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Benchmark Dose Analysis for *Bacillus anthracis* Inhalation Exposures in the Nonhuman Primate and Application to Risk-Based Decision Making

Office of Research and Development
National Homeland Security Research Center

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Disclaimer

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List of Abbreviations/Acronyms

AGI	a glass impinger
AIC	Akaike Information Criterion
$BAULA_{Dae}$	biologically active units per liter of air as function of aerodynamic diameter
BMD	benchmark dose
BMDL	benchmark dose limit
BMDS	benchmark dose software
BMR	benchmark dose response
CBRN	chemical, biological, radiological, and nuclear
CBRNIAC	Chemical, Biological, Radiological, and Nuclear Defense Information Analysis Center
CFU	colony forming unit
DIA	Defense Intelligence Agency
DoD	Department of Defense
EPA	United States Environmental Protection Agency
GSD	geometric standard deviation
L	liter
LD_{50}	lethal dose for 50% of the test population
µm	micrometer
MMAD	mean median aerodynamic diameter
NA	not applicable
NRC	National Research Council
PI-Cat	Pathogen Information Catalog
RDDR	regionally deposited dose ratio
USAPHC	United States Army Public Health Command

Foreword

Following the events of September 11, 2001, the U.S. Environmental Protection Agency's (EPA) mission was expanded to address critical needs related to homeland security. Presidential directives identify EPA as the primary federal agency responsible for the country's water supplies and for decontamination following a chemical, biological, and/or radiological attack.

As part of this expanded mission, the National Homeland Security Research Center (NHSRC) was established to conduct research and deliver products that improve the capability of the Agency in carrying out its homeland security responsibilities. One specific focus area of our research is the compilation, development and evaluation of information on the human health effects of pathogens that might be used by terrorists. Such information is critical to understanding the risks associated with biological contamination and to support the development of site specific cleanup goals.

This report demonstrated that the EPA's Benchmark Dose Software is a useful tool for evaluating microbial dose-response data, including that from *Bacillus anthracis* inhalation exposures. Furthermore, this study found that a number of disparities in the literature for *B. anthracis* lethality estimates could be traced to differences in physical characterization of the spore product, receptor-specific exposure assumptions, the calculated dose metric, and the statistical process employed to assess the data.

NHSRC has made this publication available to assist the response community to prepare for and recover from disasters involving microbial contamination. This information is intended to move EPA one step closer to achieving its homeland security goals and its overall mission of protecting human health and the environment while providing sustainable solutions to our environmental problems.

Jon Herrmann, Director

National Homeland Security Research Center

Executive Summary

The U.S. Environmental Protection Agency's (EPA) National Homeland Security Research Center helps to protect human health and the environment by the development of risk assessment methodologies for chemical, biological, and radiological threat agents. There is no current technical or regulatory consensus of an acceptable inhalation *Bacillus anthracis* dose-response relationship that is reflective of a wide range of doses and response levels. The lack of this relationship is the main challenge in the development of an overall risk-based approach for addressing *B. anthracis* releases. This study reviewed available *B. anthracis* dose-response modeling and literature for the nonhuman primate, evaluated the use of the EPA's Benchmark Dose Software (BMDS) (BMDS 2.1.1 Version 2.1.1.55) to fit mathematical models to these data, and considered the application of these dose-response data in risk-based decision making.

The review of published dose-response data for *B. anthracis* inhalation exposures identified significant variability in study design and subsequent lethality estimates. The reviewed studies varied with regard to *B. anthracis* exposure products (e.g., strain, particle size), nonhuman primates tested (e.g., rhesus versus cynomolgus monkeys), and experimental designs (e.g., animal number tested). A search was conducted to identify available *B. anthracis* dose-response data from inhalation exposures for BMDS evaluation. Three data sets were selected: U.S. Department of Defense historical data for Dugway Proving Ground outdoor studies conducted during the 1950's (Janssen 1955a, 1955b, 1955c), a U.S. Defense Intelligence Agency study conducted with multiple strains of *B. anthracis* spores in 2001 (Barnewall et al. 2001), and the classic exposure study conducted by Druett et al. (1953).

The results of the benchmark dose modeling for the Department of Defense Anthrax Data, Defense Intelligence Agency Anthrax Data, and the Druett Anthrax Data show no apparent consistency in the calculated median benchmark response levels when using the same model with different data sets and no apparent consistency with the previously published values or reanalyses of original data. BMDS outputs reported included the benchmark dose (BMD), and the benchmark dose limit (BMDL) for identified response levels, statistical measures of fit for models, and fitted parameters and intercepts. As one indication of the overall variation in results, the best fitting models yielded $BMDL_{50}$ (BMD_{50}) values for the Department of Defense, Defense Intelligence Agency, and Druett Anthrax Data Sets of 660 (530), 10,000 (4,900) and 48,000 (37,000) inhaled spores, respectively. Even with the use of criteria designed to increase the comparability among the selected studies for this review, large differences in derived values were still present. A $BMDL_{10}$ animal inhaled dose value of 550 inhaled spores from the $\log_e$ logistic mathematical model was identified as a point of departure using BMDS guidance (U.S. EPA 2008) and subsequently used in the interspecies extrapolation and human equivalent dose development. This value was derived from the Defense Intelligence Agency Anthrax Data.

The development of human equivalent doses from the animal dose-response value requires explicit evaluation of the dosimetric differences between the test animal and the human receptors to properly conduct an interspecies extrapolation. Data elements (i.e., physical characterization of the exposure product, receptor-specific exposure assumptions, and particle size-specific depositional data for both receptors of interest) that are critical to the development of the dose-response relationship are also important to the extrapolation process. In lieu of reliance on the calculated environmental air concentration as the human equivalent dose, an environmental surface concentration was developed due to its ease in sampling. However, accompanying assumptions were necessary regarding the expected deposition of airborne particles to the sampling surface, the areal extent of the sampling surface, and the efficiency at which these particles can be removed from the surface and recovered from the wipe sample. The surface concentration was then converted to an estimated viable spore number recoverable from a wipe after sampling. This hypothetical human equivalent dose was presented to illustrate the use of animal dose-response data in support of site-specific cleanup goal development but did not include other considerations typically considered as part of site-specific risk management decisions.

The study demonstrated that the EPA's BMDS is a useful tool for evaluating microbial dose-response data, including that from *B. anthracis* inhalation exposures. As with all statistical software applications, users must identify the assumptions incorporated within the software and the mathematical models it supports. However, this concept is also important for the evaluation of published dose-response data when comparing results. This study found that a number of disparities in the literature for *B. anthracis* lethality estimates could be traced to differences in physical

characterization of the spore product, receptor-specific exposure assumptions, the calculated dose metric, and the statistical process employed to assess the data. One area that consistently has received less attention in study design has been the determination of spore number per particle. The reliance on data sets using single spore particles may be an appropriate means to bypass this concern. However, lack of these data or sufficient confidence that exposure products used in exposure products are composed of single spore particles can hinder confidence in historical and even more recent data sets.

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1.0 Introduction

The United States anthrax letter attacks of 2001 highlighted the need for *Bacillus anthracis* dose-response data that are suitable for risk-based decision making (Gutting et al. 2008). While a number of publications identify 8,000 to 10,000 inhaled spores as a median range of lethality estimates for human exposures, Coleman et al. (2008) noted that these values cannot be attributed to an originating data set and are seemingly more reflective of best professional judgment. While the lack of scientific evidence for this commonly cited measure of lethality is problematic, it also is indicative of much larger knowledge gap. There are a number of published lethality estimates for identified data sets, but the published values differ greatly (e.g., Druett et al. 1953, Glassman 1966). Currently, there is no technical or regulatory consensus on an acceptable inhalation *B. anthracis* dose-response relationship that is reflective of a wide range of doses and response levels. The lack of an accepted dose-response relationship is the main challenge in the development of a risk-based decision making approach for *B. anthracis* releases.

Mathematical models (e.g., probit, exponential, beta-Poisson) used to describe chemical dose-response relationships have also been used with microbial hazards. As with chemical hazards, quantitative dose-response relationships have been developed for microbial hazards through the evaluation of mathematical models with available data by using curve-fitting techniques. Descriptions of microbial quantitative dose-response relationships using mathematical models (e.g., Armstrong and Haas 2007, FAO and WHO 2003, Haas et al. 1999) have been available for a number of years as well as comparisons of results obtained from deploying different models with individual data sets (e.g., Holcomb et al. 1999). The resulting equations for the mathematical models, percentiles of interest (e.g., Lethal Dose for 50% of the test population [LD_{50}]), and parameter values are typically reported. However, only two microbial dose-response analyses have been identified that reported results using benchmark dose concepts and terminology (Moon et al. 2004, 2005). Moon et al. (2004) compared a set of mathematical dose-response models when applied to microbial dose-response data sets and presented results in the form of benchmark dose outputs. Building on this approach, model averaging of benchmark results has been conducted as a means to incorporate recognized uncertainties in model choice (Moon et al. 2005). In contrast, Englehardt and Swartout (2006) have noted concerns with the use of confidence limits associated with classical statistics for microbial dose-response data, which would also preclude the use of the classical models associated with benchmark dose modeling for these data sets. However, this area of concern is beyond the scope of this paper.

Though originally developed for chemical hazards, the U.S. Environmental Protection Agency's (EPA) Benchmark Dose Software (BMDS) provides an accessible tool to evaluate benchmark dose approaches and facilitates the consideration of a number of mathematical models for dose-response relationships. While there have been publications describing the use of benchmark dose analysis, there are no published dose-response results for microbial analyses that have been generated utilizing the BMDS.

This report will review available *B. anthracis* dose-response modeling and literature for the nonhuman primate, evaluate the use of the EPA's BMDS to fit mathematical models to these data, and consider the application of dose-response data in risk-based decision making.

2.0 Literature Review

2.1 Modeling Exposure and Lethality from Inhalation Exposures

As with many biothreat agents, the inhalation route of exposure is the primary concern in a bioterrorist release of *B. anthracis*. Exposure products are the combination of the biothreat agent plus any additives or impurities in the preparation that would be used in the bioterrorist release or in the dosing of test animals. The exposure product would be in the form of single or multiple spore-containing particles. By design, spore products readily form aerosols that maximize airborne time prior to deposition on surfaces. Inhaled spores that deposit in the alveolar region of the lung and then survive subsequent phagocytosis by local macrophages have the potential to germinate into vegetative bacteria (Hilmas et al. 2009). Systemic and lethal illness may result when surviving bacteria are transported to the lymph nodes absent early and effective treatment.

Two frameworks have been identified to provide a comprehensive perspective for the discussion of dose-response modeling of *B. anthracis* lethality. The first framework, the National Research Council's (NRC 2008) *Framework for Assessing the Health Hazard Posed by Bioaerosols*, describes an approach to quantify the physical and biological factors driving the health hazard posed by biothreat agents during inhalation exposures. The second framework, EPA's exposure assessment process (U.S. EPA 1992), provides terminology and concepts to consistently describe the calculation and measurement of dose.

NRC (2008) identifies the physical characteristics of the aerosolized product mixture for determination of the likelihood and number of inhaled spores that deposit deep in the lung. Physical characteristics define the concentration of the product in the air medium in units of particles per unit volume, the median particle size and standard deviation about that median measure, and the spore number per particle. Biological factors include the type of biothreat agent, the viability of the biothreat agent, and the virulence of the biothreat agent. Together, these factors comprehensively describe the exposure product based on its potential to deposit in the lung and the product's innate virulence.

Of the physical characteristics, particle size is a key determinant of the potential for spore deposition in the alveolar region of the lung. Knowledge of the general relationship between particle size and *B. anthracis* lethality was identified relatively early in the *B. anthracis* dose-response literature (e.g., Druett et al. 1953). However, technology at that time lacked the capacity to identify particle measurements for complex size distributions as is commonly performed today. Early *B. anthracis* dose-response studies targeted environmental measurement of particles 5 µm and less as a proxy for respirable particles (e.g., Glassman, 1966, Janssen 1955a, 1955b, 1955c). Data were not typically measured describing the particle distribution (e.g., median particle size and associated distribution of sizes about that median [geometric standard deviation]) or the nontruncated elements of the particle size range (i.e., particles greater than 5 µm).

