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technical BRIEF

BUILDING A SCIENTIFIC FOUNDATION FOR SOUND ENVIRONMENTAL DECISIONS

Review of *Bacillus anthracis* (Anthrax) Studies for Dose-Response Modeling to Estimate Risk

INTRODUCTION

U.S. EPA's Homeland Security Research Program (HSRP) develops products based on scientific research and technology evaluations. Our products and expertise are widely used in preventing, preparing for, and recovering from public health and environmental emergencies that arise from terrorist attacks. Our research and products address biological, radiological, or chemical contaminants that could affect indoor areas, outdoor areas, or water infrastructure. HSRP provides these products, technical assistance, and expertise to support EPA's roles and responsibilities under the National Response Framework, statutory requirements, and Homeland Security Presidential Directives.

Bacillus anthracis, the causative agent of inhalation anthrax, is one of the most highly studied biological threat agents (Wilkening 2006), yet consensus is still lacking on an appropriate dose-response relationship to describe the human health effects from low dose exposures (Taft and Hines 2012). Dose-response relationships can be an important element in the development of protective actions and decontamination strategies after a bioterrorism event (Executive Office of the President 2009). For example, dose-response relationships can assist in the identification of environmental concentrations where protective actions may be necessary to arrest the development of inhalation anthrax in exposed individuals.

A commonly cited dose-response number is the infectious dose that could cause illness in 50% of the exposed population (ID_{50}). Unfortunately, due to the very high mortality associated with inhalational anthrax, there are no dose-response data available to estimate any anthrax specific infectious doses such as the ID_{50} (Leffel and Pitt 2006). There are dose-response studies in the literature that report anthrax lethal doses such as the lethal dose that could cause death in 50% of the exposed population (LD_{50}). However, all of the reported lethal dose-response data come from experimental animal exposure studies because there are no human dose-response data in the available literature. Even for those limited events where inhalation anthrax has been diagnosed (e.g., occupational settings in animal hair mills, 2001 anthrax letters event, Sverdlovsk accidental release (Wilkening 2006)), there were no corresponding environmental dose-response data collected as part of the epidemiological investigation.

This technical brief identifies current challenges in the development of a *B. anthracis* dose-response relationship specifically for low dose exposures, summarizes available dose-response data sets for inhalation exposures in commonly used animal models and observational data gathered from human epidemiological studies, and assesses the usability of the identified dose-response data sets for modeling low dose exposures.

U.S. Environmental Protection Agency
Office of Research and Development, Homeland Security Research Program

EPA/600/S-12/681
October 2012

REVIEW OF AVAILABLE DOSE-RESPONSE DATA SETS FOR MODELING LOW DOSE EXPOSURES

To support the development of a *B. anthracis* dose-response relationship for low dose exposures, a review of published or other open-source reports containing *B. anthracis* inhalation lethality dose-response data from acute exposure studies (i.e., exposures of one 24-hour period or less) of the guinea pig (Table 1), rabbit (Table 2), and nonhuman primate (Table 3) were conducted. No human dose-response data were identified for acute *B. anthracis* exposure. Data from lethality dose-response studies using multiple dose exposures (i.e., more than one dose in an exposure period greater than 24 hours) were also identified for guinea pig, rabbit, and nonhuman primate receptors; observational data from epidemiological studies of multiple dose exposures in an occupational setting were found for humans (Table 4).

The primary purpose of this evaluation was to identify published, open source dose-response data sets and provide a preliminary assessment of their data usability for modeling low dose *B. anthracis* exposures. Dose-response data were included in these tables if (1) dose-response data and/or summary statistics (i.e., LD_{50}) were reported, and (2) dose and response were concurrently measured and reported from the same study population. Tables identify the study authors and year of publication, study details, and an assessment of the data usability for modeling low dose exposures. The "Highest Dose or Highest Dose Group without Reported Death" column identifies the reported dose that represents the highest dose for which no individual in the identified dose group or lower dose groups were reported to have died from the tested (animal) or observed (human) exposure. If data were reported on an individual basis, the column identifies the highest reported dose for which no individual died at that dose or lower dose levels. The identified doses are not analogous to a No Observable Adverse Effect Level (i.e., NOAEL) as adverse effects other than death can occur with *B. anthracis* exposure and were not captured when lethality was the only measure of effect.

Dose-response data must have included a measure of environmental air concentration or inhalation dose; dose data that were derived from modeled estimates of air concentration data (e.g., Meselson et al. 1994) were not included. Re-analyses of previously published data were also not included in these data tables (e.g., Haas 2002, Bartrand et al. 2008).

Data usability for modeling low dose exposures was assessed by the development of a data usability score for each dose-response data set or summary statistic reported from the identified publication. Data usability scores were assigned into one of three categories: high data usability identified by green shading in the data usability cell of the table, medium data usability identified by yellow shading, and low data usability identified by red shading. Figure 1 identifies the scoring questions and describes the process to assign the data usability for modeling low dose-exposures. Scores were assessed using only published data. It is important to recognize that studies that received a low data usability score in this assessment may be of very high quality relative to their originally intended purpose. However, the data usability score presented here is an assessment of the applicability of the published data specific to modeling low dose exposures to support site-specific risk-based decisions.

