On the Risk of Mortality to Primates Exposed to Anthrax Spores

Charles N. Haas*

Current events have heightened the importance of understanding the risks from inhalation exposure to small numbers of spores of Bacillus anthracis. Previously reported data sets have not been fully assessed using current understanding of microbial dose response. This article presents an assessment of the reported primate dose-response data. At low doses, the risk to large populations of low doses of inhaled spores (e.g., < 100) is not insignificant.

KEY WORDS: Microbial risk assessment; dose-response assessment; anthrax; monkeys; Bacillus anthracis; bioterrorism

1. INTRODUCTION

The inhalation anthrax bioterrorism event in the United States commencing in October 2001(1) has highlighted the importance of quantifying the risks from exposure to potential bioterrorism agents such as Bacillus anthracis. Although there have been several recent reviews(2–4) of the potency of B. anthracis, these have focused on describing impact in terms of the median lethal dose (frequently mislabeled as the “infectious dose”). Since the early 1980s(5,6) a paradigm for describing risk from exposure to microorganisms has emerged in which the standard risk assessment techniques (dose-response assessment, exposure assessment) are employed to estimate risk from pathogenic microorganisms. However these techniques only have been applied to various ingestion(8–14) and dermal exposures(15). There has only been one recent attempt to apply quantitative risk assessment to inhalation exposures, in the context of bioaerosol emissions from sludge disposal operations,(16) There has been a somewhat parallel development in the industrial hygiene literature, in which an exponential model is used to assess risk.(17) Nicas(18) has modified this to incorporate interhost variability, which effectively produces a set of models similar to the beta-Poisson model.(5)

The objective of this article is to assess the dose response of primates exposed (by inhalation) to anthrax spores. While experimental work has previously been conducted, the underlying data has generally been summarized with respect to median infectious dose. For the purpose of developing formal risk assessments it is necessary to develop dose-response models. A maximum likelihood methodology was used following the approaches of prior work.(5,6,11,19,20)

2. DATA SOURCES

Three published sources of information have been found that contain quantitative observations on mortality from inhalation exposure of primates to anthrax spores and that are suitable for dose–response analysis.
Druett et al. \cite{21} exposed Rhesus (Macacus rhesus) monkeys to a one-minute bolus exposure to aerosolized (single spore) preparations of Bacillus anthracis (M36) in an exposure chamber. The aerosol size was verified microscopically by these authors. The data used in the present analysis is that for aerosols containing single spores, since larger diameter aerosols had lower potency (due to reduced airway penetration). The raw data (from Table 9 in the original source) is given in Table I. In addition, the estimated inhaled dose is given, using a respiration rate of 2.4 L/min for the monkey.

Brachman et al. \cite{23} exposed cynomolgus monkeys to Bacillus anthracis spores generated from the machine picking of contaminated goat hair. The dose was reported as the estimated inhaled dose in 5 \(\mu\)m particles. This was measured by plate counts of air samples using an impinger. Exposure occurred over variable periods of time. The data used in the analysis in this report is recapitulated in Table II (from the original Table 1 of the authors).

The third source of dose-response information is the published dose–response curve provided by Glassman \cite{24} of inhalation mortality in cynomolgus monkeys exposed to heterogeneously sized aerosol particles of sub 5 \(\mu\)m diameter containing Bacillus anthracis spores. Work was performed by J. V. Jemski. The dose-response relationship was reported to be log-probit, with an LD\(_{50}\) of 4,130 spores and a probit slope of 0.669. Although this data set reportedly encompassed an extremely large number of animals (1,236), the raw data does not appear to have been published, and the goodness of fit of the log-probit model, and other alternatives appears not to have been examined.

