., lethality for this assessment).

Uncertainty in the key events process for development of inhalation anthrax complicates the use of a disease progression approach from initiation of infection through end-stage illness. To reduce reliance on a strict disease progression interpretation, the animal model selection assessment evaluated general concordance in tissue location and pathology associated with inhalation anthrax in the animal models and the human. As the data were analyzed, time-dependency was considered to play a potential role in the relative development of pathology across hosts and was incorporated as an element of the final assessment. While the lack of serial sacrifice data for the animal models limits the ability to draw conclusions for the precise timing and relative sequence of events of inhalation anthrax pathology, the identification of differences in the appearance of pathology between animals dying earlier and later may assist in determining those elements associated with a longer duration of infection (e.g., meningitis).

Table 5-9 shows general concordance in the anthrax pathology between the rabbit and nonhuman primate with regard to presence or absence of lesions and inflammation in target tissues

associated with anthrax pathology in the human. The pathological lesions identified in the human for which the rabbit animal model differs with the nonhuman primate have a time-dependent element in their presentation, with the rabbit differing from the nonhuman primate either in the severity as defined by level of inflammation or leukocytic infiltration or general incidence (Table 5-9).

There were no identified differences between the rabbit and the nonhuman primate animal models for elements of anthrax pathology that do not have a time-dependency regarding incidence or severity in presentation. However, those elements of pathology that showed differences between the rabbit and nonhuman primate animal model preliminarily indicate that that time-dependency may be related to their pathological presentation. The results of the concordance assessment of pathology support the use of the rabbit and nonhuman primate animal models for development of dose-response data.

**Table 5-9. Key Human Histopathological Findings Relative to Time-Dependent Pathology in the Rabbit and Nonhuman Primate after Single-Dose Exposure**

| Pathology | Rabbit | Nonhuman Primate | Evidence for Time-Dependency in Severity or Incidence |
|---|---|---|---|
| Pneumonia | Yes - Zaucha et al. (1998) with noted lower incidence and severity than NHP and human | Yes - Albrink and Goodlow (1959), Fritz et al. (1995) | Yes - Progression to pneumonia is associated with inflammatory process, lower incidence, and lesser severity reported in rabbit |
| Splenic lymphoid depletion | Yes - Zaucha et al. (1998), Lovchik et al. (2012) | Yes - Fritz et al. (1995) | No - Spleen is an early disease target in inhalation anthrax |
| Meningitis | Yes – U.S. Environmental Protection Agency (2011a) in 1/25 rabbits, lower incidence than NHP and human | Yes - Fritz et al. (1995), Gleiser et al. (1963), Lever et al. (2008), Twenhafel et al. (2007), Vasconcelos et al. (2003) | Yes - Hypothesized as time-dependent in Zaucha et al. (1998), not identified in any of NHP serial sacrifices reported in Berdjis et al. (1962) |
| Hepatic hemorrhage or inflammation | No - Not reported after inhalation exposure pathology, one report after intravenous dosing in Nordberg et al. (1961) | Yes - Vasconcelos et al. (2003), Henning et al. (2012), Lever et al. (2008) | Yes – Reported as time-dependent in NHP by Vasconcelos et al. (2003) |
| Gastrointestinal hemorrhage or inflammation | Yes - Zaucha et al. (1998), U.S. Environmental Protection Agency (2012b) | Yes - Fritz et al. (1995); Vasconcelos et al. (2003) | No - Hemorrhagic spread to gastrointestinal tract seems to occur early in the disease process |
| Urogenital hemorrhage or inflammation | Yes - Zaucha et al. (1998) but noted as rare | Yes - Twenhafel et al. (2007), Vasconcelos et al. (2003) | Unknown - Evidence or reports for time-dependency are lacking |

NHP - nonhuman primate

### *5.4.2 Identification of Microbial Dose-Response Data*

A literature search was conducted for open source rabbit, nonhuman primate, and human dose-response data, including dose-response data sets, modeled $LD_{50}$ values, or reported parameter values (e.g., probit slope values). Given the scarcity of available human data, dose-response data were more broadly defined for the human to include epidemiological and qualitative dose-response data. Dose-response studies that reported either acute (i.e., less than 24-hour or single-dose) or multiple-dose exposures were identified. Dose-response studies that reported infection and/or lethal endpoints were also collected in the literature search. The search evaluated published literature from January 1950 through January 2014. However, documents of historical

relevance (i.e., pre-1950) that provided background or context for selected secondary data were also identified as part of the literature search.

A dose-response relationship describes "the relationship between a quantified exposure (dose) and the proportion of subjects demonstrating specific biologically significant changes in incidence and/or in degree of change (response)" (U.S. Environmental Protection Agency, 2011c). To model the dose-response relationship, response data must be reported for each individual or dose group. For the inhalation route of exposure, the exposure dose must have been reported as an inhaled dose or a deposited inhaled dose, or sufficient data was provided to derive an inhaled dose. For data that did not report an inhaled or deposited dose metric, an allometric equation could be used to calculate an exposure dose if environmental concentration with individual or group animal weight data were available. Oral dose-response data were collected without regard to dose metric or animal model due to the recognized scarcity of published data.

***Summary of Findings for Identification of Microbial Dose-Response Data***

- Few inhalation challenge studies were identified as Key Studies for the rabbit and nonhuman primate; there were no Key Studies or Supporting Studies identified for the human.
- There were very few single or multiple dose challenge studies using low doses.
- Dose-response data are available for the rabbit and nonhuman primate that may be suitable for development of a human dose-response relationship.
- The uncertainty associated with the use of these data may be high.
- Depending on the level of acceptable uncertainty in the analysis outputs, there may be limitations on how these data may be used in decision-making.

For the human, acceptable dose-response data were more broadly defined to include additional data types. Published epidemiological data, modeled values, and parameters developed from animal and/or human inputs or fitted parameter values, and data derived from expert elicitation processes were all targeted by the literature search. If the animal data were identified as

appropriate to apply to the human, they were evaluated for use as human dose-response data. Acceptable epidemiological data identified known exposure characteristics associated with human outbreaks of anthrax illness. Qualitative data describing the relative susceptibility of the human to anthrax infection were also collected as they were identified.

After identification by the literature search, all dose-response data sets and modeled dose-response values were evaluated using general quality criteria identified in the U.S. Environmental Protection Agency (2003) data quality guidance. Data sets that met the general quality criteria were then further evaluated using the project-specific criteria described in the next section.

#### *5.4.2.1 Categorization of Dose-Response Data*

Project-specific criteria in the form of assessment questions and defined rules for data handling were used to categorize the identified dose-response data as Key Studies, Supporting Studies, and Additional Data. The process described in U.S. Environmental Protection Agency (2012c) was the starting point for the development of assessment questions and the evaluation process. U.S. Environmental Protection Agency (2012c) evaluated published *B. anthracis* dose-response data relative to its utility for developing dose-response relationships, especially in the low-dose region. The assessment questions presented in U.S. Environmental Protection Agency (2012c) addressed: (1) the availability of raw dose-response data (i.e., original data set), (2) the availability of particle size distribution data, including reported use of single spore particles in the challenge, (3) the presence of dose groups with less than 50% lethality rate or an overall lethality rate of less than 50% when individual doses were reported, (4) the use of real-time

methods to derive inhalation rates, (5) sufficient animal numbers in individual dose groups ($n \geq 5$) or total number tested for individual dose measurements ($n \geq 12$).

The purpose of the report is to generate a comprehensive picture of available dose-response data and models for *B. anthracis*. As a result, dose-response data were sought even if an individual data set might be insufficient to derive a dose-response relationship. To incorporate this change, modifications were made to the process identified in U.S. Environmental Protection Agency (2012c): (1) dose-response data (e.g., model parameter values and outputs, epidemiological data for the human) were defined more broadly, (2) the quantitative scoring process was not used, and (3) different output assessment categories were employed. Given the potential for inhalation rates derived from allometric data to significantly under- or overestimate the actual dose (U.S. Environmental Protection Agency, 2012c), one additional modification was made to the process: the use of real-time methods (e.g., plethysmography) was a Key Study design requirement.

Using knowledge gained from the implementation of the assessment questions in U.S. Environmental Protection Agency (2012c), default rules were developed to place data in the Additional Data category. Dose-response data that consisted solely of high-dose challenge of a control group for a medical countermeasure study were automatically categorized as Additional Data. The dose levels used in the high-dose challenges are dose typically 100 to 200 times the Zaucha et al. (1998) $LD_{50}$ value. If the original dose-response data set was not available, a all modeled values (e.g., probit slope values, fitted parameter values, $LD_{50}$) were placed in Additional Data. If the original data set was identified, modeled values were reported alongside their originating data set in the summary tables. All identified epidemiological data for the human were categorized as Additional Data. Dose-response data were not quantitatively scored

as they were in U.S. Environmental Protection Agency (2012c), but were categorized based on the sufficiency of the published data for modeling dose-response relationships for low-dose exposures or for informing dose-response relationships of higher quality data.

Key Studies were defined as representative of the highest quality dose-response studies that met criteria for selection during the literature search. Quality was defined by the availability of study data, study design with real-time inhalation rate and particle size measurement, data elements including evaluation of low dose and associated response levels (i.e., between 1% and 50% lethality), and sufficient number of animal and dose group numbers to mathematically model a dose-response relationship. Supporting Studies had identifiable limitations in assessment quality indicators relative to Key Studies, yet were found to have potential in bounding potential dose-response relationship(s) as described by Key Studies. Additional Data were defined by the lack of data critical to assessing dose-response relationships (e.g., original dose and response data set) or study design elements that limit utility for development of low-dose dose-response relationships. As a result, their utility in dose-response analysis may be limited to providing corroborative support for higher quality data.

Key Studies are presented in summary text and tables in the following sections, with strengths and weaknesses relative to the use of these data in dose-response analysis also identified. Modeled dose-response values that are re-analyses of previously published primary data are associated with the primary data set, if the data set was identified. Highly relevant or often cited Additional Data were also reported in conjunction with Key Studies to provide additional context for the presented data. Summary of dose-response data that were categorized as Supporting Data

or Additional Data are provided in Appendices D and E for the rabbit and nonhuman primate, respectively.

#### *5.4.2.2 Results from Literature Search of* **Bacillus anthracis** *Dose-Response Data*

The development of a human-relevant dose-response relationship for *B. anthracis* is challenged by a lack of suitable data sets for dose-response analysis (U.S. Department of Homeland Security and U.S. Environmental Protection Agency, 2009). One area of particular concern is the limited number of low-dose exposure studies for single- and multiple-dose challenges. The majority of animal dose-response data identified through the literature search originated from single-dose studies at very high doses, sometimes as high as 200 times the identified $LD_{50}$ value. Single high-dose studies have limited value for the assessment of repeated low-dose exposure (U.S. Environmental Protection Agency, 2012c).

Few studies that reported dose-response data were designed to derive data for dose-response analysis. Reported study purposes for recent data sets included evaluation of the pathology or pathophysiology of infection, or assessment of the efficacy of medical countermeasures. These studies were often conducted using a single high-dose challenge to ensure a high likelihood of systemic anthrax infection in the challenge animals. Historical data were often developed to report an $LD_{50}$ value for use in military applications or early anthrax research and little attention was paid to representation of low doses.

Few studies were identified as Key Studies for the rabbit and nonhuman primate; there were no Key Studies or Supporting Studies identified for the human. The two Key Studies for the rabbit were the single-dose U.S. Environmental Protection Agency (2011a) study and the multiple-dose U.S. Environmental Protection Agency (2012b) study. No studies were categorized as

Supporting Studies. For the nonhuman primate, one single-dose Key Study (Lever et al., 2008) and one single-dose Supporting Study (Druett et al., 1953) were identified.

#### *5.4.2.3 Human Inhalation Data*

All identified human dose-response data for the human were categorized as Additional Data. Human dose-response data included epidemiological data, modeled data from the nonhuman primate that were identified for human application (with or without the addition of human relevant values), and specific values or ranges elicited from experts for modeled values of interest (e.g., $LD_{50}$). Dose-response data were primarily reported using the lethality endpoint. However, the ID and LD were identified as equivalent by expert elicitation (Rickmeier et al., 2001), in the presentation of a range of median infectious dose ($ID_{50}$) values, (U.S. Army Medical Research Institute of Infectious Diseases, 2011), or incorporated in modeling (Webb and Blaser, 2002; Wein et al., 2003; Craft et al., 2005; Toth et al., 2013).

No open source studies reported human dosing with *B. anthracis*. The lack of available human dose-response data has been previously reported (Taft and Hines, 2012; Toth et al., 2013). Environmental exposure or dose data were not reported with human outbreak data (e.g., Sverdlovsk, 2001 anthrax letter event). However, there was one study (Dahlgren et al., 1960), with subsequent reanalysis by Cohen and Whalen (2007), that reported two days of air measurements to which a mixture of vaccinated and unvaccinated mill workers were exposed without incidence of anthrax illness.

Primary citations of human dose-response data identified through the literature review are presented in Table 5-10. Repeated secondary citations of the same human dose-response data were not included here. For example, there were numerous citations of the Inglesby et al. (2002)

human $LD_{50}$ range. Qualitative assessments regarding relative susceptibility that were identified through the literature search are also summarized.

When reviewing Table 5-10, it is important to recognize that the $LD_{50}$ values come from a variety of data sources with varying levels of data quality and reproducibility (e.g., expert elicitation, combinations of human epidemiological and animal model challenge data), as well as variability in fundamental study design elements (e.g., animal model, strain). The literature search identified a number of incorrect citations of previously published data (i.e., secondary data). These unique values are included in Table 5-10 and identified as incorrect, but are not considered further in the report.

**Table 5-10. Additional Data for the Human**

| Published Study<br>Value or Associated Model (*B. anthracis* Strain) | Basis for Value or Model Specification |
|---|---|
| Cohen and Whalen (2007)<br>(Originating data set: Mill aerosol, unknown strain[s])<br>600 inhaled respirable spores over an 8 hour day is the "lower boundary of the maximum noninfectious dose for inhalation anthrax" in a healthy individual "who is not egregiously predisposed to anthrax or lung disease, or is immunocompromised" | Data reported in Dahlgren et al. (1960), Brachman et al. (1966), and assumptions regarding the human exposure rate were used to derive the 600 inhaled respirable spores value |
| Craft et al. (2005)<br>Age-dependent linear dose-response model to predict the probability of infection for a given age<br>(unknown strain)<br>$P(s,a) = \min\left(1, \left(\frac{s}{c_1 - c_2 a}\right)\right)$<br>$s$ = dose, $a$ = age<br>$c_1 = 38{,}000$<br>$c_2 = 450$<br>$A_{max} = 80$<br>$Age\ distribution\ U[0,A] and\ pdf\ f(a) = A^{-1}$ | Craft et al. (2005) is an independent paper by members of AMWG. Used ID values from Table 3 in Webb and Blaser (2002). Original data source for nonage-dependent ID values was Rickmeier et al. (2001) |
| Curling et al. (2010)<br>(Originating Druett et al. (1953) data set strain: M36)<br>Exponential model, fitted parameter $\lambda = 1.36 \times 10^{-5}$<br>$LD_{50}$ = approximately 51,000 spores | Druett et al. (1953) nonhuman primate data for single spore clouds, model fitted parameter and output reported in the *NATO Planning Guide for the Estimation of Chemical, Biological, Radiological, and Nuclear (CBRN) Causalities, Allied Medical Publication - 8(c)* (Curling et al., 2010) |

| Published Study<br>Value or Associated Model (*B. anthracis* Strain) | Basis for Value or Model Specification |
|---|---|
| Dahlgren et al. (1960)<br>(Originating data set: Mill aerosol, unknown strain[s])<br>Approximately 1,300 spores (510 spores in particles 5 µm and less in size) may be inhaled over 8 hours by nonimmunized individuals in an occupational setting without infection | Airborne measurements of *B. anthracis* spores taken in Pennsylvania textile mill during time period with no reported incidence of human inhalation anthrax in a population where only 33% were vaccinated |
| Defense Intelligence Agency (1986)<br>(Unknown strain)<br>$LD_{50}$ = 8,000 to 10,000 spores | Unspecified studies |
| Franz et al. (1997)<br>(Unknown strain)<br>ID = 8,000 to 50,000 spores | Unspecified studies, Franz et al. (1997) identified USAMRIID as general source of information for values, U.S. Army Medical Research Institute of Infectious Diseases (2011) identifies the same range of values for ID |
| Inglesby et al. (1999), Inglesby et al. (2002)<br>(Unknown strain)<br>$LD_{50}$ = 2,500 to 55,000 inhaled spores [sic] | Incorrect identification of Defense Intelligence Agency (1986) reported $LD_{50}$ range |
| Rickmeier et al. (2001)<br>(Unknown strain)<br>$ID_{50}$ = between 8,000 and 10,000 spores (calculated as 8,940 spores)<br>$ID_{10}$ = 1,000 to 2,000 spores (calculated as 1,135 spores)<br>$ID_{90}$ = 50,000 to 100,000 spores<br>Calculated probit slope = 1.43 probits/$\log_{10}$ dose | Subject matter expert opinion elicited for ID values and used to calculate probit slopes |
| Toth et al. (2013)<br>Exponential model with time-dependence<br>(Originating data set: Mill aerosol, unknown strain[s])<br>Simplified Equation:<br>$I(d,t) = 1 - exp\left(-rd(1 - e^{-\Theta t})\right)$<br>$r = 6.4 \times 10^{-5}$<br>(CI = $4.0 \times 10^{-5}$ to $9.5 \times 10^{-5}$)<br><br>$r$ value determined after setting the following parameters:<br>Rate of clearance ($\Theta$) = 0.07 $day^{-1}$<br>Best fit $\Gamma$ distribution shape parameter $a$ = 5.43 and scale parameter $b$ = 0.864 for assessing time-dependent elements of disease progression<br>with:<br>$I$- infection<br>$d$ – dose<br>$t$ - time<br><br>$ID_{50}$ = 11,000 spores<br>(95% CI = 7,200 to 17,000)<br>$ID_{10}$ = 1,700 spores<br>(95% CI = 1,100 to 2,600)<br>$ID_1$ = 160 spores<br>(95% CI = 100 to 250) | EISD model populated with human and nonhuman primate data sources, Brachman et al. (1966) for nonhuman primate dose-response data, Brookmeyer et al. (2005) reported value for rate of clearance ($\Theta$) = 0.07 $day^{-1}$ based on Henderson et al. (1956) nonhuman primate, and Holty et al. (2006) for human Sverdlovsk data |

| Published Study<br>Value or Associated Model (*B. anthracis* Strain) | Basis for Value or Model Specification |
|---|---|
| U.S. Centers for Disease Control and Prevention (2009)<br>(Unknown strain[s])<br>$LD_{50}$ = 4,100 to 10,000 inhaled spores | Nonhuman primate data from Glassman (1966), Peters and Hartley (2002), and Franz et al. (1997).<br><br>Note: Glassman (1966) referenced as Glassman (1965) in U.S. Centers for Disease Control and Prevention (2009). |
| U.S. Army Medical Research Institute of Infectious Diseases (2011)<br>(Unknown strain)<br>ID = 8,000 to 50,000 spores | No studies identified, same ID range as identified in Franz et al. (1997). |
| Watson and Keir (1994)<br>(Unknown strain)<br>6,000 inhaled spores as a "worst" case inhalation critical dose to man" | Brachman et al. (1960) NHP $LD_{50}$ value identified as the lowest single strain $LD_{50}$ value of 6,000 spores, assumed direct applicability to the human. |
| Webb and Blaser (2002)<br>Logit equation describing probability of infection given age ($a$) and dose ($S$), with $a[n]$ = $ID_{50}$ and $b[n]$ = $ID_{10}$ with age-specific values identified below<br>(Unknown strain)<br>$$Pr[n](S) = \frac{b[n]\left(exp\left(\frac{S}{a[n]} - 1\right)\right)}{1 + b[n]\left(exp\left(\frac{S}{a[n]} - 1\right)\right)}$$<br>$ID_{50}$ and $ID_{10}$ values by age group:<br>Less than 25 years: 15,000 and 4,500 spores<br>25–44 years: 10,000 and 3,000 spores<br>45–65 years: 6,000 and 1,800 spores<br>Greater than 65 years: 1,500 and 450 spores | Used expert elicitation values for specific $ID_x$ values as reported in Rickmeier et al. (2001) and modified to develop age-adjusted distribution. |
| Wein and Craft (2005)<br>(Unknown strain[s])<br>Probit slope value of 1.82<br>Probit slope value of 0.7 | Wein and Craft (2005) is an independent paper by members of AMWG convened by DHHS, probit slope value of 1.82 reportedly developed by Harper and Kaufmann of the AMWG, no description or formal citation for derivation, the source of the 0.7 value was Glassman (1966). |
| Wein et al. (2003) (Supporting Text)<br>Age-dependent probit slope model<br>(Unknown strain[s] in Glassman [1966])<br><br>$P(s, a) = \Phi(\alpha + \beta \log(s) + \gamma(a) + \delta(a^2))$<br>Where s = dose of spores<br>$a$ = age in years<br>$\Phi$ = standard normal distribution<br><br>Intercept ($\alpha$) = -9.733<br>Probit dose slope ($\beta$ ) = 1.025<br>Probit age slope ($\gamma$ ) = -0.016 $year^{-1}$<br>Probit age quadratic ($\delta$) = 0.0006 $year^{-2}$ | Wein et al. (2003) is an independent paper by members of AMWG, incorporated age-dependence into the Glassman (1966) probit model using Webb and Blaser (2002) infectious dose values ($ID_{50}$ and $ID_{10}$) for the ages of 15, 35, 55, and 75 years with parameter values estimated using least-squares analysis. |

AMWG – Anthrax Modeling Working Group convened by U.S. Department of Health and Human Services
CBRN – chemical, biological, radiological, and nuclear
CI – 95% confidence interval
DHHS – U.S. Department of Health and Human Services
EISD – Exposure – Infection – Symptomatic illness – Death
λ or r - fitted parameter, potency estimate in exponential dose-response model
ID – infectious dose, infective dose
$ID_x$ – infectious dose for x% of individuals
$LD_x$ – lethal dose for x% of individuals
NHP – nonhuman primate
Pdf – probability density function
USAMRIID – U.S. Army Medical Research Institute of Infectious Diseases

First reported citations for inhalation anthrax $LD_{50}$ or $ID_{50}$ values for a single dose (or less than 24-hour total exposure) ranged from 1,500 spores identified for those older than 65 years of age (Webb and Blaser, 2002) to approximately 51,000 spores presumably appropriate for a general population (Franz et al., 1997; Curling et al., 2010; U.S. Army Medical Research Institute of Infectious Diseases, 2011). The $ID_{50}$ value of 50,000 spores for the human reported in U.S. Army Medical Research Institute of Infectious Diseases (2011) (and which was also reported in previous editions) is generally consistent with nonhuman primate median lethality values reported by authors with a U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) affiliation during the 1990s (Friedlander et al., 1993; Ivins et al., 1996; Ivins et al., 1998). It is also comparable to the $LD_{50}$ value of 53,000 spores (single spore size) originally reported for the nonhuman primate by Druett et al. (1953) and the range of $LD_{50}$ values (c. 48,750 to 53,500 spores in a single spores cloud) that could be calculated from the Henderson et al. (1956) control group data. The Druett et al. (1953) data were the basis for the Curling et al. (2010) $LD_{50}$ value of approximately 51,000 spores, which is the highest value identified in Table 5-10.

The classic human $LD_{50}$ range of 8,000 to 10,000 spores was first published by the Defense Intelligence Agency (1986) and is commonly cited in the literature, but the original dose-response data set(s) and study protocol(s) remain unpublished (Coleman et al., 2008). Using a

slightly broader range of $LD_{50}$ values, the U.S. Department of Health and Human Services (DHHS) Aerosolized Anthrax Response Playbook (U.S. Centers for Disease Control and Prevention, 2009) estimated that the human $LD_{50}$ value for inhalation anthrax ranged between 4,100 and 10,000 based on the nonhuman primate study values reported in Glassman (1966), Peters and Hartley (2002), and Franz et al. (1997). However, U.S. Centers for Disease Control and Prevention (2009) acknowledged the uncertainty inherent in the range of values for the human.

Only two studies reported values for response levels less than 50%, including an $ID_{10}$ range of 1,000 to 2,000 spores derived from expert elicitation (Rickmeier et al., 2001) and an $ID_{10}$ value of 1,700 spores (95% confidence interval of 1,100 to 2,600 spores) based on the modeling of a combination of nonhuman primate and human data (Toth et al., 2013). However, response levels other than the median lethality value can easily be calculated from reported probit slope values or empirical models, such as the exponential model.

Anthrax models developed to assess human populations, which incorporated dose-response elements, are also a source of data for the modeling of human dose-response relationships for inhalation anthrax. Prior to the 2001 anthrax letter event, the DHHS convened the Anthrax Modeling Working Group (AMWG) to provide modeling support for recommendations on medical countermeasures (Hupert et al., 2009). Members of the AMWG published a series of papers, but noted that the papers were not representative of group consensus or final group outputs as indicated in Craft et al. (2005) and Wein and Craft (2005). These papers presented various models to predict necessary medical countermeasures during a disease event, with human dose-response models or model parameter values (e.g., probit slope) embedded in the

overall mathematical models. Two human dose-response models that predicted the probability of infection as a function of dose and age were developed. Wein et al. (2003) combined a probit slope model with a quadratic expression describing age-dependency in response, based on the age-based infection distributions reported in Webb and Blaser (2002). Craft et al. (2005) then developed a linear model of age dependency from the same data in Webb and Blaser (2002). Interestingly, the base data for the inhalation anthrax dose-response relationship in these AMWG members' models were derived from the expert elicitation values reported in Rickmeier et al. (2001), not dose-response data from animal challenges.

Animal model data has been used as input to semi-quantitatively assess the dose-response relationship for the human and to identify "threshold" dose levels where infection and disease may be less likely in identified or general populations. Watson and Keir (1994) identified 6,000 spores as the critical dose for inhalation anthrax infection based on their identification of the lowest single strain published $LD_{50}$ value in the nonhuman primate of 6,000 spores (Brachman et al., 1960). Cohen and Whalen (2007) reported that 600 spores "may not be sufficient to induce disease" in those exposed unless they exhibited health issues associated with increased susceptibility to inhalation anthrax. The 600 spore value was based on an estimation of human exposure using aerosol sampling results from two goat hair mills reported by Dahlgren et al. (1960) and Brachman et al. (1966). Ho and Duncan (2005) calculated a range of potential exposure doses after the handling *B. anthracis*-contaminated envelopes and reported that modeled exposure doses associated with human mortality were between 30,000 and 170,000 spores.