To Develop Data Usability for Modeling Low Dose Exposures Rating:

Assign one point for each question that can be answered "yes" for the identified data set and/or reported summary statistic and identify rating based on numerical score and answers to specific questions that trigger an automatic usability rating.

1) **Are dose-response raw data available?**
2) **Are particle size distribution data or identification of single spore particles reported based on measurements during testing?**
3) **Are there dose groups with a less than a 50% lethality rate or is there an overall lethality rate of less 50% is doses reported for individuals?**
4) **Were real-time methods used during the exposures to derive inhalation rates?**
5) **Is the dose group number ($n \geq 5$) or total number tested for individual dose measurements ($n \geq 12$) sufficient for dose-response modeling?**

Data Usability Rating for Modeling Low Dose Exposures	Question Answers that Automatically Assign Usability Rating for Modeling Low Dose Exposures	Numerical Score for Usability for Modeling Low Dose Exposures
High	Not Applicable	5
Medium	Not Applicable	3 or 4
Low	No to Question 1 or 5	<3

Figure 1. Scoring system to assign data usability score for modeling low dose exposures.

GUINEA PIG DOSE-RESPONSE STUDIES

There were no identified guinea pig dose-response studies that were assigned a high data usability score for modeling low dose exposures (Table 1). The primary areas of weakness were a lack of identified particle size (e.g., Altboum et al. 2002; Barnes 1947), a lack of real-time methods to derive inhalation rates (e.g., use of allometric equation to estimate inhalation rate in Druett et al. 1953), or a lack of dose information (e.g., Barnes 1947; Day et al. 1962; Young et al. 1946). As part of earlier efforts to evaluate available guinea pig dose-response data, the U.S. Environmental Protection Agency's (EPA) National Homeland Security Research Center in conjunction with the US Army Public Health Command developed the Pathogen Information Catalog (PI-CAT) (EPA 2009) to compile unclassified data for selected biological threat agents. A report was generated that evaluated PI-CAT data for the guinea pig and a conducted a benchmark dose analysis of selected data sets (EPA 2010a).

RABBIT DOSE-RESPONSE STUDIES

Two studies conducted by EPA (2011 and 2012) that were designed to develop dose-response data for modeling low dose exposures were the only studies receiving a high data usability rating for the rabbit animal model (Table 2) for the purpose of this evaluation. The majority of the identified studies received a medium to low data usability score for modeling low dose exposure because the dose-response data were control group data with no survivors (e.g., Little et al. 2004, Pitt et al. 2001) or doses were not reported specific for dose groups or individuals (e,g, Barnes 1947, Zaucha et al. 1998).

NONHUMAN PRIMATE DOSE-RESPONSE STUDIES

There was one published nonhuman primate study (Lever et al. 2008) that received a high usability data rating (Table 3). The main reasons for lower data usability ratings for the other identified studies were a lack of reported doses for dose groups or individuals (e.g., Estep et al. 2003; Ivins et al. 1998; Vasconcelos et al. 2003; Young et al. 1946) or a lack of reporting of the particle size distribution (e.g, Rossi et al. 2008; Twenhafel et al. 2007). Druett et al. (1953) received a medium data usability rating because the inhalation rates were only derived from allometric equations. A number of these reported dose-response data sets originated from studies designed to evaluate pathology of inhalation anthrax (e.g., Twenhafel et al. 2007; Vasconcelos et al. 2003) or efficacy of medical countermeasures (e.g., Friedlander et al. 1993; Ivins et al. 1998). By design, many of these studies would not be expected to produce data sets with the necessary characteristics to assess dose-response relationships, especially for modeling low dose exposures needed to support risk-based site-specific decision making.

Given the limited published data of high usability for modeling low dose exposures for the nonhuman primate, the PI-CAT (EPA 2009) was again queried for available data. Additional data sets were identified and evaluated for development of dose-response relationships specifically for low dose exposures; the outputs of this modeling were published (EPA 2010b; Taft and Hines 2012). However, the two dose-response data sets that were also reported in EPA (2010b) and Taft and Hines (2012) did not include identification in the publications of dose-response raw data due to distribution limitations maintained by the originators of the data.

MULTIPLE EXPOSURES DOSE-RESPONSE STUDIES

There were considerably fewer dose-response data sets published describing multiple dose exposure to *B. anthracis* for observations in human and animal dose-response data (Table 4). The EPA's (2012) multiple dose rabbit study was assigned a high data usability rating for modeling low dose exposures. The remaining identified studies were all assigned low data usability ratings due to a lack of sufficient individuals tested (e.g., Albrink and Goodlow 1959; Dahlgren et al. 1960) or a lack of real-time methods to derive inhalation rates (e.g., Albrink and Goodlow 1959; Brachman et al. 1966; Dahlgren et al. 1960). One study identifying human exposure data for a multiple dose exposure to *B. anthracis* was identified (Dahlgren et al. 1960) and included in Table 4. This epidemiological study reported *B. anthracis* air concentrations over a two-day period and the lack of inhalation anthrax observed in exposed workers was noted. However, data interpretation is complicated by the fact that approximately 30% of the exposed individuals were vaccinated at the time of the testing in one mill and 100% vaccinated in the second mill. The study was rated low data usability because of the low number of individuals exposed and a lack actual individual exposure doses and inhalation rates.

