 DOI: 10.1111/risa.12688

Modeling Rabbit Responses to Single and Multiple Aerosol Exposures of *Bacillus anthracis* Spores

Margaret E. Coleman, Harry M. Marks, Timothy A. Bartrand, Darrell W. Donahue, Stephanie A. Hines, Jason E. Comer, and Sarah C. Taft*

Survival models are developed to predict response and time-to-response for mortality in rabbits following exposures to single or multiple aerosol doses of *Bacillus anthracis* spores. Hazard function models were developed for a multiple-dose data set to predict the probability of death through specifying functions of dose response and the time between exposure and the time-to-death (TTD). Among the models developed, the best-fitting survival model (baseline model) is an exponential dose–response model with a Weibull TTD distribution. Alternative models assessed use different underlying dose–response functions and use the assumption that, in a multiple-dose scenario, earlier doses affect the hazard functions of each subsequent dose. In addition, published mechanistic models are analyzed and compared with models developed in this article. None of the alternative models that were assessed provided a statistically significant improvement in fit over the baseline model. The general approach utilizes simple empirical data analysis to develop parsimonious models with limited reliance on mechanistic assumptions. The baseline model predicts TTDs consistent with reported results from three independent high-dose rabbit data sets. More accurate survival models depend upon future development of dose–response data sets specifically designed to assess potential multiple-dose effects on response and time-to-response. The process used in this article to develop the best-fitting survival model for exposure of rabbits to multiple aerosol doses of *B. anthracis* spores should have broad applicability to other host–pathogen systems and dosing schedules because the empirical modeling approach is based upon pathogen-specific empirically-derived parameters.

KEY WORDS: Hazard function; survival analysis; time and dose dependency

1. INTRODUCTION

Available microbial dose–response models assess the likelihood of illness resulting from a single exposure and rely on experimental data from single-exposure experiments or epidemiological data from outbreaks. However, many microbial exposures are recurring. Microbial dose–response analyses[1–10] have been conducted for single- and multiple-dose data sets for inhalation anthrax, but a formal methodology potentially applicable to a wide variety of pathogens and multiple-dose exposure scenarios has yet to be introduced. Current challenges in the development of a multiple-dose methodology include uncertainty in the selection of appropriate techniques to incorporate dosing schedule, conflicting data on the assumption of independent and equal hazard posed by individual pathogens (i.e., independent action hypothesis) for single- and multiple-dose exposures, and scarcity of multiple-dose data sets suitable for microbial dose–response analysis.

National Homeland Security Research Center, U.S. Environmental Protection Agency, Cincinnati, OH, USA.
*Address correspondence to Sarah C. Taft, National Homeland Security Research Center, U.S. Environmental Protection Agency, 26 West Martin Luther King Drive, Cincinnati, OH 45268, USA; tel: +1-513-288-5460; fax: +1-513-288-5460; Taft.Sarah@epa.gov.

One approach to assess multiple-dose exposure has been to calculate an average dose and apply it over the identified exposure duration. Following the process first applied in chemical risk assessment, the approach requires a determination of the appropriate increment of time over which the dose should be averaged (e.g., dose/day). However, problems with this approach include the potential for impact of prior doses on the hazard posed by successive doses and uncertainty in the selection of appropriate techniques to incorporate dosing schedule for discontinuous doses. A second approach for the development of dose–response relationships for multiple-dose exposures has been to introduce mechanistic assumptions to partially describe physiological processes hypothesized to occur, in this case over the repeated exposures. Haas[11] used the term quasi-mechanistic to describe those mechanistic models that provide an incomplete representation of the overall physiological processes associated with disease. For example, researchers have developed dynamic models predicting the accumulated dose relative to clearance mechanisms to remove the hazard posed by inhaled spores[3] or have used a competing risk model to assume rates associated with individual elements that cause or prevent the progression of infection and subsequent disease.[2,6–8] However, there are critical gaps in knowledge of the basic pathogenesis events of infections even for host–pathogen interactions. For example, there are considerable uncertainties in the basic elements of infection initiation[12] and limited quantitative data on the kinetics of infection for inhalation anthrax. Accordingly, significant challenges for mechanistic dose–response modeling of multiple-dose data are: (1) uncertainty in the mechanisms that are being modeled and (2) the limited quantity of robust data to support mechanistic model development.

In addition to the prediction of response for microbial pathogens, there also is interest in estimating the time-to-response (e.g., incubation period [IP], time-to-death [TTD]). Time-to-event characteristics are likely dependent on complex interactions of the disease triangle (host, pathogen, and environment),[13–15] including pathogen dose and dosing schedule. For *Bacillus anthracis*, some aspects representing this biological complexity have been modeled using mechanistic approaches.[2,3,5–8,10,11] Given the complexity in the kinetic or time-based elements of pathogenesis, the modeling of time-to-response using mechanistic approaches is impeded by uncertainty in the pathogenesis events and the lack of data on the kinetics of the processes leading to infection, disease, and death of the host.

The advantage of an empirical approach over mechanistic approaches for developing dose–response models is its wide applicability to other host–pathogen systems and, given sufficient data, its lack of dependence on mechanistic assumptions. This study uses an empirical approach within a survival analysis framework to derive response and time-to-response endpoints. To allow for comparison with mechanistic approaches, existing published models are considered. Single- and multiple-dose data sets developed for *B. anthracis* inhalation exposure in the rabbit[4,16] are used to estimate parameters for evaluated models.

1.1. Multiple-Dose Phenomena

Exposure to multiple low doses of pathogens could result in different host and pathogen responses than those from a single high dose. Response differences are hypothesized to result from an effect of the host's prior exposure on the hazard functions of successive doses, with the potential for prior doses to affect the host–pathogen system responses to the continuing addition of pathogens at internal targets in the host. Dose-dependent responses are exhibited when there are host or pathogen response differences based on the administered dose; these responses include differential gene expression in pathogens via quorum sensing,[17–22] varying host immune responses for high versus low doses of pathogen,[23–25] and differing disease endpoints or times-to-response in the host.[26–28] However, available dose-dependent responses are derived from comparisons between single- and high-dose exposures and there are limited data to conduct evaluations of dose dependency in responses from multiple-dose exposure studies.

1.2. Studies of Multiple-Dose Pathogen Exposures

Statistically significant differences in host response (e.g., probability of infection, IP) for three experimental dosing schedules were reported in the hamster for the prion disease scrapie.[29] As discussed above, such a finding is expected. A literature search did not discover other published studies with designs developed to measure the impact of multiple-dose schedules on responses.

Data from single- and multiple-dose experimental and epidemiologic studies to assess time and dose dependency of inhalation anthrax in the rabbit,

nonhuman primate (NHP), and the human are summarized in Tables SI and SII. Significant knowledge gaps for human inhalation anthrax exist, including uncertainty in dose levels causing illness and incomplete details of exposure (numbers exposed; day, time, and duration of exposure) from the epidemiologic evidence. For example, data from the Sverdlovsk accidental release from a bioweapons factory[30–33] are lacking environmental exposure levels, vaccination status of those presumed to be exposed, and use of medical preventative treatments (e.g., vaccines, antibiotics).

Data for estimating IPs and TTDs for inhalation anthrax pathosystems are summarized for the rabbit and NHP as supplemental data. Low-dose data are lacking for the rabbit and sparse for the NHP (Table SI). It is unknown how the reliance on high-dose data to derive IP and TTD values will affect analysis of time and dose dependency, although both time-to-response and variability are expected to decrease with increasing dose. The respective estimates of IPs and TTDs for *B. anthracis* (Table SII) were four to six days and five to eight days for human inhalation from opened or handled contaminated mail.[34,35] Two of the most recent well-controlled rabbit studies caused 100% mortality at presumably high comparable doses.[36,37] The mean TTDs were 47 and 73 hours, including death of one rabbit within 29 hours of exposure. Slower progression to fatal inhalation anthrax was observed in multiple NHP species; mean TTDs were reported at 3–14 days [38–46] including NHP deaths at 18, 20, 25, and 50 days in independent studies. Interspecies effects noted above merit additional research.