Current publications (e.g., NRC 2008, Pitt and LeClaire 2005, U.S. EPA 2004) describe the particle size range of optimal deposition for humans to be 1 to 5 µm (measured as an aerodynamic diameter). However, it should be noted that the 5 µm value does not represent a strict cutoff for deposition as particles greater than 5 µm may still be deposited, albeit at lower rates, during normal nasal breathing patterns. Breathing pattern changes, including increases in tidal volume, breathing frequency, and the use of oral versus nasal breathing, may all increase the alveolar deposition fraction of particles in the 1 to 5 µm range as well as facilitate alveolar deposition of particles greater than 5 µm in size (U.S. EPA 2004). As a point of reference, depositional fractions in humans during nasal inhalation have been identified to be approximately 20% for 1 µm particles with a decline to 10% for 5 µm particles (U.S. EPA 2004). However, 5 µm particles may exhibit between 20 and 50% alveolar deposition during oral inhalation (U.S. EPA 2004).

A unique contribution made by the NRC (2008) framework is recognition of the importance in quantifying the spore number per particle. Knowledge of the spore number per particle, when combined with the air concentration and the particle size and associated depositional fraction, can be used to quantify the spore number deposited in the alveolar region. With noted exceptions (i.e., portions of the Druett et al. 1953 published data), data sets are not available that delineate spore number per particle when single spore particles were not identified as the exposure product.

In recognition of the multifactorial nature of particles in bioaerosol exposures, the NRC (2008) proposed

a new measure to describe particle characteristics and concentration. This measure, the Biologically Active Units per Liter of Air as a function of particle aerodynamic diameter ($BAULA_{Dae}$), incorporates the measurement of airborne particle concentration, agent number per particle, and particle distribution to capture the dosimetrically important elements of *B. anthracis* aerosolized products. The use of the $BAULA_{Dae}$ measurement process will facilitate the prediction of the deposited dose from a measured environmental air concentration.

In addition to the framework describing and quantifying characteristics of the aerosolized spore product as described by NRC (2008), traditional approaches (e.g., U.S. EPA 1992) for chemical exposure assessment provide a useful complement to consistently define exposure and the type of dose metric (Figure 1). The distinction among the various exposure and dose metrics is important in the evaluation of published dose-response values to ensure that comparisons are made at similar levels in the exposure assessment continuum.

The point of contact measurement is the environmental air concentration of *B. anthracis* (Figure 1). The environmental air concentration can then be combined with exposure assumptions describing receptor contact rates with the air medium to define the inhaled dose. Absent accompanying particle size definition for the product, this dose definition is not of sufficient rigor to allow for extrapolation to humans or to evaluate the applicability of these data to different biothreat agent products or receptor exposure scenarios.

The deposited dose, also defined as an applied dose, reflects the material available for absorption across a body boundary, or in this case, movement across the alveolar membrane. Particle deposition is driven by particle size and density. For the monkey receptor, off-the-shelf computer applications are not available to derive the particle size distribution-specific depositional fractions as are currently available for other receptors (e.g., rabbits, guinea pigs, and humans using U.S. EPA's Regionally Deposited Dose Ratio [RDDR] Model). Additionally, spore per particle data are a necessary accompaniment to particle size data to adequately describe the number of spores that are deposited. These data are not present in most published data sets, with the exception of products identified as single spore particles. As a result, the majority of historical data is of limited utility for the development of a dose metric beyond the potential dose, or inhaled dose, as identified in Figure 1. In the absence of sufficient data to derive an applied dose, the potential dose is typically used as a proxy for the applied dose (U.S. EPA 1992).

However, the inability to predict the relationship between environmental air concentration and spore deposition is a major uncertainty in the dose-response modeling of *B. anthracis* exposures (Coleman et al. 2008). The determination of a deposited dose is critically important to the extrapolation of dose-response data by allowing explicit consideration of dosimetric differences (e.g., inhalation minute volume, species-specific deposition rates) between the test animal and the human (Jarabek et al. 2005, Pitt and LeClaire 2004). This lack of data also severely limits the application of dose-response relationships for aerosolized products where the particle size composition differs from the particle size of the product for which the dose-response relationship was originally developed.

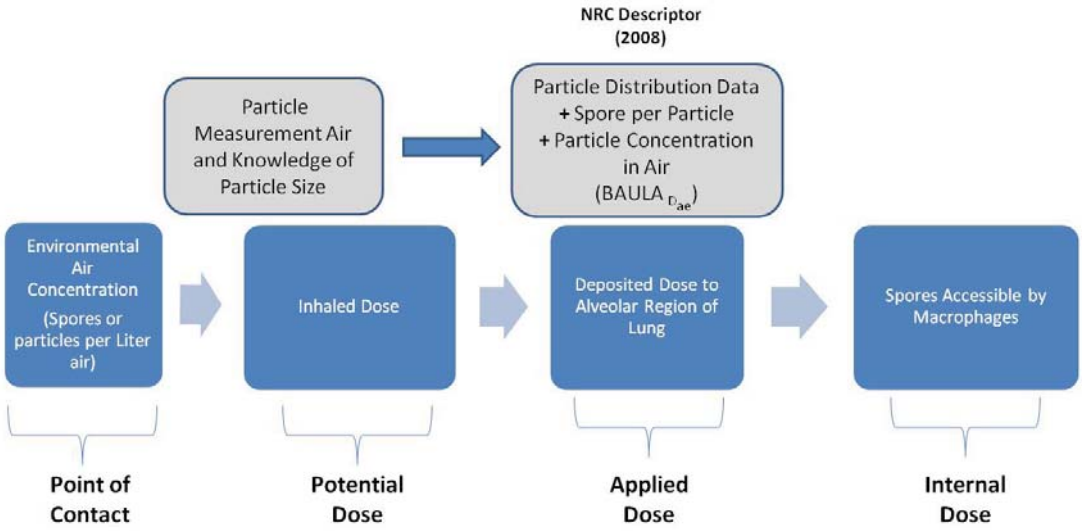

Figure 1. Exposure assessment modeling of inhalation route of exposure to aerosolized *B. anthracis*.

2.2 Available Dose-Response Data

Overall, published dose-response data for inhalation exposures exhibited significant variability in lethality estimates (Tables 1 and 2). The original studies included differences in *B. anthracis* strain and product types, particle size, nonhuman primate test animal, assumed inhalation rate, and experimental design (Table 1). Table 2 describes available published reanalyses of studies identified in Table 1.

2.2.1 Nonhuman Primate Data

Published median lethality values for nonhuman primate studies range from 4,130 (Glassman 1966) to 61,800 inhaled spores (Vasconcelos et al. 2003) (Table 1). The Glassman (1966) study design included a high number of subjects (i.e., 1,236 cynomolgus monkeys), and its calculated median lethality value is considerably lower than most other estimates. There is high interest in the data set and it is often cited, but the raw data are not available nor are statistical goodness of fit measures for the published probit slope and median lethality value. Unfortunately, the only available documentation is Glassman's (1966) brief discussion of the study and results.

Three *B. anthracis* dosing studies (i.e., Albrink and Goodlow 1959, Druett et al. 1953, Young et al. 1946,) were conducted with nonhuman primates during the 1940's and 1950's that include published dose-response data (Table 1). Median lethality estimates were provided in metrics of environmental spore concentration and inhaled spore dose. Young et al. (1946) identified a median lethality estimate of 250,000 spores per liter air. Albrink and Goodlow (1959) described individual monkey lethal doses in units of inhaled spores. Druett et al. (1953) provided a median lethality estimate of 45,000 spores per liter air and an inhaled dose of 53,000 spores. All studies cited the use of single spore particles.

It should also be noted that Haas (2002) and Bartrand et al. (2008) reanalyzed the Dreutt et al. (1953) data to assess the fit of a number of commonly used mathematical models (Table 2). Haas (2002) identified a statistically significant fit to the exponential dose-response relationship and reported an LD_{50} value of 96,800 inhaled spores. Haas (2002) compared the low dose extrapolation derived from Druett et al.'s (1953) data and the exponential model with the low dose extrapolation derived using Glassman's (1966) published probit slope value in the probit model. The estimates in the low dose regions of these models varied by almost three orders of magnitude, which is not unexpected given the typical behavior of these curves in the low dose regions (Haas 2002). Haas (2002) posited that these differences may also indicate fundamental differences between the two data sets, but that this would not be possible to ascertain absent the original data from Glassman (1966). Bartrand et al. (2008) also reanalyzed Druett et al. (1953) monkey data sets using the exponential, probit, and beta-Poisson models; the best fitting model identified was the exponential model with a calculated LD_{50} of 92,000.

More recent studies using nonhuman primates have been conducted to assess median measures of lethality. These studies often have considerably lower animal numbers (i.e., 14 or less subjects) than Glassman's (1966) study and have produced median lethality estimates that are considerably higher than Glassman's published value of 4,130 inhaled spores. Using a $\log_{10}$ probit model, Vasconcelos et al. (2003) derived an LD_{50} of 61,800 inhaled spores for 14 cynomolgus monkeys exposed to the Ames strain. In a different study using the $\log_{10}$ probit model, Estep et al. (2003) derived an LD_{50} of 10,900 inhaled spores for the Ames strain and 10,300 spores for the Vollum strain.

2.2.2 Human Data

There are no human dosing studies conducted with *B. anthracis* due to the known high lethality from inhalation exposures. The limited human dose-response data available are derived from dose reconstruction after airborne release, and these values have questionable levels of rigor relative to typically conducted animal dosing studies.

Human anthrax incidence from the 1979 Sverdlovsk *B. anthracis* release has been modeled in conjunction with doses calculated using assumptions for the amount of source material released, atmospheric dispersion modeling, and human exposure locations (Meselson 1995, Wilkening 2006). These studies have shown agreement in modeled human anthrax cases when using $\log_{10}$ probit and exponential dose-response models. In an attempt to identify a range in the amount of released source material, Meselson (1995) demonstrated potential agreement with human anthrax incidence when using Druett et al.'s (1953) LD_{50} value of 45,000 inhaled spores for rhesus monkeys as the input into an exponential model and also Glassman's (1966) $\log_{10}$ probit slope value of 0.7 as an input into a probit slope model. Using more recently available geospatial and weather data specific for Sverdlovsk in 1979, Wilkening (2006) further demonstrated that these data could also be shown to be consistent with the level of response estimated using Glassman's (1966) $\log_{10}$ probit slope value of 0.7 as well as that of an exponential model incorporating competing-risk components to replace the deterministic potency value (i.e., typically identified as k in exponential model equation).

However, the actual volume of the released source during the Sverdlovsk event remains unknown. Therefore, these studies may provide confirmation of general mathematical forms appropriate for the dose-response relationship, but the derivation of potency values is dependent on knowledge of the source amount. As a result, there is high uncertainty associated with the estimated dose used to confirm these relationships and they will, at best, serve a complimentary rather than a definitive role in any subsequent evaluations.

Table 1. Published *Bacillus anthracis* Data Sets and Corresponding Estimated Lethality Values Identified from Literature Search for Nonhuman Primate Data.

Author	Reanalysis or Original Data?	Strain	Particle Size	Animal (Total Number Exposed) / Assumed Inhalation Rate, If Dose Metric of Inhaled Spores	Model and Calculated LD_{50} (95% Confidence Interval)	Parameters
Young et al., (1946)	Original	Detrick 25	Single Spore	Unspecified Monkey (16) NA	Bliss (1935) **200,000 Spores Environmental Air Concentration**	NA
Druett et al., (1953)	Original	Vollum M36	Single Spore	Rhesus Monkey (72) 1.2 L/minute	Log_{10} Probit **45,000 Spores Environmental Air Concentration (95% 30,000 – 52,000)** **53,000 Spores Inhaled Dose**	Log_{10} Probit Slope = 3.19 with intercept of 2.91 (Based on Exposure Concentration x 10^{-4} as Dose)
Albrink and Goodlow, (1959)	Original	Vollum rB	Single Spore	Chimpanzee Monkey (4) Developed Method to Measure Minute Volume, Values Not Provided in Article	Dose-response data published, no analysis provided Inhaled Dose – Response 32,800 – Survival 34,350 – Survival 39,700 – Death 66,500 – Death	NA
Glassman, (1966)	Original Data from Personal Communication by Jemski	Original publication does not identify strain, Haas (2002) references Meselson (2001) as the source of the strain identification of Vollum	Assumed to be less than 5µm through use of preimpinger	Cynomolgus Monkey (1,236) Unknown	Log_{10} Probit **4,130 Spores Inhaled Dose** **(95% 1,980 – 8,630)**	Log_{10} Probit Slope = 0.669 No Intercept Reported
Vasconcelos et al., (2003)	Original	Ames	1 and 2 µm (Mass Median Aerodynamic Diameter)	Cynomolgus Monkey (14) Plethysmography during Challenge, Values Not Provided in Article	Log_{10} Probit **61,800 Spores Inhaled Dose** **(Fiellers* 95% Confidence Interval 34,800 – 110,000)**	Log_{10} Probit Slope = 4.21 No Intercept Reported

Author	Reanalysis or Original Data?	Strain	Particle Size	Animal (Total Number Exposed) / Assumed Inhalation Rate, If Dose Metric of Inhaled Spores	Model and Calculated LD_{50} (95% Confidence Interval)	Parameters
Estep et al., (2003)	Original	Ames	1.31 mM [sic] (Cumulative Mass Median Aerodynamic Diameter)	Rhesus Monkey / Plethysmography during Challenge, Values Not Provided in Article	Log_{10} Probit **10,900 Spores Inhaled Dose (Fiellers* 95% Confidence Interval 1,320-241,000)**	No Slope Reported; No Intercept Reported
		Vollum	1.31 mM [sic] (Cumulative Mass Median Aerodynamic Diameter)	Rhesus Monkey / Plethysmography during Challenge, Values Not Provided in Article	Log_{10} Probit **6,750 Spores Inhaled Dose (Fiellers* 95% Confidence Interval 21 – 116,000**	No Slope Reported; No Intercept Reported

*Fieller's confidence interval, as calculated using SAS™, is an inverse confidence limit describing the limit about the level of the independent variable that results in the specified result (i.e., confidence limit about the dose related to an identified response level).