SUMMARY

The reported experimental animal anthrax lethal dose-response data in the literature are highly variable with the LD_{50}s ranging from 10^2 to 10^6. Historically, the animal exposure studies were conducted to assess weapons potential or to test the effectiveness of countermeasures such as antibiotics or vaccines. Therefore, these studies were often conducted with one-time (acute) exposure at very high doses, which makes them less applicable for estimating the potential human health risk posed by repeated exposures at low doses. Different techniques are required to most accurately extrapolate animal lethal doses to corresponding human consequences. The technique that is required depends on the animal model used and whether dose estimates were derived from environmental concentrations, inhaled doses, intranasal administrations, and/or different exposure durations. It is therefore critical to evaluate what the reported historical literature dose numbers actually represent and how they were derived, as well as the limitations for the intended application.

The methods used for the experimental animal inhalation exposure study have advanced significantly, and recent dose-response studies using these contemporary techniques are more applicable for modeling low dose exposures and estimating potential human health risks. However, there has been only one recent applicable study that evaluated the dose-response of repeated low doses of *B. anthracis.* Additional dose-response studies targeting repeated exposures are still needed before the potential risk posed by repeated exposure to residual spores following anthrax contamination events can be adequately addressed.

Overall, the differences in reported experimental methodologies, strain virulence, host susceptibilities, and uncertainties extrapolating animal data to humans make the selection of one specific lethal dose or infectious dose number nearly impossible. Until adequate dose-response studies targeting repeated low doses are conducted, quantifying risks of exposure to *B. anthracis* is hampered by the lack of sufficient dose response data.

LIST OF TABLES

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Table 1. Available Dose-Response Data for Acute Exposure of the Guinea Pig

Author and Year	Strain and Particle Size	Doses Tested (Units)	Number per Dose Group	LD_{50} Reported for Study Data (95% Confidence Limit)	Highest Dose or Highest Dose Group Without Reported Death	Reported as Environmental Concentration, Inhaled Dose, or Intranasal Administration	Data Usability for Modeling Low Dose Exposures (Red, Yellow, or Green and Reason(s) for Rating)
Altboum et al. 2002	Vollum, Particle Size Not Reported	2×10^7 CFU	8	4×10^4 CFU	2×10^3 CFU	Intranasal Administration	**Medium: Particle size not reported based on measurement, Lack of real-time methods to derive inhalation rates**
		2×10^6 CFU					
		2×10^5 CFU					
		2×10^4 CFU					
		2×10^3 CFU					
		2×10^2 CFU					
	ATCC 6605, Particle Size Not Reported	3×10^6 CFU	9	8×10^4 CFU	3×10 CFU		
		3×10^5 CFU					
		3×10^4 CFU					
		3×10^3 CFU					
		3×10^2 CFU					
		3×10 CFU					
Barnes 1947	Strain Not Reported, 98% Single Spore Particles	7.5×10^5 Spores/l	20	Not Reported	Lethality in All Tested Dose Groups	Environmental Concentration	**Medium: Particle size not reported based on measurement, Lack of real-time methods to derive inhalation rates**
		6.56×10^5 Spores/l					
		7.05×10^5 Spores/l					
Druett et al. 1953	M36 (Vollum), Single Spore Particles	0.168×10^6 Single Spores – minutes/l*	32	0.34×10^6 Single Spores – minutes/l	Lethality in All Tested Dose Groups	Environmental Concentration	**Medium: Lack of real-time methods to derive inhalation rates**
		0.346×10^6 Single Spores – minutes/l					
		0.646×10^6 Single Spores – minutes/l					
		1.000×10^6 Single Spores – minutes/l					
	M36 (Vollum), 3.5 µm Particles	0.26×10^6 Organisms – minutes/l	40	0.36×10^6 Organisms – minutes/l	Lethality in All Tested Dose Groups	Environmental Concentration	**Medium: Lack of real-time methods to derive inhalation rates**
		0.44×10^6 Organisms – minutes/l	40				
		0.17×10^6 Organisms – minutes/l	20				
		0.29×10^6 Organisms – minutes/l	20				
		0.44×10^6 Organisms – minutes/l	20				
		0.52×10^6 Organisms – minutes/l	20				
		0.69×10^6 Organisms – minutes/l	20				