Despite chronic exposure of mill workers to aerosols containing *B. anthracis* spores,[47,48] human inhalation anthrax cases were rare. From 1948 until initiation of the vaccination program from 1955 to 1957, 0.6–1.4% of workers in four mills in the northeastern United States. reported anthrax,[47] predominantly cutaneous anthrax cases. Evidence was reported for clusters of inhalation anthrax cases consistent with peaks of high dust exposure (presumably with *B. anthracis*) for the human.[49,50] In addition, clusters of cases in NHPs were thought to be associated with prior peak exposures when exposed to mill aerosols during production.[38] The aerosol sampling studies conducted in the three U.S. mills suggest there was chronic exposure of mill workers to aerosolized particles containing microbial populations,[49,50] with intermittent exposure to *B. anthracis* spores. The *B. anthracis* strain isolated from a mill worker with inhalation anthrax was of similar virulence as the strains characterized in U.S. Army bioweapons programs.[49,50] These data support the conjecture that human anthrax cases were likely not associated with average daily or cumulative exposures over long periods, but rather with short-term exposures of relatively high levels.

Reports on two additional recent rabbit data sets[4,16] described subsequently in this article extend the body of evidence in the rabbit for both single- and multiple-dose studies. The purpose of these rabbit experimental studies was to evaluate physiological responses following exposures to single or multiple low doses of *B. anthracis* spores, and not specifically to estimate time- and dose-dependent parameters. These data, nevertheless, are used to develop time-dependent dose–response models presented in this article.

1.3. Recently Published Models Using Multiple Doses

Below are brief summaries and critiques of published mechanistic models for inhalation anthrax based on the multiple-dose study using naturally contaminated aerosols from mill environments[38] and/or human epidemiologic information detailed in the supplementary data. Mayer *et al.*[3] used a survival analysis framework to develop a dose–response model predicting mortality and TTD estimates for the cynomolgus monkeys in the Brachman *et al.* data set.[38] The Mayer model[3] included parameters for clearance from NHP lungs explicitly and the dynamics of immune response via a deterministic empirical function. The Mayer model[3] does not include spore germination and vegetative bacteria growth parameters, unlike other theoretical models.[5] Rather, the net *in vivo* pathogen count from a given dose is assumed to decline due to clearance from the lungs. The Mayer model[3] assumed that the likelihood of infection during a period is proportional to the number of pathogens *in vivo* during the period. Thus, the model has three mechanistic model parameters plus two additional parameters reflecting an uncertain and variable lag time between symptoms and death. Mayer *et al.*[3] estimated the average daily dose values presented graphically in Fig. 3 of Brachman *et al.*[38] after fixing the remaining parameter values in the model. Given the assumed intermittent and unquantified peak exposures in the data

set, the use of the average daily exposure estimates rather than actual exposures might contribute to biased parameter estimates. When considering the lag time parameter, Mayer *et al.*[3] integrated the likelihood function for the other parameters in the model over all assumed possible lag time values to derive the parameter estimates.

Other published models[2,5–8,10] were not developed through assumptions of hazard functions. Instead, they specified probability functions of death and durations for infection, symptoms, and death. The Wilkening model[10] extended the competing risk model of Brookmeyer *et al.*[5] and developed a seven-parameter dose–response model. The Wilkening model[10] assumed log normal distributions for lag times between spore germination, symptoms, and death. In the Wilkening[10] paper, four of the parameter values were derived using maximum likelihood estimation (MLE); literature-based values were assumed for the other three parameters, which included a parameter that represented the threshold number of cells needed for development of clinical symptoms. Models developed by Gutting *et al.*[6–8] are based on these same mechanistic assumptions. Based on the current analysis of these models described below, model outputs are extremely sensitive to the estimate of the uncertain threshold number of cells necessary to cause symptoms.

Toth *et al.*[2] presented a model based on both human epidemiologic studies and NHP studies, including the multiple-dose mill aerosol study.[38] The model is similar to that of Wilkening.[10] However, the Toth *et al.*[2] model has five parameters: the probability that an individual spore germinates prior to clearance, the rate of clearance from the lungs, a lag parameter representing the time between germination and symptoms, and two parameters for a gamma distribution of time between symptoms and death. To model the impact of multiple doses over time on infection, the Toth *et al.*[2] model assumes response based on the "accumulation" of pathogen cells over time. Toth *et al.*[2] estimated the spore germination probability and lag parameter from the Brachman cynomolgus monkey data for experimental runs 3–5[38] using daily doses visually estimated from graphs.[38] The clearance probability was estimated from rhesus monkey data, and the parameters for the gamma distribution were derived from human data reported by Holty *et al.*[51] for measurements of times between symptoms and death. Information to validate this set of parameter values is lacking in the rabbit animal model.

Mechanistic models for inhalation anthrax[2,3,5–8,10] assume a sequence of steps from progression of infection, symptomatic illness, and host death with limited *in vivo* data available to support the specified models. For each step, estimates of parameters were made from available data, although there are varying levels of rigor associated with literature-derived parameter values. The biological meaning and appropriate interpretation of the model's parameter values depend on the disease pathosystem being sufficiently and correctly defined. If the defined structures are correct, then the estimated parameters are biologically meaningful, and the models are typically assumed to have greater generalizability to settings other than those of the original data set. If the defined system is incorrect or any of the mathematical functions used for describing the pathosystem are defined incorrectly, the model parameter estimates would not represent the biology of the host–pathogen interactions and the resulting model would not be generalizable.

1.4. Survival Analysis Approach

A simple empirical approach is described herein based on formal application of survival analysis and hazard functions to explore time and dose dependencies for mortality in rabbits dosed once or multiple times with low doses of *B. anthracis* spores. The objectives of this analysis are to assess a baseline survival model and more complex models based on the rabbit data and to compare the parsimonious empirical models to published "mechanistic" models of inhalation anthrax.

2. METHODS

The development of the dose- and time-dependent survival model presented in this article relies on an empirical approach of data analysis for developing a model that predicts mortality and time-to-response rather than using mechanistic assumptions. Initially, "baseline" models are derived using simple assumptions of exponential dose response, independent hazards from multiple dosages, and hazard functions proportional to dose levels. Alternative models are then developed using more complex assumptions, as explained in Section 2.2. Measures of fit for these models are compared one to the other in order to determine the best fitting dose–time dependent survival model, termed the dose–time dependent model or baseline model.