Table 2. Published Reanalyses of Nonhuman Primate Original Data Sets Provided in Table 1.

Author	Reanalysis or Original Data?	Strain	Particle Size	Animal (Total Number Exposed) and Assumed Inhalation Rate (If Inhaled Spores Dose Metric)	Model and Calculated LD_{50}	Parameters and/or Coefficient
Haas, (2002)	Reanalysis of Druett et al. (1953)	Vollum M36	Single Spore	Rhesus Monkey (72) / 2.4 L/minute	Exponential **96,800 Spores Inhaled Dose (95% 70,700 – 136,000)**	$k=7.16 \times 10^{-6}$
Bartrand et al. (2008)	Reanalysis of Druett et al. (1953)	Vollum M36	Single Spore	Rhesus Monkey (72) / 2.4 L/minute	Exponential **92,000 Spores Inhaled Dose (95% 29,440 – 70.932) [sic]**	$k=7.16 \times 10^{-6}$

3.0 Methods

3.1 Identification of Animal Model

Nonhuman primates, by virtue of their close phylogenetic relationship to humans, have been identified as an appropriate animal model for human inhalation exposure to *B. anthracis* spores. In particular, rhesus and cynomolgus monkeys exhibit an overall disease course and pathology of anthrax illness similar to that identified in humans (Fritz et al. 1995, Zaucha et al. 1998). A comprehensive literature review for rhesus and cynomolgus monkey dose-response data was conducted to identify dose-response analyses and potential data sets for reanalysis with benchmark dose modeling techniques.

Based on the literature review, the rhesus monkey (*Macaca mulatta*) was selected as the animal model to further evaluate benchmark dose approaches because of the availability of both published analyses and suitable dose-response data for reanalysis.

3.2 Identification of Data Sources

Two unclassified data sources were consulted to identify suitable data for further dose-response analysis. The first source was the Pathogen Information Catalog (PI Cat); this data compilation was the product of a joint effort between the EPA's NHSRC and U.S. Army Public Health Command (formerly the U.S. Army Center for Health Promotion and Preventative Medicine (U.S. EPA 2010). The PI Cat was developed to identify and collect available open source and limited distribution, but unclassified, *B. anthracis* dose-response data. Data collection procedures for the PI Cat are described in U.S. APHC (2010). The second source was the Chemical, Biological, Radiological, and Nuclear Defense Information Analysis Center, also known as CBRNIAC. The CBRNIAC is a Department of Defense Information Analysis Center that is a comprehensive repository for chemical, biological, radiological, and nuclear (CBRN) technical information.

3.3 Criteria for Use of Data Sets

The primary criterion for selection of data sets was their suitability for dose-response analysis using the dose metric of inhaled spores and the measured response of lethality. In lieu of the published dose metric of inhaled spores, a dose metric of environmental air concentration was acceptable if there were body weight or measured minute volume data for the animal subjects. Suitability for dose-response analysis was evaluated through the consideration of three characteristics: 1) a description of the *B. anthracis* exposure product allowing for characterization of the physical factors described in NRC (2008), 2) sufficient animal numbers in the dose-response data set to allow for use of readily available dose-response methods (i.e., preferably 24 or more total animals), and 3) the inclusion of dose groups in the lower range of responses (e.g., data including dose groups with lethality at levels greater than 0% but less than 20%).

The identification of the NRC (2008) comprehensive set of physical factors was not available prior to the generation of the evaluated data sets and has not been routinely collected during the generation of *B. anthracis* dose-response data. At a minimum, selected studies must have defined the administered dose in a manner (e.g., particle size, quantification of spores) that allows for a calculation of inhaled dose. Biological factors were not incorporated in the identification of data sets since only limited data were available in the literature.

Three data sets were selected for benchmark dose analysis based on their meeting all elements from the identified three criteria, with the noted slight relaxation of the NRC physical factors to allow acceptance of particle size and spore quantification as sufficient exposure product information. The first data set selected was U.S. Department of Defense (DoD) historical data from Dugway Proving Ground outdoor studies (Janssen 1955a, 1955b, 1955c). Strengths of this study included an experimental design that incorporated relatively low dose exposures and the largest total number of exposed monkeys for which raw dose-response data were available. The second data set selected was a U.S. Defense Intelligence Agency study conducted with multiple strains of *B. anthracis* spores in 2001 (Barnewall et al. 2001) Strengths of this study included the direct measurement of respiratory parameters data obtained during the exposure challenge and provided a detailed particle size characterization of the exposure product. One historical study, Druett et al. (1953), was also identified for further evaluation using benchmark dose techniques. Strengths of this study included the use of a single spore dosing product, the availability of the average monkey weight for the overall study, and the large number of total exposed monkeys.

3.4 Selected Data Sets

3.4.1 Department of Defense Anthrax Data Set

The Department of Defense Anthrax Data (hereafter, DoD Anthrax Data) were developed from three outdoor studies conducted at Dugway Proving Ground, Utah (Janssen 1955a, 1955b, 1955c). These studies measured the lethality of *B. anthracis* inhalation exposures of monkeys, identified by U.S. APHC (2010) as the rhesus monkey (*Macaca mulatta*). Monkey weights ranged from 2.4 to 6.2 kilograms (Janssen 1955a, 1955b, 1955c). The *B. anthracis* strain was not identified in the available DoD trial reports, but the Vollum strain has been described in common use at the time by DoD researchers (U.S. APHC 2010). Particle size data were not available from Janssen (1955a, 1955b, 1955c).

Bacillus anthracis spores were deployed in an outdoor environment from exploding E61R4 bomblets, and the released spores traveled by natural air currents to the exposure location of the monkeys. Air samples were obtained from approximately 35 outdoor sampling locations where a group of five monkeys was co-located with three preimpinger/impinger air sampling devices. The distance and height of the impingers relative to the placement of the exposed monkeys is unknown. The exposure duration of the monkeys is also unknown. However, it was assumed that the air samplers were maintained for the same time duration as the potential exposure of the monkeys.

Air measurement devices consisted of a preimpinger and impinger. Preimpingers were used to filter out particles greater than 5 µm as this value was thought to be the upper limit on respirable particle size. Impinger fluid was then plated and colony forming units (CFU) were counted as the measurement for *B. anthracis* organisms in the air per liter.

The work was conducted during the mid-1950s, and study designs were limited by available knowledge. However, there were some shortcomings in the study design that warrant noting. The trial reports did not identify the post-exposure observation period for measurement of lethality after exposure. It is possible that a 10-day observation period was used as that length is consistent with *B. anthracis* studies of guinea pigs conducted at Fort Detrick at that time (Jemski and Phillips 1964, U.S. APHC 2010). However, Jemski and Phillips (1964) also note that an additional 3 to 5% mortality may be identified if monkeys are held for up to six months. It is now known that the incubation period for inhalation anthrax may extend up to 100 days (Inglesby et al. 2002), which would likely be most relevant for low dose exposures. The reports did not describe any steps to decontaminate the monkeys (i.e., cleaning or decontaminating of fur) after removal from the exposure environment. Particle size data were not described nor was information provided on the spore number per particle.

3.4.2 Defense Intelligence Agency Anthrax Data Set

The Defense Intelligence Agency Anthrax Data (hereafter, DIA Anthrax Data) were derived from one *B. anthracis* inhalation study conducted during 2001 (Barnewall et al. 2001). Thirty-four rhesus monkeys (*Macaca mulatta*) were exposed to aerosolized strains of *B. anthracis* spores in a head-only chamber enclosed in a Class III biological safety cabinet. The strain information is classified. A specially designed nebulizer delivered *B. anthracis* spores in aerosol droplets; the delivered particle size and their distribution were described as a mean median aerodynamic diameter (MMAD) of 1.31 µM and a geometric standard deviation (GSD) of 1.8. During testing, monkeys were physically restrained and dosed with 3 to 6 mg/kg Telazol® (a combination anesthetic and tranquilizer). Monkeys were exposed for 10 minutes to the aerosolized *B. anthracis* air mixture and then were maintained in the same chamber for an additional five minutes while clean air was flushed through the system.

The air concentration was sampled through the use of an all glass impinger (AGI)-6 impinger, pressure gauge, and a vacuum pump to pull the sample. The impinger collected air samples in sterile water, and plating was conducted after serial dilution to determine the *B. anthracis* aerosol concentration in CFU per milliliter of liquid. This value was then used to calculate the value for CFU per liter of air after incorporation of the air sampling parameters. Plethysmography was conducted during testing to measure individual-specific minute volumes. After exposure to the aerosolized *B. anthracis*, the head of the each monkey was decontaminated prior to removal from the biological safety cabinet. Monkeys were then observed for *B. anthracis*-related death for 120 days post-exposure.

The DIA Anthrax Data were developed using state-of-the-art practices for inhalation studies of aerosolized biological agents, and the exposure data (i.e., minute volume and aerosol concentration in air medium) are presumed to be accurate. However, spore number per particle was not identified in Barnewall et al. (2001).

3.4.3 Druett et al. (1953) Anthrax Data Set

The Druett et al. (1953) Anthrax Data (hereafter, Druett Anthrax Data) were derived from published dose-response data from *B. anthracis* inhalation studies. The study measured environmental air concentration (in units of single spores per liter) and associated mortality

in 7 to 14 pound rhesus monkeys. The monkey species was identified in original publication as *Macacus rhesus* (historical name for *Macaca mulatta*). Single spore cloud exposures were conducted in a Henderson apparatus. Monkeys were exposed for one minute and then were observed for three weeks post-exposure for mortality.

Since no measurements of minute volume were taken prior to or during the aerosol challenge, allometric equations relating monkey weight to minute volume were used in the original study and in this subsequent analysis. The original work does not indicate if the monkeys were tranquilized and/or sedated during the exposure challenge and also does not identify if animals were acclimatized to the testing apparatus prior to the exposure challenge. The use of tranquilizers or sedatives, or conversely monkeys experiencing high levels of stress, will affect the challenge animal's minute volume beyond that predicted by the allometric equation. An allometric equation relating weight to minute volume is based only on the measured correlation and the physiological state at which the measurements were made.

3.5 Calculation of Inhaled Dose

3.5.1 Department of Defense Anthrax Data Set

Inhaled doses were determined using the calculated minute volume inhalation rate and the environmental air concentration derived from CFU counts of germinated *B. anthracis* spores plated from the impinger fluid. The exposure duration was unknown but it is assumed that the impingers collected air samples during the same time period as the monkey exposure duration.

The environmental air concentrations provided in the original study reports (Janssen 1955a, 1955b, 1955c) were used directly and not recalculated for this study. These values were derived using Equation 1 and incorporated the impinger sampling rate and the count of *B. anthracis* colonies plated from the impinger fluid.

Equation 1.

$$Environmental\ Air\ Concentration\ (CFU\ per\ L/\text{minute}) = \left(\frac{\text{Impinger}\ Count\ (CFU)}{Impinger\ Rate\ (L/Minute)}\right)$$

The environmental air concentration (Equation 1) was used with the estimated minute volume (Equation 2) to derive the inhaled dose (Equation 3) that was used as the dose metric in this reanalysis. Janssen (1955a, 1955b, 1955c) identified the arithmetic averaged group-specific weight for each dose group. The minute volume was calculated using body weight values in the allometric equation described by EPA (1988) (Equation 2 following a unit conversion to liters per minute). Equation 2 was developed using regression analysis, with 0.81 and 0.4862 representing parameters that were fit to the data used to derive the allometric equation (U.S. EPA 1988).