Author and Year	Strain and Particle Size	Doses Tested (Units)	Number per Dose Group	LD_{50} Reported for Study Data (95% Confidence Limit)	Highest Dose or Highest Dose Group Without Reported Death	Reported as Environmental Concentration, Inhaled Dose, or Intranasal Administration	Data Usability for Modeling Low Dose Exposures (Red, Yellow, or Green and Reason(s) for Rating)
	M36 (Vollum), 4.5 µm Particles	0.597×10^6 Organisms – minutes/l	40	0.49×10^6 Organisms – minutes/l	Lethality in All Tested Dose Groups	Environmental Concentration	**Medium: Lack of real-time methods to derive inhalation rates**
		0.269×10^6 Organisms – minutes/l					
		0.374×10^6 Organisms – minutes/l					
		0.125×10^6 Organisms – minutes/l					
		1.025×10^6 Organisms – minutes/l					
		1.34×10^6 Organisms – minutes/l					
		0.385×10^6 Organisms – minutes/l					
		0.231×10^6 Organisms – minutes/l					
	M36 (Vollum), 8 µm Particles	7.32×10^6 Organisms – minutes/l	40	3.8×10^6 Organisms – minutes/l	Lethality in All Tested Dose Groups	Environmental Concentration	**Medium: Lack of real-time methods to derive inhalation rates**
		2.28×10^6 Organisms – minutes/l					
		3.39×10^6 Organisms – minutes/l					
		4.78×10^6 Organisms – minutes/l					
		1.72×10^6 Organisms – minutes/l					
	M36 (Vollum), 12 µm Particles	3.16×10^6 Organisms – minutes/l	40	5.7×10^6 Organisms – minutes/l Note: Used recalculated results for Table 8.	Lethality in All Tested Dose Groups	Environmental Concentration	**Medium: Lack of real-time methods to derive inhalation rates**
		12.19×10^6 Organisms – minutes/l					
		2.84×10^6 Organisms – minutes/l					
		1.87×10^6 Organisms – minutes/l					
		5.8×10^6 Organisms – minutes/l					
		12.8×10^6 Organisms – minutes/l					
		7.65×10^6 Organisms – minutes/l					
Druett et al. 1953	M36 (Vollum), 3.6 µm Particles with 18 Spores per Particle	1.66×10^6 Organisms – minutes/l	40	Not Reported	Lethality in All Tested Dose Groups	Environmental Concentration	**Medium: Lack of real-time methods to derive inhalation rates**
	M36 (Vollum), 8.4 µm Particles with 19 Spores per Particle	1.39×10^6 Organisms – minutes/l					
	M36 (Vollum), 11.6 µm Particles with	1.1×10^6 Organisms – minutes/l					

Author and Year	Strain and Particle Size	Doses Tested (Units)	Number per Dose Group	LD_{50} Reported for Study Data (95% Confidence Limit)	Highest Dose or Highest Dose Group Without Reported Death	Reported as Environmental Concentration, Inhaled Dose, or Intranasal Administration	Data Usability for Modeling Low Dose Exposures (Red, Yellow, or Green and Reason(s) for Rating)
	18 Spores per Particle						
Barnes 1947	Strain Not Reported, 98% Single Spore Particles	Not Reported	Not Reported	370,000 Spores	Not Reported	Inhaled Dose	**Low: Dose-response raw data not reported, Lack of real-time methods to derive inhalation rates**
Brachman et al. 1960	Strain and Particle Size Not Reported	Not Reported	Not Reported	50,000 Inhaled Spores	Not Reported	Inhaled Dose	**Low: Dose-response raw data not reported, Particle size not based on measurement**
Day et al. 1962	V1B, Particle NMD 2.5 µm	Ranged from 2×10^3 to 4×10^6 Spores	6 to 10 Animals	6.5×10^2 Spores (3.9×10^2 to 1.3×10^4 Spores) 5.8×10^4 Spores (3.6×10^4 to 2.1×10^5 Spores) 9.8×10^3 Spores (5.5×10^3 to 2.1×10^4 Spores) 3.0×10^3 Spores (2.2×10^2 to 4.2×10^4 Spores)	Not Reported	Inhaled Dose	**Low: Dose-response raw data not reported, Lack of real-time methods to derive inhalation rates**
	NH_6, Particle NMD 2.5 µm	Ranged from 2×10^3 to 4×10^6 Spores	6 to 10 Animals	4.4×10^4 Spores (1.3×10^4 to 1.5×10^5 Spores) 5.0×10^4 Spores (3.1×10^4 to 8.1×10^4 Spores)	Not Reported	Inhaled Dose	**Low: Dose-response raw data not reported, Lack of real-time methods to**

Author and Year	Strain and Particle Size	Doses Tested (Units)	Number per Dose Group	LD_{50} Reported for Study Data (95% Confidence Limit)	Highest Dose or Highest Dose Group Without Reported Death	Reported as Environmental Concentration, Inhaled Dose, or Intranasal Administration	Data Usability for Modeling Low Dose Exposures (Red, Yellow, or Green and Reason(s) for Rating)
				7.0×10^4 Spores (3.9×10^4 to 1.2×10^5 Spores)			**derive inhalation rates**
				3.8×10^4 Spores (1.7×10^4 to 6.8×10^4 Spores)			
Young et al. 1946	Detrick 25, Single Spore Particles	Not Reported	16	19×10^4 Spores/l	Not Reported	Environmental Concentration (5-Minute Exposure)	**Low: Dose-response raw data not reported, Lack of real-time methods to derive inhalation rates**