2.1. Derivation of the Baseline Survival Model

Baseline models are derived using survival analysis.[52] For a baseline model, the hazard function describing the likelihood that an exposed individual dies at time t is the conditional-specific rate of animal death at t given that the animal has survived up to t. If multiple events (exposed doses, indexed by k with $k = 0$ as the initial dose at time zero) act on the host at times t_k, where each event creates its own hazard function for the host, h_k, the total hazard function for the host at time t is the sum of the individual hazard functions at t. The total hazard function, $H(t,\ \{t_k,\ d_k\})$, to the host at t, given that the animal is exposed to K doses, d_k, at times, t_k, is:

$$H\left(t, \{t_k, d_k\}_{k=0}^{K-1}\right) = \sum_{k=0}^{K-1} h_k\left(t - t_k, d_k\right), \qquad (1)$$

where $t_0 = 0$ is assumed. One of the most common assumptions made in survival analysis is that hazard functions are proportional to independent variables that affect the system. Thus, initially, the assumption is that h_k is equivalent to $rd_kg'(t - t_k)$, where r is a constant describing the probability of the endpoint; d_k is the dose for the kth event; $g(t)$ is a cumulative distribution function for TTD; and $g'(t - t_k)$ is the derivative of the function $g(t - t_k)$. For an animal exposed to K doses at times $t_0, t_1, \ldots, t_{K-1}$, the total hazard at a given time, t, is:

$$\begin{aligned} H\left(t, \{d_i, t_i\}\right) = {} & rd_0g'(t) \\ & + rd_1g'(t - t_1) + rd_2g'(t - t_2) \\ & + \ldots + rd_{K-1}g'(t - t_{K-1}). \end{aligned} \qquad (2)$$

Once the hazard function, $H(t,\ \{t_k,\ d_k\})$, is determined, a simple mathematical transformation is used to determine the survival function, $S(t)$; the probability that the animal survives at t,

$$S(t) = \exp\left[-\int_0^t H(u)\ du\right] \qquad (3)$$

and the probability of death at time, t, is $F(t) = 1 - S(t)$. It is often advantageous to consider the logarithm of the survival function $S(t)$ versus time:

$$\ln\left[S(t)\right] = -\int_0^t H(u)\ du. \qquad (4)$$

The density function, f, is the joint probability distribution of death at time t given survival to time t. The density function is estimated from $f(t) = S(t)H(t)$. As described more fully in Section 2.4, the density function is used to generate the likelihood function to derive MLE values for parameters.

For baseline models from Equations (2) and (3), the survival function is defined as follows:

$$S(t, d) = \exp\left(-rdg(t)\right). \qquad (5)$$

In this study, g is referred to as a TTD distribution, the distribution of times between exposure and death. Equation (5) is also referred to as the exponential dose–response model with TTD distribution g. A total of eight cumulative distribution functions (Table I), including the exponential dose–response model with TTD distribution g, were evaluated in this study using the rabbit study data[4,16] (Tables II and III).

The assumption that g is a cumulative distribution function is important because this makes Equation (5) consistent with expected microbial dose–response phenomena: the level of response (% mortality) decreases with decreasing dose, and, in the limit $t \to \infty$, not all animals are assumed to die. Equation (5) has been used in prior published studies of time-to-response for single doses of pathogenic organisms[53–55] and is functionally the same as a model developed by Brookmeyer *et al.*[5] The derivation described in this article is based on survival analysis, while prior studies [2,3,6–8] are based on mechanistic assumptions.

2.2. Extensions of the Baseline Model

One type of alternative model that accounts for dependencies between effects of doses over time is obtained by multiplying hazards for successive doses by factors $\beta(\{d_i\}, n)$, where $\{d_i\}$ is the collection of previous doses and n is the number of previous doses. Time is not included as a variable of the function β because all animals in the data set used were dosed with the same schedule and the majority of the doses were administered daily except during weekends. These alternative models assume that:

$$h_k = \beta_{k-1}h_{k-1}, \qquad (6)$$

for $k = 1, \ldots., K$, where β_{k-1} is a function of the dose for the previous exposure, d_{k-1} for $k > 0$. It is assumed that β_{k-1} are between 0 and 1, reflecting the assumption that rabbit immunological systems respond to previous doses so that the hazards for subsequent doses are less than those for earlier doses. Several functional forms for the factor β are evaluated, including an exponential model, a logistic function, and a Gompertz model.

A second type of alternative to the baseline model is derived by changing the initial

Table I. Survival Model Time-to-Death (TTD) Distribution and Cumulative Dose–Response Relations

Model[a]	TTD Distribution, g	Dose–Response Relation, F
Exponential	$1 - e^{-(at)}$	$F(t) = 1 - e^{-rd\,(1-e^{-at})}$
Lagged exponential[b]	$1 - e^{-a(t-t_{\text{lag}})}$	$F(t) = \begin{cases} 0 & 0 \le t \le t_{\text{lag}} \\ 1 - e^{-rd\left(1-e^{-a\,(t-t_{\text{lag}})}\right)} & t > t_{\text{lag}} \end{cases}$
Inverse exponential	$e^{-(a/t)}$	$F(t) = 1 - e^{-rd\,e^{-(a/t)}}$
Lagged inverse exponential[b]	$e^{-a/(t-t_{\text{lag}})}$	$F(t) = \begin{cases} 0 & 0 \le t \le t_{\text{lag}} \\ 1 - e^{-rde^{-a/(t-t_{\text{lag}})}} & t > t_{\text{lag}} \end{cases}$
Weibull	$1 - e^{-(t/a)^b}$	$F(t) = 1 - e^{-rd\left[1-e^{-(t/a)^b}\right]}$
Lagged Weibull[b]	$1 - e^{-((t-t_{\text{lag}})/a)^b}$	$F(t) = \begin{cases} 0 & 0 \le t \le t_{\text{lag}} \\ 1 - e^{-rd}e^{(rd)e^{-((t-t_{\text{lag}})/a)^b}} & t > t_{\text{lag}} \end{cases}$
Inverse Weibull	$e^{-(a/t)^b}$	$F(t) = 1 - e^{-rd\,e^{-(a/t)^b}}$
Lagged inverse Weibull[b]	$e^{-[a/(t-t_{\text{lag}})]^b}$	$F(t) = \begin{cases} 0 & 0 \le t \le t_{\text{lag}} \\ 1 - e^{-rde^{-[a/(t-t_{\text{lag}})]^b}} & t > t_{\text{lag}} \end{cases}$
Mayer[c]		$F(T) = \begin{cases} 1 - e^{-\frac{s}{\gamma(2-\alpha)}\left\{d^{2-\alpha}-\left[\left\{d^{1-\alpha}-\gamma(1-\alpha)T\right\}\right]^{\frac{2-\alpha}{1-\alpha}}\right\}} & T < t_e \\ 1 - e^{-\frac{s}{\gamma(2-\alpha)}d^{2-\alpha}} & T \ge t_e \end{cases}$

[a]Dose–response model parameters are r (likelihood of an individual pathogen initiating an infectious focus and death), t (time), d (dose).
[b]In lagged models the parameter t_{lag} is an empirical parameter giving the lag time between inoculation and the initiation of death.
[c]In all of the models except the Mayer model, the parameters a and b describe the time-to-death distribution. In these time-to-death distributions, a is a scale parameter and in the Weibull and inverse Weibull time-to-death model, b is a shape parameter. In the Mayer model, α is a shape parameter associated with pathogen accumulation. In the Mayer relation, $t_e = d^{1-\alpha}/[\gamma(1-\alpha)]$.

Table II. Group-Specific Single Dose Experiment Doses and Mortality

Group ID Number	Geometric Mean Dose	Mortality; Time-to-Death by Study Day
A2	284	0/5; NA
A3	2,040	0/5; NA
A4	31,900	2/5; Day 4, 11; Mean = 7.5
A5	266,000	4/5; Day 3, 4, 6, 6; Mean = 4.8
A6	8,124,000	5/5; Day 2, 3, 4, 4, 5; Mean = 3.6

ID, identification; NA, not applicable.

dose–response function (exponential) used in Equation (5). For this evaluation, the beta-Poisson and log-probit dose–response models are used. To include time-to-response in the beta-Poisson and log-probit models, the dose was multiplied by the TTD distribution as was performed for the beta-Poisson model by Huang and Haas.[54]

2.3. Extensions Using Published Mechanistic Models

Several recently published studies provide dose–response models that were fit to the rabbit data[4,16] and compared with the baseline and alternative models described above. First, a model described by Wilkening,[10] and evaluated by Gutting *et al*. with

Table III. Group-Specific Multiple Dose Experiment Doses and Mortality

Group ID Number	Geometric Mean Dose per Exposure	Geometric Mean of Animal-Specific Sum	Mortality; Time-to-Death by Study Day
M2	271	4,300	0/7; NA
M3	1,181	17,800	1/7; Day 18
M4	11,060	138,900	4/7; Day 11, 13, 15, 21; Mean = 15

ID, identification; NA, not applicable.

rabbit data,[8] is considered. This model was based on a probability function proposed by Brookmeyer *et al.*,[5] $F_G(t, d; \theta, \lambda)$, in which anthrax illness was modeled as the outcome of the competition between spore clearance and spore germination. In the Brookmeyer "competing risk" dose–response model, θ and λ described the rates of spore germination and spore clearance, respectively. Wilkening[10] and Gutting *et al.*[8] extended the "competing risk" model by assuming that the incubation time between spore germination and generation of a threshold *in vivo* population was lognormally distributed and related to the doubling time of *B. anthracis.* This assumption introduced three additional model parameters, which are described below.