Qualitative data categorizing human dose-response relationships relative to that reported for animals were also identified in the literature search. The relatively low overall incidence of inhalation anthrax in the human in both occupational and general settings led those studying the issue in England to assert that the human had an "inferior susceptibility" as early as the 1800s (Gochenour, 1961). This was based on the recognition of many possible exposure sources for the general public and the acknowledgement of relatively higher source exposure in workers without universal illness (Gochenour, 1961). The World Health Organization (2008) classified the human as "moderately resistant" to anthrax (presumably to infection) based on epidemiological data derived from circumstantial and historical evidence for incidence in wildlife workers, and human outbreaks. Exposure sources included a mixture of natural and occupational settings, as well as accidental and intentional releases of manufactured spores. Given the mix of exposure sources, the use of a single descriptor for human susceptibility implies a generally perceived equivalence in World Health Organization (2008) in the hazard of infection posed by equivalent exposures of manufactured or naturally occurring spore products.

In contrast to the "moderately resistant" determination of the World Health Organization (2008), Lincoln et al. (1967) categorized the rabbit, rhesus monkey, and human as "susceptible" (versus resistant) to the establishment of anthrax. The susceptible category was defined by relative differences between susceptible and resistant animal models. Characteristics for placement in the susceptible category of Lincoln et al. (1967) included lower parenteral and aerosol $LD_{50}$ doses to establish anthrax, higher number of toxin units to cause lethality by intravenous injection, higher terminal concentration of bacteremia, greater inhibition of phagocytes by toxin, and differing rates of intracellular germination by spores in phagocytes in reported values relative to the resistant group. Since challenge data are unavailable, the human was placed in the

susceptible category based on consideration of *in vitro* results from human-derived cell lines and the evaluation of limited availability epidemiology data (Lincoln et al., 1967). Examples of animals identified as resistant to the establishment of anthrax included the rat, swine, and dog (Lincoln et al., 1967).

With regard to the endpoint of the available dose-response data, all data reported either lethality or modeled infection with the assumption that infection led to 100% lethality. Human survival of inhalation anthrax after development of clinical symptoms was reported, but generally after the use of antibiotics and aggressive medical treatment (Jernigan et al., 2001; Walsh et al., 2007; Griffith et al., 2014). Survival increased to 55% for those infected with inhalation anthrax as a consequence of the 2001 anthrax letter event (Inglesby et al., 2002). Historical reports of survival after inhalation anthrax are relatively rare, though Albrink et al. (1960) reported one suspected case of inhalation anthrax in the 1957 epidemic that resulted in survival of the individual. The simplifying assumption that infection is equivalent to lethality has been identified through expert elicitation (Rickmeier et al., 2001) and included in modeling for bioterrorism medical preparedness (Hupert et al., 2009) as well as human dose-response modeling (Toth et al., 2013). Given the scarcity of rigorous data regarding survival after inhalation anthrax infection in the human, lethality will be used as the endpoint for human inhalation anthrax dose-response modeling for this report.

#### *5.4.2.4 Rabbit Inhalation Data*

Two Key Studies for the rabbit animal model were identified through the literature search, the single-dose U.S. Environmental Protection Agency (2011a) study and the multiple-dose U.S. Environmental Protection Agency (2012b) study (Table 5-11). These studies used similar study

designs and were categorized as Key Studies. Each study reported data for the endpoints of infection and lethality, though dose-response calculations were evaluated for lethality only.

**Table 5-11. Single- and Multiple-Dose Key Studies for the Rabbit**

| Study Citation, Rabbit Breed, and Strain | Key Study Outputs | Modeled Data Identified for Key Study |
|---|---|---|
| **Single Dose** | | |
| U.S. Environmental Protection Agency (2011a)<br>New Zealand white rabbit<br>Ames strain | Logistic regression model fit to dose group level $log_{10}$ dose data<br>Inhaled dose $LD_{50}$ = 51,800 CFU<br>(Fieller's CI = $6.14 \times 10^3$ to $7.27 \times 10^5$ CFU) | U.S. Environmental Protection Agency (2012b)<br>Benchmark dose analysis, dichotomous-Hill model with individual animal doses<br>$BMD_{50}$ = 52,000 CFU<br>$BMDL_{50}$ = 13,000 CFU<br>$BMD_{10}$ = 5,700 CFU<br>$BMDL_{10}$ = 1,400 CFU |
| | | U.S. Environmental Protection Agency (2014d)<br>Exponential model with individual animal doses<br>$r = 7.507 \times 10^{-6}$ |
| **Multiple Dose (Number of Doses and Exposure Duration)** | | |
| U.S. Environmental Protection Agency (2012b)<br>(15 doses over 19 days)<br>New Zealand white rabbit<br>Ames strain | Logistic regression to fit $log_{10}$ transformed geometric mean inhaled dose for each individual animal using an accumulated dose metric<br>$LD_{50}$ = 8,100 CFU<br>(Fieller's CI = $2.3 \times 10^3$ to $3.6 \times 10^7$ CFU)<br>Benchmark dose analysis, $log_e$ logistic model with average daily dose<br>$BMD_{50}$ = 6,800 CFU<br>$BMDL_{50}$ = 2,600 CFU<br>$BMD_{10}$ = 760 CFU<br>$BMDL_{10}$ = 290 CFU<br>Benchmark dose analysis, $log_e$ logistic model with accumulated dose<br>$BMD_{50}$ = 120,000 CFU<br>$BMDL_{50}$ = 44,000 CFU<br>$BMD_{10}$ = 13,000 CFU<br>$BMDL_{10}$ = 4,900 CFU | U.S. Environmental Protection Agency (2014d)<br>Exponential model with individual animal accumulated daily doses<br>$r = 5.243 \times 10^{-6}$ |

$BMD_x$ - benchmark dose for response in x% of individuals
$BMDL_x$ - the 95% lower statistical confidence limit of the $BMD_x$ when the 95% lower confidence limit is applied to the estimated slope parameter value
CFU – colony forming unit(s)
CI – 95% confidence interval
$LD_{50}$ – lethal dose for 50% of individuals
r - fitted parameter, potency estimate in exponential dose-response model

The U.S. Environmental Protection Agency (2011a) challenge doses ranged from an average inhaled dose of 286 to 2.75 x $10^5$ CFU. Using logistic regression to fit $\log_{10}$ dose single-dose data at the level of the individual animal, U.S. Environmental Protection Agency (2011a) reported an $LD_{50}$ value of 51,800 CFU, with a Fieller's 95% confidence interval that spanned almost two orders of magnitude ($6.14 \times 10^3$ to $7.27 \times 10^5$ CFU) (Table 5-11). A benchmark dose (BMD) analysis of these same data in the U.S. Environmental Protection Agency (2011a) study using a dichotomous-Hill model with individual animal doses was reported in U.S. Environmental Protection Agency (2012b). The BMD value for response of 50% of the population ($BMD_{50}$) value was 52,000 CFU (U.S. Environmental Protection Agency, 2012b). When using a lethality endpoint, the $BMD_{50}$ corresponds to the $LD_{50}$ of the population. The benchmark dose limit value (BMDL) represents the 95% lower statistical confidence limit of the BMD when the 95% lower confidence limit is applied to the estimated slope parameter value for 50% response ($BMDL_{50}$) value. The $BMDL_{50}$ for the U.S. Environmental Protection Agency (2011a) was 13,000 CFU U.S. Environmental Protection Agency (2012b).

A re-analysis of the U.S. Environmental Protection Agency (2011a) data using individual animal doses in the exponential model reported an r value (fitted potency parameter for the exponential model) of $7.507 \times 10^{-6}$ (U.S. Environmental Protection Agency, 2014d), which would calculate an $LD_{50}$ value of approximately 92,000 CFU. Gutting et al. (2013) analyzed a combination of data sets [i.e., U.S. Environmental Protection Agency (2011a), Zaucha et al. (1998), and previously unpublished data] and reported a fitted potency parameter value[3] for the exponential

[3] Depending on the modeler and/or cited publication, the fitted parameter value for the exponential model is identified as an r, k, or λ parameter. Regardless of the term used to identify the fitted potency parameter for the exponential model, it represents the same value.

model of $7.22 \times 10^{-6}$, which was generally similar to that reported in U.S. Environmental Protection Agency (2014d). One possible reason for the two-fold disparity $LD_{50}$ values for the same data set is the probable lower quality fit of the exponential model relative to the dichotomous-Hill or logistic regression models. U.S. Environmental Protection Agency (2012b) evaluated the exponential model in the suite of evaluated models and this model was not the best fitting model of those assessed.

No single-dose data for the rabbit were categorized as Supporting Studies. Single-dose dose-response data categorized as Additional Data for the rabbit are provided in Appendix D.

The most cited rabbit $LD_{50}$ value of $1.05 \times 10^5$ originated from the Zaucha et al. (1998) study, though the original dose-response data set was not published until Gutting et al. (2013). The Zaucha et al. (1998) $LD_{50}$ value is based on a challenge of 50 animals with mean group doses of 98 to 713,000 spores (Gutting et al., 2013). The Zaucha et al. (1998) value has been directly cited or others have reported values that differ only by varying adjustments in the number of significant figures (see Appendix D for the Additional Data Table). The Zaucha et al. (1998) study was categorized as Additional Data due to: (1) the lack of response data in the range between 1% and 49%, (2) particle size data were not associated with the study exposures for which the $LD_{50}$ value was derived, and (3) it was assumed that the inhalation rate was determined via plethysmography but prior to the aerosol challenge. The dose spacing and the lack of responses between 0 and 50% lethality are problematic because there are insufficient data to differentiate between possible mathematical dose-response models based on the fit to the observable data.

One multiple-dose study in the rabbit was identified through the literature search. The U.S. Environmental Protection Agency (2012b) multiple-dose study in the rabbit was categorized as a Key Study. In this study, rabbits were challenged with 15 doses over 19 days (i.e., Monday through Friday dosing, with no doses over the weekend). Using logistic regression to fit $\log_{10}$ transformed geometric mean inhaled dose data for each individual animal, U.S. Environmental Protection Agency (2012b) reported an $LD_{50}$ value for the accumulated dose metric of 8,100 CFU with a Fieller's 95% confidence interval that spanned approximately four orders of magnitude ($2.3 \times 10^3$ to $3.6 \times 10^7$ CFU). Using the U.S. Environmental Protection Agency (2012b) data set and a calculated average daily dose derived using the exposure duration of the challenge, a benchmark dose analysis identified the best fitting model as the $\log_e$ logistic and reported a $BMD_{50}$ of 6,800 CFU and a $BMDL_{50}$ of $2.60 \times 10^3$ CFU (U.S. Environmental Protection Agency, 2012b). The same BMD analysis process using the $\log_e$ logistic model and an accumulated dose metric reported a $BMD_{50}$ of 120,000 CFU and a $BMDL_{50}$ of 44,000 CFU (U.S. Environmental Protection Agency, 2012b).

The U.S. Environmental Protection Agency (2012b) data were reanalyzed using individual animal accumulated doses and the exponential model; a r value of $5.243 \times 10^{-6}$ was reported (U.S. Environmental Protection Agency, 2014d). This r value would derive an $LD_{50}$ value of approximately 132,000 CFU. The $LD_{50}$ value calculated by the U.S. Environmental Protection Agency (2012b) was considerably lower than that reported for the $BMD_{50}$ value in U.S. Environmental Protection Agency (2012b) or the $LD_{50}$ value calculated from the r value reported in U.S. Environmental Protection Agency (2014d). No additional multiple-dose dose-response data for the rabbit were identified.

#### *5.4.2.5 Inhalation Data for the Nonhuman Primate*

One Key Study for the nonhuman primate was identified through the literature search (Table 5-12). Lever et al. (2008) challenged a group of 12 male and female common marmoset (*Callithrix jacchus*) with a range of inhaled doses from $1.4 \times 10^1$ to $1.9 \times 10^5$ CFU and a reported $LD_{50}$ value of $1.47 \times 10^3$ CFU (95% confidence interval of $7.19 \times 10^5$ to $2.95 \times 10^5$ CFU). The marmoset animal model was evaluated as a small animal alternative in the nonhuman primate animal model. The endpoint assessed was lethality between the exposure challenge and 10 days after exposure. Infection was not reported.

The Lever et al. (2008) data set was categorized as a Key Study. The judgment was made that the study was sufficiently close to meeting the requirement of having an overall lethality rate of less than 50% (i.e., 6 of 12 monkeys died). Though a higher number of animals in the low-dose region of exposure may have been preferred, the available data were sufficient to derive the reported $LD_{50}$ value.

**Table 5-12. Single-Dose Key Study for the Nonhuman Primate**

| Study Citation, Nonhuman Primate Species, and Strain | Key Study Outputs | Additional Data Identified for Key Study | Additional Data Outputs |
|---|---|---|---|
| **Single Dose** | | | |
| Lever et al. (2008)<br><br>Common marmoset (*Callithrix jacchus*)<br><br>Ames strain | Geometric mean $LD_{50}$ = $1.47 \times 10^3$ CFU<br><br>CI = $7.19 \times 10^5$ to $2.95 \times 10^5$ CFU | No additional data | No additional data |

CFU – colony forming unit(s)
CI – 95% confidence interval
$LD_{50}$ – median lethality value

Single dose data for the nonhuman primate characterized as Supportive Data or Additional Data are provided in Appendix E.

An additional consideration for the use of the nonhuman primate data is that the identified $LD_{50}$ values categorized as Additional Data must be carefully evaluated prior to use for informing risk assessment. It is important to recognize that most values were derived from studies with the primary purpose of evaluating pathology or medical countermeasures; the $LD_{50}$ values were generated with study designs that did not explicitly evaluate statistical considerations regarding animal and dose range to generate a representative median value. With the exception of the Vasconcelos et al. (2003) $LD_{50}$ value, the remaining identified values in the 50,000 to 62,000 CFU range were cited as a personal communication or unpublished data from an author associated with the USAMRIID laboratories. Examples of publications fitting this description include Ivins et al. (1996), Vasconcelos et al. (2003), and Coleman et al. (2008). Other examples include those directly cited by an author with USAMRIID affiliation as in the case of Henderson et al. (1956) and Friedlander et al. (1993). It is possible that multiple published citations of approximately the same $LD_{50}$ value may not represent multiple independent studies that corroborate the identified value, but may be the same study or a limited number of studies repeatedly cited.

Two multiple-dose studies (Albrink and Goodlow, 1959; Brachman et al., 1966) and subsequent re-analyses of these data were identified in the literature search (Table 5-13). Brachman et al. (1966) reported selected dose data from three multiple-dose exposure challenges of nonhuman primates to *B. anthracis*-contaminated aerosols from a picking station at a goat hair processing plant (Table 5-13). The total cumulative doses ranged from 947 to 16,962 *B. anthracis*-bearing

particles. The exposure duration varied from a low of 31 hours for Run 5 to up to 47 days for the first segment of Run 3. The endpoint reported was lethality as measured over an observation period that varied for each run; with a low of two to five days for Run 3 to up to 25 days for the first challenge in Run 5. Infection was not reported, though evidence of anthrax infection was noted in individual animals at sacrifice.

Brachman et al. (1966) graphically reported daily cumulative dosing with an accompanying identification of animal deaths from anthrax. The original raw dose-response data set was not published and has not since become available. After interpolation of the graphical data to identify values for modeling, the Brachman et al. (1966) data were reanalyzed by Haas (2002) and Mayer et al. (2011). Mayer et al. (2011) and Haas (2002) reported fitted values for the potency parameter in the exponential model that can be used to calculate $LD_{50}$ values of 19,327 spores and 28,750 spores, respectively. The higher $LD_{50}$ value of 28,750 likely resulted from an error in the calculation of the average daily dose by Haas (2002) that was identified in Toth et al. (2013).

Most reported studies identified were performed to determine the median lethality endpoint, assess efficacy of medical countermeasures, or describe the pathology resulting from lethal infection, but not to identify dose-response relationships for infection from low- or high-dose nonhuman primate study data (Albrink and Goodlow, 1959; Ivins et al., 1996; Ivins et al., 1998; Fellows et al., 2001; Rossi et al., 2008; Saile et al., 2011; Henning et al., 2012). However, survival after anthrax bacteremia in animal models appears to be rare relative to lethality in the dose ranges commonly tested. Published reports of survival after anthrax bacteremia were identified during the literature search in the unvaccinated nonhuman primate (Albrink and

Goodlow, 1959; Fellows et al., 2001; Saile et al., 2011; Henning et al., 2012) and the unvaccinated rabbit (U.S. Environmental Protection Agency, 2012b). Given the lack of research interest in the survival endpoint after infection, study designs did not incorporate statistical sufficiency to estimate the likelihood of survival after bacteremia. This would likely entail the need for significantly higher animal numbers to reliably measure prevalence. It is also unknown if there is dose-dependence in survival after infection.

**Table 5-13. Multiple-Dose Additional Data for the Nonhuman Primate**

| Study, Nonhuman Primate, and Model Parameters or Other Outputs | Other Data |
|---|---|
| Brachman et al. (1966)<br><br>Cynomolgus monkey<br>*(Macaca fascicularis*)<br><br>Reanalyzed by Haas (2002)<br>Exponential model<br>$k = 2.6 \times 10^{-5}$<br>CI = 1.3 to $1.6 \times 10^{-5}$<br><br>Reanalyzed by Toth et al. (2013)<br>EISD model<br>Assumed fixed model parameters for clearance where $\Theta = 0.07$ day$^{-1}$, shape parameter $a = 5.43$, scale parameter $b = 0.864$, and then fit an r value of $6.4 \times 10^{-5}$, and T = 2.3 days<br><br>Reanalyzed by Mayer et al. (2011)<br>Exponential model<br>$k = 3.57 \times 10^{-5}$ when assuming $\alpha = 1.0$<br><br>Also derived time-dependent modification for exponential model, $\alpha = 0.9$, $\gamma = 0.0097$ h$^{-1}$, and $s = 1.81 \times 10^{-7}$ h$^{-1}$ with $s/\gamma = 1.87 \times 10^{-5}$ where $s/\gamma$ is mathematically equivalent to the k potency estimate in exponential equation | Albrink and Goodlow (1959)<br><br>Chimpanzee<br>(*Pan troglodytes Schwarz and Pan troglodytes troglodytes*)<br><br>Melvin Dose 1: 32,800 inhaled viable spores<br>Dose 2: 90,300 inhaled viable spores with survival after Dose 2<br><br>John Dose 1: 34,350 inhaled viable spores<br>Dose 2: 112,000 inhaled viable spores with death after Dose 2<br><br>Brachman et al. (1966)<br><br>Cynomolgus monkey<br>*(Macaca fascicularis*)<br><br>Daily doses not reported, 3 exposure runs of various lengths ≤ 47 days with reported exposure data, differing exposure sources and concentrations<br><br>Run Three: 16,962 total *B. anthracis* particles over 47 days<br><br>Run Four: 4,959 total *B. anthracis* particles over 41 days<br><br>Run Five: 947 total *B. anthracis* particles over 55 hours + 1,347 total *B. anthracis* particles over 31 hours<br><br>Fatality rate of approximately 10% for exposure to approximately 1,000 *B. anthracis*-bearing particles over 3 to 5 days, with fatality rate of 20 to 25% for exposure to approximately 3,500 to 5,500 *B. anthracis*-bearing particles over a 5 days |

| Study, Nonhuman Primate, and Model Parameters or Other Outputs | Other Data |
|---|---|
| CI - 95% confidence interval<br>EISD – Exposure-Infection-Symptomatic Illness-Death<br>$ID_x$ - Infectious Dose for *x* percent exposed<br>k or r - fitted parameter, potency estimate in exponential dose-response model<br>$a$ – shape parameter | α – shaping parameter for accumulation effects<br>$b$ - scale parameter<br>γ – net per pathogen clearance rate ($h^{-1}$)<br>Θ – probability-per-time for clearance of spores from the lung<br>s – instantaneous risk to individual pathogen<br>T – delay between spore germination and initiation of symptoms |

#### *5.4.2.6 Oral Data for Multiple Animal Models*

Few published data are available for oral exposure to *B. anthracis* spores or vegetative bacteria (Table 5-14). Oral challenge dose-response data were identified for the guinea pig, rabbit, rhesus monkey, cow, mouse, pig, and human (Table 5-14). Published $LD_{50}$ values for oral exposure generally range from $10^6$ to $10^8$ spores, and include data from animals that are viewed as very susceptible to infection (World Health Organization, 2008). For example, Schlingman et al. (1956) reported that a group of three cattle challenged with oral doses of $10^7$ spores had one survivor, and exhibited a longer time-to-death after exposure than $10^8$ and $10^9$ spore doses.

**Table 5-14. Oral Dose-Response Data**

| Study<br>Animal Model<br>(Form of *B. anthracis*) | Dose-Response Data |
|---|---|
| Young Jr. et al. (1946)<br>Guinea pig (Spores) | Occasional deaths in tested guinea pigs after oral administration of $1 \times 10^8$ Detrick 25 strain spores, details not provided on death number or total tested. |
| Druett et al. (1953)<br>Guinea pig (Spores) | No infections after oral administration of $10^8$ strain spores in unspecified number of animals (assumed to be same M36 strain used in aerosol challenge). |
| Druett et al. (1953)<br>Rabbit (Spores) | No infections after oral administration of $10^8$ strain spores in unspecified number of animals (assumed to be same M36 strain used in aerosol challenge). |
| Lincoln et al. (1965)<br>Rhesus monkey (Spores) | Two monkeys each were orally challenged using infant feeding tubes at doses of $10^2$, $10^4$, $10^6$, and $10^8$ spores, all animals survived (assumed to be same V1b strain used in aerosol challenge). |
| Redmond et al. (1997)<br>Large White x Landrace Crossbred pig<br>(Spores with feed and grit) | Two of 50 pigs died that were challenged with total doses (delivered in one to three doses) of approximately $10^7$ to $10^{10}$ CFU of Ames strain or reisolates from pigs infected with same Ames strain, grit was added to feed to facilitate infection. |
| Schlingman et al. (1956)<br>Mixed dairy and Hereford breeds of cattle<br>(Spores) | One cow administered $6 \times 10^8$ Vollum strain spores in gelatin capsule exhibited an elevated temperature for days 5 through 9, then recovered. |
| | Three of 4 cattle administered $10^9$ V770-2-P strain spores in a feed pellet died, the surviving cow was rechallenged with same dose (timing unknown) and promptly died. |
| | One cow was challenged with $10^9$ Vollum strain spores, exhibited a slight febrile reaction and recovered, was rechallenged with $10^9$ V770-2-P strain spores and exhibited no evidence of infection. |
| | Three of 4 cattle administered $10^9$ V770-2-P strain spores in a feed pellet died, the survivor was rechallenged 7 days later and survived. |
| | In a rechallenge taking place an unknown time after the first exposure, two of 2 cattle died that were administered $10^9$ V770-2-P strain spores in a feed pellet. |
| | Two of 3 cattle administered $10^8$ V770-2-P strain spores in a feed pellet died, the surviving cow was noted to not chew the pellet, was rechallenged 10 days later, and survived. |
| | Two of 3 cattle administered $10^7$ V770-2-P strain spores in a feed pellet died, the surviving cow had an elevated temperature days 2 through 6 and survived. |

| Study<br>Animal Model<br>(Form of *B. anthracis*) | Dose-Response Data |
|---|---|
| | Two of 3 cows administered $1.5 \times 10^8$ V770-2-P strain spores in a feed pellet survived, the survivor was rechallenged after 7 days with the same dose of the initial challenge and died. |
| | One cow challenged with $10^9$ V770-2-P strain spores died. |
| Schlingman et al. (1956)<br>Chester White pig (Spores) | No evidence of infection after oral administration of $10^6$ V770-2-P strain spores in pigs (number assumed to be 15). |
| Schlingman et al. (1956)<br>Chester White pig<br>(Likely mixture of vegetative and spore forms) | Two of 2 pigs that were fed 1 guinea pig recently dead of anthrax infection from either V770-2-P or 1062 strain survived, with each exhibiting fever. |
| | Of the 8 swine in the control groups, all swine survived ingestion of guinea pig carcasses that died from anthrax infection with V770-2-P strain spores, though all pigs exhibited symptoms of elevated temperature, with some pigs noted to exhibit pharyngeal swelling and anorexia. |
| Xie et al. (2013)<br>A/J mouse (Vegetative bacteria) | $LD_{50} = 2.3 \times 10^7$ for Sterne strain, authors noted that dose of $2.3 \times 10^6$ vegetative bacteria can cause lethal infection. |

When designing testing, an oral dose of $1.5 \times 10^8$ spores was thought to prove fatal to most unimmunized cattle (Schlingman et al., 1956). The pig is known to exhibit a high degree of resistance to systemic anthrax infection from the inhalation and intraperitoneal challenge routes (Walker et al., 1967). Accordingly, high oral dose levels ranging from $10^7$ to $10^{10}$ CFU were associated with very low levels of lethality (2/50) in the challenged swine even with the addition of grit to the food source (Redmond et al., 1997). In two separate studies, an oral challenge dose as high as $10^8$ spores did not result in infection in the rabbit (Druett et al., 1953) or the nonhuman primate (Lincoln et al., 1965) (Table 5-14).

Of the few oral challenge studies available, most have been conducted with spores. The relative infectivity of spores versus vegetative bacteria has been characterized as unknown (World Health Organization, 2008). However, relatively new data in the mouse animal model from Xie et al. (2013) described infection lethality in doses as low as $2.3 \times 10^6$ CFU and reported an $LD_{50}$ value of $2.3 \times 10^7$ CFU. Noting increased infectivity of vegetative bacteria in subcutaneous challenge in the same animal model, Xie et al. (2013) hypothesized that vegetative bacteria toxin

production could contribute to breakdowns in the epithelial barrier and promote infection and dissemination. Data are unavailable to draw conclusions on the relative human infectivity of the spore versus vegetative bacterial forms in the human beyond that hypothesized by Inglesby et al. (2002), that large oral doses of vegetative bacteria may be necessary to result in gastrointestinal anthrax.

#### *5.4.2.7 Conclusions for Dose-Response Data Literature Review*

Few studies were identified as Key Studies for the rabbit and nonhuman primate; there were no Key Studies or Supporting Studies identified for the human. The two Key Studies for the rabbit were the single-dose U.S. Environmental Protection Agency (2011a) study and the multiple-dose U.S. Environmental Protection Agency (2012b) study. No studies were categorized as Supporting Studies. For the nonhuman primate, one single-dose Key Study (Lever et al., 2008) and one single-dose Supporting Study (Druett et al., 1953) were identified.

**Table 5-15. Summary of Number of Key Studies, Supporting Studies, and Additional Data Sources for the Human, Rabbit, and Nonhuman Primate**

| | Number of Key Studies (Table) Study Citation | Number of Supporting Studies (Table) Study Citation | Number of Sources of Additional Data (Table) |
|---|---|---|---|
| Human | 0 | 0 | 15 single dose* (Table 5-9) |
| Rabbit | 1 single dose (Table 5-10) U.S. Environmental Protection Agency (2011a) 1 multiple dose (Table 5-10) U.S. Environmental Protection Agency (2012b) | 0 | 15 Single Dose (Table Appendix D-1) |
| Nonhuman primate | 1 Single Dose (Table 5-11) Lever et al. (2008) | 1 Single Dose (Table E-1) Druett et al. (1953) | 5 Single Dose (Table Appendix E-1) 4 Multiple Dose (Table 5-12) |

* For the human Additional Data, some of the input data reported as the basis for the published data were derived using multiple dose data in full or in part. However, the Additional Data sources did not clearly specify whether the modeled values should be applied to single or multiple dose exposures.