### 3. RESULTS AND DISCUSSION

The Druett et al. data set fit the exponential dose-response model given by Equation (1). In this equation, \(d\) is the average dose, \(k\) is the dose-response parameter, and \(\pi\) is the expected proportion of exposed individuals with the effect (in this case, mortality). The estimated \(k\) was \(7.16 \cdot 10^{-6}\), with a \(p\) value for goodness of fit of 0.18, and a residual deviance of 11.25. The 95\% confidence limits estimated by likelihood ratio were \(5.1 - 9.8 \cdot 10^{-6}\). This corresponds to an LD\(_{50}\) of 96,800, with a range < 70,700, 136,000 >, which is somewhat higher than the estimate of 45,000 obtained by Druett et al. using log-probit regression. A comparison between the experimental data and the fitted dose-response relationship is given in Fig. 1. It was also ascertained that the neither the use of the beta-Poisson nor log-probit models resulted in a statistically significant improvement in fit to this data.

\[
\pi = 1 - \exp(-kd) \quad (1)
\]

By comparison, the reported dose-response relationship from Glassman (based on the underlying Jemski data) is also shown in this figure. It is clear that there is a difference in these dose-response relationships, both with respect to shape and position.

These data have been discussed in several other works but only in the context of median lethal dose. For example, Watson and Keir\cite{3} note that these two data sets lead to different values of the LD\(_{50}\). Pile et al.\cite{2} recapitulate the summary of Watson and Keir. In assessing the risk from anthrax via drinking water exposure, Burrows and Renner\cite{4} rely solely upon these LD\(_{50}\) values.

What is most interesting is that the fitted (exponential) dose-response model based on the Druett data is dramatically different at low doses from the Glassman reported dose-response function. This is illustrated in Fig. 2. For example, at an

### Table I. Dose-Response Data from Druett et al.\cite{21}

<table>
<thead>
<tr>
<th>Exposed Concentration (#/L)</th>
<th>Estimated Inhaled Dose(^{(1)})</th>
<th>Estimated Inhaled Dose(^{(2)})</th>
<th>Total Animals</th>
<th>Dead Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>29,300</td>
<td>70,320</td>
<td>8</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>32,100</td>
<td>77,040</td>
<td>8</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>45,300</td>
<td>108,720</td>
<td>8</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>57,300</td>
<td>137,520</td>
<td>8</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>64,800</td>
<td>155,520</td>
<td>8</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>67,000</td>
<td>160,800</td>
<td>8</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>100,000</td>
<td>240,000</td>
<td>8</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>125,000</td>
<td>300,000</td>
<td>8</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>166,000</td>
<td>398,400</td>
<td>8</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>

\(\text{Note: }^{(1)}\text{Calculated from a respiration rate of 2.4 L/min for the one-minute exposure time.}\)

### Table II. Dose-Response Data from Brachman et al.\cite{23}

<table>
<thead>
<tr>
<th>Group</th>
<th>Average Daily Inhaled Dose</th>
<th>Days of Exposure</th>
<th>Total Animals</th>
<th>Dead Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>530</td>
<td>47</td>
<td>32</td>
<td>14</td>
</tr>
<tr>
<td>B</td>
<td>198</td>
<td>41</td>
<td>31</td>
<td>7</td>
</tr>
<tr>
<td>C</td>
<td>413</td>
<td>2.29</td>
<td>28</td>
<td>2</td>
</tr>
<tr>
<td>D</td>
<td>1041</td>
<td>1.29</td>
<td>22</td>
<td>0</td>
</tr>
</tbody>
</table>

\这项是沃伦的B. anthracis.\cite{22}
average inhaled dose of 10 spores, there is almost a three order of magnitude difference in the resulting risk estimates. This difference may arise from the differences in the underlying experimental data (which in the absence of the raw data for the Glassman result is impossible to judge) and/or the very different low-dose extrapolation properties of the exponential and log-probit models.

The Druett and Glassman dose-response relationships were used to analyze the data collected by Brachman et al. For each group in the Brachman data set (Table II), the average daily risk of mortality (for each day of exposure) was computed by assuming that every day presented an independent and identical risk. An exponential model was then used to fit the daily risk. The fitted “k” value was $2.6 \cdot 10^{-5}$ (95% confidence interval $1.3 - 1.6 \cdot 10^{-5}$). This is compared to the observed mortality in each group (over the entire period of the study) in Fig. 3. Also shown are the projected mortalities using the Druett and the Glassman dose-response functions.