Equation 2.

$$Daily\ Inhalation\ Volume\ (m^3/Day) = 0.81 \times (\text{Body Weight}_{kg})^{0.4862}$$

Equation 3.

$$Inhaled\ Dose\ (CFU) = Minute\ Volume\ (L/\text{minute}) \times Environmental\ Air\ Concentration\ (CFU\ per\ L/\text{minute})$$

3.5.2 Defense Intelligence Agency Anthrax Data Set

The DIA Anthrax Data study design directly measured the monkey respiratory parameters and used an active sampling approach to derive *B. anthracis* environmental air concentrations. Inhaled dose was calculated by Barnewall et al. (2001) using the AGI sample concentration, sampling parameters, and exposure duration (Equation 4). The originally published inhaled doses were used as the doses in this reanalysis.

Equation 4.

$$Inhaled\ Dose\ (CFU) = \frac{(AGI\ Concentration\ (CFU/ml) \times AGI\ Sampler\ Volume\ (ml) \times Minute\ Volume\ (L/Minute)}{AGI\ Sampling\ Rate\ (L/Minute)}$$

3.5.3 Druett et al. (1953) Anthrax Data Set

Druett et al. (1953) gathered environmental air concentration data using impingers described by Henderson (1952). Druett et al. (1953) described the range of body weights (i.e., 7 to 14 pounds) for all monkeys used in the overall study, and the midpoint of this range (i.e., 10.5 pounds) was used after conversion as the input for Equation 2. The calculated minute volume of 1.2 L was used for all dose groups. The inhaled dose was then derived by multiplying the minute volume and the environmental air concentration (Equation 3).

3.6 Benchmark Dose Analysis

The overall goal of benchmark dose analysis is to fit a mathematical function that best describes the dose-response relationship in the observable low dose region of the data to enable extrapolation to doses lower than those tested. Benchmark dose analysis estimates the dose, termed a benchmark dose (BMD), for a specified

level of benchmark dose response (BMR) observed. The BMR is defined as the level of change in the response rate. For example, a BMR of 10% would be equivalent to a 10% response rate of the endpoint of interest. The BMDS allows for the change in response rate to be calculated as one of added or extra risk. Extra risk is the increase in risk relative to the available risk; the use of the extra risk calculation is recommended when conducting benchmark dose analysis (U.S. EPA 2008). However, the output of calculations for extra or added risk is identical when conducting microbial dose-response analysis when the background risk is assumed to be zero.

EPA (2008) recommends a BMR value of 0.10 for use with dichotomous data sets when deriving a point of departure value, although users may make data-specific determinations to select other values. For this assessment, BMRs of 0.50, 0.10, and 0.01 were selected for comparison of different model estimates at various points in the dose-response relationship. These values correspond to estimates of 50% lethality (i.e., LD_{50}), 10% lethality, and 1% lethality, and the resulting BMDs would be written BMD_{50}, BMD_{10}, and BMD_{01}, respectively. The lower levels of lethality (i.e., 1% and 10% levels) are more appropriate for the selection of a human equivalent dose than higher levels of response, such as that exhibited by a BMD_{50}. The primary BMDS outputs of interest are the BMD and the benchmark dose limit (BMDL). The BMD is the dose that produces a response at the level of the BMR. 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.

For the benchmark dose evaluation, the current version of EPA's BMDS (BMDS 2.1.1 Version 2.1.1.55) (U.S. EPA 2009a) was used to fit models to the dose-response data. Models from the BMDS dichotomous and dichotomous-alternative model suites were used for analysis: 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, $\log_e$ probit-background response, logistic-background response, and $\log_e$ logistic-background response. The background parameter was directly specified as zero for those models allowing this selection (i.e., $\log_e$ logistic, $\log_e$ probit, Weibull, and Weibull run as exponential) and the g parameter was specified as zero for the dichotomous Hill model to ensure model fits did not incorporate a background incidence of lethality.

The BMDS software places a number of default restrictions on the slope and power values for some models. These restrictions operate in the slope parameter for the $\log_e$ probit and $\log_e$ logistic models, where the value of the slope parameter is restricted to be equal or greater than one, and in the power term for the gamma, Weibull, $\log_e$ logistic, and $\log_e$ probit models, where the value of the power term is restricted to be greater than or equal to one. These restrictions prevent the modeling of supra-linear response in the low dose region. All default slope and parameter restrictions were maintained in this analysis. Restrictions were maintained based on recognition that historically used microbial dose-response models (i.e., exponential, beta-Poisson) are typically linear in the low dose region and are mathematically precluded from displaying supra-linear behavior in this region. To test the potential impact of unrestricted BMDS slope and model parameters on model fits and output lethality values, Appendix A compares results when varying the use of default restrictions for the DIA Anthrax Data.

Statistically valid model fits and BMD values for a given data set were identified using EPA guidance (U.S. EPA 2008). For each model, two BMDS outputs describing the fit of an individual model to the data were evaluated: the global goodness of fit as measured by the model-calculated Chi-square p-value and the scaled residuals calculated for each dose group. The p-value reflects the overall goodness of fit, and a p-value of greater than 0.1 was used to identify a statistically valid fit. The scaled residual is the difference between the model estimate of response for an individual dose group relative to its measured value. Scaled residuals closest to the BMD are of most concern for benchmark dose analysis as they indicate the fit of the model to the data in the dose region of greatest interest.

When comparing the fit of different models with valid statistical fits and equivalent restrictions, the lowest BMDL was selected when the calculated BMDLs were not within a three-fold range (U.S. EPA 2008). However, if the BMDLs were within a three-fold range, the model with the lowest calculated value of the Akaike Information Criterion (AIC) was selected. Since the Chi-square p-values cannot be used to compare the fits among different families of models or model with differing numbers of parameters, the AIC value is more appropriately used to compare fits across models. The AIC value is calculated using the log-likelihood at the maximum likelihood estimates for the model parameters and the number of model degrees of freedom.

4.0 Results

4.1 Statistical Description of Dose-Response Sets

4.1.1 Department of Defense Anthrax Data Set

For each of the three studies comprising the DoD Anthrax Data, there were 35 stations (with the exception of one test using 36 stations), and a total of 285 monkeys had the potential for exposure to released *B. anthracis* spores. However, only those monkeys where the co-located measurement devices captured *B. anthracis* spores were included in the dose-response analysis. Additionally, available copies of the original reports obtained through CBRNIAC included some data points that were no longer legible. These data points were removed from the data set if they could not unequivocally be identified. Additional data were removed at the higher doses (i.e., greater than 1,000 inhaled spores) to limit the inhaled doses to within a 2-log range to facilitate model fits to the data.

The selected data set consisted of 24 dose groups; 23 dose groups of five monkeys and one dose group of four monkeys. To provide an indication of the distribution of doses used for the benchmark dose analysis (i.e., 21 to 941 spores), inhaled doses were binned and displayed in histogram form (Figure 2). The dose groups were not evenly distributed across the range of doses, and the higher doses in the range were underrepresented relative to lower doses. Approximately 58% of the dose groups in this data set were less than 400 spores. It was assumed that the presence of these lower dose groups facilitated more reliable dose-response fits in the lower response regions of the curve.

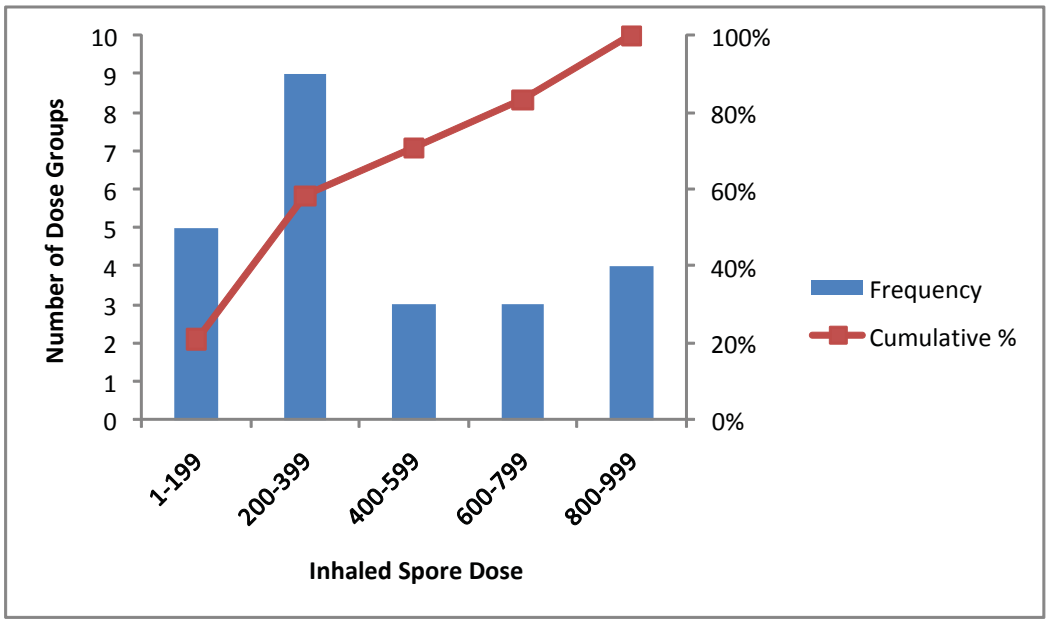

Figure 2. Histogram and cumulative curve showing the frequency and cumulative percentage of the inhaled doses in the DoD Anthrax Data.

4.1.2 Defense Intelligence Agency Anthrax Data Set

Individual dose-response data were provided for each of the 34 monkeys in the study. The range of doses was from 337 to 878,000 inhaled spores. To provide an indication of the distribution of doses in the benchmark dose analysis, the individual monkey inhaled doses were binned and displayed in histogram form (Figure 3). In sharp contrast to the DoD Anthrax Data, only 2.9% of the individual doses were less than 400 spores.

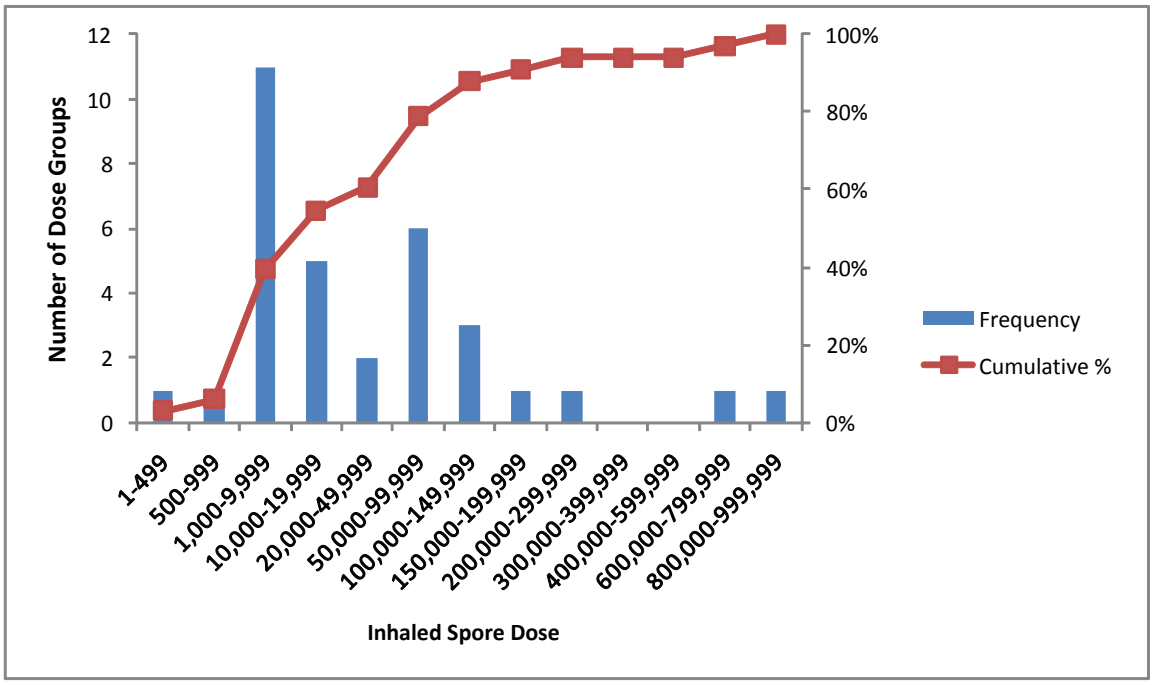

Figure 3. Histogram and cumulative curve showing the frequency and cumulative percentage of the inhaled doses in the DIA Anthrax Data.