The results of the literature search indicate that the lethality endpoint is the only endpoint that can be supported with identified data for inhalation anthrax in the rabbit, nonhuman primate, and human. This is identified for the following reasons: (1) the high concordance between infection and death for challenge studies in animal models and human epidemiological reports, (2) very few studies that report infection data, and (3) lack of appropriate study design to capture the incidence of nonlethal infection.

Table 5-16 reviews the Twenhafel (2010) key human histopathological findings relative to the pathology reported in the Rabbit and Nonhuman Primate Key Studies. There was not a good concordance between the key human histopathological findings and identified Key Studies for the rabbit and nonhuman primate, with 3/6 of the histopathological findings reported in the rabbit and 2/6 of the histopathological findings reported in the nonhuman primate. The lack of concordance may include differing protocols for the pathology evaluations and/or relatively small animal numbers of the animals evaluated for pathology in the dose-response studies. The Key Studies identified for the rabbit did not report findings for pathology in the spleen. Given that the spleen is one of the earlier involved organs in the disease process, it is unexpected that the spleen did not show early signs of lesions or other pathologies, even if it had not progressed to splenic lymphoid depletion. However, the protocol triggered histopathology on those organs that exhibited gross pathology at necropsy. This could explain the lack of even initial stages of splenic pathology reported.

When comparing the results contained in Table 5-16 with

Table 5-9, the Zaucha et al. (1998) study for the rabbit reported concordance with 4/6 of the histopathology findings. However, the Zaucha et al. (1998) also had the largest animal number examined for pathology (n=22 for aerosol challenged rabbits). This is less than the six nonhuman primates that died of inhalation anthrax in Lever et al. (2008) and eleven and five rabbits in the U.S. Environmental Protection Agency (2011a) U.S. Environmental Protection Agency (2012b) that died of inhalation anthrax. It is possible that the low animal numbers evaluated may affect the presentation of specific pathologies in inhalation anthrax, as noted by Lever et al. (2008) with regard to the lack of meningitis with hemorrhage in the study. Given that some of the Twenhafel (2010) histopathological findings may be infrequent in the human and having variability in appearance in studies, the low animal numbers in the studies may be a compelling explanation.

**Table 5-16. Identification of Twenhafel (2010) Key Human Histopathological Findings in Rabbit and Nonhuman Primate Key Studies**

| Histopathology | Rabbit Key Studies<br>• U.S. Environmental Protection Agency (2011a)<br>• U.S. Environmental Protection Agency (2012b) | Nonhuman Primate Key Study<br>• Lever et al. (2008) | Evidence for Time-Dependency Indicated in Table 5-9? |
|---|---|---|---|
| Pneumonia | Yes* | Not reported in Key Study | Yes - Progression to pneumonia is associated with inflammatory process, lower incidence, and lesser severity reported in rabbit. |
| Splenic lymphoid depletion | Not reported in Key Studies | Not reported in Key Study | No - Spleen is an early disease target in inhalation anthrax. |
| Meningitis | Yes | Yes | Yes - Hypothesized as time-dependent in Zaucha et al. (1998), not identified in any of NHP serial sacrifices reported in Berdjis et al. (1962). |
| Hepatic hemorrhage or inflammation | Not reported in Key Studies | Yes | Yes – Reported as time-dependent in NHP by Vasconcelos et al. (2003). |
| Gastrointestinal hemorrhage or inflammation | Yes | Not Reported in Key Study | No - Hemorrhagic spread to gastrointestinal tract seems to occur early in the disease process. |

| Histopathology | Rabbit Key Studies<br>• U.S. Environmental Protection Agency (2011a)<br>• U.S. Environmental Protection Agency (2012b) | Nonhuman Primate Key Study<br>• Lever et al. (2008) | Evidence for Time-Dependency Indicated in Table 5-9? |
|---|---|---|---|
| Urogenital hemorrhage or inflammation | Not reported in Key Studies | Not Reported in Key Study | Unknown - Evidence or reports for time-dependency are lacking. |

[*] Reported suppurative inflammation in pulmonary interstitium
NHP – nonhuman primate

## 5.5 Model the Dose-Response Relationship

There are a number of considerations necessary to model a dose-response relationship for *B. anthracis* once determinations have been made regarding suitable animal models and available dose-response data gathered. Table 5-17 indicates the key questions and associated report sections in which available data and dose-response analysis processes are reviewed for *B. anthracis*. The following sections will consider identification of appropriate dose metrics, empirical and mechanistic approaches for modeling dose-response relationships for *B. anthracis*, and mathematically modeling the dose-response relationship.

**Table 5-17. Development of Microbial Dose-Response Relationships**

| Step in Microbial Dose-Response Analysis | Key Questions | Report Section |
|---|---|---|
| Model the dose-response relationship<br>(Section 5.5) | What dose metrics can be supported based on available disease pathogenesis and other dose-response data?<br><br>What assumptions are associated with a given dose metric? | Section 5.5.1 Determination of Dose Metric |
| | What types of empirical and mechanistic models may be suitable for *B. anthracis*?<br><br>Can mechanistic models be supported by available dose-response data for *B. anthracis*? | Section 5.5.2 Empirical and Mechanistic Modeling Approaches |
| | What approaches can be used to mathematically model the dose-response relationship and estimate the POD? | Section 5.5.3 Mathematically Modeling the Microbial Dose-Response Relationship |

### *5.5.1 Determination of Dose Metric*

A dose metric is the mathematical description of the challenge study dose that is used to model the dose-response relationship and conduct the interspecies extrapolation. The preferred dose metric is the internal dose that can be most closely mechanistically or otherwise correlated with the biological endpoint of interest (Jarabek et al., 2005). A dose metric is associated with a specified exposure duration and can also be expressed as a time-normalized measurement (e.g., CFU/day) (U.S. Environmental Protection Agency, 2014b). Dose metrics may also include a "biologically motivated" normalization factor that assesses the dose magnitude over an identified tissue area or cell number (e.g., number of macrophages contacting the particle) (Jarabek et al., 2005).

There are a range of potential dose metrics for inhalation *B. anthracis* exposure ranging from administered dose to differing measures of internal dose (e.g., deposited dose, dose accessible by macrophages) (U.S. Environmental Protection Agency, 2010a). The dose metric selected for the single-dose *B. anthracis* dose-response studies (U.S. Environmental Protection Agency, 2011a; Gutting et al., 2013) and the multiple-dose study (U.S. Environmental Protection Agency, 2012b) was an inhaled dose metric.

Uncertainty in the most appropriate internal dose for the endpoint of lethality poses a challenge in the selection of dose metrics. If it is assumed that initiation of infection is the key event most closely associated with the endpoint of lethality and that initiation of infection takes place in the alveolar lung region (e.g., Trojan horse model), one

***Summary of Findings for Determination of Dose Metric***

- There is a lack of mechanistic data relating dose to the lethality endpoint.
- Uncertainty in the initiation of infection adds to the difficulty in dose metric selection.
- There is uncertainty in the selection of an appropriate dose metric when evaluating multiple-dose exposure of microbial pathogens, including *B. anthracis*.

appropriate measure of the internal tissue dose is the deposited dose in the alveolar region. If infection is assumed to initiate across a variety of respiratory tract tissues and the likelihood of initiation of infection across tissue or regions is unknown (e.g., jailbreak model), multiple-dose metrics may be appropriate for consideration. Though not evaluated to date for *B. anthracis*, dose metric selection can incorporate a normalization factor. For evaluation of inhaled particulate chemical hazards, normalization factors have described the magnitude of dose relative to the number of contacting cells with potential to initiate infection (e.g., macrophage) or surface area available for uptake of chemical (Jarabek et al., 2005).

There is also uncertainty in the selection of an appropriate dose metric when evaluating multiple-dose exposure of microbial pathogens, including *B. anthracis*. The U.S. Environmental Protection Agency (2012b) multiple-dose study reported dose-response relationship evaluations using two dose metrics: accumulated inhaled dose and average daily inhaled dose. An accumulated dose metric assumes an equivalent hazard whether the intake is in the form of one dose or in many doses over that same time (i.e., the hazard assumed per spore is equivalent regardless of the dosing schedule) (Mayer et al., 2011).[4] The independent action hypothesis, also termed the independent event hypothesis, may have relevance for the determination of dose metrics for multiple-dose *B. anthracis* exposure studies (U.S. Environmental Protection Agency, 2014d). Independent action of pathogens was described by Druett (1952) as a constant relationship between response and the product of administered dose (e.g., environmental concentration) and exposure time. In the derivation of the independent action hypothesis, Druett

[4] Druett (1952) independently described the microbial-equivalent of Haber's Law. Haber's Law, reported in the early 1900s, also described a constant concentration-time relationship between exposure and mortality response for exposure to inhalation exposure to volatile chemicals. Since that time, Haber's Law has been updated to include a fitted exponent on the concentration term to better fit tested chemicals (ten Berge et al., 1986). Likewise, a fitted exponent may also be found appropriate for the mathematical description of independent action.

(1952) assumed the following: (1) a constant probability for each organism to cause the identified response (i.e., mortality or infection) in the host, (2) independent action of each organism (e.g., no immune system activation), (3) an $LD_{50}$ value that can be determined, and (4) a large homogenous experimental population (Druett, 1952). The independent action hypothesis has been further defined to indicate that the probability of survival of each individual organism is the same (Haas et al., 1999b) and that the probability of an individual organism causing infection is independent of the number ingested or inhaled (Buchanan et al., 2009). Relevant to the consideration of multiple-dose exposure, the definition of the independent action hypothesis has been expanded to include a lack of effect of prior doses on subsequent dose (U.S. Environmental Protection Agency, 2014d).

If the independent action hypothesis were correct, the accumulated (or total) dose would be an appropriate dose metric for a *B. anthracis* and there is no biological justification for consideration of a daily average dose. However, a limitation to the exposure duration over which independent action could be assumed (e.g., short enough to preclude immune system activation) was noted by Druett (1952) in the original formulation of the hypothesis. Though Druett (1952) developed the hypothesis with single-dose data, the concept should be equally relevant to multiple-dose assessments. The independent action hypothesis allows for the use of an aggregate dose metric only if the exposure time over which the daily doses are aggregated does not exceed the time duration associated with dose independence.

Potential dependencies by time, dose, or route of exposure may affect consistency with the independent action hypothesis. The magnitude of exposure or exposure duration (Mayer et al., 2011; U.S. Environmental Protection Agency, 2014d) where independent doses can be delineated from dependent doses have not been explicitly evaluated to date. Dose-dependencies

may be present in the expression of independent action if larger doses could affect response to subsequent doses when overloading of clearance or other innate immune functions are affected (Mayer et al., 2011). If overloading can occur, this implies that the presence of independent action could vary by route of exposure if varying innate response levels are present (e.g., dermal exposure versus inhalation). The timing of the exposures relative to the dose and clearance capabilities is also a critical exposure consideration relative to the selection of dose metrics (Mayer et al., 2011).

The determination of a theoretical time point separating independent and dependent doses may be considerably more complicated for inhaled pathogens that have the potential to persist in the lungs (U.S. Environmental Protection Agency, 2014d). For example, spore persistence in the lung and subsequent inhalation anthrax has been reported in one nonhuman primate that died 58 days after exposure after initially receiving 30 days of antibiotic treatment starting on the exposure day (Friedlander et al., 1993).

Though there is uncertainty in the identification of the most appropriate dose metric, this should not limit the evaluation of dose-response relationships. Relevant dose metrics should be identified and a justification provided for those that are evaluated. With regard to selection of the regional deposition location(s) for the deposited dose, multiple-dose metrics can be evaluated. Given that the differences in deposition may be small relative to other components of the inhalation dose calculation, the actual difference in the modeled dose-response relationship may be of limited magnitude. The documentation for the dose-response relationship should include a transparent identification of the basis for selection of the dose metric(s) considered. There should also be a qualitative discussion of the uncertainty associated with the dose metric selection in the risk characterization element of the risk assessment.

### *5.5.2 Empirical and Mechanistic Modeling Approaches*

Two fundamental types of dose-response modeling approaches are available to derive microbial dose-response relationships. Empirical models, also termed fitted models (Gutting et al., 2008), rely on statistical curve-fitting techniques to fit mathematical models to dose-response data. Depending on the model, parameter values fit by these models may not have biological meaning or bear precise relationships to measurable biological parameters (Andersen et al., 1999). It is recommended that microbial dose-response models exhibit biological plausibility, which is defined as a biological basis for the mathematical representation of the model (Haas et al., 1999a). However, the ability to precisely describe biological plausibility may be limited due to lack of basic mechanistic data for microbial pathogenesis (Taft and Hines, 2012).

***Summary of Findings for Empirical and Mechanistic Modeling Approaches***

- There is insufficient mechanistic data for comprehensive mechanistic dose-response models for *B. anthracis*.
- Parsimony in model selection will lead to the continued use of empirical models and limited or nominally mechanistic models.

Empirical models have considerable utility in dose-response modeling because they allow for the description of a wide variety of curve shapes, provide a general assessment of potency (or virulence for pathogens), and can assess time-based elements of the test system (Andersen et al., 1999). Empirical models can interpolate within the original range of the study data, but may provide unreliable extrapolations to lower or higher doses (Buchanan et al., 2000; Gutting et al., 2008). The primary value of empirical models is to provide a first step in the identification of dose-response relationships when scarce mechanistic and parameter value data limit the use of other approaches.

Most of the microbial dose-response modeling conducted for *B. anthracis* to date has relied on empirical modeling approaches. The probit slope and median lethality values reported by Druett et al. (1953) are an early example of empirical dose-response modeling. Empirically derived dose-response relationships using either inhaled or deposited dose metric have since been reported for *B. anthracis* inhalation exposure in the nonhuman primate (Glassman, 1966; Haas, 2002; Bartrand et al., 2008; Weir and Haas, 2011; Taft and Hines, 2012) and rabbit (U.S. Environmental Protection Agency, 2011a, 2012b). Hybrid models of empirically fit parameters combined with expert elicited dose-response values were published in population-based anthrax models for the human (Webb and Blaser, 2002; Wein et al., 2003; Wein and Craft, 2005). Likewise, empirically fit models have been developed using a survival analysis framework to incorporate time-dependencies in dosing and/or response (Mayer et al., 2011; U.S. Environmental Protection Agency, 2014d).

In contrast to empirical models, mechanistic models incorporate known or hypothesized biological mechanisms to derive an estimate of predicted response (U.S. Environmental Protection Agency, 2011c). Mechanistic models can be extremely data-intensive and rely upon significant mechanistic knowledge of the microbial pathogen and host (Gutting et al., 2008). However, mechanistic models offer a unique advantage over empirical models as they can allow for more robust extrapolation across species and dose ranges of interest (Gutting et al., 2008). The biologically based dose-response (BBDR) model is a mechanistic model, but has the distinguishing trait where the probability of response to an administered dose is modeled as a function of biological variable(s) that are mechanistically associated with the adverse response (Crump et al., 2010).

There are differences of technical opinion in the microbial dose-response community as to whether the exponential and beta-Poisson dose-response models should be identified as empirical or mechanistic (U.S. Environmental Protection Agency, 2010b). The determination that exponential and beta-Poisson models are mechanistic has been used as the basis to exclude consideration of empirical models. However, uncertainty in the basic pathogenesis of *B. anthracis* and conflicting evidence for the presence of identified disease pathogenesis characteristics used to define the model as mechanistic (e.g., independent action, no threshold, assumed particle distribution) should prompt consideration of both empirical and mechanistic dose-response modeling approaches to reduce the potential impact of model uncertainty (Taft and Hines, 2012).

To facilitate clarity in the discussion of mechanistic models, a hierarchy of mechanistic models is presented that defines models relative to the level of biological knowledge incorporated in the model. The conceptual basis for the three-part delineation is based on the dose-response model description described in Andersen et al. (1999). The hierarchy of mechanistic models, terminology for category of model, and published models for each category are identified in Figure 5-2 and summarized below:

(1) Nominally mechanistic models incorporate simple biological representations, but biological measurements or modeling cannot inform parameter values; all parameter values are derived through empirically fitting the dose-response data to a mathematical model; an example is the exponential model described by Haas et al. (1999a),

(2) Limited mechanistic models, including BBDR models, incorporate mechanistic assumptions and data that can be derived or informed by biological measurements,

examples include the competing risk model of Gutting et al. (2008) and biokinetic model of Huang and Haas (2011), and

(3) Comprehensive mechanistic models incorporate mechanistic assumptions and data to fully describe biodynamic and biokinetic elements, the earliest conceptualization of a microbial-equivalent to the physiologically based pharmacokinetic [PBPK] model generated for chemical hazards was proposed by Coleman and Marks (1998), and a compartmental and data description for a physiologically based biokinetic [PBBK] model specific for *B. anthracis* was subsequently described by Gutting et al. (2008).

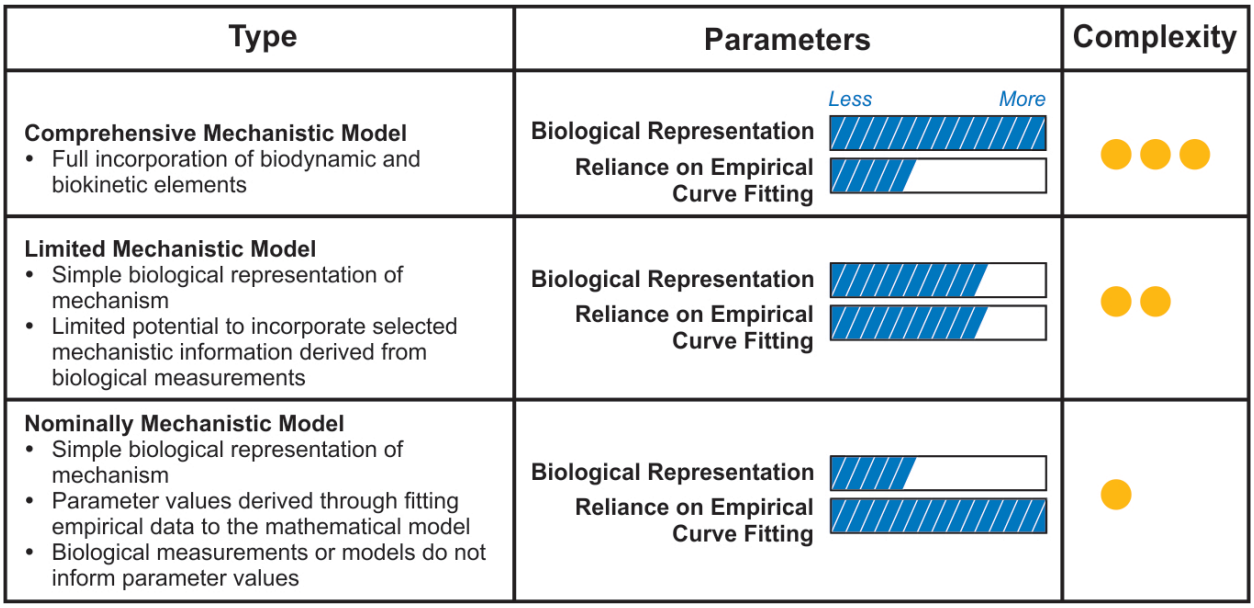


**Figure 5-2. Comparison of mechanistic models relative to biological representation, empirical curve-fitting, and complexity.**

Nominally mechanistic dose-response models (i.e., exponential and beta-Poisson) were evaluated for inhalation exposure to *B. anthracis* in the nonhuman primate (Haas, 2002; Bartrand et al., 2008; Weir and Haas, 2011; Taft and Hines, 2012) and the rabbit (U.S. Environmental Protection

Agency, 2012b, 2014d). Using the competing risk mathematical model to describe the likelihood of successful spore germination versus clearance (Brookmeyer et al., 2003; Brookmeyer et al., 2005), limited mechanistic BBDR models were generated for the rabbit (Gutting et al., 2013) and the human (Toth et al., 2013) using a mixture of human and nonhuman primate sourced data. Table 5-18 summarizes the types of mathematical models that have been reported for the rabbit, nonhuman primate, or human by type of mechanistic model and presence of threshold.

**Table 5-18. Examples of Mathematical Dose-Response Models for Inhalation Anthrax in the Rabbit, Nonhuman Primate, or Human by Type of Model**

| Type of Model | Dose-Response Model | Does Model Exhibit Threshold? | Reported Dose-Response Relationship Using Model |
|---|---|---|---|
| Empirical | Probit or Log Probit | No, unless a background or threshold parameter is included. | Druett et al. (1953)<br>Glassman (1966)<br>Taft and Hines (2012) |
| | Logistic or Log Logistic | | Taft and Hines (2012)<br>U.S. Environmental Protection Agency (2012b) |
| | Weibull | | |
| | Dichotomous Hill | Yes | |
| | Gamma | Yes | |
| | Survival Models | Varies | U.S. Environmental Protection Agency (2014d) |
| Nominally Mechanistic | Exponential | No | Haas (2002)<br>Bartrand et al. (2008)<br>Taft and Hines (2012) |
| | Beta Poisson | No | Bartrand et al. (2008)<br>Taft and Hines (2012) |
| Limited Mechanistic | Competing Risk Model | Yes | Gutting et al. (2008) and Gutting et al. (2013), incorporating base competing risk model of Brookmeyer et al. (2003); Brookmeyer et al. (2005) |
| | Cumulative Dose Model | No | Pujol et al. (2009) |
| | In-vivo Delivered Dose Model | Depends on the model from which the dose variable is being revised to represent delivered dose | Weir and Haas (2011) |
| | Novel EISD Model (Expansion of Competing Risk Model) | No | Toth et al. (2013) |
| | Time-Dependent Dose-Response Model with Survival Analysis Model | No | Mayer et al. (2011) |
| Comprehensive Mechanistic | None to Date | N/A | None to Date |

EISD – Exposure – Infection – Symptomatic Illness – Death
N/A not applicable

Mechanistic models have been reported to exhibit greater validity than empirical models (Haas et al., 1999a). However, mechanistic models only consistently outperform empirical models to the extent that the mathematical and statistical assumptions correctly and sufficiently capture the modeled biological setting (Portier and Lyles, 1996). Not all mechanistic models are sufficiently rigorous relative to the actual biology that they can be reliably assumed to outperform empirical models, especially when there are insufficient data to support the development of a mechanistic model (Taft and Hines, 2012). There are also the twin concerns of scarce and uncertain mechanistic data. The lack of specific mechanistic data (i.e., quantitative impacts of dose-dependency, time dynamics of response) has been identified as a rationale for the selection of simpler models, including the exponential model (Toth et al., 2013). There is also the potential tradeoff from increasing the complexity of mechanistic models where any potential advantages of introducing more biological or mechanistic realism then has the potential "to be lost in a sea of statistical uncertainty" of the model outputs (Crump et al., 2010).

With regard to selection of models specifically to support risk-based decision-making, the term "mechanistic-enough" models has been coined to acknowledge that there is utility in models other than comprehensive mechanistic models for use in risk-based decision-making for microbial pathogens (U.S. Environmental Protection Agency, 2010b). Models only need to be sufficiently mechanistic to allow for confidence in the decisions made using its outputs (U.S. Environmental Protection Agency, 2010b). The lack of necessary mechanistic data for comprehensive mechanistic dose-response models for *B. anthracis* and a preference for parsimony in model selection will continue to favor the types of models currently in use: empirical models, nominally mechanistic models, and possibly limited mechanistic models.

### *5.5.3 Mathematically Modeling the Microbial Dose-Response Relationship*

Benchmark dose (BMD) analysis empirically fits models to dose-response data and identifies the dose associated with a specific response level for an identified endpoint (U.S. Environmental Protection Agency, 2012a). The dose-response models selected for evaluation must be appropriate based on the type of data. In the case of *B. anthracis*, the lethality endpoint will be used for dose-response analysis and is typically reported as a percentage or fraction of the individuals that die at a given dose. This allows for the use of dichotomous dose-response models (e.g., exponential, probit).

***Summary of Findings for Mathematically Modeling the Microbial Dose-Response Relationship***

- Benchmark dose analysis empirically fits models to dose-response data.
- A science policy gap for the use of benchmark dose analysis is guidance on the selection of the BMR and POD for a given data set.
- Selection of the BMR for *B. anthracis* is challenged by the reliance on lethality endpoints in most data sets.

BMD analysis is distinguished from other approaches for empirical curve fitting due to its clear terminology to describe the overall process and associated reporting of results. The POD is then generated based on an identified BMD that has associated with it a specified response level and has an identified lower limit of the BMD value at the specified response level. The discussion of mathematical modeling will focus on the BMD approach for empirical modeling of dose-response relationships because it adds necessary structure to the reporting of empirical modeling results.

Benchmark dose analysis estimates the dose, termed a BMD, for an identified response level, the benchmark dose-response (BMR) (U.S. Environmental Protection Agency, 2012a). The BMD is the model's best estimate of the dose that produces a response at the level of the BMR. A BMR of 10% would be equivalent to a 10% increase in the response rate of the endpoint of interest (i.e., extra risk) (U.S. Environmental Protection Agency, 2012a). Ideally, the response level of interest is within or near the lowest end of the observable range of the dose-response data set

(U.S. Environmental Protection Agency, 2012a). The POD is then determined using the identified BMR value. The POD is the dose-response point from where the low-dose extrapolation can be performed when necessary.

When modeling dichotomous data for chemical hazards, BMR values of 0.50, 0.10, and 0.01 are identified as standardized reporting values. When using a lethality endpoint, these values correspond to BMD estimates of 50% lethality (i.e., $LD_{50}$), 10% lethality, and 1% lethality, with the resulting BMDs written as $BMD_{50}$, $BMD_{10}$, and $BMD_{01}$, respectively. An identified BMR value, or a range of BMR values, specific for microbial data to support risk-informed decision-making from BMD outputs or for standardized reporting is not available. The lack of BMR guidance limits the ability to define a statistically-based POD from the fitted dose-response model.

However, the determination of the appropriate BMR values may require a unique evaluation relative to the values for chemical agents due to the reliance on lethality endpoints in *B. anthracis* dose-response data sets, high lethality levels associated with exposure levels of concern, and limited statistical power of most dose-response data sets. The selection of the BMR value is data-dependent, but also incorporates science policy determinations when setting the value. The identification of the BMR range of values or guidance for their selection is a science policy gap for microbial dose-response analysis.

When empirically fitting models, there are many methods to fit the models to a data set (e.g., methods of maximum likelihood, nonlinear least squares, and generalized estimating equations [GEE]) (U.S. Environmental Protection Agency, 2012a). U.S. Environmental Protection Agency (2012a) should be consulted for more details on how best to evaluate the method of fitting the

model. Empirical curve-fitting is appropriate for microbial dose-response analysis of empirical models, nominally mechanistic models, and some limited mechanistic models depending on the form of the model.

To capture the statistical variability associated with the calculated BMD value, the benchmark dose limit (BMDL) value identified. The BMDL is the 95% lower statistical confidence limit of the BMD when the 95% lower confidence limit is applied to the estimated slope parameter value (U.S. Environmental Protection Agency, 2012a). The BMDL is the lowest dose that is supportable from the modeling when the BMR is within or near the lower end of the observable range of dose-response data. The modeled BMDL values are then evaluated to select the POD(s) as a starting dose value for an interspecies or low-dose extrapolation (U.S. Environmental Protection Agency, 2012a).