The rabbit data[4,16] were also fit to the model given in Mayer *et al.*[3] This model assumed a hazard function for an endpoint of infection at time t equal to the product of a parameter, s, and the number of viable anthrax cells in the host at time t, $P(t)$. The function $P(t)$ was derived assuming $dP(t)/dt = -\gamma[P(t)]^{\alpha}$. For multiple doses, the function $P(t)$ was calculated sequentially, where the contribution of the kth dose at time t_{k-1} is added to determine $P(t_{k-1})$. The Mayer[3] model has three mechanistic descriptive parameters: $s > 0$, $\gamma > 0$, and $\alpha \in [0, 1]$, reflecting the shape of the pathogen–survival curve. Mayer *et al.*[3] introduced what the authors called a "fixed population lag period," defined as the time between infection ("take-off") and death. Values of the lag parameter may depend on many factors, including the dose levels, dose strain, and host species.[3] Mayer *et al.*[3] assumed that this parameter was distributed as a discrete uniform distribution over 1–4 days for cynomolgus monkeys. Rather than estimating a value, the authors integrated it out of the likelihood. Thus, the Mayer[3] model has five parameters: the three mechanistic descriptive parameters and two additional parameters defining the range of the lag period estimates. The impact of values of the latter two parameters on values of the three mechanistic parameters was not explored in the Mayer *et al.*[3] paper.

The Gutting model[8] assumed that once spore germination occurs within the host, there was a time-to-symptom distribution. Therefore, the probability of symptoms was computed directly (numerically) by calculating the convolution (integral) of the probability function of spore germination (Equation (8) in Gutting model[8]) and the assumed symptom endpoint distribution (Equation (12) in Gutting model[8]). The latter was a function of three parameters: the standard deviation of the natural log-transformed IP (time), the doubling time for *B. anthracis* vegetative cell growth, and a threshold parameter representing the number of vegetative bacteria present in the host before illness. The full Gutting model has five parameters: the three parameters above plus two additional parameters related to the spore germination and clearance rates. Gutting *et al.*[8] estimated values of the doubling time using two data sets, one from high-dose exposure as measured using culture-based measurements and the second from low-dose exposure as measured using polymerase chain reaction (PCR) measurements. The application of the Gutting model in this article assumes that TTD coincides with the time-to-symptom observation, reflecting the short times observed between initial disease expression and death in rabbits.[36]

2.4. Model Fitting

Model parameter values are estimated via MLE. In deriving the likelihood function, TTD is treated as a continuous variable because TTD is recorded to the nearest minute for the single- and multiple-dose experiments. For the jth animal succumbing at time t_j, for which t_j is less than the experimental observation time, t_e, the likelihood of mortality is given by:

$$L_j = f(t_j) = S(t_j)\ H(t_j), \tag{7}$$

where the dependencies on other variables and parameters besides time are not noted for simplicity. When the animal does not die during the experiment, the likelihood for the animal is:

$$L_j = S(t_{e,j}). \tag{8}$$

The likelihood function for all animals is:

$$L = \prod_{j=1}^{J} L_j. \tag{9}$$

The logarithm of this likelihood is maximized with respect to all identified parameters using R computer program routines.[56] In addition to using graphical analyses for evaluating goodness of fit, statistical inferences were drawn about the goodness of fit of individual models, the difference in fit of nested models, and the difference in fit of nonnested models. The deviance of the fit (−2 times the log of the likelihood function evaluated at the MLE values) is used for comparison of nested models. The corrected Akaike information criterion (cAIC) measure[57] is used for comparison of nonnested models to select the best-fitting model. The best-fitting model is associated with the lowest deviance or the lowest cAIC values. For all estimates reported, the MLE procedure obtains convergence using different starting values and a nonsingular Hessian matrix, when setting a relative tolerance of 1×10^{-8} as the convergence criterion. Plotting the likelihood function and bootstrapping are performed to examine stability of the estimates. For the latter, individual animals are used as the primary sampling unit. Further details of the calculations can be obtained from the authors.

3. RESULTS

In this section, results are presented for a preliminary analysis and survival analysis of baseline and alternative time–dose dependent models.

3.1. Preliminary Analysis

The single-dose data set[16] consists of five groups, A2–A6, each with five animals exposed to single doses of *B. anthracis* aerosols. The multiple-dose data set[4] consists of three groups, M2–M4, each with seven animals that were exposed to *B. anthracis* aerosols once a day, except for weekends, for 15 days over a 19-day exposure period. Control groups A1 and M1 are not included in the figure and tables as there were no rabbit deaths in the control groups that received irradiated spores.

Summaries of the mortality results are given in Tables II and III. For Tables II and III, the group-specific geometric means of the animal-specific doses are listed. For Table III, group-specific geometric means of the animal-specific daily doses and the geometric mean of the animal-specific sums of daily doses are provided. TTD values given in the tables are rounded to the nearest day, though for all statistical analyses, results to the nearest minute were used.

The coefficients of variation (CV) for the TTD for the groups A5, A6, and M4 are all about 30%; for group A4, the two animals that died had TTDs of 4 and 11 days, and the CV is about 65%. More variable TTD at low dose than at high dose has been observed in other microbial dose–response studies.[58] The data from group A4 have substantial influence on the models that were fit to the data, contributing to the uncertainty of the predicted TTDs for given doses.

Death rates (i.e., proportion of animals succumbing) were expected to be an increasing function of daily doses and TTD to be a decreasing function of daily doses. Based on the observed death rates and the geometric means of daily doses given in Tables II and III, both data sets exhibited a positive dose-dependent relationship for lethality. For the mean TTD in the multiple-dose data set, the number of samples and deaths are too small to determine a relationship. For the single-dose data set, there is an observed negative correlation between dose and TTD; however, this is primarily due to the one animal that died 11 days after exposure.

It is also plausible that animals exposed to multiple doses as compared to a single dose of approximately the same daily dose amounts would die earlier, assuming the animal dies. The comparison of TTD results between groups A4 and M4 (respective mean $\log_{10}$ daily doses of 4.5 and 4.0, approximately the same order of magnitude) is inconsistent with this expected relationship: the mean TTD for the M4 group of animals that died is about twice that of the corresponding mean for the A4 group. It might be that such a large difference in mean TTD values for the two groups would be due to the small difference in mean $\log_{10}$ daily dose. If true, TTD would be sensitive to even small dose-level differences. Otherwise, the observation of greater mean TTD for multiple-dosing animals suggests the possibility that multiple doses over time induce a protective effect

Table IV. Baseline Survival Models and Fit Statistics (Best-Fitting Model in Bold)

Time-to-Death Model	Time-to-Death Function	cAIC for MLE Fit to Pooled Data
Exponential	$1 - e^{-at}$	146.05
Lagged exponential	$1 - e^{-a(t-t_{\text{lag}})}$	148.29
Inverse exponential	$e^{-a/t}$	121.48
Lagged inverse exponential	$e^{-a/(t-t_{\text{lag}})}$	123.77
Weibull	$\mathbf{1 - e^{-(t/a)^b}}$	**118.16**
Lagged Weibull	$1 - e^{-[(t-t_{\text{lag}})/a]^b}$	120.50
Inverse Weibull	$e^{-(a/t)^b}$	123.60
Lagged inverse Weibull	$e^{-[a/(t-t_{\text{lag}})]^b}$	126.01
Mayer *et al.*	Likelihood of event ~ *in vivo* (cumulative) dose assuming a lag of 1 day for response	134.09

cAIC, corrected Akaike information criterion measure; MLE, maximum likelihood estimation.

that enables survival for a longer period. However, it is difficult to discern a relationship for higher mean TTDs for the M4 group compared to that of the A4 group due to large variability and small sample size. A *t*-test assuming equal group-specific variances of a group effect of TTD has p-value = 0.126 (two-sided, four degrees of freedom), suggesting a possible, though not statistically significant, group effect for dose on TTD. This example shows that more animals are needed to determine these types of relationships and efforts to better characterize variability in TTD are necessary.