The dose-response relationship from the Druett study underpredicts the mortality observed in the Brachman work. The dose-response relationship given by Glassman dramatically overpredicts the mortality risk. It should be noted, however, that if the underlying Jemski data were to have been adequately described by a beta-Poisson or by an exponential model (rather than log-probit), then it is possible that better consistency between the data sets would have been noted. Unfortunately, in the absence of the Jemski data it is impossible to test this possibility. It should be stressed that the average daily exposures in the Brachman experiments were substantially lower (maximum 1,041 spores) than the lowest experimental data point of Druett (70,320 spores), so the fact that the Druett data is within a factor of two to three of the low-dose risk is encouraging. This slight difference could be explained by exposure heterogeneity.

There may be a number of reasons for the differences between the data sets. First, Druett used Rhesus monkeys, while the other studies used cynomolgus monkeys. There may be species differences...
in inherent susceptibility. Both the Druett and the Jemski work used the *B. anthracis* Vollum strain.(22) The Brachman studies used an aerosol generated by manipulation of goat hair, which may have contained mixed strains, as well as additional respiratory pathogens (and chemical antagonists). There may also have been differences in the surface properties of the spore preparations, perhaps influenced by the presence of surface active agents, which could influence virulence of a spore preparation.(27)

It is interesting to note that Glassman presented the Jemski work as a discussion to the Brachman paper, and suggested that the Jemski and Brachman data were consistent. This reanalysis suggests that the indicated finding may not have been appropriate.

There are some important practical considerations to this work. First, since a dose-response model fitting the underlying data (either the Glassman or the curve derived from the Druett study) is nonzero at any average dose, there would appear to be a possibility of illness derived from exposure (of a sufficiently large population) to even a small dose. This is consistent with prior findings for other infectious agents.(5,6,8–12,14,28–30)

To use this information to assess human risks, it is necessary to assume that the potency to humans is reasonably well estimated from the primate potency experiments. This assumption, in the case of inhalation anthrax, has been made by others.(2,3,31) There does not appear to be human data that would be necessary to test this assumption.3

Meselson *et al.* computed that the dose resulting in 2% fatalities in the Sverdlovsk incident would correspond to an inhaled dose of nine spores if the Glassman dose-response curve is valid at low dose and is applicable to the exposed human population.(31,32) This yielded their estimate of $4 \cdot 10^9$ spores released in the incident. They further noted that use of the LD$_{50}$ reported in the Druett paper would result in an estimate (of spore dose and total release) of 146 times greater if it were used in an exponential (independent-action) dose-response relationship. If the exponential fit to the Druett paper is used, then it is estimated that the dose used to produce a 2% fatality rate would be approximately 2,300 spores, and thus the estimate for release would be 256 times greater than that from the Glassman curve.

The shape and location of the dose-response curve at the low-dose range has major implications for the assessment of risk, and for the implementation of protective regimes to reduce that risk (e.g., facility cleanup). For example, if it is desired to keep the risk associated with a single exposure to under 1/1,000 (i.e., one case in 1,000 persons so exposed) then the Glassman (Jemski) dose-response curve would predict that the dose needs to be kept below 0.1 spore (i.e., on average out of 10 persons one person inhales one spore). If the Druett dose-response relationship were used, the “acceptable” dose at this risk level would be 140 spores. This difference implies different levels of stringency against a possible challenge.

4. CONCLUSIONS

Based on this analysis, it is concluded that inhalation anthrax dose response (in nonhuman primates) can be described by the exponential dose-response model. There is some difference with a prior published dose-response relationship (presented by U.S. Army investigators). However, in the absence of the underlying data, it is hard to ascertain the basis, if any, for such differences. This work does show that, in accordance with other microorganisms examined, a low-dose exposure is predicted to produce a *nonzero* low risk of disease.

ACKNOWLEDGMENTS

The author thanks the reviewers for useful comments. In addition, he is highly appreciative of the comments and clarifying information received from Professor Matthew Meselson (Harvard University).

REFERENCES

3. Watson, A. and D. Keir, “Information on Which to Base Assessments of Risk from Environmental Contaminated with