*Druett et al. (1953) uses the term “dosage” (Nt) to describe the product of environmental concentration and period of exposure (e.g., Nt × 10^{-6} = 0.168); for ease in reading table, this term has been recorded as Nt (e.g., 0.168 × 10^6)
CFU – Colony forming unit
l - Liter
LD_{50} – Lethal Dose for 50% of the Tested Population
NMD – Number Median Diameter
µm – micron

Table 2. Available Dose-Response Data for Acute Exposure of the Rabbit

Author and Year	Strain and Particle Size	Doses Tested (Units)	Number per Dose Group	LD_{50} Reported for Study Data (95% Confidence Limit)	Highest Dose or Highest Dose Group Without Reported Death	Reported as Environmental Concentration or Inhaled Dose (Comment)	Data Usability for Modeling Low Dose Exposures (Red, Yellow, or Green and Reason(s) for Rating)
U.S. Environmental Protection Agency 2011	Ames, MMAD (GSD) for Each Dose Group 0.96 µm (1.33), 0.82 µm (1.48), 0.92 µm (1.57), 0.87 µm (1.59), 1.12 µm (1.33), and 1.12 µm (1.31), Respectively	2.00 CFU/animal	5	5.18×10^4 CFU (95% Fieller's Confidence Interval, 6.14×10^3 CFU to 7.27×10^5 CFU)	2.06×10^3 CFU	Inhaled Dose	**High: All data usability elements present**
		2.86×10^2 CFU/animal					
		2.06×10^3 CFU/animal					
		2.54×10^4 CFU/animal					
		2.75×10^5 CFU/animal					
		8.27×10^6 CFU/animal					
Barnes 1947	Strain Not Reported, 98% Single Spore Particles	Not Reported	Not Reported	600,000 Spores	Not Reported	Inhaled Dose	**Low: Dose-response raw data not reported, Lack of real-time methods to derive inhalation rates**
Little et al. 2004	Ames, Particle Size Not Reported	Compilation of 4 Separate Experiments with Reported Average Doses Not Distinguished Between Control and Treatment Groups, 166.2 ± 95.77 (Mean $\pm$ SD) $\times LD_{50}$, 467.4 ± 379.7 (Mean $\pm$ SD) $\times LD_{50}$, 156.7 ± 97.5 (Mean $\pm$ SD) $\times LD_{50}$, 228.7 ± 106.0 (Mean $\pm$ SD) $\times LD_{50}$ where $LD_{50} = 1.1 \times 10^5$ Spores	31	Not Calculable	No Survivors in Control Groups	Inhaled Dose	**Low: Dose-response raw data not reported, Lack of real-time methods to derive inhalation rates, 100% lethality**
		Average Dose of 269.4 ± 258.9 (Mean $\pm$ SD) $\times LD_{50}$ where $LD_{50} = 1.1 \times 10^5$ Spores	8				
Pitt et al. 2001	Ames, Particle Size of MMAD 1.2 µm	Reported Average Dose for Both Control and Treatment Groups of 133 ± 51 (Mean $\pm$ SD) $\times LD_{50}$ where $LD_{50} = 1.1 \times 10^5$ Spores	8	Not Calculable	No Survivors in Control Groups	Inhaled Dose	**Low: Dose-response raw data not reported, Lack of real-time methods**

Author and Year	Strain and Particle Size	Doses Tested (Units)	Number per Dose Group	LD_{50} Reported for Study Data (95% Confidence Limit)	Highest Dose or Highest Dose Group Without Reported Death	Reported as Environmental Concentration or Inhaled Dose (Comment)	Data Usability for Modeling Low Dose Exposures (Red, Yellow, or Green and Reason(s) for Rating)
		Reported Average Dose for Both Control and Treatment Groups of 84 $\pm$ 42 (Mean $\pm$ SD) × LD_{50} where $LD_{50} = 1.1 \times 10^5$ Spores	10				**to derive inhalation rates, 100% lethality**
Zaucha et al. 1998	Strain and Particle Size Not Reported	Not Reported	Not Reported	1.05×10^5 CFU	Not Reported	Inhaled Dose	**Low: Dose-response raw data not reported, LD_{50} reported for different data set than discussed in publication**

CFU – colony forming unit
GSD – Geometric Standard Deviation
µm – micron
LD_{50} - Lethal Dose for 50% of the tested population
MMAD – Mass Median Aerodynamic Diameter
SD – Standard Deviation