3.2. Survival Analysis for Baseline Models

Single-dose data, multiple-dose data, and pooled data are fit to survival models using the TTD distributions shown in Table IV. The models do not include interdose dependence (i.e., the parameter β in Equation (6) is equal to 1) and are termed the "baseline" survival models. On the basis of the cAIC, the survival model using the Weibull TTD distribution provides the best fit to the single- and multiple-dose data sets (not shown) and the pooled data set (Table IV). Incorporation of a lag parameter did not yield a significant improvement in fit over the non-lagged versions of the TTD distributions.

For the Mayer[3] model, when the lag time is treated as a single variable-value model parameter rather than a fixed parameter, convergence is not obtained using MLE. By fixing the lag time, convergence is obtained using MLE for the other three parameters. The estimated values of these parameters, however, are greatly impacted by the assumed value of the lag time. For example, the value of the specific rate of death for an individual pathogen(s) changed by one $\log_{10}$ with a change of assumed lag time from 1 to 1.5 days. In the models with lag presented in Table IV, a value of 1 day is used for the lag (based on the shortest observed TTD of 1.7 days).

For the Gutting[8] model with five parameters, convergence is not obtained for the single-dose data. Thus, for this article, some of the parameters are fixed and the other parameters are estimates using MLE. The solutions are very sensitive to estimates of the assumed value of the threshold bacteria count at which responses occurred. For some assumed values of the threshold count, no estimates of other parameters could be obtained, and for other values, the model predictions are not realistic (i.e., all animals died at all doses or all animals survived all doses). In addition, estimates for the rate of clearance of spores from lungs were orders of magnitude greater than the value proposed by Gutting *et al.*[8] These results for the Gutting model indicate that the data sets are too small to fit the model without some fixed parameter value and that fitting a reduced version of the model by assuming fixed values for a subset of the parameters produces widely varying parameter estimates.

Parameter estimates for the survival models with the Weibull TTD distribution are presented in Table V. The results for the pooled data set incorporate the assumption that no data set effect exists and that the parameter values would be the same regardless of whether the animal receives a single dose or multiple doses. The estimated values of the parameters r and

Table V. Parameter Estimates for the Baseline Best-Fitting Weibull Incubation Survival Model

Data Set	r	a	b
Multiple	5.280×10^{-6}	10.911	376.7
Single	5.949×10^{-6}	8.815	4.119
Pooled	5.58×10^{-6}	9.25	3.905

a (a scale parameter for the TTD distribution) obtained when fitting the single and multiple dose data sets separately do not vary widely among the fits to the single- and multiple-dose data sets, whereas the estimated values of the parameter b (a shape parameter for the TTD distribution) have a statistically significant difference (p-value = 0.0002).

The best-fit Weibull baseline survival model predictions are shown in Fig. 1. Two curves are shown for each dose group. The dotted line curve shows observed mortality and the solid line curves show predicted mortality. Qualitatively, the best-fit baseline model provides a good fit to both the single-dose and multiple-dose data. The apparent large difference for the predicted and observed results for group A4 is not statistically significant, and is due to the small number of animals in the group. Although there is a statistical difference between the values of b when estimated separately for the two data sets the model predictions are not sensitive to the values of b used. Further, the differences in predicted TTDs using the pooled value versus the data-set–specific values (4.119 for single-dose data, 376.7 for multiple-dose data, and 3.905 for pooled data) are not of practical significance. Therefore, for this article, the baseline model is assumed to have an exponential dose–response function and a Weibull TTD distribution.

3.3. Survival Analysis of More Complex Alternative Models

As described in Section 2, two types of alternative models are explored. In the first type, the hazard function is scaled between successive doses as shown in Equation (6). Three functions are used for the scale parameter β: an exponential, a logistic, and a Gompertz function. The MLE parameter estimates and best-fit model deviances are reported in Table IV. Because each of the models constitute a nested set with the baseline Weibull model, the significance of the additional parameter(s) is assessed based on the differences in deviance for the baseline model and the models with scaled hazard functions. None of the models with hazard function scaling produces a statistically significant improvement in fit over the baseline model.

The second type of alternative model assumes a more complex doseresponse than the exponential dose–response function. Alternative functional forms common in microbial dose–response modeling include log-probit and approximate beta-Poisson. In these alternatives, the dose is scaled by the TTD distribution, as described in Section 2, and the resulting models are fit to the pooled data set using MLE, assuming parameter values do not depend on data set. The model fits were compared to the baseline model via their cAICs because the models are not nested. The resulting cAIC values are presented in Table VII. Lagged models are not included in this analysis because the lag term is not significant in any of the baseline model analyses. The exponential dose–response model with a Weibull TTD yields the best fit (lowest cAIC) among all alternative combinations of framework and TTD models, indicating that the more complex alternative dose–response models do not yield a statistically significant improvement in fit over the simpler exponential dose–response model, although the differences of the cAIC values are not great.

4. DISCUSSION

Models for predicting both response and time-to-response when hosts are exposed to multiple pathogen doses are developed in this article using a survival analysis framework. The functions and parameter values of the baseline model are derived using empirical data analysis on data sets of single- and multiple-dose data[4,16] consisting of mortality and TTD for *B. anthracis* exposure in the rabbit. The best-fitting model among all of the models evaluated was derived using a one-parameter exponential dose–response function for death and a two-parameter Weibull distribution for the TTD distribution. A visual inspection of the observed and predicted TTD relative to mortality probability by dose group shows that this best-fitting baseline model provided a generally good fit to the pooled data set for outputs (Fig. 1). More complex survival models accounting for dose–effect dependencies over time and different dose–response functions did not yield statistically significant improvements in fits for the rabbit data TTD observations over the best-fitting three-parameter survival (baseline)

Table VI. Summary of Fits for Models with Hazard Function Scaling Between Doses

Model	Scale Parameter Functional Form	Parameter Estimates	Deviance	Δ Deviance	p-Value
Baseline Weibull model		$r = 5.58\times10^{-6}$ $a = 9.25$ $b = 3.905$	111.593		
Exponential dependence on prior dose	$\beta(d_i; w) = e^{-w d_{i-1}}$	$r = 7.29\times10^{-6}$ $a = 9.76$ $b = 3.98$ $w = 7.50\times10^{-6}$	110.676	0.917	0.338
Logistic dependence on prior dose	$\beta(d_i; i, v) = \frac{1}{1+e^{u\ln d_{i-1}+v}}$	$r = 6.24\times10^{-6}$ $a = 9.48$ $b = 3.93$ $u = 0.00938$ $v = -3.55$	111.454	0.139	0.933
Gompertz dependence on prior dose	$\beta(d_i; \eta, \chi) = 1 - e^{-e^{\eta\ln d_{i-1}+x}}$	$r = 6.45\times10^{-6}$ $a = 9.53$ $b = 3.94$ $w = 0.0361$ $x = -3.575$	111.433	0.163	0.922

model. The baseline model predictions for TTD are consistent with the observed 2- to 3-day TTD in other published rabbit data sets[36,37,59] at high doses.