4.1.3 Druett et al. (1953) Anthrax Data Set

The data set consisted of a total of nine dose groups of eight monkeys. The environmental air concentrations tested ranged from 29,300 to 166,000 single spores per liter air. To provide an indication of the distribution of doses used for the benchmark dose analysis (i.e., 35,000 – 198,000 spores), inhaled doses were binned and displayed in histogram form (Figure 4). The Druett Anthrax Data included inhaled doses considerably higher than the other data sets. As a result, there were no doses less than 400 inhaled spores and 22% of the doses were less than 49,999 inhaled spores.

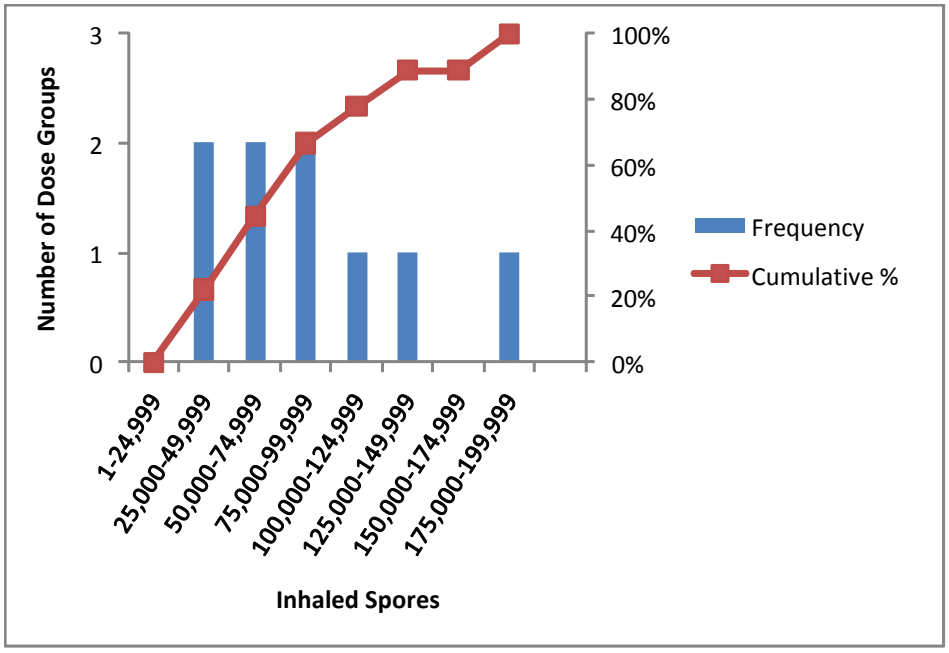

Figure 4. Histogram and cumulative curve showing the frequency and cumulative percentage of the inhaled doses in the Druett Anthrax Data.

4.2 Benchmark Dose Analysis Results

4.2.1 Department of Defense Anthrax Data Set

The following BMDS models exhibited acceptable fits as measured by p-values and scaled residuals of interest: dichotomous hill, $\log_e$ logistic, Gamma, Weibull, $\log_e$ probit, and Weibull run as exponential. Among those models with acceptable fits, the calculated $BMDL_{50}$ and $BMDL_{10}$ values did not vary by more than three-fold and the $\log_e$ probit model exhibited the lowest AIC value. As a result, the $\log_e$ probit model was identified as the best fitting model. The $\log_e$ probit model calculated a $BMDL_{50}$ of 530 inhaled spores and a $BMDL_{10}$ of 150 inhaled spores. Model parameters, confidence limits, and measures of fit are provided in Table 3. Calculated BMDs and BMDLs for identified BMRs are provided in Table 4. Figure 5 shows the visual fit of the $\log_e$ probit model to the data.

Table 3. Model Parameters, Standard Errors, 95% Conf dence Limits, and AIC Values for the Statistically Signif cant Mathematical Model Fits to the DoD Anthrax Data.

Model	Slope (Standard Error) 95% Conf dence Limit	Intercept (Standard Error) 95% Conf dence Limit	Power (Standard Error) 95% Conf dence Limit	v and g Parameters (Standard Error) 95% Conf dence Limit	AIC Values	Value of Scaled Residual Closest to BMD_{10}
Dichotomous Hill (p=0.11)	2.85 (1.38) 0.144 to 5.55	-16.5 (7.48) -31.1 to -1.82	Parameter Not in Model	v: 0.576 (0.126) 0.329 to 0.822 g: Specified as 0	129.178	-0.7457
Log_e Logistic (p=0.13)	1.44 (*) *	-9.44(*) *	Parameter Not in Model	Parameters Not in Model	128.651	0.603
Gamma (p=0.11)	0.00130 (0.000749) -0.000167 to 0.00277	Parameter Not in Model	1.24 (0.448) 0.368 to 2.12	Parameters Not in Model	129.175	0.557
Weibull (p=0.12)	0.000385 (0.000688) -0.000963 to 0.00173	Parameter Not in Model	1.14 (0.284) (0.582 to 1.70)	Parameters Not in Model	129.274	0.516
Log_e Probit (p=0.11)	1 (NA†) (NA)	-6.49 (0.131) -6.74 to -6.23	Parameter Not in Model	Parameters Not in Model	126.775	-0.748
Weibull as Exponential (p=0.18)	0.000913 (0.000154) 0.000610 to 0.00122	Parameter Not in Model	Parameter Specified as 1	Parameters Not in Model	127.518	-0.595

*Standard Error not calculated by BMDS due to recognized error in its calculation

† NA signifies that the Standard Error and associated Confidence Limit were not calculated as parameter has hit a boundary condition.

Table 4. The BMD and BMDL at Identif ed BMRs for the DoD Anthrax Data.

	BMR = 0.50	BMR = 0.10	BMR = 0.01
Dichotomous Hill	BMD_{50} = 630 $BMDL_{50}$ = 400	BMD_{10} = 190 $BMDL_{10}$ = 98	BMD_{01} = 78 $BMDL_{01}$ = 10
Log_e Logistic	BMD_{50} = 700 $BMDL_{50}$ = 540	BMD_{10} = 150 $BMDL_{10}$ = 76	BMD_{01} = 28 $BMDL_{01}$ = 6
Gamma	BMD_{50} = 720 $BMDL_{50}$ = 560	BMD_{10} = 140 $BMDL_{10}$ = 89	BMD_{01} = 21 $BMDL_{01}$ = 8
Weibull	BMD_{50} = 720 $BMDL_{50}$ = 570	BMD_{10} = 140 $BMDL_{10}$ = 89	BMD_{01} = 17 $BMDL_{01}$ = 8
Log_e Probit	BMD_{50} = 660 $BMDL_{50}$ = 530	BMD_{10} = 180 $BMDL_{10}$ = 150	BMD_{01} = 64 $BMDL_{01}$ = 51
Weibull as Exponential	BMD_{50} = 760 $BMDL_{50}$ = 580	BMD_{10} = 120 $BMDL_{10}$ = 88	BMD_{01} = 11 $BMDL_{01}$ = 8

4.2.2 Defense Intelligence Agency Anthrax Data Set

The log_e logistic and the dichotomous Hill BMDS models exhibited acceptable fits as measured by p-values and scaled residuals at BMDLs of interest (Table 5). The calculated BMDL values did not vary by more than three-fold at either the $BMDL_{10}$ or the $BMDL_{50}$ measures for these models, and the log_e logistic model exhibited the lowest AIC value (Table 5). The loge logistic model calculated a $BMDL_{50}$ of 4,900 inhaled spores and a $BMDL_{10}$ of 550 inhaled spores (Table 6). Model parameters, confidence limits, and measures of fit are provided in Table 5. Calculated BMDs and BMDLs for identified BMRs are provided in Table 6. Figure 6 shows the visual fit of the log_e logistic model to the data.

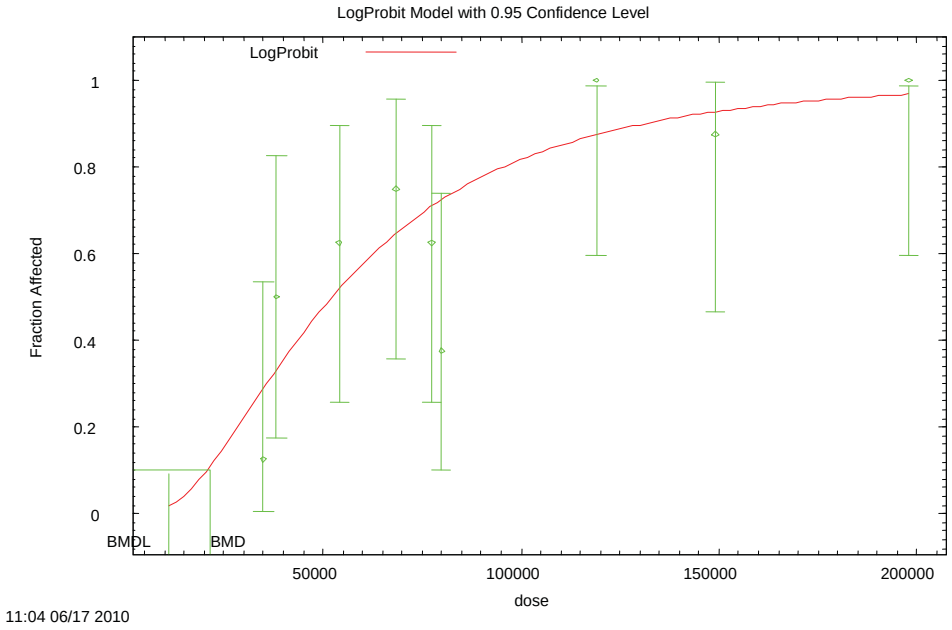

Figure 5. Visual fit of probit model to the DoD Anthrax Data.

Table 5. Model Parameters, Standard Errors, 95% Conf dence Limits, and AIC Values for the Statistically Signif cant Mathematical Model Fits to the DIA Anthrax Data.

Model	Slope (Standard Error) 95% Conf dence Limit	Intercept (Standard Error) 95% Conf dence Limit	v and g Parameters (Standard Error) 95% Conf dence Limit	AIC Values	Value of Scaled Residual Closest to BMD_{10}
Log_e Logistic (p=0.34)	1 (*) *	-9.23(*) *	Parameters Not in Model	36.814	-0.316
Dichotomous Hill (p=0.48)	1 (NA)† (NA)	-9.00 (0.706) -10.4 to -7.62	v: 0.944 (0.135) 0.679 to 1.21 g: Parameter Specified as 0	38.636	-0.330

*Standard Error not calculated by BMDS due to recognized error in its calculation

† NA signifies that the Standard Error and associated Confidence Limit not calculated as parameter has hit a boundary condition

Table 6. The BMD and BMDL at Identif ed BMRs for the DIA Anthrax Data.

	BMR = 0.50	BMR = 0.10	BMR = 0.01
Log_e Logistic	BMD_{50} = 10,000 $BMDL_{50}$ = 4,900	BMD_{10} = 1,100 $BMDL_{10}$ = 550	BMD_{01} = 100 $BMDL_{01}$ = 49
Dichotomous Hill	BMD_{50} = 9,200 $BMDL_{50}$ = 3,500	BMD_{10} = 960 $BMDL_{10}$ = 300	BMD_{01} = 87 $BMDL_{01}$ = 26

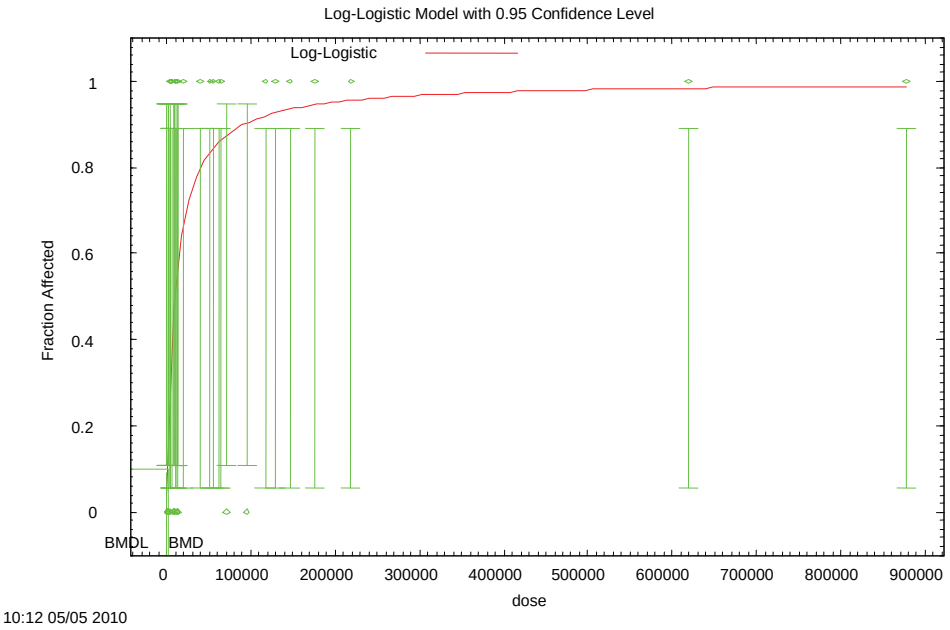

Figure 6. Log_e Logistic model for the DIA Anthrax Data.