Table 3. Available Dose-Response Data for Acute Exposure of the Nonhuman Primate

Author and Year	Strain, Particle Size, and Animal Model	Doses Tested (Units)	Number per Dose Group	LD_{50} Reported for Study Data (95% Confidence Limit)	Highest Dose or Highest Dose Group Without Reported Death	Reported as Environmental Concentration or Inhaled Dose (Comment)	Data Usability for Modeling Low Dose Exposures (Red, Yellow, or Green and Reason(s) for Rating)
Lever et al. 2008	Ames, Particle Size in the 1 to 3 µm Range, Marmoset	1.9×10^5 CFU	1	1.47×10^3 CFU (7.19 to 2.95×10^5 CFU)	1.4×10^1 CFU	Inhaled Dose	**High: All data usability elements present**
		1.1×10^5 CFU					
		1.4×10^5 CFU					
		1.6×10^4 CFU					
		1.5×10^4 CFU					
		1.2×10^4 CFU					
		2.4×10^4 CFU					
		3.7×10^3 CFU					
		2.5×10^3 CFU					
		2.3×10^2 CFU					
		4.2×10^2 CFU					
		1.4×10^1 CFU					
Druett et al. 1953	M36 (Vollum), Single Spore Particles, Rhesus Macque	0.0293×10^6 Single Spores – minutes/l†	8	0.045×10^6 Single Spores – minutes/l	Lethality at All Tested Dose Levels	Environmental Concentration	**Medium: Lack of real-time methods to derive inhalation rates**
		0.0321×10^6 Single Spores – minutes/l					
		0.0453×10^6 Single Spores – minutes/l					
		0.0573×10^6 Single Spores – minutes/l					
		0.0648×10^6 Single Spores – minutes/l					
		0.0670×10^6 Single Spores – minutes/l					
		0.1000×10^6 Single Spores – minutes/l					
		0.1250×10^6 Single Spores – minutes/l					
		0.1660×10^6 Single Spores – minutes/l					
	M36 (Vollum), 12 µm Particles, Rhesus Macque	0.251×10^6 Organisms – minutes/l	8				
		0.320×10^6 Organisms – minutes/l	8				
		0.422×10^6 Organisms – minutes/l	8				
		0.615×10^6 Organisms – minutes/l	8				
		0.682×10^6 Organisms – minutes/l	8				
		1.760×10^6 Organisms – minutes/l	7				
		3.310×10^6 Organisms – minutes/l	6				
		3.74×10^6 Organisms – minutes/l	8				
Twenhafel et al. 2007	Ames, Particle Size Not	204 CFU	1	Not Calculable	Lethality in Lowest Tested	Inhaled Dose	**Medium: Particle size not reported**
		2.2×10^3 CFU					

Author and Year	Strain, Particle Size, and Animal Model	Doses Tested (Units)	Number per Dose Group	LD_{50} Reported for Study Data (95% Confidence Limit)	Highest Dose or Highest Dose Group Without Reported Death	Reported as Environmental Concentration or Inhaled Dose (Comment)	Data Usability for Modeling Low Dose Exposures (Red, Yellow, or Green and Reason(s) for Rating)
	Reported, African Green Monkey	3.2×10^3 CFU 4.9×10^3 CFU 5.5×10^3 CFU 9.8×10^3 CFU 2.2×10^4 CFU 2.6×10^4 CFU 2.8×10^4 CFU 3.5×10^4 CFU 9.8×10^6 CFU 1.0×10^7 CFU			Individual Dose of 204 CFU		**based on measurement, Lack of real-time methods to derive inhalation rates**
Albrink and Goodlow 1959	Vollum Particle Size Ranged from 1.05 to 1.4 NMD, Chimpanzee	32,800 Viable Spores 34,350 Viable Spores 39,700 Viable Spores 66,500 Viable Spores	1	Not Reported	34,350 Viable Spores	Inhaled Dose	**Low: Number of total individuals from which dose measurements were obtained less than 12, Lack of real-time methods to derive inhalation rates**
Glassman 1966	Strain Not Reported, Particle Size Reported to be ≤ 5 µm, Cynomolgus Monkey	Not Reported	Not Reported	4,130 Spores (95% Confidence Interval of 1,980 to 8,630 Spores)	Not Reported	Inhaled Dose	**Low: Dose-response raw data not reported, Lack of real-time methods to derive inhalation rates**
Brachman et al. 1960	Strain and Particle Size Not Reported, Unspecified Monkey	Not Reported	Not Reported	6,000 Inhaled Spores	Not Reported	Inhaled Dose	**Low: Dose-response raw data not reported, Particle size not reported based on measurement**
Estep et al. 2003	Ames, Cumulative	Not Reported	Not Reported	10,900 CFU (Fieller's 95%	Not Reported	Inhaled Dose	**Low: Dose-response raw data**