Further refinement of the empirically-based methodology using the rabbit study data would require the ability to find statistically significant multiple-dose effects. However, the small number of animals and the experimental designs of the rabbit studies limit the statistical power of the data set. In particular, the timing of doses was essentially the same for all animals receiving multiple doses, and there were small numbers of animals at doses that produced fractional death proportions (not 0% or 100%). Consequently, the lack of improved fit of more complex models compared with the baseline model could be a limitation of the data, and not reflective of the "true" underlying phenomena for this pathosystem. The analysis of the rabbit single- and multiple-dose data sets also highlighted the need for appropriate statistical power in the measurement of both response and time-to-response. There has been little study in the variability and contributors to variability in TTD for inhalation anthrax, although knowledge of this variability could inform important inputs in survival models using time-to-response parameters.

The empirical approach used in this article for developing models uses minimal mechanistic assumptions, whereas published mechanistic modeling approaches[2,3,6–8] partially define a series of disease progression steps using multiple parameters. The specification of fully mechanistic models can require significant amounts of data to estimate parameter values and validate the models. Because these data do not exist for the inhalation anthrax pathosystem, researchers used simplifications to specify the models and/or parameter values that may not appropriately describe the system. Without validation of the mechanistic system assumptions, the estimated parameters have uncertain biological meaning; thus, even though the models may provide satisfactory fit for the available animal model data sets, the predictions from them to regions outside the data would not be justified. In addition, the mechanistic models examined in this article may be overparameterized relative to available data, possibly leading to a large degree of uncertainty in model predictions. The empirical approach used in this article avoided that problem by identifying parsimonious models (i.e., models using the fewest necessary parameters) that still produce the best statistical fit for the rabbit studies[4,16] data sets.

The empirical modeling approach demonstrated for the rabbit inhalation anthrax pathosystem has merit for assessment of multiple-dose effects in other host–pathogen scenarios, particularly where the use of alternative modeling techniques is limited. The modeling approach is flexible and can use pathogen-specific empirically-derived parameters for elements

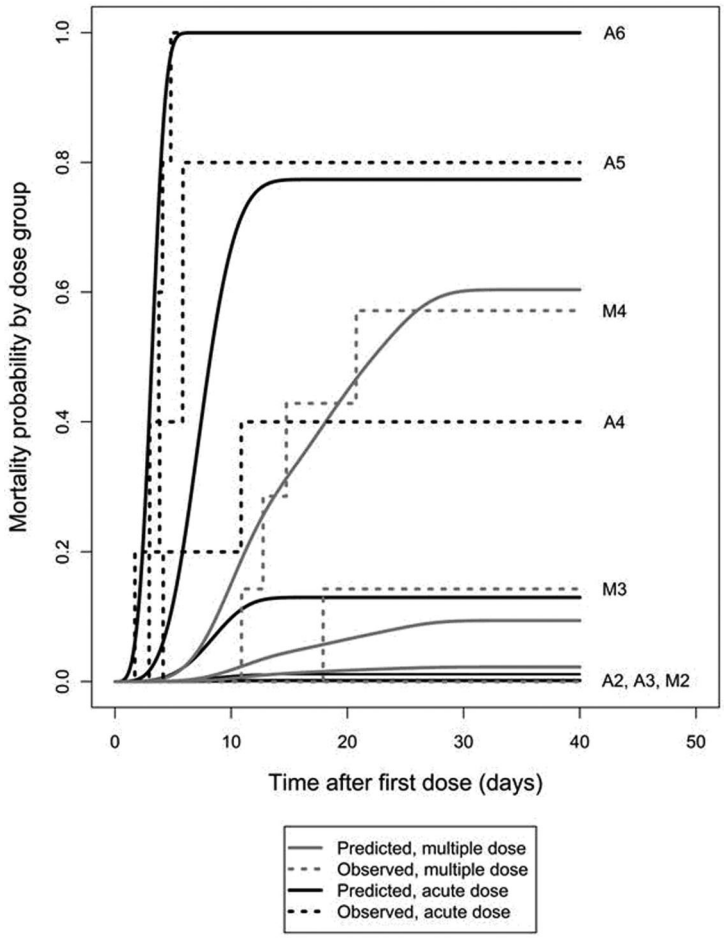

Fig. 1. Best-fit baseline survival model for pooled single- and multiple-dose data set.

Table VII. Model Fit Statistics for Models with Alternative Frameworks

	Underlying Dose–Response Model and cAIC		
Incubation Model	Exponential	Log-Probit	Approximate Beta-Poisson
	$P(d) = 1 - e^{-rd}$	$P(d) = \varphi\left(\frac{1}{q_2} \ln \frac{d}{q_1}\right)$	$P(d) = 1 - \left[1 + \left(\frac{d}{N_{50}}\right)(2^{1/\alpha} - 1)\right]^{-\alpha}$
Exponential	146.04	141.74	143.54
Inverse exponential	121.48	119.68	120.82
Weibull	**118.16**	**119.04**	**118.97**
Inverse Weibull	123.60	120.35	121.90

cAIC, corrected Akaike information criterion measure.

that are typically default judgments that are universally applied across pathogens (e.g., interdose dependence or independence). This broadens the applicability of the methodology across pathogens, hosts, and exposure scenarios with pathogen-specific data on some host–pathogen characteristics (e.g., multiple-dose data with evidence for dependent action in prions[29]).

Model-directed research initiatives have proven essential to develop and test mechanistic models for other pathosystems,[60–63] with resulting data from these efforts aiding in both model specification and parameter identification. These approaches can be of considerable value for future development of dose–response data sets specifically designed to assess potential multiple-dose effects on response and

time-to-response. Mayer *et al.*[3] identified considerations in the design of studies to measure multiple-dose effects for an identified host–pathogen system. The range of doses providing fractional response (e.g., some death) and time-to-response is an important element in study design because of the unknown cumulative effect of multiple doses.[3] The range of doses that causes fractional death proportions (i.e., between 0% and 100%) may be different for multiple doses and depend on the time pattern of the exposures as well as the time scale of response. Study design should incorporate different time patterns and levels of doses (e.g., fixed total doses while varying dose, number, and timing).[3] Mayer *et al.*[3] identified that large sample sizes may be necessary to obtain reliable estimates of parameters. Survival models could be developed following the approach presented in this article for newly generated data sets on time and dose patterns for any pathosystem of interest, as also demonstrated in another pathosystem by Gravenor *et al.*[29] for three experimental dosing schedules using multiple doses.

5. CONCLUSIONS

The article presents a simple baseline model (exponential dose–response function and Weibull distribution of TTDs) that fits the pooled single- and multiple-dose rabbit data sets[4,16] for inhalation anthrax and generates TTD predictions consistent with reported TTDs from three independent rabbit data sets.[36,37,59] More complex alternative models do not yield a statistically significant improvement in fit over the simple baseline model.

To advance the science for dose–response analysis of multiple-dose exposures, more experimental research is needed regarding the effect of different time and dose patterns for multiple doses, multiple hosts, and multiple pathogens to inform empirical and mechanistic model development. More robust and generalizable response estimates can ultimately be developed using mechanistic models once the quantitative knowledge of the underlying pathogenesis is available. This will necessitate a great deal of additional research. For example, as shown in this article, more experimental data are needed to determine the nature of dose dependencies over time, particularly in the low-dose region.