Appendix F identifies the process to perform BMD, available software, and potential considerations when modeling dose-response relationships of microbial pathogens.

### 5.6 Conduct Interspecies Extrapolation

The purpose of the interspecies extrapolation process is to account for potential differences in kinetics and dynamics between the human and the animal models from which the dose-response data were obtained. Specifically, the POD is converted to a HED via this process. Table 5-19 identifies the key questions that must be assessed as part of the interspecies extrapolation process.

**Table 5-19. Conduct Interspecies Extrapolation**

| Steps in Microbial Dose-Response Analysis | Key Questions | Report Section |
|---|---|---|
| Conduct Interspecies Extrapolation to a HED (Section 5.6) | What is a general framework that can be used for interspecies extrapolation of *B. anthracis*? | Section 5.6.3. Proposed Framework for Interspecies Extrapolation for *B. anthracis* |
| | What data for the rabbit, nonhuman primate, and human are available to evaluate the kinetics and dynamics of *B. anthracis* pathogenesis? | Section 5.6.4 Available Kinetic Data<br><br>Section 5.6.5 Available Dynamic Data |
| | How can available data be incorporated in the extrapolation process? | Section 5.6.6 Summary of Extrapolation Framework for *B. anthracis* |

HED -- human equivalent dose

### *5.6.1 Review of Interspecies Extrapolation Approaches for Chemical Agents*

The interspecies extrapolation process estimates a HED by accounting for differences in response between the animal model and the human to the same level of external exposure. The HED is derived to have the same "magnitude of effect" as the POD of the animal model (U.S. Environmental Protection Agency, 2011c). Comprehensive guidance for interspecies extrapolation of chemical dose-response data is available and is routinely applied in the generation of toxicity values for chemicals with sufficient data. However, the development of microbial dose-response approaches to address interspecies extrapolation lags significantly behind that of the chemical agents.

The interspecies extrapolation process for microbial dose-response analysis lacks a framework, defined terminology, and published approaches to comprehensively describe an interspecies extrapolation process. The lack of accepted interspecies extrapolation approaches has been widely identified as a knowledge gap to be addressed (U.S. Environmental Protection Agency, 1994a.; International Life Sciences Institute (ILSI), 2000; U.S. Environmental Protection Agency, 2014c). Given the progress made for interspecies extrapolation of chemical dose-response analysis, these frameworks should be evaluated for applicability to microbial agents.

The interspecies extrapolation process for chemical agents identifies two factors that contribute to variability in response between the

***Summary of Findings for Conduct Interspecies Extrapolation***

- Interspecies extrapolation process for microbial dose-response analysis lacks a framework, defined terminology, and published frameworks.
- The interspecies extrapolation process for chemical agents is an appropriate starting framework for interspecies extrapolation process of biological agents.
- There are sufficient data and available approaches to conduct the dosimetric adjustment element of the interspecies extrapolation process for inhaled and deposited dose metrics.
- Knowledge gaps that currently limit the quantitative assessment of dynamic differences between the animal model and the human.

animal model and the human: kinetics and dynamics. Kinetics considers the dosimetry associated with the movement and transformation of the administered dose to an internal dose, whereas dynamics evaluates how differences in concentration at the identified target tissue may be associated with the same level of response in both the test animal and human (U.S. Environmental Protection Agency, 2014b). The common element of kinetics and dynamics is the focus on the internal dose: the factors determining the internal dose from an administered dose that dominate kinetics or the factors that define the response from a given internal dose level that are describing dynamics (U.S. Environmental Protection Agency, 2014b).

Kinetics is the "determination and quantification of the time course and dose-dependency of adsorption, distribution, metabolism, and excretion (ADME) of chemicals" (U.S. Environmental Protection Agency, 2014b). One adjustment for the kinetics of inhalation exposure is a categorical dosimetric adjustment factor (DAF) that explicitly considers differences (i.e., anatomical, physiological) between species, physical differences between particles and gases, and whether the toxicity is anticipated to be limited to the portal-of-entry or will have a systemic presentation (U.S. Environmental Protection Agency, 2014b). The process for development of reference concentration values detailed the derivation and application of DAF values (U.S. Environmental Protection Agency, 1994a).

Dynamics is the "determination and quantification of the sequence of cellular and molecular events leading to a toxic response" (U.S. Environmental Protection Agency, 2014b). Dynamics evaluates the interaction of the "biologically active chemical" with the target site and subsequent events that are associated with toxicity (U.S. Environmental Protection Agency, 2014b). The measure of the "biologically active chemical" at the target site is termed the internal dose. The internal dose should be the measurement at a specified tissue location that is most closely

associated with the response endpoint of (Jarabek et al., 2005). The evaluation of dynamics requires some level of mechanistic knowledge, including key events and mode of action leading to the toxicological endpoint of interest (U.S. Environmental Protection Agency, 2014b).

U.S. Environmental Protection Agency (2014b) identifies a hierarchy of extrapolation techniques to model kinetics and dynamics. The hierarchy ranges from data-intensive modeling to default values consisting of PBPK modeling, data-derived extrapolation factors (DDEF), and default factors (U.S. Environmental Protection Agency, 2014b). The recommended approach for extrapolation is based upon the availability of data and supporting models (U.S. Environmental Protection Agency, 2014b).

The least data-intensive approach is the use of default values, such as Uncertainty Factors (UF) (e.g., 10-fold UF values used in toxicity values for chemical hazards). The UF values are used when there are very limited or no chemical-specific data (U.S. Environmental Protection Agency, 2014b). The following UF values are identified: interspecies UF, intraspecies UF, lowest observable adverse effect level (LOAEL) to lowest observable adverse effect level (NOAEL) UF, and Database UF, and Subchronic to Chronic UF, with the recommendation that the total UF should not exceed 3,000 (U.S. Environmental Protection Agency, 2002). The UF values account for both uncertainty and variability (U.S. Environmental Protection Agency, 2014b). The maximum UF value is 10 (i.e., one order of magnitude), with a half-power value ($10^{0.5}$) of approximately 3. A UF factor of up to 10 is assigned to interspecies differences, with ½ of 10 (i.e., $10^{0.5}$) applied for interspecies kinetic differences and ½ of 10 (i.e., $10^{0.5}$) assigned for dynamics differences (U.S. Environmental Protection Agency, 2014b). The UF values were defined specifically for chemical agents, with evolution in their interpretation over time and data generated showing the values could be supported through evaluation of chemical-specific animal

and human data (Renwick, 1993). Renwick (1993) evaluated chemical-specific ADME data for the animal and human relative to the UF value of 10 and found the value to be generally appropriate for that element of an interspecies extrapolation. The use of the UF value of 10 combined for kinetics and dynamics for interspecies extrapolation has not been assessed for microbial pathogens.

The most data-intensive approach for extrapolation involves the use of PBPK modeling. U.S. Environmental Protection Agency (2014b) identifies this as the preferred approach if sufficient chemical-specific mechanistic data and models are available. The PBPK model is a type of compartment model that incorporates consideration of both tissue volume and blood flow information. Models are individually developed for the animal model and the human to predict internal doses and responses (U.S. Environmental Protection Agency, 2006).

The remaining method of extrapolation in the hierarchy is the use of DDEF values. A DDEF approach is based on two fundamental assumptions: (1) the endpoint of interest results from the interplay of kinetic and dynamic elements, and (2) relevant kinetic and dynamic elements can be quantified in animals and humans (U.S. Environmental Protection Agency, 2014b). In contrast to UFs, DDEF values address variability only (U.S. Environmental Protection Agency, 2014b). They may reduce uncertainty through the incorporation of chemical-specific data, but they do not explicitly include an uncertainty component (U.S. Environmental Protection Agency, 2014b). U.S. Environmental Protection Agency (2014b) identifies three forms of data necessary to derive the DDEF value: (1) mode of action, including key events through endpoint of interest and identification of “toxicologically active chemical species,” (2) target tissue, and (3) an appropriate dose metric for measurement of exposure (U.S. Environmental Protection Agency, 2014b). For chemicals with some kinetic and dynamic data, the DDEF values provide a data-

driven middle ground between comprehensive PBPK models and default approaches for extrapolation of chemical data.

### *5.6.2 Published Approaches for Interspecies Extrapolation of B. anthracis*

A partial interspecies extrapolation for *B. anthracis* was conducted using a "dosimetric adjustment" to evaluate species differences in inhalation and deposition for the nonhuman primate and the human (U.S. Environmental Protection Agency, 2010a). The "microbial equivalent of dynamics" was identified as a component of an interspecies extrapolation, but was noted to be beyond the scope of that particular assessment (U.S. Environmental Protection Agency, 2010a). The same dosimetric adjustment approach was later applied in the rabbit animal model using an average daily dose metric for the multiple-dose *B. anthracis* data set (U.S. Environmental Protection Agency, 2012b).

Stochastic mass balance modeling of inhalation and particle deposition rates was used to evaluate species differences between identified animal models (i.e., guinea pig, nonhuman primate) and the human for *B. anthracis* inhalation exposure as described in Weir and Haas (2011). An alternative approach for interspecies extrapolation of a different microbial pathogen, *Legionella* spp. was the preferential selection of animal models to maximize similarity for a subset of host immune responses in the human (Armstrong and Haas, 2007). Modeling results were then compared with human epidemiological data to evaluate model outputs suitability for the human (Armstrong and Haas, 2007). However, the extremely low incidence of human inhalation anthrax and lack of epidemiological data would preclude use of this approach for *B. anthracis*.

### *5.6.3 Proposed Framework for Interspecies Extrapolation for* B. anthracis

The interspecies extrapolation process for chemical agents is an appropriate starting framework to begin development of an interspecies extrapolation process for inhalation exposure to *B. anthracis* spores. This general framework is consistent with and will build upon the approach initially described in U.S. Environmental Protection Agency (2010a) and U.S. Environmental Protection Agency (2012b). These approaches incorporated EPA exposure assessment practices and some terminology from the interspecies extrapolation framework for chemical agents (e.g., dosimetric adjustment).

To address the dosimetric elements of kinetics, U.S. Environmental Protection Agency (2010a) and U.S. Environmental Protection Agency (2012b) evaluated inhalation rate and deposition rate to derive an internal dose for the animal model. This is equivalent to the use of the DAF described in U.S. Environmental Protection Agency (1994a) in guidance for the development of the inhalation reference concentration. Due to a lack of dynamic data, it was assumed that an equivalent internal dose was associated with the level of response in the animal model and the human. While this assumption was made to simplify the previous assessment, the potential to assess dynamics for *B. anthracis* requires further evaluation. For example, significant differences in species sensitivity were reported across a variety of animal models to intravenous challenge with anthrax toxin Lincoln et al. (1967). Additionally, population variation in cellular sensitivity to anthrax toxin was reported from *in vitro* studies of human cells (Martchenko et al., 2012). The key challenge will be sufficient mechanistic knowledge to quantitatively link these various measures to both dose and endpoints of interest.

For inhalation of *B. anthracis* spores, the internal dose evaluation, at a minimum, should consider both an inhaled dose and deposited dose(s) to the region(s) associated with initiation of infection. Though it was not explicitly stated, U.S. Environmental Protection Agency (2010a)

and U.S. Environmental Protection Agency (2012b) assumed that the *B. anthracis* spore is the biologically active form of the pathogen. It can be argued that the vegetative bacterium should be considered the biologically active form as the spore is not pathologically active until it germinates. However, the initial host-pathogen interaction takes place between the spore and the host tissue (e.g., phagocyte, epithelial cell, lymphoid tissue). It is this first contact that is the opportunity point for the spore to germinate or to lose viability based on the action of the host immune system (e.g., phagocytosis by macrophage).

For this proposed framework, an initial point of delineation between kinetic and dynamic processes is the interface of the spore and the environment associated with initiation of infection. Assumptions must be made regarding the host tissue most closely associated with initiation of infection to select appropriate dose metrics for dose-response relationship development and the interspecies extrapolation. It was identified in Section 0 that multiple-dose metrics should be evaluated for the development of dose-response relationships. If an internal dose was not used as part of the dose-response modeling, it is reasonable to assess multiple internal doses as part of the interspecies extrapolation process to see if there is a substantial difference in outputs.

For microbial dose-response analysis of *B. anthracis* spores, the kinetics process can be described in two parts. The first part represents host contact with the administered dose (e.g., air concentration) or delivered dose (e.g., inhaled dose) through the spore transport to the target internal tissue where germination may first take place. However, U.S. Environmental Protection Agency (2012b) notes the challenge in a clear delineation between kinetics and dynamics because of the interplay between the two processes. Given this reasoning, a second conceivable kinetics element for interspecies differences might be the proliferation rate of vegetative bacteria in the blood based on recently reported species differences among the human, nonhuman

primate, and rabbit for spore germination and vegetative proliferation rates (Bensman et al., 2012).

To address the dynamic elements for the interspecies extrapolation, the interaction between the host and the biologically active *B. anthracis* form must be described through the key events leading to the endpoint of the assessment (U.S. Environmental Protection Agency, 2014b). There must be sufficient data on the key events and quantitative mechanistic information to link them with internal dose and the endpoint (U.S. Environmental Protection Agency, 2014b). The dynamic element of the extrapolation may be appropriately modeled with BBDR or other dynamic models (U.S. Environmental Protection Agency, 2014b). This is the area of greatest challenge for the development of a microbial interspecies extrapolation process. For *B. anthracis*, there is currently an insufficient mechanistic understanding of the key events from initiation of infection through bacteremia and toxemia to conduct a full dynamic evaluation. However, it may be possible to begin to evaluate initial host-pathogen interactions to develop a better understanding of dynamics associated with initiation of infection as a starting point for species differences.

There are elements of the hierarchy used with chemical dose-response analysis that are difficult to implement with microbial dose-response data. It would entail considerable effort to develop comprehensive default values (e.g., UF values), which may not be appropriate across the diverse group of microbial pathogens of interest. There would be considerable effort associated with the development of UF values for microbial pathogens as there is not an equivalent set of data for microbial pathogens relative to chemical agents to support selection of UF values. There is a lack of general data describing variability in response for microbial pathogens as a group, and *B. anthracis* specifically, to support development of interspecies and intraspecies UF values. The

concept of uncertainty has not been considered outside of general qualitative statements. It is unknown if a pathogen-wide default value is biologically appropriate given potential differences among pathogens. The initial dose-response modeling approach to use BMD models in lieu of identification of NOAEL and LOAEL values negates the use of that UF value. The adjustment for subchronic studies that is applied for chronic values also does not have available the same body of data that was used for chemical dose-response (i.e., the vast majority of *B. anthracis* challenge studies are single-dose).

### *5.6.4 Available Kinetic Data*

The two categories of kinetics data relevant for inhalation of *B. anthracis* spores are inhalation rate and deposition rate. Most currently performed animal challenge studies with *B. anthracis* use plethysmographic data to determine the inhalation rate (e.g., volume/time) during the challenge study. However, care should be taken if allometric equations are used to derive the animal model inhalation rate if plethysmographic data are not available. When allometric equations are used to estimate minute volume, they do not consider the physiological state of the test animal (e.g., stress, tranquilizers) and may not accurately reflect the actual inhalation rate during the challenge (Taft and Hines, 2012). However, human inhalation data for a variety of activity levels are readily available from the *Exposure Factors Handbook* (U.S. Environmental Protection Agency, 2011b) and will not be further considered here.

#### *5.6.4.1 Experimental Sources of Deposition Data*

For the rabbit, published particle deposition data and modeled data are available describing whole or lung region-specific values (Raabe et al., 1988; Gutting et al., 2012; Gutting et al., 2013) (Table 5-20). However, the reliability and precision of the measurement techniques raise potential issues for their application in modeling. Potential biases in measurement approaches are

described in Table 5-20. For example, historical data derived from inhalation of radiological aerosols is compromised by the lack of real-time inhalation data to estimate dose (Raabe et al., 1988) and bronchoalveolar lavage may undercount deposited particles due to potential translocation within the lungs (Gutting et al., 2012).

**Table 5-20. Summary Table of *B. anthracis* Deposition Data for the Rabbit**

| Study | Reported Value | Measurement | Potential Bias |
|---|---|---|---|
| Gutting et al. (2013) | Pooled value of 4.63% from two data set values: 4.33% (±2.2%) and 4.93 % (±0.8%), represents whole lung deposition | Homogenization of New Zealand white rabbit lung tissue and extrapolation to the whole lung after inhalation exposure to *B. anthracis* spores, particle size MMAD[a] of 1.0 µm ± 0.3 µm. | Potential for underestimation of deposition if epithelial cell internalization of deposited particles is rapid, see Jenkins and Xu (2013) data for mouse animal model. |
| Gutting et al. (2012) | 3.07% ± 0.9% and 1.33% ± 0.2%, represents whole lung deposition | Bronchoalveolar lavage to wash out deposited *B. anthracis* spores in New Zealand white rabbit, particle size MMAD of 1.0 µm ± 0.3 µm. | Deposited doses reported from bronchoalveolar lavage may be biased low if inability to wash out all deposited spores or rapid transport across epithelial cell lining takes place (Gutting et al., 2012). |
| Raabe et al. (1988)[a] | Ranging from 6.6 ± 0.6 % at 0.97 µm to 1.1 ± 0.2 % at 4.86 µm,[a] pulmonary deposition only | Measurement of deposition to pulmonary region of the rabbit after inhalation of monodisperse $^{169}$Yb aluminosilicate aerosol with aerodynamic resistance diameters of particles ranging from 0.18 to 8.65 µm. | Use of Guyton's formula to estimate minute volume for calculation of deposition would bias results if actual animal inhalation rate differed (Raabe et al., 1988). |

MMAD – Mass Median Aerodynamic Diameter

[a] Raabe et al. (1988) data were the basis for U.S. EPA's RDDR model as described in U.S. Environmental Protection Agency (1994b)

#### *5.6.4.2 RDDR Modeling*

The EPA's Regionally Deposited Dose Ratio (RDDR) model (U.S. Environmental Protection Agency, 1994b) provides estimates for the fractional regional depositional efficiency in the lung for inhalation of particulates for laboratory animal species and the human (U.S. Environmental Protection Agency, 1994a). The model output, the RDDR, is the "ratio of the deposited dose in a respiratory tract region (r) for the laboratory animal species of interest ($RDD_A$) to that of humans ($RDD_H$)" (U.S. Environmental Protection Agency, 1994a). This ratio can be used as a DAF for

the kinetics portion of an interspecies extrapolation (U.S. Environmental Protection Agency, 1994a). At a minimum, the inputs include the particle air concentration, the Mass Median Aerodynamic Diameter (MMAD) and geometric standard deviation (GSD) value for the particle distribution, and the animal model body weight from the challenge study. One caveat to the use of the RDDR model is that the animal deposition modeling incorporates data from Raabe et al. (1988), which relied on an allometric equation to determine the inhalation rate necessary to determine deposition.

However, the RDDR model can be used with supplied inhalation rate data (e.g., plethysmographic data) to generate regional surface area ($SA_r$) and regional fraction deposition ($F_r$) values specific to the lung region of interest. If this change is not made, allometric body weight equations will be used in the model to generate the minute volume ($V_e$). For distributions of particle sizes with a known MMAD and GSD, one advantage of the RDDR software is that the software scales the $F_r$ value to the specified $V_e$ (U.S. Environmental Protection Agency, 1994a).

The equations of the RDDR model can be used with study-specific data to generate a type of dosimetric adjustment factor, the RDDR value. Figure 5-3 shows the calculation of the RDDR DAF that can be used to account for interspecies differences in inhalation and deposition for inhaled particles. As shown in Figure 5-3, the DAF can be multiplied by the POD from the animal study to derive a HED that accounts for interspecies differences in inhalation and deposition.

$$\mathbf{RDDR} = \frac{\left(\frac{\mathbf{V_e}}{\mathbf{SA_r}} \times \mathbf{F_r}\right)_{\mathbf{animal}}}{\left(\frac{\mathbf{V_e}}{\mathbf{SA_r}} \times \mathbf{F_r}\right)_{\mathbf{human}}}$$

**Dosimetric Adjusted Dose for Human: POD × RDDR**

**Where:**

**POD = Point of Departure from animal study data**

**RDDR = Regional Deposited Dose Ratio of the animal to the human**
**(Also termed a dosimetric adjustment factor [DAF])**

$\mathbf{V_e} = \mathbf{Minute\ Volume\ (mL)}$

$\mathbf{SA_r} = \mathbf{Surface\ Area\ of\ Region_r\ (cm^2)}$

$\mathbf{F_r} = \mathbf{Fractional\ Deposition\ of\ Region_r\ (Unitless)}$

**Figure 5-3. Calculation of an RDDR-based dosimetric adjustment factor.**

#### *5.6.4.3 CFD Modeling*

Due to advances in computational modeling, particulate deposition models for the lung have become highly developed (Kleinstreuer et al., 2008) and provide the ability to track patterns of deposition through the pulmonary system as a function of the morphology, breathing parameters, and particle characteristics. Building on these advances, Kabilan et al. (2015) developed the first particle deposition model using physiologically realistic, image-based 3D airway geometries of the human and rabbit with computational fluid dynamics (CFD) airflow modeling coupled with Lagrangian particle tracking methods. The CFD model was developed using particle size distributions, concentrations, and rabbit plethysmographic data from the EPA single-dose challenge study for the rabbit (U.S. Environmental Protection Agency, 2011a). The CFD model predicts the inhalation and deposition of *B. anthracis* spores during transient breathing. Table

5-21, originally from Kabilan et al. (2015), reports the modeled deposition efficiencies for the respiratory tract regions in the rabbit and the human. The modeled deposition values for the deep lung are considerably higher for both the rabbit and the human than previous deposition measurements or modeled results; further corroboration may be appropriate prior to use.

**Table 5-21. Deposition Efficiencies for Different Annotated Regions in the Rabbit and the Human**

| Modeling Case | MMAD (μm) | Concentration (Spores/$m^3$) | Location | % Deposition Based on Exposure | |
|---|---|---|---|---|---|
| | | | | Rabbit | Human |
| Case 1 | 1.12 | 3.97E+11 | Nose | 12.61 | 3.21 |
| | | | Pharynx | 0.03 | 0.12 |
| | | | Larynx | 0.13 | 0.33 |
| | | | Trachea | 0.07 | 0.01 |
| | | | Bronchi & Bronchioles | 1.44 | 5.70 |
| | | | Deep Lung | 54.34 | 62.08 |
| Case 2 | 0.92 | 1.18E+08 | Nose | 7.05 | - |
| | | | Pharynx | 0.01 | - |
| | | | Larynx | 0.16 | - |
| | | | Trachea | 0.06 | - |
| | | | Bronchi & Bronchioles | 1.49 | - |
| | | | Deep Lung | 58.94 | - |

*The total particle deposition for the rabbit and the human was 68.62% and 71.45%, respectively for Case 1.

### *5.6.5 Available Dynamic Data*

Though the pathophysiology of anthrax in the human has been deemed "well characterized" for over one hundred years (Ioannidis, 2012), these data were not generated to mechanistically describe the origin and magnitude of potential response differences between the test animal and the human. Currently, there are insufficient mechanistic knowledge and associated modeling approaches to assess dynamic contributions to potential interspecies differences. The evaluation of dynamics requires mechanistic knowledge of key events associated with the host-pathogen interactions at a quantitative level and in association with internal dose. *B. anthracis* is not unique in lacking these data, as sufficiently detailed mechanistic knowledge for an interspecies

extrapolation is likely lacking for most if not all microbial pathogens for which microbial risk assessment is conducted. Given these data challenges, it is not recommended that a generic default value be developed for use.

As a first step in developing a framework for dynamics of *B. anthracis* response, a conceptual mapping of contributors to potential species differences in response should be conducted. Though their approach employed a qualitative evaluation for *Legionella* spp., Armstrong and Haas (2007) described a systematic approach to compare early immune system response between an animal model and the human. The initiation of infection of *Legionella* spp. is associated with inhalation and uptake by the alveolar macrophage. Armstrong and Haas (2007) identified mechanisms that could be associated with species differences (e.g., macrophage uptake and replication, macrophage "bactericidal mechanism responses") and compared responses for the human and guinea pig. However, Armstrong and Haas (2007) used the assessment qualitatively to determine sufficient similarity between the animal model and the human. This approach could be easily applied to *B. anthracis* to map potential host-pathogen interactions with the goal of identifying potential contributors to species differences in response and gathering of potentially relevant data. The ultimate goal would be development of a quantitative assessment factor.

### *5.6.6 Summary of Extrapolation Framework for* B. anthracis

An interspecies extrapolation framework that considers both kinetic and dynamic elements as potential contributors to species differences is a viable approach for microbial pathogens, including *B. anthracis*. For the kinetics element of the process, the dosimetric adjustment process for assessment previously described in U.S. Environmental Protection Agency (2010a) and U.S. Environmental Protection Agency (2012b) provides a good starting foundation. The availability of new CFD data (Kabilan et al., 2015) modeled with the U.S. Environmental Protection Agency

(2010a) adds the knowledge base for deposition of spore particles in the rabbit. The dosimetric adjustment factor equation (Figure 5-3) as used in the RDDR model provides a mathematical approach that can use currently available data for general species-specific elements (e.g., particle deposition) and study-specific data (e.g., animal-specific inhalation rate during the challenge) to conduct the kinetics portion of the interspecies extrapolation.

However, there are knowledge gaps that currently limit the quantitative assessment of dynamic differences between the animal model and the human. One starting recommendation is to map host-pathogen interactions associated with initiation of infection for *B. anthracis* with the goal of identifying potential contributors to species differences in response. Available data can then be evaluated relative to the sufficiency to quantitatively evaluate species differences in the context of key events and endpoints of interest.

# 6 Conclusion

The primary purpose of this report is to provide open source data and analysis approaches that can be used to develop a site-specific HHRA for *B. anthracis*. The report presents the results of an agent-specific planning activity for *B. anthracis* that evaluated published dose-response data, identified data and process gaps for microbial dose-response analysis of the agent, and identified science policy decisions that may be necessary to conduct a HHRA for this agent. The results of the report are summarized by answering the science questions posed in Section 2 as shown in Figure 6-1.

- *What natural history data for B. anthracis are available to inform development of a site-specific CSM for the identified exposure scenario?*

Source materials associated with potential exposure to *B. anthracis* spores include contaminated animal products, cross-contamination of materials by contaminated animal products, or manufactured spore products that are intentionally or unintentionally released. With the exception of the deliberate release of manufactured spores, anthrax illness is relatively rare in developed countries and most often results from contact with infected animals or contaminated animal products (Passalacqua and Bergman, 2006). Published reports of anthrax infection support the potential for the released *B. anthracis* spores to result in inhalation, ingestion, and dermal exposure with potential disease transmission associated with these routes of exposure. Inhalation anthrax is associated with severe life-threatening illness and a quantitative HHRA could be developed with existing data. However, there is the potential for high levels of uncertainty associated with the quantitative HHRA outputs from limitations in dose-response data. The ingestion and dermal pathways are also likely to be complete but there are insufficient

data to conduct a quantitative HHRA. As a result, a qualitative assessment is recommended for these exposure pathways.