Author and Year	Strain, Particle Size, and Animal Model	Doses Tested (Units)	Number per Dose Group	LD_{50} Reported for Study Data (95% Confidence Limit)	Highest Dose or Highest Dose Group Without Reported Death	Reported as Environmental Concentration or Inhaled Dose (Comment)	Data Usability for Modeling Low Dose Exposures (Red, Yellow, or Green and Reason(s) for Rating)
	MMAD for Both Strains 1.31 mm [sic] and GSD of 1.8, Rhesus Macaque			Confidence Interval of 1,320 to 241,000)			**not reported**
	Vollum, Cumulative MMAD Collectively for Both Strains 1.31 mm [sic] GSD of 1.8, Rhesus Macaque	Not Reported	Not Reported	6,750 CFU (Fieller's 95% Confidence Interval of 21 to 116,000)	Not Reported	Inhaled Dose	**Low: Dose-response raw data not reported**
Friedlander et al. 1993	Vollum, MMAD 1.2 µm, Rhesus Macque	$4.0 \pm 1.6 \times 10^5$ (Mean ± SD) Spores	10	Not Reported	9/10 Control Animals Died	Inhaled Dose	**Low: Dose-response raw data not reported, Lack of real-time methods to derive inhalation rates**
Ivins et al. 1996	Ames, Particle Size Not Reported, Rhesus Macque	Not Reported	Not Reported	5.5 x 104 Spores	Not Reported	Inhaled Dose	**Low: Dose-response raw data not reported, LD_{50} reported for different data set than discussed in publication**
Ivins et al. 1998	Ames, Particle Size Not Reported, Rhesus Macque	93 ± 63 (Mean ± SD) × LD_{50} where $LD_{50} = 5.5 \times 10^4$ Spores	3	Not Calculable	No Survivors in Control Group	Inhaled Dose	**Low: Dose-response raw data not reported, Lack of real-time methods to derive inhalation rates**
Rossi et al. 2008	Ames, Particle Size Not	210,000 CFU 210,000 CFU	1	(1.1×10^4 CFU LD_{50} reported	Lethality at All	Inhaled Dose	**Low: Total number of**

Author and Year	Strain, Particle Size, and Animal Model	Doses Tested (Units)	Number per Dose Group	LD_{50} Reported for Study Data (95% Confidence Limit)	Highest Dose or Highest Dose Group Without Reported Death	Reported as Environmental Concentration or Inhaled Dose (Comment)	Data Usability for Modeling Low Dose Exposures (Red, Yellow, or Green and Reason(s) for Rating)
	Reported, African Green Monkey	520,000 CFU 630,000 CFU 750,000 CFU 11,200,000 CFU 12,800,000 CFU 15,900,000 CFU 18,900,000 CFU		for different data set than discussed in publication)	Tested Dose Levels (1 Survivor and 1 Death at 210,000 CFU)		**animals tested less than 12, Particle size not reported based on measurement, Lack of real-time methods to derive inhalation rates**
Vasconcelos et al. 2003	Ames, Particle Size Between 1 and 2 µm MMAD, Cynomolgus Monkey	Not Reported	14 Total Animals	61,800 CFU (95% Confidence Interval of 34,000 to 110,000)	Not Reported	Inhaled Dose	**Low: Dose-response raw data not reported, Lack of real-time methods to derive inhalation rates**
Young et al. 1946	Detrick 25, Single Spore Particles, Unspecified Monkey	Not Reported	16	20×10^4 Spores	Not Reported	Environmental Concentration (5-Minute Exposure)	**Low: Dose-response raw data not reported, Lack of real-time methods to derive inhalation rates**

†Druett et al. (1953) uses the term "dosage" (Nt) to describe the product of environmental concentration and period of exposure (e.g., Nt × 10^{-6} = 0.168); for ease in reading table, this term has been recorded as Nt (e.g., 0.168 × 10^{6})

CFU – colony forming unit
GSD – Geometric Standard Deviation
l – Liter
LD_{50} – Lethal Dose for 50% of the Tested Population
mm – millimeter
MMAD – Mass Median Aerodynamic Diameter
NMD – Number Median Diameter
SD – standard deviation
µm - micron

Table 4. Available Dose-Response Data for Multiple Dose Exposures of Guinea Pigs, Rabbits, Nonhuman Primates, and Humans

Author and Year	Strain and Particle Size (Receptor)	Doses Tested [for animal receptor] or Doses Observed [human receptor] (Units) and Number of Exposures	Number per Dose Group	LD_{50} Reported for Study Data	Highest Dose or Highest Dose Group Without Reported Death	Reported as Environmental Concentration or Inhaled Dose (Comment)	Data Usability for Modeling Low Dose Exposures (Red, Yellow, or Green and Reason(s) for Rating)
U.S. Environmental Protection Agency 2012	Ames, MMAD (GSD) for Each Dose Group 0.79 µm (1.52), 0.82 µm (1.53), 0.86 µm (1.49), Respectively; New Zealand White Rabbit	2.91×10^2 CFU with 15 Exposures	7	Accumulated LD_{50} of 8.1×10^3 CFU (95% Fieller's Confidence Interval, 2.3×10^3 CFU to 3.6×10^7 CFU), Average Daily Dose $BMDL_{50}$ of 2.60×10^3 CFU, Accumulated Dose $BMDL_{50}$ of 4.40×10^4 CFU	2.91×10^2 CFU Dose Group, 1.12×10^3 CFU Highest Individual Daily Mean Dose Not Associated with Death	Inhaled Dose	**High: All data usability elements present**
		1.22×10^3 CFU with 15 Exposures					
		1.17×10^4 CFU with 15 Exposures					
Albrink and Goodlow 1959	Vollum rB, Particle Size NMD of 1.4 µm, Chimpanzee	Dose 1: 32,800 Viable Spores Dose 2: 90,300 Viable Spores	1	Not Reported	Dose 1: 32,800 Viable Spores Dose 2: 90,300 Viable Spores	Inhaled Dose	**Low: Total number of animals tested less than 12, Lack of real-time methods to derive inhalation rates**
		Dose 1: 34,350 Viable Spores Dose 2: 112,000 Viable Spores	1				