4.2.3 *Druett et al. (1953) Anthrax Data Set*

The tested BMDS models that exhibited acceptable fits as measured by p-values and similar AIC values are shown in Table 7. All tested BMDS models exhibited acceptable fits to the data, with the exception of the logistic-background response model. The calculated $BMDL_{50}$ and $BMDL_{10}$ values did not vary by more than three-fold across models with acceptable p-values (p>0.1); therefore the model with the lowest AIC was selected. This approach identified the Weibull model run as exponential as the best fitting model. The Weibull run as exponential calculated a $BMDL_{50}$ of 37,000 inhaled spores and a $BMDL_{10}$ of 5,600 inhaled spores. Model parameters, confidence limits, and measures of fit are provided in Table 7. Calculated BMDs and BMDLs for identified BMRs are provided in Table 8. Figure 7 shows the visual fit of the log-logistic model to the data.

Table 7. Model Parameters, Standard Errors, 95% Conf dence Limits, and AIC Values Associated with a Statistically Signif cant Model Fit to the Druett Anthrax Data.

Model	Slope (Standard Error) 95% Conf dence Limit	Intercept (Standard Error) 95% Conf dence Limit	Power (Standard Error) 95% Conf dence Limit	v and g Parameters (Standard Error) 95% Conf dence Limit	AIC Values	Value of Scaled Residual Closest to BMD_{10}
Dichotomous Hill (p=0.21)	2.30 (0.630) 1.06 to 3.53	-25.0 (6.98) -38.7 to -11.3	Parameter Not in Model	v: 1 (NA†) NA g: Parameter Specified as 0	78.4946	-0.9428
Log_eLogistic (p=0.21)	2.30 (*) *	-25.0 (*) *	Parameter Not in Model	Parameters Not in Model	78.4946	-0.943
Gamma (p=0.25)	2.77E-05 (1.47E-05) -1.05E-06 to 5.64E-05	Parameter Not in Model	1.84 (0.914) 0.0510 to 3.63	Parameters Not in Model	77.9141	-1.07
Weibull (p=0.25)	1.64E-07 (6.64E-07) -1.14E-06 to 1.47E-06	Parameter Not in Model	1.40 (0.359) 0.693 to 2.10	Parameters Not in Model	77.855	-1.106
Log_eProbit (p=0.23)	1.39 (0.358) 0.687 to 2.09	-15.1 (3.99) -22.9 to -7.32	Parameter Not in Model	Parameters Not in Model	78.1857	-0.953
Weibull Run as Exponential (p=0.32)	1.44E-05 (2.35E-06) 9.81E-06 to 1.90E-05	Parameter Not in Model	Parameter Specified as 1	Parameters Not in Model	77.1788	-1.568
Probit – Background Response (p=0.26)	1.76E-05 (5.03E-06) 7.70E-06 to 2.74E-05	-1.0049(0.396) -1.78 to -0.228	Parameter Not in Model	Parameters Not in Model	77.9739	-1.325

*Standard Error not calculated by BMDS due to recognized error in its calculation

† NA signifies that the Standard Error and associated Confidence Limit not calculated as parameter has hit a boundary condition

Table 8. The BMD and BMDL at Identif ed BMRs for the Druett Anthrax Data.

	BMR = 0.50	BMR = 0.10	BMR = 0.01
Dichotomous-Hill	BMD_{50} = 54,000 $BMDL_{50}$ = 40,000	BMD_{10} = 21,000 $BMDL_{10}$ = 8,500	BMD_{01} = 7,200 $BMDL_{01}$ = 1,400
Log_e Logistic	BMD_{50} = 54,000 $BMDL_{50}$ = 40,000	BMD_{10} = 21,000 $BMDL_{10}$ = 8,500	BMD_{01} = 7,200 $BMDL_{01}$ = 1,400
Gamma	BMD_{50} = 55,000 $BMDL_{50}$ = 40,000	BMD_{10} = 16,000 $BMDL_{10}$ = 6,000	BMD_{01} = 4,200 $BMDL_{01}$ = 580
Weibull	BMD_{50} = 56,000 $BMDL_{50}$ = 40,000	BMD_{10} = 14,000 $BMDL_{10}$ = 6,100	BMD_{01} = 2,700 $BMDL_{01}$ = 580
Log_e Probit	BMD_{50} = 54,000 $BMDL_{50}$ = 40,000	BMD_{10} = 21,000 $BMDL_{10}$ = 11,000	BMD_{01} = 10,000 $BMDL_{01}$ = 3,900
Weibull as Exponential	BMD_{50} = 48,000 $BMDL_{50}$ = 37,000	BMD_{10} = 7,300 $BMDL_{10}$ = 5,600	BMD_{01} = 700 $BMDL_{01}$ = 540
Probit Background-Response	BMD_{50} = 68,500 $BMDL_{50}$ = 57,000	BMD_{10} = 17,000 $BMDL_{10}$ = 13,000	BMD_{01} = 2,000 $BMDL_{01}$ = 1,400

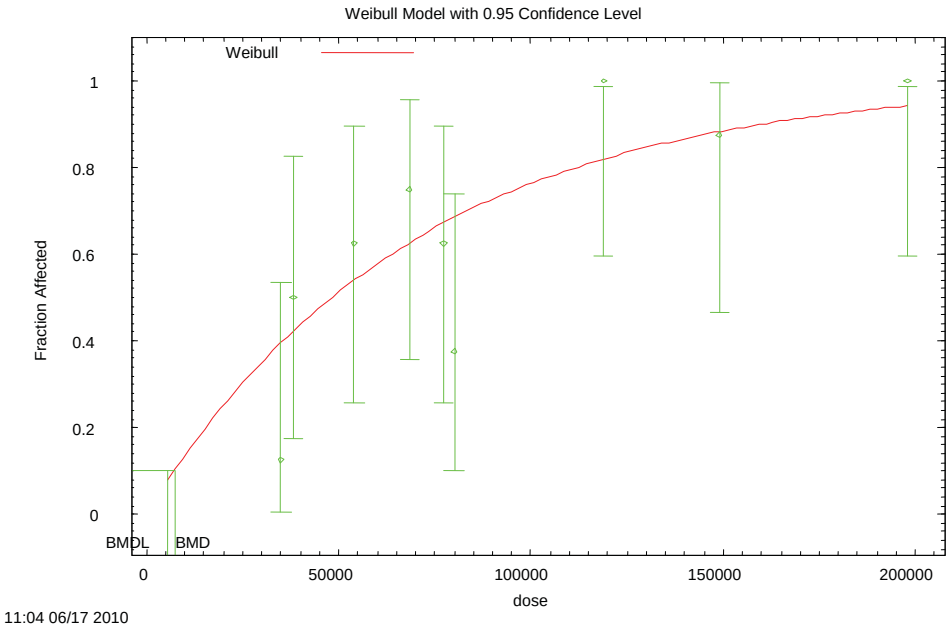

Figure 7. Weibull run as exponential for the Druett Anthrax Data.

5.0 Discussions

5.1 Variation in Dose-Response Lethality Estimates

The results of the benchmark dose modeling for the DoD Anthrax Data, the DIA Anthrax Data, and the Druett Anthrax Data show no apparent consistency in the calculated median benchmark response levels when using the same model with different data sets and show no apparent consistency with the previously published values (Table 1) or reanalyses of original data (Table 2). While obvious similarities in outputs of the probit and logistic models can be shown in the results, these two models often fit similarly to the same dichotomous data set (U.S. EPA 2008). As one indication of the overall variation in results, the best fitting models yielded BMD_{50} ($BMDL_{50}$) values for the DoD, DIA, and Druett Anthrax data sets of 660 (530), 10,000 (4,900) and 48,000 (37,000) inhaled spores, respectively. Even with the use of criteria designed to increase the comparability among the selected studies for this review, large differences in derived values are still present.

To further understand reasons for these differences in study results, a systematic approach to further evaluate each element in the dose-response assessment process is proposed (Figure 8). Elements of the dose-response assessment process are defined to include: 1) physical characterization of the spore product, 2) determination of receptor-specific exposure assumptions, 3) selection of dose metric and calculation of dose, 4) statistical assessment of dose-response data, and 5) selection of the dose-response relationship and identification of point of departure values. The physical characterization of the spore product and the determination of receptor-specific exposure assumptions are conducted to inform selection of the dose metric and the calculation of the dose. After statistically testing the fit of mathematical models to the dose-response data, the best fitting model can be identified and the dose associated with the point of departure identified.

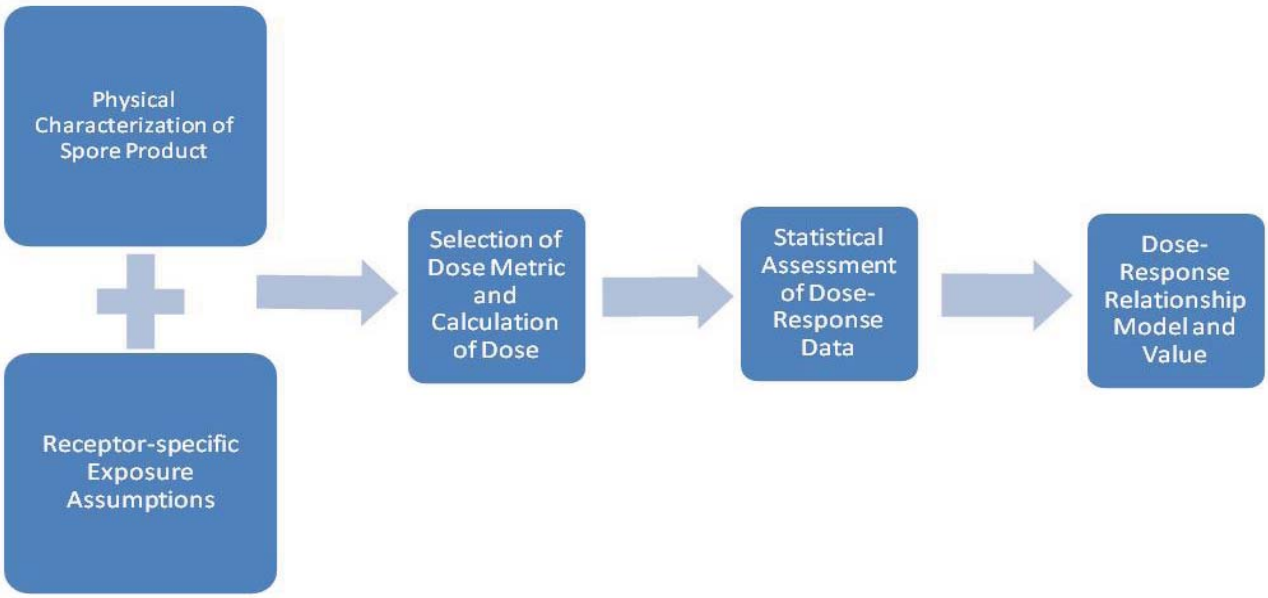

Figure 8. Dose-response assessment steps in the development of dose-response relationships.

5.1.1 Physical Characterization of Exposure Product

To evaluate identified *B. anthracis* dose-response relationships across studies that tested different exposure products, the products must be sufficiently described to be comparable. The information must allow the determination that the products are either sufficiently similar relative to the dosimetry of the receptor(s) being evaluated, or the products must be characterized to allow for the quantification of inhaled doses and deposited doses.