The available natural history data are sufficient to generate a site-specific CSM with regard to identification of potential sources of *B. anthracis* exposure, fate and transport mechanisms, potential exposure pathways, the likelihood of completed exposure pathways, and the ability to perform a quantitative or qualitative assessment.

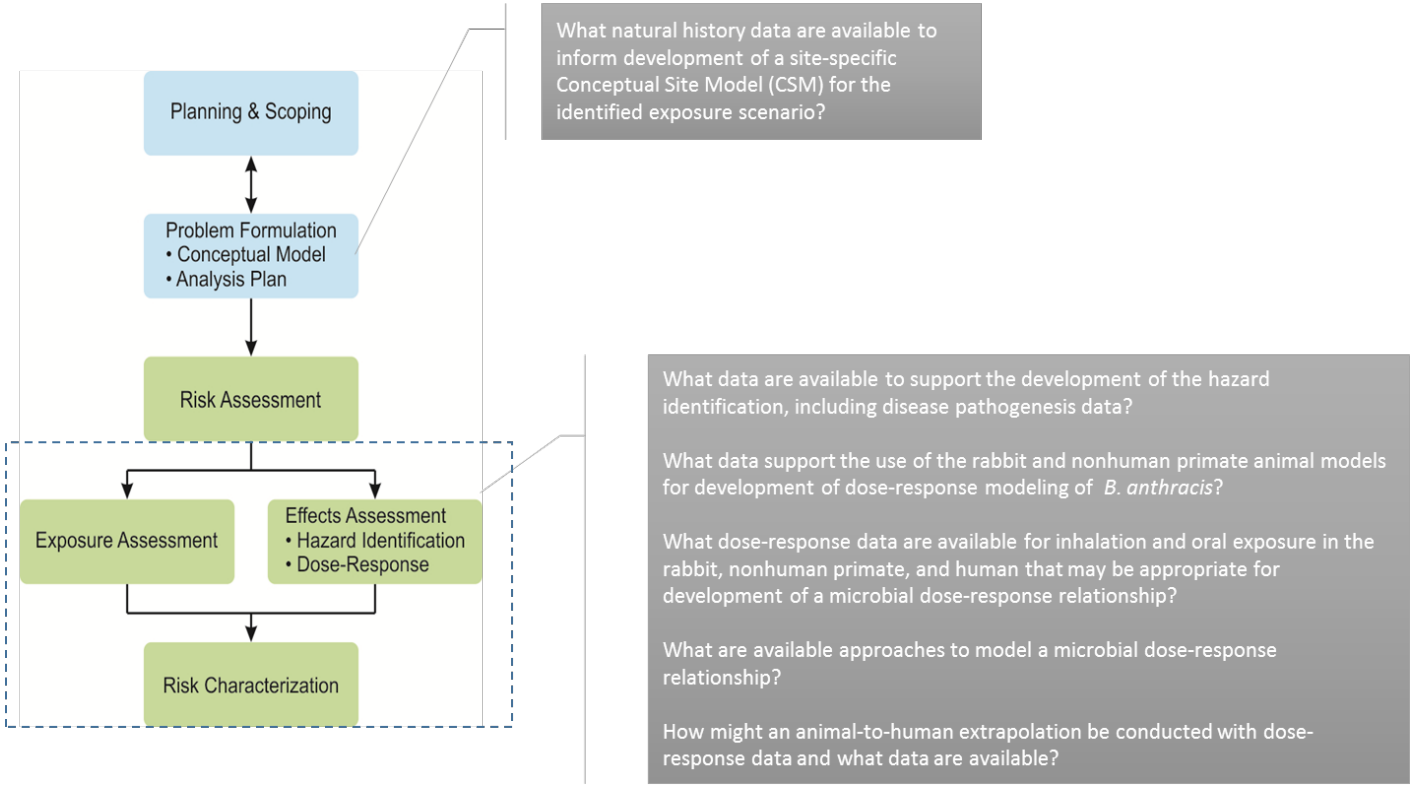


**Figure 6-1. Science questions and associated elements of the U.S. Environmental Protection Agency (2014a) human health risk assessment framework.**

- *What data are available to support the development of the hazard identification, including disease pathogenesis data?*

The hazard posed by exposure to *B. anthracis* spores is documented by published reports, including the transmission of inhalation anthrax from contaminated animal products or the intentional or accidental release of spores. Though each type of anthrax illness can progress to a fulminant infection, inhalation anthrax poses the greatest threat of lethality because it is difficult to diagnose during early stages of illness and becomes rapidly lethal after development of severe symptoms (Inglesby et al., 2002). Even with modern medical treatment and early diagnosis, the case fatality rate of those with inhalation anthrax during the 2001 anthrax letter event was 45% (Inglesby et al., 2002). However, the fatality rate is generally estimated to be almost twice as high without antibiotics or intensive medical treatment (Inglesby et al., 2002; Hilmas et al., 2009).

The disease pathogenesis process for inhalation anthrax is well described relative to key events. However, there is still considerable uncertainty in the mechanistic details of the disease process. There is not a clear link between mechanistic pathway(s) or tissue dose(s) associated with the lethality endpoint. There is also uncertainty regarding the mechanistic process for the initiation of the infection. There are two models that currently describe the initiation of infection using slightly different assumptions regarding the role of identified tissues and *B. anthracis* toxin in the initial stages of infection: the Trojan horse model of Guidi-Rontani (2002) and the jailbreak model of Weiner and Glomski (2012). Knowledge of the pathway(s) by which infection is initiated is critical for many aspects of the dose-response modeling process.

There are sufficient natural history data to generate the hazard identification element of an HHRA. However, there are significant data gaps associated with disease pathogenesis knowledge. This uncertainty has ramifications for multiple areas in the HHRA including the selection of dose metric(s) for generation of dose-response relationships and the interspecies extrapolation process.

- *What data support the use of the rabbit and nonhuman primate animal models for development of dose-response modeling of* B. anthracis*?*

Animal model suitability for development of a *B. anthracis* dose-response relationship was determined by an assessment of general concordance in anthrax pathology between the human and the rabbit and nonhuman primate animal models. Twenhafel (2010) evaluated human pathology data from Sverdlovsk (Abramova et al., 1993; Grinberg et al., 2001) and the 2001 anthrax letter event (Jernigan et al., 2001) to generate the list of key human pathological findings. The Twenhafel (2010) list was used to assess anthrax pathology of the rabbit and nonhuman primate relative to that of the human.

The rabbit and nonhuman primate exhibit many commonalities in the type of lesions and tissues associated with inhalation anthrax pathology in the human. The principal anthrax lesions of edema, hemorrhage, and necrosis are present in a variety of common tissues in the rabbit, nonhuman primate, and human. However, this constellation of pathology is generally consistent with descriptions of animal models susceptible to fulminant inhalation anthrax infection (Gleiser et al., 1963) and is not unique to the rabbit and nonhuman primate animal models. Lesion differences among susceptible animals are manifested by differing levels of inflammation and infiltration of leukocytic elements into existing lesions (U.S. Food and Drug Administration,

2002), whereby less susceptible animals exhibit greater inflammation and leukocytic infiltration than more susceptible animals, which rapidly succumb to illness.

There were no identified differences between the rabbit and the nonhuman primate animal models for elements of anthrax pathology that do not have a time-dependency for incidence or severity in presentation. However, there are preliminary indications that time-dependency may be contributing to the identified differences in pathology.

The results of this pathology assessment support the continued use of the rabbit and nonhuman primate animal models for development of dose-response data for *B. anthracis*.

- *What dose-response data are available for inhalation and oral exposure in the rabbit, nonhuman primate, and human that may be appropriate for development of a microbial dose-response relationship for* B. anthracis*?*

Dose-response data were categorized into three categories: Key Data, Supporting Data, and Additional Data. Key Studies were defined as representative of the highest quality dose-response studies that met criteria for selection during the literature search. Supporting Studies had identifiable limitations in assessment quality indicators relative to Key Studies, yet were found to have potential in bounding the potential dose-response relationship(s) as described by Key Studies. Additional Data were defined by the lack of data critical to assessing dose-response relationships (e.g., original dose and response data set) or study design elements that limit utility for development of low-dose dose-response relationships.

A literature search was conducted for the inhalation route of exposure for each animal model and dose-response data were categorized. Few inhalation challenge studies were identified as Key Studies for the rabbit and nonhuman primate; there were no Key Studies or Supporting Studies

identified for the human. The two Key Studies for the rabbit were the single dose U.S. Environmental Protection Agency (2011a) study and the multiple dose U.S. Environmental Protection Agency (2012b) study. No studies were categorized as Supporting Studies. For the nonhuman primate, one single dose Key Study (Lever et al., 2008) and one single dose Supporting Study (Druett et al., 1953) were identified.

One area of particular concern is the limited number single or multiple dose challenge studies using low doses. Most animal dose-response data identified through the literature search originated from single dose studies at very high doses, sometimes as high as 200 times an identified $LD_{50}$ value. Single high-dose studies have limited value for the assessment of repeated low-dose exposure (U.S. Environmental Protection Agency, 2012c). Few studies that reported dose-response data were designed to derive data for dose-response analysis. Study purposes for recent data sets included evaluation of the pathology, pathophysiology, or assessment of the efficacy of medical countermeasures. These studies were often conducted using a single high-dose challenge to ensure a high likelihood of systemic anthrax infection in the challenge animals. Historical data were often developed to report an $LD_{50}$ value for use in military applications or early anthrax research with little representation of low doses.

Dose-response data are available for the rabbit and nonhuman primate that may be suitable for development of a human dose-response relationship. However, the uncertainty associated with the use of these data may be high and is associated with a lack of corroborative data to increase confidence in their use. Depending on the level of acceptable uncertainty in the analysis outputs, there may be limitations on how these data may be used in decision-making. There may be value in conducting additional dose-response challenge studies that are designed with appropriate

statistical power for modeling and gather necessary data to inform the animal-to-human extrapolation process.

- *What are available approaches to model a microbial dose-response relationship for* B. anthracis*?*

### *Empirical and Mechanistic Models*

Empirical and mechanistic models have been used for microbial dose-response modeling of *B. anthracis*. To aid in model evaluation, a hierarchy of mechanistic models was proposed to describe the relative level of biological representation and complexity in the models. The simplest models are nominally mechanistic models that incorporate simple biological representations, but biological measurements or modeling cannot inform parameter values. All parameters are estimated empirically. Limited mechanistic models are the next level of model; they incorporate mechanistic assumptions and data that can be derived or informed by biological measurements. The most complex models are comprehensive mechanistic models that incorporate mechanistic assumptions and data to fully describe biodynamic and biokinetic elements. The lack of necessary mechanistic data for comprehensive mechanistic dose-response models for *B. anthracis* and a preference for parsimony in model selection (i.e., models with as few parameters as necessary) will lead to the continued use of empirical models and limited or nominally mechanistic models.

### *Determination of Dose Metric and Other Modeling Assumptions*

A dose metric is the mathematical description of the challenge study dose that is used to model the dose-response relationship and conduct the interspecies extrapolation. The preferred dose metric is the internal dose that can be most closely mechanistically or otherwise correlated with

the biological endpoint of interest (Jarabek et al., 2005). A dose metric is associated with a specified exposure duration and can also be expressed as a time-normalized measurement (e.g., CFU/day) (U.S. Environmental Protection Agency, 2014b).

The selection of dose metrics for multiple-dose exposure of *B. anthracis* introduces questions regarding the time duration to which the dose should be applied. The U.S. Environmental Protection Agency (2012b) multiple-dose study reported dose-response relationship evaluations using two dose metrics: accumulated inhaled dose and average daily inhaled dose. An accumulated dose metric assumes an equivalent hazard whether the intake is in the form of one dose or in many doses over that same time (Mayer et al., 2011). The independent action hypothesis may have relevance for the determination of dose metrics for multiple-dose *B. anthracis* exposure studies (U.S. Environmental Protection Agency, 2014d). Potential dependencies by time, dose, or route of exposure may affect consistency with the independent action hypothesis. The magnitude of exposure or exposure duration (Mayer et al., 2011; U.S. Environmental Protection Agency, 2014d) where independent doses can be delineated from dependent doses have not been explicitly evaluated to date.

Though there is uncertainty in the identification of the most appropriate dose metric, this should not limit the evaluation of dose-response relationships. Relevant dose metrics should be identified and a justification provided for those that are evaluated. With regard to selection of the regional deposition location(s) for the deposited dose, multiple-dose metrics can be evaluated. Given that the differences in deposition may be small relative to other components of the inhalation dose calculation, the actual difference in the modeled dose-response relationship may be of limited magnitude. The documentation for the dose-response relationship should include a transparent identification of the basis for selection of the dose metric(s) considered. There should

also be a qualitative discussion of the uncertainty associated with the dose metric selection in the risk characterization element of the risk assessment.

***Benchmark Dose Modeling***

Benchmark dose modeling can be used to fit dose-response data to mathematical models. However, one science policy gap in the use of BMD for microbial pathogens is the lack of guidance on the selection of a BMR for microbial data. The determination of a BMR should be based upon the intended use of the BMD outputs, the statistical features of the data set, and biological basis of the modeled disease process (U.S. Environmental Protection Agency, 2012a). A BMR value (or range of BMR values) to standardize reporting or to support BMD decision-making using microbial data is not available. However, the determination of a suggested range of appropriate BMR values may require a unique evaluation relative to the values used for chemical agents. This is due to the reliance on lethality endpoints in *B. anthracis* dose-response data sets, high lethality levels associated with exposure levels of concern, and limited statistical power of most dose-response data sets.

- *How might an animal-to-human extrapolation be conducted with* B. anthracis *dose-response data and what data are available?*

The interspecies extrapolation process is designed to account for differences between the animal model and the human that could affect the human response to environmental exposures. However, the development of microbial dose-response approaches to address interspecies extrapolation lags significantly behind that of chemical dose-response analysis. The interspecies extrapolation process for microbial dose-response analysis lacks a framework, defined terminology, and published approaches that comprehensively describe an interspecies

extrapolation process. Using the interspecies extrapolation process for chemical agents as a starting framework, an interspecies extrapolation framework that considers both kinetic and dynamic elements as potential contributors to species differences should be a viable approach for microbial pathogens, including *B. anthracis*. The use of a dosimetric adjustment process to assess the initial elements of kinetics for *B. anthracis* has been described previously in U.S. Environmental Protection Agency (2010a) and U.S. Environmental Protection Agency (2012b). There are sufficient data and available approaches to conduct the dosimetric adjustment element of the interspecies extrapolation process for inhaled and deposited dose metrics.

However, there are knowledge gaps that currently limit the quantitative assessment of dynamic differences between the animal model and the human. One starting recommendation is to map host-pathogen interactions associated with initiation of infection for *B. anthracis* with the goal of identifying potential contributors to species differences in response. Available data can then be evaluated relative to the potential to quantitatively evaluate species differences in the context of key events and associated endpoints. While there do not appear to be sufficient mechanistic knowledge and quantitative data to fully evaluate dynamic elements of the extrapolation at present, the approach should be increasingly attainable over time with continued evaluation and directed data generation.

*Summary*

A considerable body of knowledge is now available for the development of a site-specific HHRA for *B. anthracis*. There are sufficient data to develop the CSM, generate the hazard identification, data and methods to generate a dose-response relationship for *B. anthracis*, and conduct a partial interspecies extrapolation. While there are sufficient data to generate a quantitative HHRA, data

quality and the presence of data gaps may contribute to potentially high levels of uncertainty in the risk assessment outputs. Depending on the intended use of the risk assessment outputs, these data may not be acceptable for all types of risk-based decision-making. Microbial risk assessors who are assisting in the initial planning and scoping element of the HHRA should take care to communicate these potential data limitations to decision-makers early in the process.

Table 6-1 summarizes the identified data gaps and science policy gaps by risk assessment element. The most significant data gap relates to the lack of high quality dose-response data, defined as possessing sufficient quality to be categorized as Key Data. This clearly affects the rigor of the risk assessment. An additional data gap is the lack of basic mechanistic data for the initiation of infection and dynamics of the early infection process. These mechanistic data would greatly assist in the confirmation of appropriate dose metrics and inform the interspecies extrapolation process. However, alternative dose metrics can be assessed for substantive differences in outputs and the interspecies extrapolation process can be conducted in part to address kinetic elements.

Science policy gaps also affect current readiness to generate a site-specific HHRA for *B. anthracis* inhalation exposure. The selection of appropriate BMR targets for reporting and risk-based decision-making for microbial pathogens is a current policy gap. While technical knowledge may inform BMR selection relative to known data set characteristics for BMD modeling, selection of values for reporting and risk-based decision-making may incorporate numerous policy considerations. An additional science policy gap is the management of uncertainty in the interspecies extrapolation given the current inability to address dynamic differences between the animal model and the human. In addition to a statement of this

uncertainty in the risk characterization, a default adjustment factor could be considered for use until further data or methodologies are available.

**Table 6-1. Summary Table for Data Gaps and Science Policy Gaps**

| Use of Microbial Dose-Response Data | Data Gaps | Science Policy Gaps |
|---|---|---|
| Hazard Identification, including Disease Pathogenesis | • Identification of BMR values or ranges<br>• Mechanistic data for the initiation of infection and dynamics of the early infection process necessary for dose metric selection | |
| Evaluation of Microbial Dose-Response Data | • High quality dose-response data for the rabbit and nonhuman primate<br>• Mechanistic data for the initiation of infection and dynamics of the early infection process necessary for dose metric selection | • Identification of BMR values or ranges to select POD for microbial pathogens |
| Conduct Interspecies Extrapolation | • Lack of data to support inter-species and intra-species UF values | • Management of uncertainty in the interspecies extrapolation given the current inability to address dynamic differences between the animal model and the human |

BMR – benchmark dose response
POD – point of departure
UF – uncertainty factor

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**Report:** Review of *Bacillus anthracis* Dose-Response Data for Human Health Risk Assessment
**(Rev. 3 Draft, October 2015)**

**Appendices**

# Appendix A - Transmission and Pathogenesis Considerations for Biological Threat Agents

## Introduction

Interest in the development of microbial dose-response relationships for biological threat agents (BTA[s]) is currently high (U.S. Department of Homeland Security and U.S. Environmental Protection Agency, 2009). The BTAs are a group of microbial pathogens that are capable of producing significant illness, death, or incapacitation in people or animals when they are released in a manner to facilitate specific types of exposure. Numerous dose-response relationships for individual BTAs have been published (Haas, 2002; Bartrand et al., 2008; Weir and Haas, 2009; Tamrakar et al., 2011; Teske et al., 2011; Weir and Haas, 2011; Taft and Hines, 2012). However, further progress is challenged by the lack of an overarching methodology for microbial dose-response analysis or alternatively, a dose-response modeling methodology specifically developed to facilitate progress for the BTA group.

Current microbial risk assessment protocols, frameworks, or other publications have identified transmission and pathogenesis considerations recommended for inclusion in microbial risk assessment and dose-response modeling (Haas et al., 1999a; International Life Sciences Institute [ILSI], 2000; Food and Agriculture Organization and World Health Organization [FAO and WHO], 2003; Parkin, 2008; Interagency Microbiological Risk Assessment Guideline Workgroup, 2011; U.S. Environmental Protection Agency, 2014).

The transmission and pathogenesis considerations that have been identified include:

- secondary transmission (Parkin, 2008; Interagency Microbiological Risk Assessment Guideline Workgroup, 2011; U.S. Environmental Protection Agency, 2014),
- propagation of the pathogen in the host (Haas et al., 1999a; Interagency Microbiological Risk Assessment Guideline Workgroup, 2011; U.S. Environmental Protection Agency, 2014),
- immunity and susceptibility of the exposed population (Parkin, 2008; Interagency Microbiological Risk Assessment Guideline Workgroup, 2011; U.S. Environmental Protection Agency, 2014),
- use of threshold versus non-threshold models (International Life Sciences Institute [ILSI], 2000; Food and Agriculture Organization and World Health Organization [FAO and WHO], 2003; Interagency Microbiological Risk Assessment Guideline Workgroup, 2011), and
- potential variation in virulence exhibited by individual strains, variants, or isolates (International Life Sciences Institute [ILSI], 2000; Parkin, 2008; Interagency Microbiological Risk Assessment Guideline Workgroup, 2011).

Addressing these transmission and pathogenesis considerations in a manner suitable for all microbial pathogens and across potential end uses of the risk assessment outputs represents a significant technical challenge. Microbial risk assessment frameworks, including Haas et al. (1999b) and the International Life Sciences Institute [ILSI] (2000), have been available for 15 years. The difficulty in addressing these considerations may help to explain the relatively slow

progress in the development of microbial dose-response methodologies even though microbial risk frameworks have been available for over 10 years.

Microbial pathogens are recognized to exhibit significant diversity in transmission and pathogenesis characteristics. However, little attention has been focused on the collective identification of BTAs that was initially based on a unique assemblage of transmission and pathogenesis characteristics. The characteristics provide an intentional aerosol release of agent to drive atypical routes of transmission relative to natural disease transmission (i.e., inhalation) and greater severity of outcomes than typical natural routes of exposure (Roy et al., 2010). More recently, changes in the desired end use of BTA dose-response relationship data also introduces unique elements into the microbial dose-response analysis for these pathogens relative to traditional pathogens. For example, dose-response relationships for BTAs may be used in the development of clearance goals after an intentional or accidental release (U.S. Department of Homeland Security and U.S. Environmental Protection Agency, 2009). This development of clearance goals could drive the need for low-dose evaluations either on the outer boundaries of the exposure areas or in areas where remedial technologies have been applied and residual levels may remain.

This appendix will evaluate a defined set of BTAs relative to the transmission and pathogenesis considerations that have been identified and consider the relevance for microbial dose-response modeling when using empirical or mechanistic modeling approaches.

## Characteristics of Biological Threat Agents

The infectivity of BTAs can be characterized as an opportunistic airborne transmission capability, with enhanced virulence resulting from the inhalation route of exposure when

compared to typical routes of exposure associated with natural exposure (e.g., inhalational versus cutaneous anthrax) (Roy et al., 2010). The BTA group exhibits a unique capability for persistence when released as respirable microbial aerosols (Eitzen, 2007) and subsequent infectivity from inhalation exposure relative to most microbial bacteria and viruses (Roy et al., 2010). It is hypothesized that commonalities in the biological mechanisms allowing for aerosol persistence and infectivity may also mediate similarities in the early interactions between host and the pathogen. Interestingly, the macrophage or other phagocytic cells are associated with initiation of infection and/or preferential replication sites for a number of bacterial BTAs (e.g., *Bacillus anthracis* (Inglesby et al., 2002)*; Burkholderia spp.* (Whitlock et al., 2007); *Franscisella tularensis* (Ketavarapu et al., 2008)). In natural environments, many of these same bacterial BTAs also utilize an amoebic niche which may be a training ground for successful invasion of host phagocytic cells. Interestingly, reliance on the amoebic niche is not unique to BTAs and has been associated with traits that also facilitate the successful invasion of host phagocytic cells, including the macrophage, by *Legionella* spp as described by Swanson and Hammer (2000).

## Evaluation of Transmission and Pathogenesis Considerations for Dose-Response Modeling of Biological Threat Agents

The BTA group for the evaluation is the "traditional" BTAs identified in the U.S. Centers for Disease Control and Prevention (CDC) historic Select Agent Category A and B lists (Rotz et al., 2002). The BTAs were defined to include bacterial agents (i.e., *B. anthracis, B. mallei, B. pseudomallei, F. tularensis, Yersinia pestis, Coxiella burnetii*) and viral agents (i.e., filovirus and arenavirus hemorrhagic fever viruses, Variola major virus [smallpox virus]).

The identification of modeling considerations necessary for microbial dose-response analysis was based on fundamental elements of infectious disease transmission and illness, with the initial focus on points of difference between chemical toxicity and microbial pathogenesis.

The following modeling considerations were evaluated for the group of BTAs that have been identified:

- secondary transmission,
- propagation of the pathogen in the host,
- immunity and susceptibility of the exposed population,
- determination of threshold in response, and
- potential variation in virulence exhibited by individual strains, variants, or isolates.

## Secondary Transmission

For many infectious diseases, transmission has been modeled as a dynamic process where infected individuals become the source of pathogens to which others can be exposed (Eisenberg et al., 2002), either directly or indirectly. Secondary transmission has been defined in various ways in the literature; this paper defines direct secondary transmission as the communicability, or transmission, of disease directly from a primary to secondary case. Direct secondary transmission can occur from person-to-person airborne transmission or direct contact with infectious bodily fluids. Indirect secondary transmission is defined as the transmission of disease through indirect means following a human-environment-human pathway, as occurs when contact with a fomite contaminated by the primary case transmits infection to a secondary case(s) (U.S. Environmental Protection Agency, 2007).

For pathogens that exhibit secondary transmission, a population-based microbial dose-response estimate based solely on the first transmission of disease can be biased low relative to the actual response due to the potential "multiplier" effect of initial cases not explicitly included in the model (i.e., successive cases that originate from transmission of the first case). This multiplier effect has led to the assertion that infectious disease risk is appropriately assessed as a population-based risk using a dynamic process for these pathogens (Eisenberg et al., 2002). Dynamic models contrast with the use of static modeling approaches such as the empirical dose-response models that are commonly used for chemical dose-response analysis.

However, most BTAs in this evaluation do not exhibit direct secondary transmission. Traditional BTAs were preferentially selected to minimize the potential for direct person-to-person spread to allow for containment of the disease spread by those releasing the agents (Eitzen, 2007). Differences exist in the communicability of the bacterial and viral BTAs that have been identified. A number of viral BTAs are considered communicable: the smallpox virus (Henderson et al., 1999) and hemorrhagic fever viruses (e.g., arenaviruses, filoviruses, Lassa viruses) (Borio et al., 2002). With the exception of the communicable pneumonic form of *Y. pestis* (Inglesby et al., 2000), the remaining bacterial BTAs are noncommunicable or rarely communicable.

In summary, the following BTAs are identified as (1) noncommunicable: *B. anthracis* (Inglesby et al., 2002), *F. tularensis* (Dennis et al., 2001), and *C. burnetii* (Azad, 2007), or (2) rarely communicable by humans: *B. mallei* (Whitlock et al., 2007) and *B. pseudomallei* (Cheng and Currie, 2005). Some viral hemorrhagic fevers have been described as communicable "predominantly" by physical contact with bodily fluids, and there is less compelling evidence

that person-to-person airborne transmission has occurred for others absent contact (e.g., filoviruses) (Borio et al., 2002). However, it is recommended that new literature from the 2014 Ebola outbreak continue to be evaluated to ensure current data are incorporated into assumptions regarding this pathogen, especially for the potential for person-to-person transmission absent intense and/or aerosol exposure to contaminated bodily fluids.