An iterative process can be used whereby empirical modeling provides critical data to inform development of mechanistic models, and mechanistic model outputs can then be used to identify relevant parameters and refine their estimates for empirical models.[60–63] If models are incorrectly defined or highly uncertain, further experimental research can be designed to illuminate the knowledge gaps for the host–pathogen systems. As an example, the development and validation of mechanistic models of host–pathogen interactions for *Mycobacterium tuberculosis* have been advanced by multifaceted approaches to modeling immune response to tuberculosis over multiple time and spatial scales using complementary mathematical, computational, and experimental studies.[60–63] Such iterative processes involving experimental research and modeling for inhalation anthrax pathosystems would advance knowledge and increase confidence in predicting responses and time-to-responses for assessing, communicating, and managing risks associated with exposures to *B. anthracis*.

ACKNOWLEDGMENTS

The U.S. Environmental Protection Agency through its Office of Research and Development funded and managed the research described here under Contract No. SP0700-00-D-3180/CB-11-0232 to Battelle, under Purchase Order No. US001-0000318847 to Coleman Scientific Consulting, and Contract No. EP-C-14-001 to ICF International, under Battelle contract 13KJBO0004 Task Order WA-1-24. It has been subjected to the Agency's review and has been approved for publication. Note that approval does not signify that the contents necessarily reflect the views of the Agency. Mention of trade names, products, or services does not convey official EPA approval, endorsement, or recommendation. Dr. M. Louise M. Pitt (U.S. Army Medical Research Institute of Infectious Diseases [USAMRIID]) generously provided a data set for inhalation anthrax in the African green monkey to aid in the analysis. Dr. Terra Haxton, Dr. David Farrar, Ms. Eletha Brady-Roberts, Dr. Tonya Nichols, and Dr. Emily Snyder provided helpful comments to a previous draft of this article.

REFERENCES

1. Haas CN. On the risk of mortality to primates exposed to anthrax spores. Risk Analysis, 2002; 22(2):189–193.
2. Toth DJ, Gundlapalli AV, Schell WA, Bulmahn K, Walton TE, Woods CW, Coghill C, Gallegos F, Samore MH, Adler FR. Quantitative models of the dose-response and time course of inhalational anthrax in humans. PLoS Pathogens, 2013; 9(8):e1003555.

3. Mayer BT, Koopman JS, Ionides EL, Pujol JM, Eisenberg JN. A dynamic dose-response model to account for exposure patterns in risk assessment: A case study of inhalation anthrax. Journal of the Royal Society Interface, 2011; 8(57):506–517.
4. U.S. Environmental Protection Agency. Multiple daily low-dose *Bacillus anthracis* Ames inhalation exposures in the rabbit. EPA/600/R-11/145. Washington, DC: U.S. Environmental Protection Agency, 2012.
5. Brookmeyer R, Johnson E, Barry S. Modelling the incubation period of anthrax. Statistics in Medicine, 2005; 24(4):531–42.
6. Gutting B. Deterministic models of inhalational anthrax in New Zealand white rabbits. Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science, 2014; 12(1):29–41.
7. Gutting BW, Channel SR, Berger AE, Gearhart JM, Andrews GA, Sherwood RL, Nichols TL. Mathematically modeling inhalation anthrax. Microbe, 2008; 3(2):78–85.
8. Gutting BW, Marchette D, Sherwood R, Andrews GA, Director-Myska A, Channel SR, Wolfe D, Berger AE, Mackie RS, Watson BJ, Rukhin A. Modeling low-dose mortality and disease incubation period of inhalational anthrax in the rabbit. Journal of Theoretical Biology, 2013; 329:20–31.
9. Huang Y. Incorporating Time to Response into Dose-Response Models Used in Quantitative Microbial Risk Assessment. Ph.D. Thesis. Philadelphia, PA: Drexel University, 2010.
10. Wilkening DA. Modeling the incubation period of inhalational anthrax. Medical Decision Making, 2008; 28(4):593–605.
11. Haas CN. Microbial dose response modeling: Past, present, and future. Environmental Science & Technology, 2015; 49(3):1245–1259.
12. Cote CK, Welkos SL, Bozue J. Key aspects of the molecular and cellular basis of inhalational anthrax. Microbes and Infection, 2011; 13(14–15):1146–1155.
13. Leffel EK, Pitt MLM. Characterization of new and advancement of existing animal models of *Bacillus anthracis* infection. Ppl 81–98 in JR Swearengen (Ed). Biodefense Research Methodology and Animal Models. New York: CRC Press, 2012.
14. National Research Council. Overcoming Challenges to Develop Countermeasures against Aerosolized Bioterrorism Agents: Appropriate Use of Animal Models. Washington, DC: National Academies Press, 2006.
15. Dembek ZF, Pavlin JA, Kortepeter MG. Epidemiology of biowarfare and bioterrorism. Pp. 39–68 in Dembek ZF (Ed). Medical Aspects of Biological Warfare. Washington, DC: Office of the Surgeon General, U.S. Army Medical Department Center and School, Borden Institute, 2007.
16. U.S. Environmental Protection Agency. Acute low dose *Bacillus anthracis* Ames inhalation exposures in the rabbit. EPA/600/R-11/075. Cincinnati, OH: U.S. Environmental Protection Agency, 2011.
17. Jones MB, Jani R, Ren D, Wood TK, Blaser MJ. Inhibition of *Bacillus anthracis* growth and virulence-gene expression by inhibitors of quorum-sensing. Journal of Infectious Diseases, 2005; 191(11):1881–1888.
18. Jones MB, Peterson SN, Benn R, Braisted JC, Jarrahi B, Shatzkes K, Ren D, Wood TK, Blaser MJ. Role of luxS in *Bacillus anthracis* growth and virulence factor expression. Virulence, 2010; 1(2):72–83.
19. Rumbaugh KP, Trivedi U, Watters C, Burton-Chellew MN, Diggle SP, West SA. Kin selection, quorum sensing and virulence in pathogenic bacteria. Proceedings of the Royal Society: Biological Sciences, 2012; 279(1742):3584–3588.
20. Antunes LCM, Ferreira RBR, Buckner MMC, Finlay BB. Quorum sensing in bacterial virulence. Microbiology, 2010; 156(8):2271–2282.
21. Sircili MP, Walters M, Trabulsi LR, Sperandio V. Modulation of enteropathogenic *Escherichia coli* virulence by quorum sensing. Infection and Immunity, 2004; 72(4):2329–2337.
22. Zhu J, Miller MB, Vance RE, Dziejman M, Bassler BL, Mekalanos JJ. Quorum-sensing regulators control virulence gene expression in *Vibrio cholerae*. Proceedings of the National Academy of Sciences of the United States of America, 2002; 99(5):3129–3134.
23. Ruthel G, Ribot WJ, Bavari S, Hoover TA. Time-lapse confocal imaging of development of *Bacillus anthracis* in macrophages. Journal of Infectious Diseases, 2004; 189(7):1313–1316.
24. Marois I, Cloutier A, Garneau É, Richter MV. Initial infectious dose dictates the innate, adaptive, and memory responses to influenza in the respiratory tract. Journal of Leukocyte Biology, 2012; 92(1):107–121.
25. Wei G, Bull H, Zhou X, Tabel H. Intradermal infections of mice by low numbers of African trypanosomes are controlled by innate resistance but enhance susceptibility to reinfection. Journal of Infectious Diseases, 2011; 203(3):418–429.
26. Schricker IL, Eigelsbach HT, Mitten JQ, *et al*. Pathogenesis of tularemia in monkeys aerogenically exposed to *Francisella tularensis* 425. Infection and Immunity, 1972; 5(5):734–744.
27. Rockx B, Brining D, Kramer J, Callison J, Ebihara H, Mansfield K, Feldmann H. Clinical outcome of henipavirus infection in hamsters is determined by the route and dose of infection. Journal of Virology, 2011; 85(15):7658–7671.
28. Capuano III SV, Croix DA, Pawar S, Zinovik A, Myers A, Lin PL, Bissel S, Fuhrman C, Klein E, Flynn JL. Experimental *Mycobacterium tuberculosis* infection of cynomolgus macaques closely resembles the various manifestations of human *M. tuberculosis* infection. Infection and Immunity, 2003; 71(10):5831–5844.
29. Gravenor MB, Stallard N, Curnow R, McLean AR. Repeated challenge with prion disease: The risk of infection and impact on incubation period. Proceedings of the National Academy of Sciences of the United States of America, 2003; 100(19):10960–10965.
30. Alibek K. Biohazard: The Chilling True Story of the Largest Covert Biological Weapons Program in the World—Told from Inside by the Man Who Ran It. New York: Dell Publishing, 1999.
31. Guillemin J. Anthrax: The Investigation of a Deadly Outbreak. Berkeley, CA: University of CA Press, 1999.
32. Stroud C, Viswanathan K, Powell T, Bass RR. (Eds). Prepositioning Antibiotics for Anthrax. Washington, DC: National Academies Press, 2012.
33. Wampler RA, Blanton TS. Anthrax at Sverdlovsk, 1979. September 11th Sourcebooks, v. 5; National Security Archive Electronic Briefing Book No. 61:1–16. Washington, DC: National Security Archive; 2001. Available at: http://nsarchive.gwu.edu/NSAEBB/NSAEBB61/, Accessed on April 17, 2014.
34. Jernigan JA, Stephens DS, Ashford DA, Omenaca C, Topiel MS, Galbraith M, Tapper M, Fisk TL, Zaki S, Popovic T, Meyer RF. Bioterrorism-related inhalational anthrax: The first 10 cases reported in the United States. Emerging Infectious Diseases, 2001; 7(6):933–944.
35. National Research Council. Reopening Public Facilities after a Biological Attack. Washington, DC: National Academies Press, 2005.
36. Comer JE, Ray BD, Henning LN, Stark GV, Barnewall RE, Mott JM, Meister GT. Characterization of a therapeutic model of inhalational anthrax using an increase in body temperature in New Zealand white rabbits as a trigger for treatment. Clinical and Vaccine Immunology, 2012; 19(9):1517–1525.
37. Yee SB, Hatkin JM, Dyer DN, Orr SA, Pitt ML. Aerosolized *Bacillus anthracis* infection in New Zealand white rabbits: Natural history and intravenous levofloxacin treatment. Comparative Medicine, 2010; 60(6):461–468.
38. Brachman PS, Kaufmann AF, Dalldorf FG. Industrial inhalation anthrax. Bacteriological Reviews, 1966; 30(3):646–657.