Author and Year	Strain and Particle Size (Receptor)	Doses Tested [for animal receptor] or Doses Observed [human receptor] (Units) and Number of Exposures	Number per Dose Group	LD_{50} Reported for Study Data	Highest Dose or Highest Dose Group Without Reported Death	Reported as Environmental Concentration or Inhaled Dose (Comment)	Data Usability for Modeling Low Dose Exposures (Red, Yellow, or Green and Reason(s) for Rating)
Brachman et al. 1966	Strain Not Reported, Particle Size Reported to be ≤ 5 µm, Cynomolgus Monkey	Daily Doses Not Reported, 3 Exposure Runs of Various Lengths ≤ 47 Days, Differing Exposure Sources and Concentrations First Run: 16,962 Total *B. anthracis* Particles over 47 Days Second Run: 4,959 Total *B. anthracis* Particles over 41 Days Third Run: 947 Total *B. anthracis* Particles over 55 Hours + 1,347 Total *B. anthracis* Particles over 31 Hours	1st Exposure Run: 32 Monkeys, 2nd Exposure Run: 31 Monkeys 3rd Exposure Run: 28 Monkeys with Sacrifice of 6 Monkeys During Exposure Period	Not Reported	Not Reported	Inhaled Dose	**Low: Dose-response raw data not reported, Particle size not reported based on measurement Lack of real-time methods to derive inhalation rates**
Brachman et al. 1966	Strain Not Reported, Particle Size Reported to be ≤ 5 µm, Guinea Pig	947 Total *B. anthracis* Particles over 55 hours + 1,347 Total *B. anthracis* Particles over 31 hours (Dose Reported as Inhaled Dose of Monkey) 2 Multiple Day Exposures	47	Not Calculable	All Survivors	Inhaled Dose	**Low: Dose-response raw data not reported, Particle size not reported based on measurement Lack of real-time methods to derive inhalation rates**

Author and Year	Strain and Particle Size (Receptor)	Doses Tested [for animal receptor] or Doses Observed [human receptor] (Units) and Number of Exposures	Number per Dose Group	LD_{50} Reported for Study Data	Highest Dose or Highest Dose Group Without Reported Death	Reported as Environmental Concentration or Inhaled Dose (Comment)	Data Usability for Modeling Low Dose Exposures (Red, Yellow, or Green and Reason(s) for Rating)
Dahlgren et al. 1960	Strain Not Reported, Environmental Concentration Reported for All Particles and Particles ≤ 5 µm, Human	Inhaled Dose Day 1: Pennsylvania 1,300 Viable Particles in 8 Hours with 510 Viable Particles ≤ 5 µm in Size Day 2: Pennsylvania 620 Viable Particles in 8 Hours with 410 Viable Particles ≤ 5 µm in Size 2-Day Exposure	Not Reported	Not Reported	Asserted that 1,300 Inhaled Spores for All Particle Size Measurement or 510 Inhaled Spores for Particles ≤ 5 µm or Less for an 8-hour Exposure Was Not Associated with Human Inhalation Anthrax	Inhaled Dose	**Low: Lack of real-time methods to derive inhalation rates, Total number of individuals tested less than 12**
		Inhaled Dose Day 1: New Hampshire 620 Viable Particles in 8 Hours with 140 Viable Particles ≤ 5 µm in Size Day 2: New Hampshire 2,200 Viable Particles in 8 Hours with 690 Viable Particles ≤ 5 µm in Size 2-Day Exposure	Not Reported	Not Reported			

Human data collected in epidemiological, observational studies in an occupational setting.
$BMDL_{50}$ – lower limit of a one-sided 95% confidence interval on the benchmark dose at a benchmark response level of 50%
CFU – colony forming unit
GSD – Geometric Standard Deviation
LD_{50} – Lethal dose for 50% of the tested Population
MMAD – Mass Median Aerodynamic Diameter
NMD – Number Mean Diameter
µm – micron

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- U.S. Environmental Protection Agency (EPA). (2012). Multiple daily low dose *Bacillus anthracis* inhalation exposures in the rabbit. Washington, DC: U.S. Environmental Protection Agency. EPA/600/R-11/145
- Taft SC and Hines SA. (2012). Benchmark dose analysis for *Bacillus anthracis* inhalation exposures in the nonhuman primate*.* Accepted in *Risk Analysis*. Published online at doi: 10.1111/j.1539-6924.2012.01808.x
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