Fomites are the primary concern for indirect secondary transmission of illness. However, contamination from bioaerosols produced by infected individuals is constrained by concentration limits imposed by the natural disease process (Roy et al., 2010), and the chain of transmission is fairly limited for bacterial BTAs that are not communicable in their natural disease process. Most traditional BTAs are zoonotic pathogens where humans are not the primary infectious target (i.e., humans as an incidental or dead-end host) (Eitzen, 2007).

Therefore, human illness may result from the high exposure concentration associated with the intentional or accidental release of BTAs, but the potential for secondary transmission potential then returns to the potentially normally exhibited during natural infections. There may be variability in indirect secondary transmission among the viral BTAs. Indirect secondary transmission has been documented for the smallpox virus; this includes transmission from books as reported by Ferson (2001) and letters as identified by Ambrose (2005). During an Ebola outbreak in 2000, there was limited evidence of secondary transmission and a lack of measurable contamination on common fomite surfaces tested in a hospital setting during the 2000 Ebola virus outbreak (Bausch et al., 2007).

For BTAs identified as noncommunicable or rarely communicable, traditional static dose-response mathematical models are appropriate. Some viral BTAs identified as potentially

communicable may require a fairly significant level of contact with infected individuals (e.g., intimate contact [Bausch et al., 2007]) or bodily fluids (e.g., blood, vomit in health care settings [Bausch et al., 2007]) to produce transmission. Further evaluation of the applicability of assumed secondary transmission may be appropriate for these viral BTAs, especially if infectivity endpoints are used to derive the original dose-response estimates.

## Pathogenic Propagation in the Host

The propagation of pathogens in the host is a key process in disease pathogenesis and can signal the transition from infection to illness for some pathogens. As a differentiator between chemical and microbial risk assessment, the multiplication of the pathogen is noted as a distinct characteristic of microbial risk assessment as toxicants are not assumed to increase in concentration or reproduce (U.S. Environmental Protection Agency, 2014). Pathogenic propagation for microbial dose-response analysis may confound the relationship between the exposure dose and response due to multiplication of pathogens in the host. The multiplication of pathogens could result in a higher exposure dose to the target tissue associated with illness than if no multiplication took place. Chemicals may form toxic metabolites and the metabolites responsible for toxicity may increase in concentration over time. However, toxic metabolite formation can be predicted from the chemical dose when kinetic relationships between the chemical, enzyme, and metabolite are known.

A complication in the assessment of microbial dose-response relationships is the recognition that larger doses of pathogens are not always associated with a higher probability of response or severity of illness (U.S. Department of Homeland Security and U.S. Environmental Protection

Agency, 2009). Dose-dependency in incubation periods has been preliminarily identified for some microbial pathogens, including BTAs (e.g., *B. anthracis* [Wilkening, 2006]).

## Immunity and Susceptibility in Population

Immunity and susceptibility result from host characteristics that affect the host-pathogen interaction. Susceptibility, inclusive of all host-related contributors[1] to variability in response, is defined as "the extent to which a host is vulnerable to infection, taking into account a host's intrinsic and/or acquired traits that influence infection" (U.S. Environmental Protection Agency, 2007). Immunity results from immunization, previous exposure, or other host-related characteristics and can provide partial or complete protection from exposure (U.S. Environmental Protection Agency, 2007). Variability in susceptibility can modify response through prevention of infection or illness or enhancement of susceptibility due to variation or suboptimal functioning of the immune system. Susceptibility may also include variation in response to toxins produced by pathogens that are toxico-infectious. For example, variation in response to anthrax toxin has been identified for *B. anthracis* (Inglesby et al., 2002).

Variation in susceptibility has been identified as a critical element in the modeling of microbial dose-response relationships (Food and Agriculture Organization and World Health Organization [FAO and WHO], 2003; Interagency Microbiological Risk Assessment Guideline Workgroup, 2011; U.S. Environmental Protection Agency, 2014). Susceptibility considerations in dose-response analysis are important to ensure that dose-response modeling allows for evaluation of interindividual variability in response, including potentially sensitive subpopulations (e.g., health compromises) or life stages (e.g., elderly). An additional concern for the modeling of infectious disease is the potential for transmission to result from the interaction of susceptible and infected

[1] However, susceptibility as defined in this paper does not extend to differential exposure as contributing to response variation (e.g., Section 3.5.1 of U.S. Environmental Protection Agency [2004]).

individuals. Susceptibility differences resulting from immunity can be exhibited as individuals shift from susceptible to immune after transmission of illness, with the result that dynamic dose-response modeling approaches may become necessary (Eisenberg et al., 2002).

Susceptibility in infection and/or illness is known to vary across populations for microbial pathogens (e.g., *Cryptosporidium* sp. in Teunis et al. [2002], Norwalk virus in Teunis et al. [2008]). There are preliminary indications that infectivity and illness exhibit greater variability than the variability described for chemicals when compared on an absolute scale (Hattis, 1997). General factors such as age, immune status, or co-existing health conditions have been identified as contributing to susceptibility differences (Teunis et al., 2002). While data are emerging on potential associations of genetic variation and modified susceptibility for some well-studied pathogens (e.g., allelic variation and associated tuberculosis susceptibility across Canadian Aboriginal populations in Larcombe et al. [2008]), the mechanistic incorporation into a dose-response model has not been described. Susceptibility may also be expressed in a dose-dependent manner whereby pathogens act as frank pathogens at higher doses but opportunistic pathogens in more susceptible populations at lower doses (e.g., 2001 Connecticut anthrax case as evaluated by Cohen and Whalen [2007]). Additionally, population variation in the sensitivity at the cellular level to pathogenic toxins (e.g., anthrax toxin in Martchenko et al. [2012]) has also been demonstrated in recently published in-vitro studies. However, there are critical knowledge gaps for mechanistic process and associated quantitative data that limit the current capability to model variation in susceptibility.

Historically, the selection of BTAs incorporated a preference for pathogens for which the targeted population exhibits a lack of immunity (Fothergill, 1960; Eitzen, 2007) and presents

uniformity in susceptibility. There has also been a desire for use of BTAs that have vaccines available, but where the population is not routinely vaccinated (Eitzen, 2007). For these reasons, BTAs can be modeled without the assumption of immunity. However, BTAs are not unique among the microbial pathogens in the potential for the host to exhibit variation in susceptibility. While it can be hypothesized that the variation in susceptibility may be limited in expression at higher dose levels, the evaluation of low level dose-response relationships will benefit from consideration of the susceptibility differences in individuals. Potential approaches to evaluate variation in susceptibility include development of data from animal models selected for their ability to mimic susceptible subpopulation conditions (e.g., disease, age, immunosuppressant drugs). General approaches to apply uncertainty factors to account for this variability have been suggested for microbial dose-response analysis (U.S. Environmental Protection Agency, 2008, 2010), but have yet to be described and published. Modeling to include variation in response has utilized tolerance-based dose-response models (e.g., probit slope in Wein et al. [2003]) where all contributions to variation in response are mathematically aggregated into one normally distributed value.

## Threshold Versus Non-Threshold Models

A threshold model incorporates the assumption that there is a "dose or exposure below which no deleterious effect is expected to occur" (U.S. Environmental Protection Agency, 2011). A non-threshold model assumes that, even with the dose of one microorganism, there is a small nonzero probability of infection and subsequent illness (Food and Agriculture Organization and World Health Organization [FAO and WHO], 2003). From a practical perspective, the presence of a pathogenic threshold cannot be determined experimentally or empirically (Food and Agriculture

Organization and World Health Organization [FAO and WHO], 2003). It has been suggested that nonthreshold mathematical models should be preferentially evaluated, but these models should have sufficient inherent flexibility to allow high or low curvature at low doses allowing for the mimicking of a "threshold-like" or sublinear response (Food and Agriculture Organization and World Health Organization [FAO and WHO], 2003). However, a full range of models (e.g., threshold, non-threshold) should be considered to avoid the uncertainty introduced with selection of one specific model assumption (Coleman and Marks, 2000).

## Potential Strain, Allelic, or Variant Differences in Virulence

Virulence is defined as "the degree of intensity of the disease produced by a microorganism as indicated by its ability to invade the tissues of the host and the ensuing severity of illness" (International Life Sciences Institute [ILSI], 2000). Strain, allelic, or variant differences in virulence for BTAs are relevant because of the potential for a mismatch between the virulence of the BTA for which the dose-response relationship was derived versus the virulence of the BTA to which the relationship is applied.

High variability in strain virulence has been described for common bacterial pathogens, including *Salmonella* sp. (Coleman et al., 2004) and *Campylobacter jejuni* (Coleman et al., 2004) and animal studies for BTAs, including *B. anthracis* (Fellows et al., 2001). Pathogenic virulence can also be modified, either decreased or increased, in response to passage through multiple

hosts (Roy et al., 2010). However, quantification of the variation in virulence is not well characterized.[2]

BTAs do not differ from the larger group of pathogens with regard to this consideration. However, there has been a preference for BTA selection based on a demonstration of greatest virulence (Eitzen, 2007), whether the endpoint is lethality (e.g., inhalation anthrax) or incapacitation (e.g., Q fever). If the concern regarding the exhibited variability is primarily related to the possibility of underestimating virulence as part of the dose-response process, one approach could include modeled strains with the presumed greatest virulence (i.e., a dose-response equivalent of the Kuhn et al. [2011] approach).

## Summary of Modeling Considerations for Biological Threat Agents

The lack of secondary transmission exhibited by bacterial BTAs and some viral BTAs allows for the use of static dose-response models for these microbial pathogens (Table A-1). For the remaining modeling process considerations, each can be addressed to varying degrees within currently available dose-response models. While the remaining considerations can be properly viewed as mechanistic, approaches are available to include these elements as part of empirical or mechanistic models. Processes can be defined that allow for a modification of the dose-response outputs (e.g., uncertainty factor, data-derived extrapolation factor) of empirical, nominally mechanistic, or limited mechanistic models. Likewise, considerations can also be explicitly modeled in increasingly mechanistic models as data are available. These considerations involve content areas for which there is acknowledged high uncertainty and very limited data, as well as

[2] Product formulation and associated practices may also affect the virulence. Further information on a Bayesian assessment conducted for the guinea pig is available in Mitchell-Blackwood et al. (2012)

the potential for extremes in variability to be exhibited. Chemical dose-response modelers struggled with similar data and methodological challenges (e.g., interindividual variability in susceptibility), and the chemical dose-response approaches may be leveraged for addressing data gaps, variability, and uncertainty.

**Table A-1. Summary of Transmission and Pathogenesis Considerations and Relevance for Modeling**

| Transmission and Pathogenesis Consideration | Universal for Microbial Pathogens or Limited Relevance for BTAs | Mechanistic Modeling Consideration and Potential Means to Address in Dose-Response Modeling | Potential Means to Address in Dose-Response Modeling in Empirical Modeling |
|---|---|---|---|
| **Immunity and Susceptibility in Population** | Immunity not relevant for BTAs, noting limited immunity as defining characteristic of BTAs<br>Variation in susceptibility universal for microbial pathogens | Yes, consider modeling element mechanistically as interindividual variability in susceptibility | Address as animal model or human dose-response input data decision, use of data derived extrapolation factor or uncertainty factor for adjustment after development of dose-response relationship |
| **Secondary Transmission** | Not relevant for bacterial BTAs; relevant for some viral BTAs | Yes for viral BTAs, dynamic dose-response models or multiplier adjustment to static estimate of response to reflect additional transmission may be potential means to address | Dynamic dose-response models or multiplier adjustment to static estimate of dose-response relationship to reflect additional transmission may be potential means to address |
| **Pathogen Propagation** | Universal for microbial pathogens | Yes, incorporate bacterial kinetics of identified compartment or other target tissues | Not an element of an empirical model |
| **Strain, Allelic, or Variant Differences in Virulence** | Universal for microbial pathogens | Possibly, as more data are available may be able to mechanistically link identified virulence differences with known elements of strains, alleles, or variants | Differences in virulence may be addressed by selection of target strain, allele, or variant for dose-response data set |
| **Threshold or Nonthreshold Determination** | Universal for microbial pathogens | No, structure of mathematical model pre-determines whether threshold or non-threshold is modeled | Consider evaluation of mathematical models that vary in the assumption of threshold to address uncertainty resulting from model selection |

Table A-2 identifies mathematical dose-response models and published examples of BTA dose-response relationships using the identified model. Existing dose-response models can be used or

new models developed. It is recommended that a variety of dose-response models be evaluated with regard to incorporation of mechanistic elements and the presence or absence of a threshold.

**Table A-2. Mathematical Dose-Response Models by Type of Model and Referencing Publication**

| Type of Model | Examples of Mathematical Dose-Response Model | Published BTA Dose-Response Relationship Using Model |
|---|---|---|
| **Empirical** | Probit or Log Probit | Taft and Hines (2012) |
| | Logistic or Log Logistic | |
| | Weibull | |
| | Dichotomous Hill | |
| | Gamma | |
| **Nominally Mechanistic** | Exponential | Bartrand et al. (2008)<br>Teske et al. (2011)<br>Taft and Hines (2012) |
| | Beta Poisson | |
| **Limited Mechanistic** | Competing Risk Model | Gutting et al. (2008) |
| | Time-Dose-Response Model | Huang and Haas (2009) |
| | In-vivo Growth Model | Huang and Haas (2011) |
| | Time-Dependent Dose-Response Model | Mayer et al. (2011) |
| | Cumulative Dose Model | Pujol et al. (2009) |
| | In-vivo Delivered Dose Model | Weir and Haas (2011) |
| | Age –Dose-Response Model | Weir and Haas (2009) |
| **Comprehensive Mechanistic** | None to Date | None to Date |

## Applicability to Microbial Pathogens Other than Biological Threat Agents

There is wide potential applicability of this microbial dose-response methodology for microbial pathogens other than BTAs. The following methodology is most appropriate for non-BTA pathogens that do not exhibit secondary transmission and exhibit initiation of infection through the inhalation route of exposure.

However, it is important to recognize that this evaluation may be appropriately applied to pathogens described as BTAs (e.g., emerging BTAs) that do not exhibit pathogenesis and transmission characteristics similar to those described for the traditional BTAs. The traditional BTAs were selected from the larger universe of pathogens based on the recognized potential infectivity for a large number of individuals when released and maximization of lethality or

incapacitation to those exposed. While pathogens identified to be of greatest concern for bioterrorism have typically been assumed to be the same as the traditional BTAs, the goal to maximize adverse health effects may be overtaken by the achievement of other ends (e.g., salad bar tampering with *Salmonella* in Oregon to disrupt elections [Torok et al., 1997]). For these emerging BTAs, unusual exposure scenarios identified uniquely for bioterrorism (versus traditional BTAs and the inhalation route of exposure) warrant further review before routine applicability of this methodology.

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# Appendix B - Historical Approaches to Microbial Dose-Response Relationship Development for *Bacillus anthracis*

## Introduction

A long history of publications describing anthrax infection in man and livestock dates back to the original publications by Koch and Pasteur first describing the *Bacillus anthracis* organism and disease transmission in the late 1800s (Hilmas et al., 2009). Early descriptions focused on disease pathogenesis and livestock vaccination strategies, with little research effort spent describing relationships between dose and effect in humans. However, there was a significant change in research focus when *B. anthracis* was evaluated as a potential bioweapon after World Wars I and II. Since that time, a body of literature has developed to model and report dose-response data of relevance to the human from intentional or accidental release of spores of *B. anthracis*.

This review will consider historical approaches to model microbial dose-response relationships for *B. anthracis* in the United States and United Kingdom starting at the end of World War II. During the 1940s, open source publications first began to identify animal model data to define lethality values for animal models or relative estimates of human susceptibility. Since that time, there has been an evolution in the development of microbial dose-response data for *B. anthracis*. Early research on *B. anthracis* focused on military applications to evaluate general potency or support preliminary development of medical countermeasures that lead to early biological models of disease pathogenesis. An apparent slowdown in research progress occurred as interest in *B. anthracis* waned after the Biological Weapons Convention in the 1970s, as there were no

published refinements in the biological models in the open literature. However, the discovery of biological weapons in Iraq during the Gulf War in the1990s followed by the 2001 anthrax letter event again accelerated basic research and medical countermeasure efforts.

The approaches used to model microbial dose-response relationships for *B. anthracis* can be described by the themes in research: (1) Determination of median lethality values, (2) Early attempts to generate biologically-based models, (3) Modeling the initiation of infection, (4) Consideration of the independent event hypothesis, and (5) Current approaches to modeling *B. anthracis* pathogenesis and dose-response relationships. The themes do not reflect a strict historical timeline as some of the themes reflect current questions in the field (e.g., modeling the initiation of infection or consideration of the independent event hypothesis). This appendix will provide a brief review of each theme while considering the overall state of progress in modeling dose-response data for *B. anthracis*.

## Determination of Median Lethality Values

Microbial pathogenesis or dose-response data for *B. anthracis* from the 1940s through the 1960s was typically associated with state-sponsored laboratories, principally the U.S. Army Chemical Corps laboratories (e.g., Fernelius et al. [1960], Lincoln et al. [1965], Lincoln et al. [1967a], Lincoln et al. [1962], Klein et al. [1966], Jones et al. [1967]) or the United Kingdom's Porton Down facility (e.g., Barnes [1947], Henderson [1952], Druett et al. [1953], Widdicombe et al. [1956], Ross [1957]). Published dose-response relationship data for the rabbit and nonhuman primate during this time primarily focused on reporting of median lethality values (e.g., Young et al. [1946], Druett et al. [1953], Barnes [1947]; Henderson et al. [1956]). In the case of Young et

al. (1946) and Barnes (1947), these values were published absent the initial data set or the calculation of the value, with a primary focus of the articles associated with studies describing pathogenesis or treatment of disease. The measurement of the median lethality value was the primary output for most studies, with little consideration for the evaluation of other values or describing the relationship between dose and response overall.

The Druett et al. (1953) study and associated dose-response data set was unique relative to its contemporaries for a number of reasons. The stated purpose of the paper was to elucidate lung regions associated with infection by testing various particle sizes. However, the study design yielded an excellent data set to evaluate dose-response relationships (i.e., sufficient numbers of animals, detailed study design description, reported all raw data). The study design also evaluated the dose-response data using probit analysis allowing for identification of different response levels than the median lethality values.

Most studies reporting median lethality values after the 1960s were typically designed for purposes other than dose-response (e.g., pathology, medical countermeasures). In these studies, high dose challenges (e.g., 100 to 200 times current estimates of median lethality values) were conducted to ensure a high likelihood of systemic anthrax infection in the challenge animals. In addition to reporting the strain, the reporting of the lethality value can provide an assessment of the general “potency” or “virulence” of the test material. Depending on the application of the data, current users of median lethality values include modelers for population hazard prediction, planners, and human health risk assessors (Gutting et al., 2015).

## Early Attempts to Generate Biologically-Based Models

As an advance from the direct calculation of median lethality ($LD_{50}$) values or probit-based empirical modeling to generate dose-response relationships, a biologically-based model of anthrax illness was first developed in the 1960s. These early mechanistic models modeled bacteremia or even lethality, but they cannot be termed dose-response models because they did not predict the probability of response.

A biologically-based mathematical model was developed to describe the kinetics of bacteremia after intravenous administration of *B. anthracis* spores through death (Lincoln et al., 1962). The first mechanistic model describing anthrax infection evaluated kinetic data to model biological events but stopped short of developing a predictive dose-response relationship because there was no mathematical association determined between the dose, either administered or internal, with a probability of response endpoint (e.g., lethality). Bacteremia concentration was modeled over time with boundary assumptions for identified parameter values and a mathematical expression evaluating dose, net bacterial growth rate, and host resistance (i.e., passive and active resistance). Active resistance, defined as phagocytosis and other immune reactions (e.g., fever), was modeled using a negative exponential function with resistance assumed to go toward zero for later values of time after infection.

Using these results and other study data developed at Fort Detrick's U.S. Army Biological Laboratories group, Klein et al. (1963) conceptually described the resistance to establishment of anthrax infection as being the collective outcome of two distinct and competing host-pathogen

interactions: (1) the ability to establish bacterial growth and infection versus (2) the susceptibility of the host to toxins produced during bacterial growth.

Using data from various animal models, an inverse relationship was identified for the resistance to infection and susceptibility to toxin (Lincoln et al., 1967b). Resistance to infection was measured by spore germination in phagocytes and/or parenteral dose to establish anthrax; susceptibility to toxin was defined by lethality after intravenous administration.

As reported in Lincoln et al. (1967b), Kashiba et al. (1959) assessed inhibition of phagocytes by terminal guinea pig serum, but American researchers could not replicate the results. As a result, American researchers then focused their efforts on other spore-phagocyte interactions including intracellular germination relative to spore numbers per phagocyte. Continued research efforts on the inhibition of phagocytes by toxin was possibly delayed by decades in the United States as a result.

Within the same U.S. laboratories, modeling of *B. anthracis* pathogenesis focused on elucidation of a primarily systemic mode of action for the toxins, as evidenced by a number of studies in the 1960s that evaluated $LD_{50}$ values for toxins administered intravenously or intraperitoneally (e.g., Klein et al. [1963], Lincoln et al. [1967b]). Evidence for toxemia as the cause of anthrax mortality was based on the elicitation of anthrax symptoms and lethality reported after toxin challenge studies. Decades later, data linking immunity to a component of the toxin (specifically, the protective antigen [PA] component of both lethal toxin [LT] and edema toxin [ET]) with conferred protection from anthrax infection also strengthened the association of toxemia with lethality (Moayeri and Leppla, 2009; Coggeshall et al., 2013).

## Modeling the Initiation of Infection

The Trojan horse model is the first and most currently cited model for initiation of inhalation anthrax since its publication in 2002 (Weiner and Glomski, 2012). The Trojan horse model is principally based on the Ross (1957) description of spore engulfment and germination in the alveolar macrophage combined with the Lincoln et al. (1965) reporting of transport of vegetative bacteria to the lymphatic system. The continued availability of in-vitro and in-vivo cellular techniques generated increasingly detailed mechanistic data on a potential role for the macrophage in anthrax infection (Shafa et al., 1966; Hanna et al., 1993; Guidi-Rontani et al., 1999b; Dixon et al., 2000). Most of the early in-vitro mechanistic work cited in the initial proposal of the Trojan horse model utilized the mouse animal model or murine-derived cell lines (Hanna et al., 1993; Guidi-Rontani et al., 1999a; Dixon et al., 2000), though Shafa et al. (1966) evaluated macrophages from the rabbit. Using these mechanistic data, the Trojan horse model hypothesizes the establishment of inhalation anthrax infection as an intracellular competition between the *B. anthracis* spore, host macrophage, and toxins expressed by vegetative *B. anthracis* (Guidi-Rontani, 2002). In the Trojan horse model, infection is initiated through engulfment of the spore by alveolar macrophages and subsequent spore germination either during transport to or upon arrival in the lymph node (Guidi-Rontani, 2002).

Using the Trojan horse model as a conceptual approach to model the initiation of infection, the first dose-response models incorporating host-pathogen interaction were not published until the 2000s, nearly 40 years after the Fort Detrick group developed their kinetic model. This interaction was conceptualized differently from the interaction presented by Klein et al. (1963) with the two competing outcomes defined at a more basic fundamental level: (1) successful spore

germination allowing proliferation of vegetative bacteria (i.e., germination) versus (2) removal and/or destruction of the spore and associated vegetative bacteria (i.e., spore clearance). Accordingly, a competing risk model to biologically model host-pathogen dynamics for inhalation anthrax at the level of an individual spore was first described in Brookmeyer et al. (2005) and Brookmeyer et al. (2003). Though the purpose of the Brookmeyer et al. (2005) and Brookmeyer et al. (2003) models was to mechanistically model the incubation period for human inhalation anthrax, a dose-response function was embedded within the overall model that could be parameterized with human and/or animal model data. Using the competing risk mathematical concept described in Brookmeyer et al. (2003) and Brookmeyer et al. (2005), a biologically-based dose-response (BBDR) model was then published for the rabbit (Gutting et al., 2013) and the nonhuman primate (Toth et al., 2013). For the Gutting et al. (2013) and Toth et al. (2013) BBDR models, a comparison of the BBDR model outputs with empirical models or study data was provided. However, statistical measures of model fit for each model type to allow comparison with empirical modeling approaches were not included.

After the Trojan horse model was published, additional phagocytic cell types capable of transporting *B. anthracis* spores to lymph nodes were identified through in-vitro studies of human dendritic cells[3] (Brittingham et al., 2005) and murine B cells (Rayamajhi et al., 2012). Spore germination outside phagocytic cells in a murine animal model after inhalation and oral exposure was reported in the lymphoid tissue of the respiratory tract and Peyer's patch tissues of the intestine, respectively (Glomski et al., 2007; Lowe et al., 2013). Spore translocation into lung

[3] Dendritic cells were identified in the original article describing the Trojan horse model as possibly providing a vehicle for transport to the lymphatic system and subsequent germination location (Guidi-Rontani, 2002).

epithelial cells was also reported from an in-vivo murine study, providing a route whereby the spores could have a direct intracellular route to the lymphatic system (Russell et al., 2008).

To accommodate these new data, the jailbreak model expanded the Trojan horse model in three important ways: (1) increased emphasis on the host-pathogen interactions in lymphoid and epithelial tissues, (2) broadened the role of alveolar macrophages to include important elements of host defense, and (3) expanded the number of potential cellular carriers to initiate infection (Weiner and Glomski, 2012). The model is unique because it provides a conceptually consistent approach to model the early stages of infection across the three natural routes of exposure: inhalation, gastrointestinal, and cutaneous anthrax (Weiner and Glomski, 2012). Multiple pathways by which inhalation anthrax may be initiated from the same route of exposure were identified (Weiner and Glomski, 2012). Weiner and Glomski (2012) note that multiple distinct pathways for initiation of infection have been identified for other microbial pathogens (e.g., salmonellae, shigellae, *Listeria monocytogenes*).

New concepts introduced in the jailbreak model include the potential for extracellular germination of spores that do not require an intracellular phagocytic location for germination while still allowing for subsequent transport to the lymph system (Weiner and Glomski, 2012). The differing role for toxins in early infection is also notable. In the jailbreak model, spores germinate in an extracellular environment and toxins are necessary to damage the integrity of cellular barriers to facilitate access to the lymph system (Weiner and Glomski, 2012). In contrast, toxins in the Trojan horse model facilitate successful intracellular germination through modulation of the oxidative burst process within the phagocytic cells (Weiner and Glomski,

2012). A subsequent paper notes that the identification of these multiple pathways does not imply that mediastinal lymph node-initiated infections are not occurring in the murine or other animal models, but that alternative or additional pathways may not be recognized absent sensitive test methods and study approaches designed to capture these other pathways (Lowe et al., 2013).

There are important differences between the Trojan horse and jailbreak models with regard to the action of toxins. The Trojan horse model (Guidi-Rontani, 2002) described a localized action for toxins as facilitating successful intracellular germination in the phagocyte and then allowing for proliferation of vegetative bacteria. Alternately, the jailbreak model of Weiner and Glomski (2012) identified toxin damage to endothelial or epithelial tissues as important to breaking key barriers necessary for establishment of infection.