39. Fritz DL, Jaax NK, Lawrence WB, Davis KJ, Pitt ML, Ezzell JW, Friedlander AM. Pathology of experimental inhalation anthrax in the rhesus monkey. Laboratory Investigation, 1995; 73(5):691–702.
40. Lever MS, Stagg AJ, Nelson M, Pearce P, Stevens DJ, Scott EA, Simpson AJ, Fulop MJ. Experimental respiratory anthrax infection in the common marmoset (*Callithrix jacchus*). International Journal of Experimental Pathology, 2008; 89(3):171–179.
41. Rossi CA, Ulrich M, Norris S, Reed DS, Pitt LM, Leffel EK. Identification of a surrogate marker for infection in the African green monkey model of inhalation anthrax. Infection and Immunity, 2008; 76(12):5790–5801.
42. Vasconcelos D, Barnewall R, Babin M, Hunt R, Estep J, Nielsen C, Carnes R, Carney J. Pathology of inhalation anthrax in cynomolgus monkeys (*Macaca fascicularis*). Laboratory Investigation, 2003; 83(8):1201–1209.
43. Pitt M Louise M (U.S. Army Medical Research Institute of Infectious Diseases [USAMRIID], Pathology Division, Fort Detrick, MD). Dataset "Inhalation anthrax in African green monkey" to: Margaret E. Coleman (Coleman Scientific Consulting).
44. Gleiser CA, Berdjis CC, Hartman HA, Gochenour WS. Pathology of experimental respiratory anthrax in *Macaca mulatta*. British Journal of Experimental Pathology, 1963; 44:416–426.
45. Gochenour WS Jr., Gleiser CA, Tigertt WD. Observations on penicillin prophylaxis of experimental inhalation anthrax in the monkey. Journal of Hygiene (London), 1962; 60(1):29–33.
46. Twenhafel NA, Leffel E, Pitt MLM. Pathology of inhalational anthrax infection in the African green monkey. Veterinary Pathology, 2007; 44(5):716–721.
47. Brachman PS, Gold H, Plotkin SA, Fekety FR, Werrin M, Ingraham NR. Field evaluation of a human anthrax vaccine. American Journal of Public Health and the Nation's Health, 1962; 52(4):632–645.
48. World Health Organization. Anthrax in Humans and Animals, 4th ed. Geneva, Switzerland: Geneva World Health Organization, 2008.
49. Brachman PS, Plotkin SA, Bumford FH, Bumford FH, Atchison MM. An epidemic of inhalation anthrax: The first in the twentieth century. II. Epidemiology. American Journal of Hygiene, 1960; 72:6–23.
50. Dahlgren CM, Buchanan LM, Decker HM, Freed SW, Phillips CR, Brachman PS. *Bacillus anthracis* aerosols in goat hair processing mills. American Journal of Hygiene, 1960; 72:24–31.
51. Holty J-EC, Bravata DM, Liu H, Olshen RA, McDonald KM, Owens DK. Systematic review: A century of inhalational anthrax cases from 1900 to 2005. Annals of Internal Medicine, 2006; 144(4):270–280.
52. Oakes D. Biometrika centenary: Survival analysis. Biometrika, 2001; 88(1):99–142.
53. Huang Y, Bartrand TA, Haas CN, Weir MH. Incorporating time postinoculation into a dose-response model of *Yersinia pestis* in mice. Journal of Applied Microbiology, 2009; 107(3):727–735.
54. Huang Y, Haas CN. Time-dose-response models for microbial risk assessment. Risk Analysis, 2009; 29(5):648–661.
55. Watanabe T, Teske SS, Haas CN. Classic dose-response and time postinoculation models for *Leptospira*. Risk Analysis, 2014; 34(3):465–484.
56. R Core Development Team. R Statistical Programming Language. Vienna, Austria: R Foundation for Statistical Computing, 2013. Available at: https://www.R-project.org, Accessed on April 17, 2012.
57. Hurvich CM, Tsai C-L. Regression and time series model selection in small samples. Biometrika, 1989; 76(2):297–307.
58. Meynell GG, Meynell EW. The growth of microorganisms *in vivo* with particular reference to the relation between dose and latent period. Journal of Hygiene, 1958; 56(3):323–346.
59. Zaucha GM, Pitt MLM, Estep J, Ivins BE, Friedlander AM. The pathology of experimental anthrax in rabbits exposed by inhalation and subcutaneous inoculation. Archives of Pathology and Laboratory Medicine, 1998; 122(11):982–992.
60. Kirschner DE, Linderman JJ. Mathematical and computational approaches can complement experimental studies of host-pathogen interactions. Cell Microbiology, 2009; 11(4):531–539.
61. Marino S, Hogue IB, Ray CJ, Kirschner DE. A methodology for performing global uncertainty and sensitivity analysis in systems biology. Journal of Theoretical Biology, 2008; 254(1):178–196.
62. Marino S, Linderman JJ, Kirschner DE. A multifaceted approach to modeling the immune response in tuberculosis. Wiley Interdisciplinary Reviews. Systems Biology and Medicine, 2011; 3(4):479–489.
63. Wigginton JE, Kirschner D. A model to predict cell-mediated immune regulatory mechanisms during human infection with *Mycobacterium tuberculosis*. Journal of Immunology, 2001; 166(3):1951–1967.