From the perspective of test product characterization for those data sets on which benchmark dose modeling was conducted, the DoD Anthrax Data appears to be the most suspect. Of the reviewed historical studies, the Glassman (1966) data likely also suffer from the same measurement deficiencies as a similar process was used in their study design. The only available particle size data is the measurement of spore-containing particles that were 5 μM or smaller in size. However, neither data set provides information to ascertain the median particle size and associated distribution, the aerodynamic diameter (i.e., no density information) or the spore per particle measures.

Interestingly, the Glassman (1966) data and the BMDS reanalysis of the DOD Anthrax Data provide the lowest estimates for LD_{50} and BMD_{50} values shown in Tables 1, 4, 6 and 8, respectively. One possible explanation for these lower values is that particles greater than 5 μM in size are contributing to exposure, but they are not being counted by the filtered impinger measurement process. Another potential explanation is that there is an undercounting of colonies from plates due to colony masking (Chang et al. 1994), but a similar process was used for the DoD Anthrax Data, Druett Anthrax Data, and Druett et al. (1953) environmental air concentration measurements. Therefore, colony masking is not likely to be causing the differences in relative values observed for these data. For both of the DoD Anthrax Data and Glassman (1966) data, there is little confidence in the generated values absent more definitive particle size data.

The DIA Anthrax Data is an example of a current research design that goes the furthest among the reviewed studies in describing the physical characteristics identified in the NRC framework (2008). Specifically, the particle size of the aerosolized product was measured in units of aerodynamic particle diameter with values provided for a mass median aerodynamic particle size and an associated geometric standard deviation. Given the reported particle diameter, it is highly likely that the exposure product utilized in the DIA Anthrax Data consisted of primarily single spores.

The physical characterization and single spore nature of the exposure product used to dose the animals is also likely replicated by Vasconcelos et al. (2003) as the studies were conducted within the same facility and similar protocols were used to produce the material. The Druett Anthrax Data, originally published in Druett et al. (1953), were also obtained using a single spore preparation based on information provided by the original authors. When there is strong certainty that the products were single spore with minimal aggregation of spores during dosing, the use of single spore particles obviates the need for particle measurement comparisons among data sets.

When tested in the same animal species under similar exposure scenarios, there should be relatively small differences in the dosimetric potential, and correspondingly, dose of the single spore products. If the tested *Bacillus* strains have relatively similar potencies, it should be expected that the calculated doses associated with a given response level should be relatively consistent. However, the Druett Anthrax Data BMD_{50} value was approximately 4.7 times higher than that of the DIA Anthrax Data. The calculated lethality values in the DIA and Druett Anthrax Data should be representative of the single spore dose associated with lethality, if assertions regarding the single spore nature of the particles are correct. The next section will consider the possibility that receptor-specific exposure assumptions may also be contributing to differences in the identified lethality values.

5.1.2 Receptor-specific Exposure Assumptions

The exposure assumptions for the minute volume used to calculate inhaled dose varied considerably among those studies reporting inhaled dose metrics. The assumed minute volume varied from 1.2 liters per minute (Druett et al. 1953) to 2.4 liters per minute (Bartrand et al. 2008, Haas 2002) for studies described in Table 1 or Table 2. The Druett Anthrax Data BMDS analysis assumed a minute volume of 1.2 liters per minute (Equation 2). In contrast, recent published studies (i.e., DIA Anthrax Data, Estep et al. 2003, Vasconcelos et al. 2003) used plethymosgraphic measurement techniques during testing that accurately measured minute volume for each animal.

In three reanalyses of the original Druett et al. (1953) data, estimates of the median lethality value varied greatly. When comparing these estimates, the source of the minute volume value should be carefully considered (Table 9). For example, the minute volume of 2.4 liters per minute assumed by Haas (2002) and Bartrand et al. (2008) in their reanalysis was two times greater than that the 1.2 liters per minute assumed for the BMDS Druett Anthrax Data. The median lethality value when

fitting the exponential model was 48,000 inhaled spores (BMD_{50}) using BMDS and ranged from 96,800 to 92,000 inhaled spores as published by Haas (2002) and Bartrand et al (2008), respectively. The Haas (2002) and Bartrand et al. (2008) median lethality values are 101% and 91% greater than that of the Druett Anthrax Data BMDS estimate for the same model, respectively. Differing exposure assumptions can be shown to account for a significant portion of the differences between the Druett Anthrax Data BMDS results, Haas (2002), and Bartrand et al. (2008) estimates.

Table 9. Comparison of Median Lethality Estimate and Assumed Minute Volume.

	LD_{50} or BMD_{50} Value Using Exponential Model (Inhaled Spores)	Minute Volume Assumption (L/minute)
BMDS Druett Anthrax Data	48,000	1.2
Druett et al. (1953)	53,000	1.2
Bartrand et al. (2008)	92,000	2.4
Haas (2002)	96,800	2.4

In addition, it should be noted that allometric relationships used to estimate minute volume do not incorporate potential impacts on minute volume due to the physiological state of the test animal (e.g., intense stress, chemical restraint with tranquilizers). Since the 1950's, most studies conducted using nonhuman primates utilized some element of restraint (e.g., chemical, physical, or a combination of the two). An early study (Berendt 1968) on the effect of straitjacket physical restraints measured increases in the minute volume of up to 200% beyond that derived by application of Guyton's (1947) allometric equation.[1] This increase was also corroborated by Jemski and Phillips (1964) who described measured inhalation rates of chimpanzees that were almost six times that of the values estimated through use of Guyton's (1947) equation. These inhalation rates were measured in animals that were not sedated and were "securely restrained in holding boxes specifically fabricated for the size and species of animal involved." Conversely, the use of chemical restraints (i.e., specifically, Telazol®) is known to decrease the measured minute volume from that calculated using Guyton's (1947) equation by as much as 50% (Besch et al. 1996). Druett et al. (1953), and by extension the Druett Anthrax Data BMDS analysis, does not report the use or nonuse of any restraints or acclimatization of the study animals. The use of Guyton's, or any other allometric equation that is not specific to the physiological state of the test animal, may substantially underestimate the minute volume of animals that are restrained and not sedated. The underestimation of the minute volume is a significant concern due to the resulting overestimate of the calculated inhaled spore number associated with a given level of response.

5.1.3 Selection of Dose Metric

Two dose metrics have been primarily used to describe lethality, environmental spore concentration and inhaled spore dose. Earlier studies (i.e., Dreutt et al. 1953, Young et al. 1946) provided most, if not all, of their lethality measures in dose units of environmental air concentration. A number of studies, often conducted more recently, used inhaled spores as the reported dose metric for at least some of the reported data sets (Druett et al. 1953, Estep et al. 2003, Glassman 1966, Vasconcelos et al. 2003) (Table 1). Additionally, there are reanalyses of older data sets that calculated inhaled dose from available data (Table 2) (Bartrand et al. 2008, Haas 2002). The presence of both dose metrics in the literature has sometimes led to inappropriate comparisons of different dose metrics. For example, Table 1 of Coleman et al. (2008) included lethality values with both the dose metric of environmental air concentration (Druett et al. 1953, Young et al. 1946) and the dose metric of inhaled spores (Albrink and Goodlow 1959, Estep et al. 2003) identified as an inhaled dose. However, Young et al. (1946) described the dose metric as the "number of spores per unit of cloud when exposed for 5 minutes" and the original Druett et al. (1953) publication identified the dose metric as the "organisms-minutes per liter of air." These two dose metrics as described are environmental air concentrations. Overall, the magnitude of difference between environmental air concentration and inhaled dose lethality estimates will vary based on the minute volume used to derive the inhaled dose.

[1] The allometric equation (Equation 2) used to derive minute volume is based on Guyton's (1947) original data plus the addition of other data sets developed after Guyton.

5.1.4 Statistical Assessment of Dose-Response Relationship

The published values for lethality estimates and associated dose-response relationships (Tables 1 and 2), and the BMDS results (Tables 3 through 8) were derived using numerous approaches. Points of difference in the approaches included the selection of mathematical models (e.g., probit versus exponential) and the use of the same mathematical model with different statistical approaches (e.g., Finney's [1947] original probit equation) or software (e.g., BMDS versus SAS®) to fit model parameters with a given mathematical model. It is expected that different mathematical models will derive different parameter values and outputs for evaluated dose-response relationships with an individual data set or across different data sets. However, care should also be exercised even when evaluating estimates developed using the same mathematical model. For example, probit slope models are supported in the BMDS software and were also utilized by early researchers, including Druett et al. (1953). However, the equations and approaches to derive slope parameters and coefficients are considerably different between the BMDS software and the original Finney equations (Figure 9). Druett et al. (1953), using the approach described in Finney (1947), fit $\log_{10}$ transformed dose-response data to a probit model. The output of the probit equation was in probit units and the estimated parameter values were fit to the units of the dependent variable. In contrast, the BMDS software model incorporates a standard normal density function in the equation used to fit the parameters and the response variable units are in probability, or percentage. The use of the standard normal density function does incorporate an element of stochasticity in the BMDS modeling process; values are selected from the density distribution as the model iterates until the data set is fit to the model. While the distinction between the deterministic approach of the original Finney (1947) approach and the stochastic approach of BMDS may result in some relatively minor differences in model results, it is likely that the fundamental differences in the mathematical equation structure are driving the identified differences in fitted parameters and intercepts.

When evaluating the use of fitted parameters for application outside the original data set and statistical approach, the dose data must be handled in a similar manner as the original statistical analysis. For example, the fitted slope parameter reflects the dose transformation of the data originally used to derive it. A $\log_{10}$ dose transformation will yield a different fitted parameter for the same data as a $\log_e$ or no dose transformation. While these different dose transformations will yield equivalent estimates once the dose is back-transformed to its original units, the value of the slope factor cannot be used to derive response estimates unless the slope factor value is used in a similarly transformed equation. These two previous considerations highlight the potential hazard of using fitted parameter values generated using one statistical approach as inputs into another statistical approach, even when the same mathematical model is to be used.

Equation used by Druett et al. (1953):
Response (Measured in Probits) = m(dose) + b
where m = slope and b = intercept
with use of companion table to transform probits to response percentage (Finney, 1947)

Equation used by BMDS software (U.S. EPA, 2009):
Response (Measured as Probability) = ϕ (α + β (*dose*))
where α = intercept, β = Slope, and ϕ = standard normal density function

Figure 9. The probit model equation used by Druett et al. (1953) and the BMDS software (U.S. EPA, 2009a) to fit dose-response data.

Additionally, it should be noted that individual statistical platforms (i.e., BMDS versus SAS®) may incorporate differing default assumptions when fitting the same mathematical model and that assumptions may not be easily discerned when results are reported. For example, BMDS model fits for the probit model may assume a background incidence of response or may incorporate a default restriction that the value of the slope parameter is greater than or equal to one (e.g., BMDS results for DIA Anthrax Data, BMDS results for Druett Anthrax Data). In contrast, the SAS probit model allows the user to explicitly select for any model formulation whether a background incidence is modeled and does not default to any restriction of fitted parameter values. As a result, published parameter and lethality estimates should be carefully evaluated in concert with the statistical application and assumptions used to generate the estimates.

5.2 Using Available Nonhuman Primate Data to Derive a Human Equivalent Dose

Providing an adequate experimental animal dose-response relationship is available, human equivalent doses can then be developed. Human equivalent doses typically take the form of a medium-specific concentration (e.g., environmental air concentration) or a surrogate measurement that serves as a proxy for the medium of interest (e.g., surface concentration that can be related to an air concentration). Depending on the cleanup authority used, human equivalent doses will support the development of site-specific cleanup goals that will also reflect both technical and other considerations (e.g., CERCLA's nine criteria for actions conducted under the National Contingency Plan). The following example will describe one potential technical approach to derive a human equivalent dose but will not consider other criteria that are also integral to the development of site-specific or situation-specific cleanup goals and risk management decisions. Additionally, the use of uncertainty or variability factors, as is typically used by EPA for reference dose or reference concentration determination from experimental animal data, has not been explicitly incorporated in this example calculation. As such, the resulting calculation should not be construed as standard setting or as a determination of the appropriate level of *B. anthracis* risk management.

There are two technical elements of a human equivalent dose that will be assessed in the development of a cleanup goal subsequent to an aerosolized release of *B. anthracis* spores. The first element is an interspecies extrapolation process to account for dosimetric differences between the test animal and human receptor. The output of this process is the medium-specific concentration (i.e., environmental air concentration) that is assumed to produce an equivalent response in humans as that exhibited by the test animal. The second element is the conversion of the derived environmental air concentration to a measurement more amenable for use in sampling. It has been assumed that a surface concentration, as measured by viable recoverable spores from a surface wipe, will be used for *B. anthracis* spore surface sampling. Figure 10 traces the general approach to combine the interspecies extrapolation with the subsequent derivation of the sampling wipe measurement.