The identification of the new pathways for infection associated with the jailbreak model were identified using bioluminescent techniques with the mouse small animal model and *B. anthracis* spores of attenuated virulence. Of most relevance for this assessment, data are unavailable to support or contraindicate the functional presence of these pathways in large animal models. A key challenge for the development of these data is a technology comparable to the bioluminescent techniques previously used in small animals (Glomski et al., 2007; Sanz et al., 2008; Dumetz et al., 2011) that can precisely delineate the locations involved in the earliest stages of infection in large animal models, such as the rabbit or nonhuman primate.

A key modeling determination for mechanistic models is the definition of infection. Differences have arisen over time in the definition of anthrax infection, definitions ranging from conceptual

to analytical. When developing conceptual models for microbial dose-response analysis, Buchanan et al. (2009) characterized infection as the state where a pathogen can "actively multiply" inside the host. More analytically-oriented definitions include seroconversion as measured by a humoral response to protective antigen (PA) (U.S. Environmental Protection Agency, 2011, 2012), confirmation of *B. anthracis* bacteremia via culture, or a combination of these measurements. Henning et al. (2012) defined infection as the presence of a positive *B. anthracis* blood culture combined with an electrochemiluminescent measurement of circulating PA, with diagnostic measures noted to be observed earlier in the disease process than nonspecific clinical signs. Boyer et al. (2009) confirmed the presence of infection using a combination of bacteremia, blood differentials, and detection of the PA gene via polymerase chain reaction (PCR) analysis.

The definition has evolved based on basic knowledge of the disease process, available technology (e.g., analytical targets, detection limit), and desired end-use of the data (e.g., modeling, confirming presence/absence of anthrax infection, assessment of kinetics of disease). Any definition will continue to be subject to modification as more sensitive measurement technologies of potential biomarkers or new insights related to the infection process are developed.

## Consideration of the Independent Action Hypothesis

Druett (1952) provides the first articulation of the independent action hypothesis. Parts of the mathematical derivation of the independent action hypothesis were previously presented in Bald (1937) and were built upon by Druett (1952). However, the model was not termed independent

action until Meynell and Stocker (1957) (U.S. Environmental Protection Agency, 2014). The model is also referred to as the independent event hypothesis. Independent action among pathogens was described by Druett (1952) as a constant relationship between response and the product of administered dose (e.g., environmental concentration) and exposure time.[4] Druett (1952) reported general consistency between the probit slope value derived from a mathematical model of the independent action hypothesis and the calculated probit slope values from single dose challenge studies reporting *B. anthracis*[5] and *Brucella suis* inhalation exposure and mortality. The following assumptions were made in the mathematical derivation: a constant probability for each organism to cause the identified response (i.e., mortality or infection) in the host, independent action of each organism (e.g., no immune system activation), an $LD_{50}$ value that can be determined, and a large homogenous experimental population (Druett, 1952).

A literature review conducted by the U.S. Environmental Protection Agency (2014) found a number of studies that described their data as consistent with the independent action hypothesis. However, rigorous experimental evidence to distinguish between independent and inter-dependent action hypotheses was limited for most host-pathogen systems (U.S. Environmental Protection Agency, 2014).

[4] Druett (1952) independently described the microbial equivalent of Haber's Law. Haber's Law, reported in the early 1900s, also described a constant concentration-time relationship between exposure and mortality response for exposure to inhalation exposure to volatile chemicals. Since that time, Haber's Law has been updated to include a fitted exponent on the concentration term to better fit tested chemicals (ten Berge et al., 1986). Likewise, a fitted exponent may also be found appropriate for the mathematical description of independent action.

[5] The *B. anthracis* dose-response data were subsequently published in Druett et al. (1953).

The independent action hypothesis may be relevant for dose-response modeling in two primary ways: the selection of appropriate dose-response models (Haas et al., 1999; Food and Agriculture Organization and World Health Organization (FAO and WHO), 2003) and the determination of dose metrics for multiple dose exposures (U.S. Environmental Protection Agency, 2014). When defined as mechanistic models, the exponential and beta-Poisson models are consistent with the independent action hypothesis and therefore, some researchers have identified them as preferable for microbial dose-response modeling (Haas et al., 1999; Food and Agriculture Organization and World Health Organization (FAO and WHO), 2003). However, the use of empirical models does not require a mechanistic interpretation of the model parameters and therefore a broader consideration of available mathematical models for microbial dose-response analysis has also been identified as appropriate (Holcomb et al., 1999; Coleman and Marks, 2000; Taft and Hines, 2012).

Independent action may not be a trait universally expressed among microbial pathogens at all times, but may present some dependencies based on microbial pathogen, route of exposure, magnitude of dose, or timing of doses. If the independent action hypothesis were correct, the total dose would be an appropriate dose metric for a *B. anthracis*, and there would be no biological rationale for consideration of a daily average dose. However, a limitation to the exposure duration over which independent action could be assumed (e.g., short enough to preclude immune system activation) was noted by Druett (1952) in the original formulation of the hypothesis. Though Druett (1952) developed the hypothesis with single dose data, the concept should be equally relevant to multiple dose assessments. The independent action hypothesis should allow for the use of an aggregate dose metric only if the exposure time over

which the daily doses were aggregated did not exceed the time duration associated with dose independence. Mayer et al. (2011) also noted that dose-response models lacking consistency with independent action assumptions may be warranted under conditions of time-dependency of doses where independent action may be less likely to occur (e.g., exposures with multiple closely spaced doses in *B. anthracis*).

The magnitude of exposure or exposure duration (Mayer et al., 2011; U.S. Environmental Protection Agency, 2014) where independent doses can be delineated from dependent doses has not been evaluated explicitly to date. Dose-dependencies may be present in the expression of independent action whereby larger doses could affect response to subsequent doses if overloading of clearance or other innate immune functions were affected (Mayer et al., 2011). If overloading can occur, this implies that the presence of independent action could vary by route of exposure if varying innate response levels are present (e.g., differential innate response for dermal versus inhalation routes of exposure). The timing of the exposures relative to the dose and clearance capabilities is also a critical exposure consideration relative to the selection of dose metrics (Mayer et al., 2011).

The determination of a theoretical time point separating independent and dependent doses may be considerably more complicated for inhaled pathogens that have the potential to persist in the lungs (U.S. Environmental Protection Agency, 2014). For example, spore persistence in the lung with subsequent inhalation anthrax has been reported in one nonhuman primate that died 58 days after exposure after initially receiving 30 days of antibiotic treatment starting on the exposure

day (Friedlander et al., 1993). In this context, a total accumulated dose could be an appropriate dose metric.

## Current Approaches to Modeling *B. anthracis* Pathogenesis and Dose-Response Relationships

Empirical dose-response relationships continue to be used for the modeling of dose-response relationships in the nonhuman primate (Haas, 2002; Bartrand et al., 2008; Weir and Haas, 2011; Taft and Hines, 2012) and rabbit (U.S. Environmental Protection Agency, 2011, 2012). The availability of statistical software capable of fitting dose-response data to mathematical models has considerably broadened the models available for evaluation. The U.S. Environmental Protection Agency (2011, 2012) studies were designed to include representation of low-dose exposure ranges. The purpose of the EPA studies was to design studies and derive dose-response relationships relevant to the assessment of residual biological contamination present after application of decontamination technologies. Data gaps identified during remediation after the 2001 anthrax letter event provide an impetus for new dose-response studies and identified the need for reliable means to assess risk in the low-dose range (Gutting et al., 2008).

Hybrid models of empirically fit parameters combined with expert elicited dose-response values have been included as elements of population-based anthrax models for the human (Webb and Blaser, 2002; Wein et al., 2003; Wein and Craft, 2005). Likewise, empirically fit models have been developed using a survival analysis framework to incorporate time dependencies in dosing and/or response (Mayer et al., 2011; U.S. Environmental Protection Agency, 2014).

Recently published biologically-based models for anthrax infection and illness evaluate the timing, type, and likely success of medical countermeasures (Kumar et al., 2008), develop a better understanding of early infection dynamics (Day et al., 2011), evaluate the incubation period (Brookmeyer and Blades, 2003; Brookmeyer et al., 2003; Brookmeyer et al., 2005; Wilkening, 2008), assess the spatial and temporal concordance of anthrax cases from the Sverdlovsk outbreak (Wilkening, 2006), and evaluate time-dependence in dose-response analysis of multiple doses (Mayer et al., 2011). Clearance of inhaled *B. anthracis* spores currently plays a key role in mechanistic modeling approaches for infection and response to exposure. However, the relationship between external exposure and clearance has been identified as a major uncertainty in *B. anthracis* dose-response prediction (Coleman et al., 2008). These biologically based models may provide important components of a comprehensive biologically-based dose-response model if linkages are made between dose, model components, and response endpoint(s) of potential interest. However, the mechanisms associated with dose-dependence in outcomes exhibit significant uncertainty (U.S. Environmental Protection Agency, 2014).

## Conclusion

As the primary end users for *B. anthracis* microbial dose-response outputs have broadened after the 2001 anthrax letter event, an additional focus for modeling *B. anthracis* dose-response relationships has included the prediction of the hazard posed by low-dose exposure. This additional focus has led to a renewed interest in biologically-based dose-response models that can incorporate dose-dependent mechanisms associated with low response levels and assist in predicting response differences between the animal model and the human. Much progress has

been made from the early emphasis on $LD_{50}$ values to a more comprehensive understanding of the disease pathogenesis and its translation to mathematical models.

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# Appendix C - Data Summary Table for End-stage Inhalation Anthrax Pathology of the Human, Nonhuman Primate, and Rabbit

**Table C-1. Data Summary Table for End-stage Inhalation Anthrax Pathology of the Human, Nonhuman Primate, and Rabbit**

| System | Rabbit | Nonhuman Primate | Human |
|---|---|---|---|
| **Immune System Including Lymph Nodes (LNs), Spleen, Thymus, and Gut-associated Lymphoid Tissue** | Hemorrhagic lymphadenitis, most often mediastinal and submandibular LN, with lymphoid necrosis in draining LN (Zaucha et al., 1998; U.S. Environmental Protection Agency, 2011; Lovchik et al., 2012); lymphoid depletion (Zaucha et al., 1998); presence of fibrin,(U.S. Environmental Protection Agency, 2011; Lovchik et al., 2012; U.S. Environmental Protection Agency, 2012); edema (Lovchik et al., 2012; U.S. Environmental Protection Agency, 2012)<br><br>Mediastinal lesions, less severe than noted in human (Zaucha et al., 1998); connective tissue and fat displaying edema and hemorrhage (Lovchik et al., 2012)<br><br>Lesions in gut-associated lymphoid tissues of sacculus rotundus (Zaucha et al., 1998); cecal appendix (Zaucha et al., 1998) and ileum (Zaucha et al., 1998); lymphocyte necrosis and depletion in lymphoid tissue of sacculus rotundus and cecal appendix (Lovchik et al., 2012)<br><br>Hemorrhage and necrosis in appendix (U.S. Environmental Protection Agency, 2012)<br><br>Lymphoid atrophy and edema in thymus (U.S. Environmental Protection Agency, 2011) or lymphocyte necrosis and depletion in thymus (Lovchik et al., 2012)<br><br>Splenomegaly, with acute fibrinous splenitis (Zaucha et al., 1998; Yee et al., 2010; Lovchik et al., 2012); necrosis (Zaucha et al., 1998; Yee et al., 2010; Lovchik et al., 2012); hemorrhage (Zaucha et al., 1998; Lovchik et al., 2012); | Hemorrhagic, enlarged and/or edema in mediastinal LN (Albrink and Goodlow, 1959; Twenhafel et al., 2007; Lever et al., 2008; Henning et al., 2012); Necrosis in mediastinal LN (23/23) (Dalldorf et al., 1971); tracheobronchial LN (Albrink and Goodlow, 1959; Fritz et al., 1995; Twenhafel et al., 2007; Lever et al., 2008); intrathoracic LN with some necrosis (Gleiser et al., 1963); axillar and inguinal LN (Fritz et al., 1995; Twenhafel et al., 2007); mesenteric LN (Twenhafel et al., 2007); cervical LN engorged with neutrophils (16/23); with some necrosis (4/21) (Dalldorf et al., 1971)<br><br>Secondary follicular development including focal fibrin deposition (Lever et al., 2008); edema (Middleton and Standen, 1961; Fritz et al., 1995; Twenhafel et al., 2007); depletion and necrosis of lymphocytes (Middleton and Standen, 1961; Fritz et al., 1995; Henning et al., 2012); sinus histiocytosis (Middleton and Standen, 1961; Fritz et al., 1995); infiltration by neutrophils (Albrink and Goodlow, 1959)<br><br>Mediastinal tissues with edema and/or hemorrhage (Gleiser et al., 1963; Vasconcelos et al., 2003); massive hemorrhagic mediastinitis not observed (Gleiser et al., 1963); acute suppurative inflammation (4/14) (Vasconcelos et al., 2003)<br><br>Mesenteric LN with hemorrhage and/or edema (Fritz et al., 1995)<br><br>Splenomegaly (Albrink and Goodlow, 1959; Middleton and Standen, 1961; Gleiser et al., 1963; Lever et al., 2008); though with low incidence identified from one study (3/13) (Fritz | Mediastinal LN with hemorrhage (Barakat et al., 2002; Gill and Melinek, 2002; Guarner and del Rio, 2011); necrosis (Barakat et al., 2002; Gill and Melinek, 2002; Guarner and del Rio, 2011) lymphocytosis (Guarner and del Rio, 2011) infiltration by neutrophils and immunoblasts (Guarner and del Rio, 2011) and hemorrhagic necrosis of thoracic LN (Abramova et al., 1993)<br><br>Hilar and peribronchial LNs enlarged, necrotic, with hemorrhage (Mina et al., 2002)<br><br>Mediastinitis with hemorrhage (Albrink et al., 1960; Suffin et al., 1978; Inglesby et al., 2002; Mina et al., 2002); necrosis (Suffin et al., 1978; Inglesby et al., 2002) and acute inflammation (Suffin et al., 1978) or edema (Albrink et al., 1960)<br><br>Mesenteric lymphadenitis in limited number of cases (9/42); with less severe involvement than thoracic LN (Abramova et al., 1993)<br><br>Splenomegaly with hemorrhage (Albrink et al., 1960); congestion (Suffin et al., 1978); necrosis (Barakat et al., 2002; Guarner et al., 2003); moderate to marked lymphocytolysis, minimal atrophy of follicles, thickening of Bilroth cords (Grinberg et al., 2001) |

| System | Rabbit | Nonhuman Primate | Human |
|---|---|---|---|
| | lesions, lymphocyte necrosis and depletion (Lovchik et al., 2012) | et al., 1995) or described as mild (Twenhafel et al., 2007); with diffuse hepatic congestion, fibrin deposition, and expanded germinal center (Lever et al., 2008); lymphocytic depletion (Fritz et al., 1995); histiocytosis (Fritz et al., 1995) with hemorrhage in splenic marginal zone (Fritz et al., 1995); necrosis of lymph follicles and/or necrosis of red and white pulp with hemorrhage (21/23) (Dalldorf et al., 1971) | |
| **Respiratory System** | Necrotizing hemorrhagic pulmonary lesions, with lower incidence of pneumonia than human (Zaucha et al., 1998)<br><br>Congestion of alveolar capillaries with large numbers of bacteria, interstitial edema, and minimal to mild perivascular infiltration of heterophils (Zaucha et al., 1998); or occasional edema, presence of fibrin, and hemorrhage (Lovchik et al., 2012)<br><br>Congestion, edema, fibrin, and bacteria in lamina propria and submucosa of trachea (Yee et al., 2010)<br><br>Suppurative inflammation in lung (U.S. Environmental Protection Agency, 2011, 2012)<br><br>Potential indirect exposure effect reported as infiltration of multi-nucleated giant cells in response to foreign body (U.S. Environmental Protection Agency, 2011) | Hemorrhagic pneumonia (Albrink and Goodlow, 1959; Lever et al., 2008); low incidence of pneumonia (2/13) but presence of hemorrhages (Fritz et al., 1995)<br><br>Pleural effusions (Albrink and Goodlow, 1959; Dalldorf et al., 1971; Vasconcelos et al., 2003; Twenhafel et al., 2007); though not reported in rhesus macaque (Twenhafel et al., 2007)<br><br>Edema of the trachea and bronchial mucosa (Albrink and Goodlow, 1959)<br><br>Hemorrhage of varying severity in the lung (Gleiser et al., 1963; Vasconcelos et al., 2003; Twenhafel et al., 2007), alveoli filled with edema often mixed with fibrin, hemorrhage, macrophages, and neutrophils (Twenhafel et al., 2007); acute suppurative inflammation (4/14) (Vasconcelos et al., 2003) | Necrotizing, hemorrhagic pneumonia with primary foci present (Abramova et al., 1993)<br><br>Pleural effusions (at autopsy or drained prior to death) (LaForce et al., 1969; Jernigan et al., 2001; Barakat et al., 2002; Mina et al., 2002; Guarner et al., 2003)<br><br>Perihilar interstitial pneumonia (Grinberg et al., 2001); acute bronchial pneumonia (Grinberg et al., 2001)<br><br>Pulmonary edema (Abramova et al., 1993; Mina et al., 2002), including intra-alveolar and interstitial edema with focal hemorrhage and fibrin deposition (Barakat et al., 2002)<br><br>Hemorrhage and edema in laminae propriae of the major bronchi and trachea, with lymph nodes and connective tissue adjacent to bifurcation of the trachea hemorrhagic and edematous (Albrink et al., 1960) |
| **Cardiovascular System Including Heart and Blood Vessels** | Necrotizing hemorrhagic lesions in myocardium (Zaucha et al., 1998)<br><br>Mild myodegeneration, necrosis, and subacute inflammation, with histiocytes, mononuclear cells, and heterophils (Note: Reported from study administering lethal toxin only) (Lawrence et al., 2011) | Hemorrhage in myocardium (2/13) (Fritz et al., 1995) and (4/14) (Vasconcelos et al., 2003), with acute myocarditis (1/13) (Fritz et al., 1995) and acute suppurative inflammation (4/14) (Vasconcelos et al., 2003)<br><br>Pericardial effusions (Twenhafel et al., 2007) | Evidence of hematogenous spread of disease (Grinberg et al., 2001)<br><br>Vasculitis, with necrosis of arteries and veins (Grinberg et al., 2001)<br><br>High and low pressure hemorrhages (Grinberg et al., 2001); with high pressure hemorrhages more |

| System | Rabbit | Nonhuman Primate | Human |
|---|---|---|---|
| | | | frequently identified in Sverdlovsk than Amerithrax victims (Guarner et al., 2003)<br><br>No specific cardiac microscopic findings (Grinberg et al., 2001)<br><br>Pericardial effusions (Jernigan et al., 2001; Mina et al., 2002); wall of left ventricle increased in thickness (Albrink et al., 1960) and moderate subendocardial hemorrhage of left ventricle (Albrink et al., 1960) |
| **Gastrointestinal System** | Hemorrhage, necrosis, and lymphoid depletion in appendix (U.S. Environmental Protection Agency, 2012)<br><br>Edema, hemorrhage, and necrosis in cecum (U.S. Environmental Protection Agency, 2012) | Liver congestion (Albrink and Goodlow, 1959; Lever et al., 2008)<br><br>Acute inflammation/leukocytosis (13/14) and acute necrosis (5/14) in liver (Vasconcelos et al., 2003); sinusoidal leukocytosis (9/10); necrosis (6/10) and acute inflammation (4/10) (Henning et al., 2012)<br><br>Foci of hemorrhage in pancreas (1/13) (Fritz et al., 1995)<br><br>Hemorrhages of varying severity in the small and large intestine serosa and esophagus mucosa (Fritz et al., 1995) or stomach mucosa and/or submucosal (Fritz et al., 1995; Vasconcelos et al., 2003) with acute colitis with necrotizing vasculitis (1/13) (Fritz et al., 1995); necrosis of villus tips in ileum or jejunum (9/14) (Vasconcelos et al., 2003); or stomach with inflammation (2/14) or ulceration (1/14) (Vasconcelos et al., 2003)<br><br>Edema, congestion, and hemorrhage in the gastrointestinal tract (Twenhafel et al., 2007) | Gastrointestinal submucosal lesions (Abramova et al., 1993; Inglesby et al., 2002)<br><br>Necrosis, hemorrhage, and edema of the ileum (Albrink et al., 1960) |
| **Central Nervous System** | Brain and/or meningeal lesions with no leukocytic infiltrate (Zaucha et al., 1998) | Meningeal hemorrhage (Gleiser et al., 1963; Dalldorf et al., 1971; Fritz et al., 1995; Vasconcelos et al., 2003; Twenhafel et al., 2007; | Meningitis (Inglesby et al., 2002) including hemorrhagic meningitis (Plotkin et al., 2002); "Cardinal's Cap" (Inglesby et al., 2002) from |

| System | Rabbit | Nonhuman Primate | Human |
|---|---|---|---|
| | Bacilli in meninges (Peterson et al., 2007)<br><br>Meningitis with suppurative inflammation (U.S. Environmental Protection Agency, 2011) | Lever et al., 2008); including relatively minor levels of hemorrhage (Lever et al., 2008); higher incidence in high versus low-dose groups (Gleiser et al., 1963), low overall incidence (1/10) (Henning et al., 2012);<br>hemorrhage over entire surface of cerebrum, cerebellum, and brain stem (Twenhafel et al., 2007); and necrotizing vasculitis (2/14) (Vasconcelos et al., 2003)<br><br>Meningeal edema (Dalldorf et al., 1971; Vasconcelos et al., 2003)<br><br>Parenchymal hemorrhage in the brain (3/13) (Lever et al., 2008)<br><br>Meningitis (9/21) (Dalldorf et al., 1971); suppurative meningitis (10/13) (Fritz et al., 1995)<br><br>Edema in brain without hemorrhage (Albrink and Goodlow, 1959; Gleiser et al., 1963); or with acute hemorrhage (1/10) (Henning et al., 2012)<br><br>Occasional neuronal necrosis, spongiosis, gliosis, hemorrhage, neutrophils, and edema in cerebrum and cerebellum (Twenhafel et al., 2007)<br><br>Localized necrosis with accompanying cellular changes and overall decrease in number of glia (Henning et al., 2012) | hemorrhage of leptomeninges, more frequently identified from Sverdlovsk than 2001 anthrax letter event victims (Guarner and del Rio, 2011)<br><br>Subarachnoid hemorrhage, extensive at times including covering frontal, parietal, temporal, and occipital lobes (Suffin et al., 1978) or fully covering both cerebral hemispheres (Albrink et al., 1960) |

| System | Rabbit | Nonhuman Primate | Human |
|---|---|---|---|
| **Other Systems (e.g., Urogenital, Reproductive, etc.)** | Adrenal hemorrhage (Zaucha et al., 1998)<br><br>Ovarian hemorrhage (Zaucha et al., 1998) | Foci of hemorrhage in the kidney (1/13) (Fritz et al., 1995)<br><br>Adrenal hemorrhages (Gleiser et al., 1963), with extensive hemorrhage of cortex and medulla of adrenal glands (1/4) (Albrink and Goodlow, 1959); cortical necrosis (2/14) (Vasconcelos et al., 2003); and extravasation of blood in the cortex with thrombi in veins (8/23) (Dalldorf et al., 1971)<br><br>Periovarian or peritesticular congestion and/or hemorrhages (Twenhafel et al., 2007)<br><br>Ovarian hemorrhage and necrosis (1/14) (Vasconcelos et al., 2003)<br><br>Retroperitoneal hemorrhages (Gleiser et al., 1963)<br><br>Laryngeal inflammation and edema (1/14) (Vasconcelos et al., 2003) | Minimal cortical atrophy, occasionally minimal cortical hemorrhage in adrenal glands (Grinberg et al., 2001)<br><br>Hemorrhagic thyroiditis (Albrink et al., 1960) |

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# Appendix D - *Bacillus anthracis* Dose-Response Data for the Rabbit Characterized as Supportive Data or Additional Data

This appendix identifies and reviews the dose-response data sets for the rabbit categorized as Supporting Data or Additional Data for development of an inhalation dose-response relationship for *B. anthracis* spores. The literature search and the criteria used to categorize each data set are provided in the main body of the report (Section 5.3.2). The categorization of the dose-response data was based on a determination of the suitability of the data set for the development of *B. anthracis* dose-response relationships.

Key Studies were defined to be representative of the highest quality dose-response studies that met criteria for selection during the literature search. Key Studies identified for the rabbit are provided in the main body of the report (Section 5.4.2.4). Supporting Studies had identifiable limitations in assessment quality indicators relative to Key Studies, yet were found to have potential in bounding the dose-response relationship(s) as described by Key Studies. As noted previously, Additional Data were defined by missing data points critical to assessing dose-response relationships (e.g., original dose and response data set) or study design elements that limit utility for development of low-dose dose-response relationships. As a result, their utility in dose-response analysis may be limited to providing corroborative support for higher quality data.

***Supporting Studies***

No single dose-response data for the rabbit were categorized as Supporting Studies.

***Additional Data***

Table D-1 identifies the single dose dose-response data categorized as Additional Data for the rabbit. Studies are presented in alphabetical order by the first study author. The most cited rabbit $LD_{50}$ value of $1.05 \times 10^5$ originated from Zaucha et al. (1998) study, though the original dose-response data set was not published until Gutting et al. (2013) (Table D-1). The Zaucha et al. (1998) $LD_{50}$ value is based on a challenge of 50 animals with mean group doses of 98 to 713,000 spores (Gutting et al., 2013). The Zaucha et al. (1998) value has been cited directly or others have reported values that differ only by varying adjustments in the number of significant figures (Table D-1). The Zaucha et al. (1998) study was categorized as Additional Data due to the lack of response data in the range between 1% and 49%. Particle size data were not associated with the study exposures for which the $LD_{50}$ value was derived, and the inhalation rate was assumed to be determined via plethysmography but prior to the actual aerosol challenge. The dose spacing and the lack of responses between 0 and 50% lethality are problematic because there are insufficient data to differentiate between possible mathematical dose-response models based on the fit to the observable data. Given the interest in the low-dose region of the *B. anthracis* dose-response relationship, it is important to select the mathematical model appropriately to maximize the reliability of a low dose extrapolation.

One seemingly outlier value of 600,000 single spore particles (Barnes, 1947) was identified as an inhaled dose. Additional $LD_{50}$ values were identified that were derived from intranasal (Peterson et al., 2006; Weiss et al., 2006; Peterson et al., 2007) or bronchoscopic (Lovchik et al., 2012) administration. However, these values are not directly comparable to inhaled $LD_{50}$ values absent evaluation of potential modifications to ensure dosimetric equivalence to an inhaled dose metric.

## Table D-1. Single Dose Additional Data for the Rabbit

| **Study Citation, $LD_{50}$ or Other Modeled Values,* Rabbit Breed, and Strain(s)** | **Empirical Model Parameters and/or Outputs** |
|---|---|
| Barnes (1947)<br>$LD_{50}$ = 600,000 single spore particles<br>Unspecified rabbit<br>Unknown strain | Gutting et al. (2013)<br><br>Note: Analysis combined New Zealand white rabbit dose-response data sets reported in Zaucha et al. (1998), U.S. Environmental Protection Agency (2011), and previously unpublished data<br><br>Exponential model<br>$k = 7.223 \times 10^{-6}$<br>Exponential model predicted attack rate (i.e., probability of disease for given dose) for 10 spores = $7.22 \times 10^{-5}$<br><br>Competing risks model<br>$\frac{\lambda}{(\lambda + \Theta)} = 6.605 \times 10^{-6}$<br><br>Competing risks predicted attack rate = $6.61 \times 10^{-5}$ |
| Lovchik et al. (2012)<br>Bronchoscopic dose $LD_{50} = 10^{3.98}$ spores<br>SE ($log_{10}$) = ± 0.19<br>New Zealand white rabbit<br>Ames strain | |
| Peterson et al. (2006)<br>Intranasal $LD_{50} = 1 \times 10^5$ CFU<br>Unspecified rabbit<br>Peterson et al. (2007)<br>Intranasal $LD_{50} = 1.125 \times 10^5$ CFU<br>Dwarf Dutch-belted rabbit<br>Ames strain | |
| Weiss et al. (2006)<br>ATCC 14578 (Vollum) strain intranasal dose $LD_{50} = 3 \times 10^5$ spores<br>ATCC 6605 strain intranasal dose $LD_{50} = 2 \times 10^4$ spores<br>New Zealand white rabbit | |
| Zaucha et al. (1998)<br>$LD_{50}$ = 105,000 CFU<br>$LD_{99}$ = 136,000 CFU<br>New Zealand white rabbit<br>Ames strain<br>Dose-response data set published in Gutting et al. (2013)<br>Note: This $LD_{50}$ value is the most commonly cited value after adjusting for differing significant figures.<br>Fellows et al. (2001) $LD_{50} = 10^5$ spores<br>Little et al. (2004) $LD_{50} = 1.1 \times 10^5$ spores<br>Little et al. (2006) $LD_{50} = 1.1 \times 10^5$ spores<br>Pitt et al. (2001) $LD_{50} = 1.1 \times 10^5$ spores | |

* Inhaled dose metric unless otherwise noted
$\lambda$ – hazard rate, risk per unit time that spore will germinate
$\Theta$ – clearance rate, hazard rate, risk per unit time that an ungerminated spore will be cleared from lung 6
ATCC – American Type Culture Collection
CFU – colony forming unit(s)
k - fitted parameter, potency estimate in exponential dose-response model
$LD_{50}$ – median lethality value
SE – standard error

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# Appendix E - *Bacillus anthracis* Dose-Response Data for the Nonhuman Primate Characterized as Supportive Data or Additional Data

The classic Druett et al. (1953) study presents the only data categorized as a Supporting Study for the nonhuman primate (Table E-1). Druett et al. (1953) aerosol challenged rhesus macaque monkeys with the M36 strain *B. anthracis* single spores and 12 µm particles in nine and eight dose groups of eight monkeys, respectively. This study was identified as a Supporting Study due to the presence of raw dose-response data, particle size data, presence of low-dose groups, and sufficient animal numbers for modeling. However, the lack of real-time determination of inhalation rates was the primary reason that this study was categorized as a Supporting Study. The Druett et al. (1953) paper was unclear on the length of observation post-challenge but did identify that the experiments for "each particle size were completed within a period of two to three weeks." The infection endpoint was not reported.

The inhaled dose $LD_{50}$ value reported for single spore particles was 53,000 spores (Druett et al., 1953). Re-analyses of the Druett et al. (1953) data set reported $LD_{50}$ or equivalent $BMD_{50}$ values ranging from 96,800 (Haas, 2002) to approximately 50,000 (Curling et al., 2010; U.S. Environmental Protection Agency, 2010; Taft and Hines, 2012; Toth et al., 2013) (Table E-2). The reason for the difference in published $LD_{50}$ values has been attributed to the two-fold higher inhalation rate used by Haas (2002) and Bartrand et al. (2008) in lieu of the inhalation value identified by Druett et al. (1953) (Curling et al., 2010; U.S. Environmental Protection Agency, 2010; Taft and Hines, 2012; Toth et al., 2013).

## Table E-1. Single Dose Supporting Studies for the Nonhuman Primate

| Study Citation, Nonhuman Primate Species, and Strain | Supporting Study Outputs | Reanalysis Studies | Additional Data Outputs |
|---|---|---|---|
| **Single Dose** | | | |
| Druett et al. (1953)<br>Rhesus macaque (*Macaca mulatta*)<br>M36 strain | Environmental concentration associated with 50% mortality:<br>$Nt^{*} = 0.045 \times 10^{-6}$ Single spores – minutes/L<br>(Inhaled dose $\times 10^{-5}$ single spores = 0.53 [53,000])<br><br>$Log_{10}$ probit slope = 3.19 with intercept of 2.91 using exposure concentration $\times 10^{-4}$ as dose<br><br>Environmental concentration associated with 50% mortality:<br>$Nt = 0.64 \times 10^{-6}$ 12 µm Spore particles – minutes/L$^{*}$<br>(Inhaled dose $\times 10^{-5}$ 12 µm spores = 7.6 [760,000]) | Haas (2002) | Exponential model<br>$LD_{50}$ = 96,800 single spores<br>(CI = 70,700 to 136,000)<br>$k = 7.16 \times 10^{-6}$<br>(CI = $5.1 \times 10^{-6}$ to $9.8 \times 10^{-6}$) |
| | | Bartrand et al. (2008) | Exponential model<br>$LD_{50}$ = 92,000 single spores<br>(CI = 29,440 to 70.932) [sic]<br>$k = 7.16 \times 10^{-6}$ |
| | | Curling et al. (2010) | Exponential model<br>$LD_{50}$ = approx. 51,000 spores<br>$\lambda = 1.36 \times 10^{-5}$ |
| | | U.S. Environmental Protection Agency (2010)<br>Taft and Hines (2012) | Exponential model<br>$k = 1.44 \times 10^{-5}$<br>(CI = 9.81E-6 to 1.9E-5)<br>$BMD_{50}$ = 48,000 single spores<br>$BMDL_{50}$ = 37,000 single spores<br>$BMD_{10}$ = 7,300 single spores<br>$BMDL_{10}$ = 5,600 single spores<br>$BMD_{1}$ = 700 single spores<br>$BMDL_{1}$ = 540 single spores |
| | | Toth et al. (2013) | Exponential model<br>$r = 1.43 \times 10^{-5}$<br>$ID_{50}$ = 48,000 single spores<br>$ID_{10}$ = 7,400 single spores<br>$ID_{1}$ = 700 single spores |

$^{*}$Druett et al. (1953) used the term "dosage" (Nt) to describe the product of environmental concentration and period of exposure (e.g., $Nt \times 10^{-6} = 0.168$); for ease in reading the table, this term has been recorded as Nt (e.g., $0.168 \times 10^{-6}$), all exposures were of one minute duration

BMDx - benchmark dose for response in x% of individuals
BMDLx - the 95% lower statistical confidence limit of the BMD when the 95% lower confidence limit is applied to the estimated slope parameter value
CI - 95% confidence interval
$ID_x$ - infectious dose for *x* percent exposed, Toth et al. (2013) assumed $ID_{50} = LD_{50}$
k, λ, or r - fitted parameter, potency estimate in exponential dose-response model
$LD_{50}$ – median lethality value
Nt - dosage

A significant amount of nonhuman primate dose-response data was categorized as Additional Data (Table E-2). The majority of these data were in the form of reported inhaled dose $LD_{50}$ values or ranges with little or no accompanying data. One exception was the Young et al. (1946) $LD_{50}$ value of 200,000 that utilized an environmental concentration dose metric. The remaining data for $LD_{50}$ values or ranges in Table E-2 tended to group into two main ranges. The low end of the range was between approximately 4,000 and 11,000 CFU or spores (Brachman et al., 1960; Glassman, 1966; Peters and Hartley, 2002; Estep et al., 2003; Leffel and Pitt, 2006; Rossi et al., 2008) and a high-end range was between approximately 50,000 to 62,000 CFU or spores (Henderson et al., 1956; Ivins et al., 1996; Vasconcelos et al., 2003; Coleman et al., 2008). A range of historical $LD_{50}$ values for rhesus monkeys (30,000 to 172,000 CFU) was also identified by Leffel and Pitt (2006).

However, the identified $LD_{50}$ values should be evaluated carefully prior to use for informing risk assessment. It is important to recognize that most values were derived from studies with the primary purpose of evaluating pathology or medical countermeasures; the $LD_{50}$ values were generated with study designs that did not explicitly evaluate statistical considerations regarding animal and dose range to generate a representative median value.

With the exception of the Vasconcelos et al. (2003) $LD_{50}$ value, the remaining identified values in the 50,000 to 62,000 CFU range were cited as a personal communication or unpublished data from an author associated with the USAMRIID laboratories (e.g., Ivins et al. (1996), Vasconcelos et al. (2003), Coleman et al. (2008)) or were directly cited by an author with USAMRIID affiliation (e.g., Henderson et al. [1956] in Friedlander et al. [1993]). It is possible that multiple published citations of approximately the same $LD_{50}$ value may not represent

multiple independent studies that corroborate the identified value, but may be the same study or a limited number of studies repeatedly cited.

**Table E-2. Single Dose Additional Data for the Nonhuman Primate**

| **Study and $LD_{50}$ Value,* Nonhuman Primate Species, and Strain** | **Study for Data Set, Nonhuman Primate Species, Reanalysis Study, Model Parameters or Outputs, and Strain** | **Other Data, Nonhuman Primate Species, and Strain** |
|---|---|---|
| **Single Dose** | | |
| Brachman et al. (1960)<br>$LD_{50}$ = 6,000 spores†<br>Unspecified NHP<br>Goat hair mill aerosol, unknown strain(s) | Glassman (1966)<br>Cynomolgus monkey<br>*(Macaca fascicularis*)<br>Reanalyzed by Peters and Hartley (2002) using the reported probit slope = 0.67 per $log_{10}$ dose spores and $LD_{50}$ = 4,100 spores, each value rounded to two significant figures<br><br>$LD_{10}$ = 50 spores<br>$LD_2$ = 4 spores<br>$LD_1$ = 1 spore<br><br>Unknown strain | Albrink and Goodlow (1959)<br>Chimpanzee<br>(*Pan troglodytes* [*Schwarz*] *and Pan troglodytes troglodytes*)<br><br>Single dose administered to 4 animals:<br><br>Melvin: 32,800 inhaled viable spores - survived<br><br>John: 34,350 inhaled viable spores - survived<br><br>Grove: 39,700 Inhaled viable spores - died<br><br>Bill: 66,500 inhaled viable spores – died<br><br>Vollum rB strain |
| Coleman et al. (2008)<br>59,000 unspecified units†<br>Rhesus monkey<br>(*Macaca mulatta*)<br>Unknown strain | Barnewall et al. (2001)<br>Rhesus monkey<br>(*Macaca mulatta*)<br>Reanalyzed by U.S. Environmental Protection Agency (2010) and Taft and Hines (2012)<br><br>$BMD_{50}$ = 10,000 CFU<br>$BMDL_{50}$ = 4,900 CFU<br><br>$BMD_{10}$ = 1,100 CFU<br>$BMDL_{10}$ = 550 CFU<br><br>Unknown strain | |
| Estep et al. (2003)<br>Ames strain $LD_{50}$ = 10,900 CFU†<br>(Fieller's CI = 1,320 to 241,000)<br><br>Vollum strain $LD_{50}$ = 6,750 CFU†<br>(Fieller's CI = 21 to 116,000)<br><br>Rhesus monkey<br>(*Macaca mulatta*) | Janssen (1955a), Janssen (1955b), and Janssen (1955c)<br><br>Original studies did not identify nonhuman primate species, assumed to be *Macaca mulatta* by Taft and Hines (2012)<br><br>Reanalyzed by U.S. Environmental Protection Agency (2010) and Taft and Hines (2012) | |

| **Study and $LD_{50}$ Value,* Nonhuman Primate Species, and Strain** | **Study for Data Set, Nonhuman Primate Species, Reanalysis Study, Model Parameters or Outputs, and Strain** | **Other Data, Nonhuman Primate Species, and Strain** |
|---|---|---|
| Henderson et al. (1956)<br>$LD_{50}$ = approximately 50,000 spores<br>(Originally reported three individual results as 4 LNt50 ~ $2.14 \times 10^5$ spores, 8 LNt50 ~ $3.9 \times 10^5$ spores, and 4 LNt50 ~ $2 \times 10^5$ spores) †<br>Rhesus monkey<br>(*Macaca mulatta*)<br>M36 strain | $BMD_{50}$ = 660 CFU<br>$BMDL_{50}$ = 530 CFU<br><br>$BMD_{10}$ = 180 CFU<br>$BMDL_{10}$ = 150 CFU<br><br>Strain not identified in original study reports, but Vollum identified in use by U.S. Department of Defense researchers at that time by U.S. Environmental Protection Agency (2010) and Taft and Hines (2012) | |
| Glassman (1966)<br>$LD_{50}$ = 4,130 spores†<br>CI = 1,980 to 8,630 spores<br>Probit slope = 0.669 probits/log dose<br>CI = 0.520 to 0.818<br>Cynomolgus monkey<br>(*Macaca fascicularis*)<br>Unknown strain | | |
| Ivins et al. (1996)<br>Rhesus monkey<br>(*Macaca mulatta*)<br>$LD_{50}$ = 55,000 CFU†<br>Ames strain | | |
| Leffel and Pitt (2006)<br>Historically reported range of $LD_{50}$ values for unspecified strain: 30,000 to 172,000 CFU<br>Rhesus monkey<br>(*Macaca mulatta*)<br><br>$LD_{50}$ values from head-to-head test of same Ames spore lot:<br>Rhesus monkey = 7,200 CFU†<br>African green monkey = 8,300 CFU† | | |
| Peters and Hartley (2002)<br>$LD_{50}$ = approximately 8,000 CFU†<br>Cynomolgus monkey<br>*(Macaca fascicularis*)<br>Unknown strain | | |
| Rossi et al. (2008)<br>$LD_{50}$ = 11,000 CFU†<br>CI = $2.9 \times 10^3$ to $8.1 \times 10^4$<br>African green monkey<br>*(Chlorocebus aethiops)*<br>Ames strain | | |
| Sharp and Roberts (2006)<br>$LD_{50}$ value = c. 5,000 to 8,000 CFU†<br>Cynomolgus monkey<br>*(Macaca fascicularis*)<br>Unknown strain | | |

| Study and $LD_{50}$ Value,* Nonhuman Primate Species, and Strain | Study for Data Set, Nonhuman Primate Species, Reanalysis Study, Model Parameters or Outputs, and Strain | Other Data, Nonhuman Primate Species, and Strain |
|---|---|---|
| Vasconcelos et al. (2003)<br>$LD_{50}$ = 61,800 CFU<br>95% CI = 34,800 to 110,000 CFU<br>Probit slope = 4.21<br>Cynomolgus monkey<br>*(Macaca fascicularis*)<br>Ames strain | | |
| Young et al. (1946)<br>$LD_{50} = 20 \times 10^{-4}$ spores<br>(Note: Dose metric for $LD_{50}$ value is an environmental concentration for a 5-minute exposure)<br>Unspecified NHP<br>Detrick 25 strain | | Twenhafel et al. (2007)<br>African Green Monkey<br>*(Chlorocebus aethiops)*<br><br>Data describing low-dose lethality at the lowest tested dose of 204 CFU<br>Ames strain |

* Inhaled dose unless otherwise noted

† $LD_{50}$ value cited from unpublished data or personal communication

BMDx - benchmark dose for response in x% of individuals

BMDLx - the 95% lower statistical confidence limit of the BMD when the 95% lower confidence limit is applied to the estimated slope parameter value

CFU - colony forming unit(s)

CI - 95% confidence interval

$LD_x$ – lethality value for x% of individuals

NHP – nonhuman primate

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# Appendix F - Conducting Benchmark Dose Analysis for Microbial Pathogens

## Introduction

Benchmark dose (BMD) analysis empirically fits models to dose-response data and identifies the dose associated with a specific response level (U.S. Environmental Protection Agency, 2012).

The following section describes the process and special considerations for the use of BMD modeling with microbial pathogens. While there is a focus on the use of EPA's Benchmark Dose Software (BMDS) in some of the examples, the process description is applicable to other software capable of conducting the empirical modeling and reporting the necessary outputs.

## Conducting the BMD Analysis

BMD analysis is conducted using the following general steps:

- Evaluate the data set,
- Fit selected dose-response models,
- Identify the best fitting mathematical model(s), and
- Report the modeling results.

The following sections discuss each step in the process and identify potential considerations when modeling dose-response relationships of microbial pathogens.

## Evaluate the Data Set

Prior to use in BMD modeling, the dose-response data should be assessed for the sufficiency of the data for BMD analysis. This step is distinct from a quality assessment that evaluates the study design, documentation, and development of the data set. The minimum data set

requirements for BMD analysis are: (1) a dose-related trend in the assessment endpoint (either statistical and/or biological significance), (2) a data set with data points between the maximum response levels in control or higher-level dose groups and no response levels, and (3) typically more than one dose group (U.S. Environmental Protection Agency, 2012). However, two dose groups may also be insufficient to evaluate some models based on parameter number and may affect the ability to evaluate model uncertainty (U.S. Environmental Protection Agency, 2012). There should be at least as many dose groups as model parameters to estimate mean response and confidence levels (U.S. Environmental Protection Agency, 2012).

As with all analyses based on curve-fitting, there is a preference for studies that have more dose groups as well as a graded monotonic response with regard to dose (U.S. Environmental Protection Agency, 2015). However, many of the available dose-response data sets for *B. anthracis* reported dose-response data, but their original purpose was not derivation of dose-response data (e.g., pathology studies that also report median lethality [$LD_{50}$] values). Current dose-response data sets that are generated for inhalation challenge studies typically use plethysmographic inhalation data that allow for reporting both individual-specific inhalation doses and targeted dose group data. In these instances, individual dose-response data can be used instead of dose group-level data. Additionally, many of these data sets may have limited coverage below the $LD_{50}$ value, which limits the lower end of the observable range and may affect selection of statistically appropriate benchmark response (BMR) values. Accordingly, the use of these data in empirical model curve-fitting approaches may be associated with higher levels of uncertainty for lower dose levels than the levels typically found in analyses of chemical dose-response data sets with better low-dose coverage. This is not to suggest that BMD may not

be a useful modeling approach for the microbial data sets, but that the uncertainty associated with the BMD outputs from these data sets should be acknowledged.

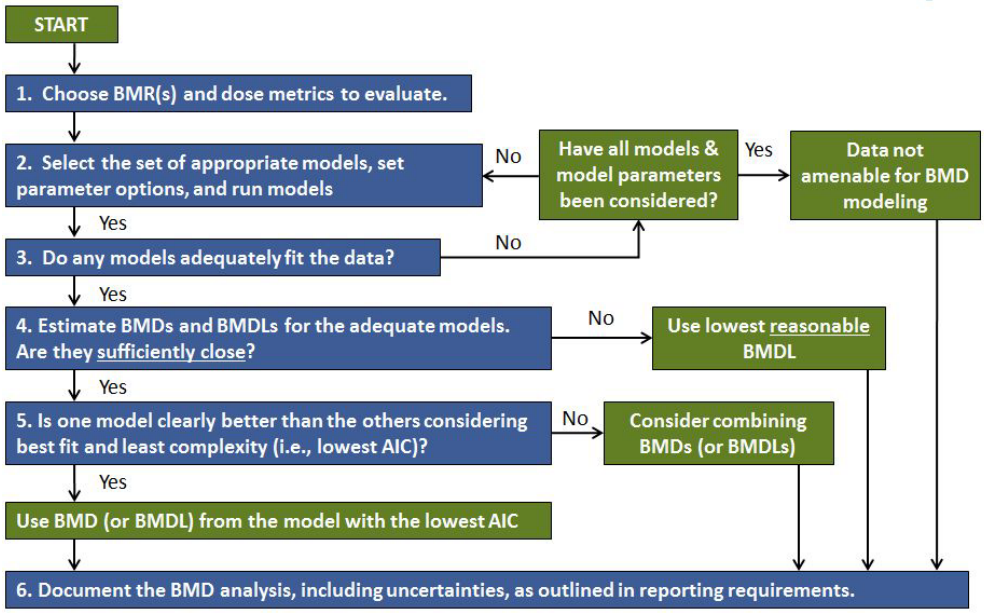
## Fit Selected Dose-Response Models

The EPA does not advocate use of any specific BMD or curve-fitting software package (U.S. Environmental Protection Agency, 2012), but recommends that selected software have a sufficiently documented methodology to evaluate the statistical algorithms used for model fit and the development of outputs. The BMDS (available from the product web page (http://www.epa.gov/ncea/bmds/) is one option to conduct BMD. The BMDS can be an important tool to evaluate commonly used empirical dose-response models for microbial pathogens, including *B. anthracis* (Taft and Hines, 2012). The BMDS was originally developed for chemical agents, but the empirical curve-fitting process employed in BMD has relevance for microbial agents (Taft and Hines, 2012). U.S. Environmental Protection Agency (2012) addresses considerations for benchmark dose analysis of chemical agents, but there is a gap in technical guidance for the use of BMD for microbial dose-response analysis.

A second software with BMD modeling capabilities is the PROAST software package (National Institute for Public Health and the Environment [RIVM], 2014). PROAST was developed by the National Institute for Public Health and the Environment (RIVM, The Netherlands) for the statistical analysis of dose-response and other similarly structured data sets. The software can be used to fit mathematical models, report goodness of fit (GOF) measures, and generate graphics (National Institute for Public Health and the Environment [RIVM], 2014). Potential advantages of PROAST may include the possibility of statistically comparing dose-response relationships

among subgroups in the data set and greater flexibility in plotting that was used to develop the BMDS software.

Figure F-1 (U.S. Environmental Protection Agency, 2015) shows a decision tree to assist in conducting the BMD modeling and determining the best fitting model(s). The first two determinations are selection of the BMR and the dose metric(s) for modeling. Considerations for the selection of the dose metric were discussed previously in Section 5.3.4 of the main report.




BMD – benchmark dose
BMDL – benchmark dose limit
AIC – Akaike Information Criterion

**Figure F-1. BMD decision tree from U.S. Environmental Protection Agency (2015).**

The BMR is the level of change in the response rate (e.g., a BMR of 10% would be equivalent to a 10% increase in the response rate of the endpoint of interest) that forms the basis for the reported BMD value. A BMR value of 10% is identified for chemical hazards and dichotomous data to standardize reporting of the benchmark dose limit (BMDL) values, but the value is not to

be interpreted as a default value (U.S. Environmental Protection Agency, 2012). The determination of a BMR should be based upon the intended use of the BMD outputs, the statistical features of the data set, and the biological basis of the modeled disease process (U.S. Environmental Protection Agency, 2012). An identified BMR value, or a range of BMR values, specific for microbial data to support risk-informed decision-making from BMDS outputs or for standardized reporting is not available. In chemical dose-response analysis, the reporting of BMDS outputs for *B. anthracis* data sets has also included the 10% BMR value for the BMDL value (e.g., Taft and Hines [2012]). However, the determination of the appropriate BMR values may require a unique evaluation relative to the values for chemical agents due to the reliance on lethality endpoints in *B. anthracis* dose-response data sets, high lethality levels associated with exposure levels of concern, and limited statistical power of most dose-response data sets. The identification of the BMR range of values or guidance for their selection is a science policy gap for microbial dose-response analysis.

A prior analysis using the BMDS and *B. anthracis* dose-response data sets evaluated the fit of the data to the following models: the Weibull model, the Weibull model run as exponential (with the power coefficient fixed as one), probit, $\log_e$ probit, logistic, $\log_e$ logistic, Gamma model, dichotomous Hill, probit-background response, and logistic-background response (Taft and Hines, 2012). The rationale for evaluation of a diverse group of empirical models was to minimize the model uncertainty associated with selection of one model and its associated assumptions (e.g., threshold, nonthreshold) (Taft and Hines, 2012).

When using modeling software for dose-response analysis, care should be taken to identify all assumptions or default restrictions placed on model parameters (Taft and Hines, 2012). There

should be sufficient information to allow an individual to recreate the dose-response model outputs from the input identified data set. For example, the BMDS places the default restriction on the slope and power terms to ensure that they do not have values greater than or equal to one. This prevents supralinear behavior in the low-dose region of the dose-response curve (U.S. Environmental Protection Agency, 2012). Since historically used microbial dose-response models (e.g., exponential, beta-Poisson) are linear in the low-dose region (Haas et al., 1999), the identified restrictions on term values are appropriate for microbial pathogens. The BMDS also includes a suite of models that allows for setting the background incidence to zero (e.g., alternative dichotomous models) if an individual lacks this fundamental assumption. This is appropriate for *B. anthracis* since it should be assumed that there is no background incidence in the challenge studies.

## Identify the Best Fitting Mathematical Models

There are no differences in the assessment of goodness of fit (GOF) for microbial dose-response analysis and chemical dose-response analysis. The Chi-square statistical test is used to evaluate the overall GOF for an individual model (U.S. Environmental Protection Agency, 2012). An insignificant p-value ($p > 0.1$) does not allow for the rejection of the null hypothesis ($H_o$) and indicates that the tested model fits the data. If the estimated BMDs and BMDLs are "sufficiently close" (as determined by decision-making needs) for models that have acceptable statistical fits to the data, the model with the lowest Akaike Information Criterion (AIC) value will be considered to have the best fit (U.S. Environmental Protection Agency, 2012). However, it should be noted that an AIC comparison should not be made across models with different restrictions in the slope, power, or background parameters (U.S. Environmental Protection

Agency, 2012). From this model with the lowest AIC value, the point of departure (POD) can be determined from the BMDL associated with the selected BMR value. An evaluation of visual fit as well as scaled residuals near the BMR(s) of interest should also be conducted (U.S. Environmental Protection Agency, 2012).

The selection of the POD(s) for use in the interspecies extrapolation process can involve additional steps to focus the model review to ensure that there is adequate statistical fit to the data and visual fit, especially in the low-dose regions (U.S. Environmental Protection Agency, 2012). Detailed analysis for determination of the POD across multiple suitably fitting models should be done in consultation with statistical experts (U.S. Environmental Protection Agency, 2012).

## Report the Modeling Results

Guidance is available on preferred reporting for BMD outputs that is applicable regardless of the platform used to conduct the analysis. If using the BMDS, it is recommended that summary reporting capability provided by the BMDS Wizard be used to facilitate reporting of BMD model fit and outputs. As with all dose-response modeling, the restriction of any model parameters (e.g., slope, power) should be clearly identified. If varying dose metrics were generated, the base assumptions and data used to calculate the dose metric should be clearly identified. For situations where multiple models exhibit a statistically significant fit, the rationale for model selection should be transparent and clearly describe the basis for selection.

When colony-based counting methods (e.g., bacterial plate counts) are used for the measurement of challenge doses for *B. anthracis*, care must be taken in reporting dose-response outputs. It is generally recognized that these analytical methods are only precise to two significant digits.

Accordingly, dose-response model outputs for BMD and BMDL values are reported to an equivalent number of significant figures.

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