The interspecies extrapolation process combines a dosimetric evaluation to derive the applied dose from the environmental air concentration for the animal receptor with an accompanying dosimetric evaluation to derive the environmental air concentration for the human receptor from the applied dose. The dosimetric adjustment process for inhalation exposures (Figure 10) exhibits considerable overlap with elements of the EPA (1992) exposure assessment process (Figure 1). While there is considerable uncertainty in the interspecies extrapolation process for microbial hazards, dosimetric adjustment begins to address some known elements of uncertainty in interspecies extrapolation. However, the adjustment does not explicitly consider differential susceptibility among species or differences in the sequence of disease events at the cellular or molecular levels (i.e., the microbial equivalent of the toxic dynamic elements of uncertainty for chemical hazards). This area is ripe for further evaluation, but was identified as beyond the scope of this current study.

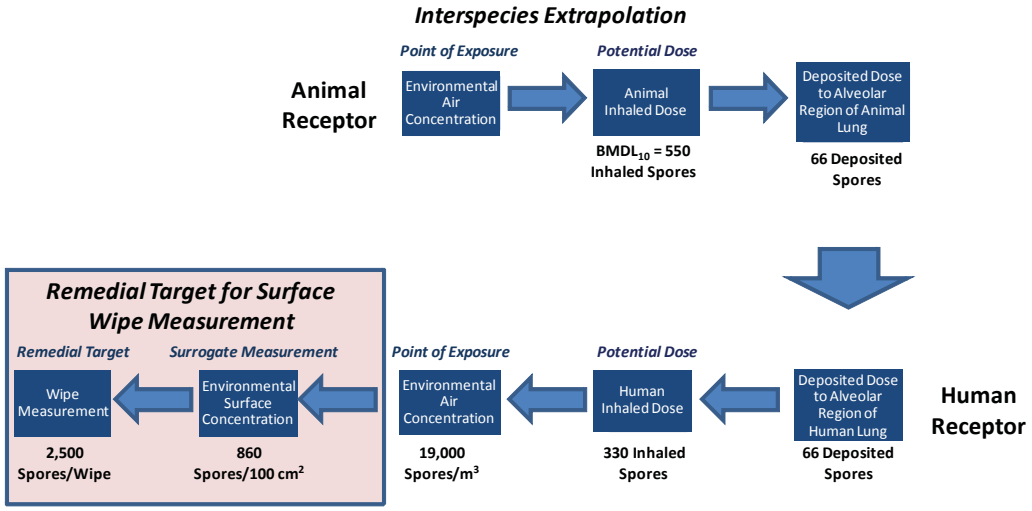

Figure 10. Generalized approach to calculate a sampling wipe measurement from animal dose-response data for inhaled *B. anthracis* spores.

The DIA Anthrax Data was selected for use in the calculation of a human equivalent dose and wipe measurement. The $\log_e$ logistic model was identified as the best fitting model for these data, and the $BMDL_{10}$ value of 550 inhaled spores (Table 6) was selected as the point of departure. This data set was chosen because it was conducted with a superior study design that included a 120 day observation period subsequent to exposure and state-of-the-art measurement technologies for both inhalation rate and particle size. The identified point of departure value was then used for the animal inhaled dose of the interspecies extrapolation. Although the benchmark dose analysis did not utilize the dose metric of deposited dose, the deposited dose was included in the following discussion since its consideration is an important component of the interspecies extrapolation process.

For the monkey, a particle depositional value of 12% for 1 to 2 μM particles was assumed (Cheng et al. 2008). This percent deposited value allowed for the calculation of the deposited dose of 66 spores from the animal inhaled dose of 550 spores. It was assumed that the same deposited dose in the monkey and human would result in equivalent levels of response. The deposited dose of 66 spores for the human receptor was then used to derive the human inhaled dose, using a human-specific depositional rate of 20%. The depositional value of 20% was based on the higher end of the range of human depositional values for 1 to 2 μM particles (Figure 6-6 in U.S. EPA 2004). Depositional rates may differ based on the animal receptor used in the exposure studies (e.g., guinea pig versus monkey), particle size of the aerosolized product, inhalation rate, and mode of breathing (e.g., oral versus nasal) (Jarabek et al. 2005, U.S. EPA 2004).

The conversion of the human inhaled dose to an environmental air concentration requires the assumption of an exposure duration and minute volume. For simplicity, the exposure duration assumed was 1 minute. The human receptor minute volume assumed in the human equivalent dose calculation was 17 liters per minute and was reflective of human adults undertaking light activity (Table 5-23 in U.S. EPA 1997). Given known relationships between age, size, activity level, and minute volume; the selected human minute volume should be representative of the exposure conditions in which the human equivalent dose will be applied. In contrast to the 17 liters per minute value assumed for human receptors, the measured minute volume value for sedated rhesus monkeys in the DIA Anthrax Data Set was 0.5 to 1.0 liters per minute (Barnewall et al. 2001). The calculated environmental air concentration resulting in the same level of response would also be expected to vary considerably between these receptors given the difference in minute volume rates between humans and rhesus monkeys.

With the assumption of a human minute volume of 17 liters per minute, the inhaled human dose of 330 inhaled spores was then converted to an environmental air concentration specific for the human receptor. The resulting environmental air concentration was 19,000 spores per cubic meter. In lieu of reliance on the calculated environmental air concentration as the human equivalent dose, an environmental surface concentration as measured by a wipe was selected. The relative ease in sampling the surface concentration when compared to the air medium drives the choice of the environmental surface concentration value as a human equivalent dose. To derive the environmental surface concentration, assumptions are necessary regarding the expected deposition of airborne particles to the sampling surface, the areal extent of the sampling surface, and the efficiency at which these particles can be removed from the surface and recovered from the wipe sample. There are a number of different modeling approaches that can be used to estimate surface concentration from environmental air concentration (e.g., Price 2009). However, it is beyond the scope of this assessment to evaluate the available deposition and resuspension models. A conservative proxy method to derive the surface concentration from the known air concentration has been described as one means to derive a surface concentration. Using this approach, 90% of the airborne spores, as measured by the environmental air concentration, are assumed to drop to a horizontal surface and be available for surface wipe sampling. The remaining 10% of the spores are assumed to deposit on vertical surfaces or remain suspended in the air. With the assumption of an air volume of 75 cubic meters, the calculated surface concentration using this approach would be 860 spores per 100 square centimeters.

The surface concentration can then be converted to an estimated spore number that can be recovered from a wipe after sampling a 930 cm^2 (i.e., 12 inch by 12 inch) surface area of a specified material type. Estill et al. (2009) identified a 31% recovery efficiency for *B. anthracis* deposited spores on a stainless steel surface when using a moistened wipe. The 31% recovery efficiency is used here to calculate a recovered spore count from a surface wipe of 2,500 spores based on the surface concentration of 860 spores per 100 square centimeters. The resulting surface wipe measurement is indicated by a recovered viable spore count from the surface wipe of 2,500 spores.

6.0
Conclusion

In summary, it has been shown that the EPA's BMDS is an important tool for evaluating microbial dose-response data, including that of *B. anthracis* inhalation exposures. As with all statistical software applications, users must identify the assumptions incorporated within the software and the mathematical models it supports. However, this concept is equally important for the evaluation of published dose-response data and the application of these data to the development of human equivalent doses to support site-specific cleanup goals. This study found that a number of disparities in the literature for *B. anthracis* lethality estimates could be traced to differences in physical characterization of the spore product, receptor-specific exposure assumptions, the calculated dose metric, and the statistical process employed to assess the data. One area that consistently has received less attention in study design has been the determination of spore number per particle. The reliance on data sets using single spore particles may be an appropriate means to bypass this concern. However, lack of these data or sufficient confidence that exposure products are indeed single spore particles can hinder confidence in historical and even more recent data sets. Knowledge of these contributors to the variability in published estimates may facilitate common agreement on a dose-response relationship based on a data set that best characterizes these elements. As has been noted previously, the NRC (2008) framework would provide an excellent guide for proper characterization of products used in the development of ideal dose-response data. As a companion to the product characterization, the receptor-specific exposure assumptions and dosimetric evaluation should also receive equivalent consideration in the study design of the ideal dose-response data set.

With an accepted *B. anthracis* inhalation animal dose-response relationship, human equivalent doses can then be developed. The development of these human equivalent doses from test animal dose-response data requires explicit evaluation of the dosimetric differences between the test animal and the human receptors to properly conduct an interspecies extrapolation. Again, those data elements (i.e., physical characterization of the spore product, receptor-specific exposure assumptions, and particle-size specific depositional data for both receptors of interest) that are critical in the development of the dose-response relationship are also important to the extrapolation process.

7.0
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Appendix A

Evaluation of Benchmark Dose Software Results with Unrestricted versus Restricted Slope Values and Zero versus Nonzero Background Values

Two BMDS modeling assumptions were tested with the DIA Anthrax Data using EPA's BMDS (BMDS Beta Version 2.1.0.4) (U.S. EPA 2009). The following models were used in the evaluation: probit, logistic, $\log_e$ probit, $\log_e$ logistic, Weibull, Dichotomous-Hill, and Weibull run as exponential. The first modeling assumption tested was the impact of allowing for a nonzero background value in the incidence of lethality for those models that allowed it (i.e., probit and logistic). Statistically significant model fits to the data were identified and the modeled $BMDL_{50}$ and $BMDL_{10}$ values were generally higher than estimates that did not allow for a background incidence (Tables D-1 and D-2). However, a direct comparison of models that were able to be fit both with and without an assumed background incidence is not available. The probit and logistic models that could be fit with a nonzero background were not able to fit to the data when the background was set at zero.

The second modeling assumption tested was a comparison of the lethality estimates obtained when restricting the value of the slope parameter to 1.0 or less. For the DIA Anthrax Data, model fits were able to be identified for models with and without the restricted slope value (Tables D-1 and D-2). Depending on the individual model compared (i.e., $\log_e$ logistic versus Dichotomous-Hill), differences between these estimates varied by a factor of 2 or less.

Table A-1. Unrestricted and Restricted BMDS[2] Model Results for the BMD_{50} and $BMDL_{50}$ Lethality Values for the DIA Anthrax Data

Data Set		Probit Nonzero Background Unrestricted	Logistic Nonzero Background Unrestricted	Log_e Probit Zero Background Restricted Slope	Log_e Probit Zero Background Unrestricted Slope	*Log_e Logistic* *Zero Background* *Restricted Slope*	Log_e Logistic Zero Background Unrestricted Slope	Weibull Zero Background	Dichotomous Hill Zero Background Unrestricted Slope	*Dichotomous Hill* *Zero Background* *Restricted Slope*	Weibull Run as Exponential Zero Background
DIA Anthrax Data Set	BMD_{50}	58,800	55,900	NF	10,100	***10,200***	9,820	NF	9,820	***9,160***	NF
	$BMDL_{50}$	34,300	30,200	NF	3,340	***4,940***	3,270	NF	3,270	***3,550***	

* NF = No overall goodness of fit (i.e., $p < 0.10$)

Table A-2. Unrestricted and Restricted BMDS Model Results for the BMD_{10} and $BMDL_{10}$ Lethality Values for the DIA Anthrax Data

Data Set		Probit Nonzero Background Unrestricted	Logistic Nonzero Background Unrestricted	Log_e Probit Zero Background Restricted Slope	Log_e Probit Zero Background Unrestricted Slope	*Log_e Logistic* *Zero Background* *Restricted Slope*	Log_e Logistic Zero Background Unrestricted Slope	Weibull Zero Background	Dichotomous Hill Zero Background Unrestricted Slope	*Dichotomous Hill* *Zero Background* *Restricted Slope*	Weibull Run as Exponential Zero Background
DIA Anthrax Data Set	BMD_{10}	11,800	11,200	NF	777	***1,140***	690	NF	690	***963***	NF
	$BMDL_{10}$	6,830	6,030	NF	26	***549***	17	NF	17	***302***	

* NF = No overall goodness of fit (i.e., $p < 0.10$)

[2]BMDS Version:

U.S. EPA, 2009. Benchmark Dose Software. BMDS 2 .1 Beta Version 2.1.0.4. United States Environmental Protection Agency, National Center for Environmental Assessment. Accessed from: http://www.epa.gov/ncea/bmds.htm.

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