This article was downloaded by: [US EPA Library]
On: 06 December 2011, At: 05:44
Publisher: Taylor & Francis
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK

Human and Ecological Risk Assessment: An International Journal
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/bher20

Quantification of the Effects of Age on the Dose Response of *Variola major* in Suckling Mice

Mark H. Weir [a] & Charles N. Haas [a]
[a] Department of Civil Architectural and Environmental Engineering, Drexel University, Philadelphia, PA, USA

Available online: 19 Nov 2009

To cite this article: Mark H. Weir & Charles N. Haas (2009): Quantification of the Effects of Age on the Dose Response of *Variola major* in Suckling Mice, Human and Ecological Risk Assessment: An International Journal, 15:6, 1245-1256

To link to this article: http://dx.doi.org/10.1080/10807030903304906

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.tandfonline.com/page/terms-and-conditions

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae, and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand, or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

Human and Ecological Risk Assessment, 15: 1245–1256, 2009
Copyright © Taylor & Francis Group, LLC
ISSN: 1080-7039 print / 1549-7860 online
DOI: 10.1080/10807030903304906

Quantification of the Effects of Age on the Dose Response of *Variola major* in Suckling Mice

Mark H. Weir and Charles N. Haas
Department of Civil Architectural and Environmental Engineering,
Drexel University, Philadelphia, PA, USA

Downloaded by [US EPA Library] at 05:44 06 December 2011

ABSTRACT

Dose-response data for *Variola major* (*V. major*), the causative agent of smallpox, were obtained from the open literature, summarized, and fitted with three dose-response models. It is known from prior outbreak experience that there is generally a difference in infectivity of the agent and its subsequent mortality depending on the age of the patient. A source of animal dose-response data were found with age delineation for the exposure group (suckling mice, intraperitoneal exposure). This delineation was used to adapt current dose-response models to include an age dependency parameter. The degree of the models' fit to the data was ascertained using maximum likelihood estimation (MLE). The effect of host age could be described quantitatively using modifications to the beta Poisson and exponential dose-response models. The modifications improvement in the accuracy of risk prediction by 72% for the beta Poisson model and 7% for the exponential model, compared to the original (unmodified) models.

Key Words: *Variola major*, dose response, age dependency, smallpox, bioterrorism.

INTRODUCTION

Smallpox is thought to have originated more than 3,000 years ago in India or Egypt, and is a highly virulent and contagious disease (Koplow 2003; WHO undated). Despite no effective treatment, smallpox was declared eradicated in 1980 due to the worldwide vaccination program (WHO undated; WHO 1968). The use of *V. major* as a bioterrorist agent is of great concern due to the high mortality, communicability, and lack of effective treatment. Added to these concerns are the current state, availability, and condition of the worldwide reserve stocks of vaccine (WHO 2005a, b). The consequences of a malicious release of *V. major* would be widespread and deadly, making further progression to a human risk analysis for *V. major* exposure necessary.

Received 2 December 2008; revised manuscript accepted 19 May 2009.
Address correspondence to Mark H. Weir, Department of Civil Architectural and Environmental Engineering, Drexel University, 3141 Chestnut St., Curtis 251, Philadelphia, PA 19104, USA. E-mail: mw88@drexel.edu

V. major is a double-stranded DNA virus, and considered a very large viral genome with 186,102 base pairs (Massung *et al.* 1994). Animal models have been difficult to develop due to the high doses and different routes of infection required to induce disease in cynomolgus monkeys, which results in bypassing the incubation and early symptom periods seen in humans (WHO 2005b). Due to the difficulties in performing animal studies as well as the mandate from the World Health Organization (WHO) that "no one other than two collaborating centers in the U.S. and Russian Federation have in their possession . . . viral DNA for *V. major*" (WHO undated; WHO 1968), animal model data is scarce.

Source of Dose–Response Data

To obtain data necessary for the conduct of the dose-response analysis, an extensive literature search was performed. In order to ensure the quality of the data, the following criteria were used:

- The animal model results must be published in a peer-reviewed journal.
- If the animal model study is a vaccine or prophylaxis trial, there must be more than one control with a response other than one or zero.
- Exposure route must be clearly defined and justifiable.
- The dose must be clearly defined in known units (in this case pock forming unit).
- Endpoint of the study must be clearly defined (*i.e.*, host death, infection, *etc.*).
  - If endpoint is death, then the number of any euthanized animals must be reported.
- The stated number of subjects at each dose administered must be reported.

Through the literature search, data were discovered and subsequently used in the dose-response analysis (Marshall and Gerone 1961). Suckling mice were infected with *V. major* intraperitoneally and intracerebrally. The intraperitoneal route contained the age delineation, therefore, this dose-response data was used to develop this work. Since suckling mice do not typically show resistance to *V. major* (Rabonivitz and Bernkopf 1952), the authors were attempting to describe susceptibility to *V. major*. Marshall and Gerone (1961) showed that mice developed resistance to *V. major* "within a few days after birth"; this determination of resistance development was the goal of the researchers. A functional form of the age–dose relationship was not developed by Marshall and Gerone (1961); this work intends to close this gap and develop this functional form.

Suckling mice are generally not considered a good analog to humans when developing an animal model (Marshall and Gerone 1961; Rabonivitz and Bernkopf 1952). However, this study can be used when considering that the age analysis is considering the extremes of age. Humans are generally exposed by direct contact or inhalation of the virus (Wehrle *et al.* 1970), which is greatly different from the intraperitoneal exposure used by Marshall and Gerone. Despite this difference in exposure route, the specific parameters reported here will likely require some modification for application to human health effect assessment. From prior outbreaks the very elderly and the very young were the worst affected groups, thus looking at the effect of age at the one extreme of the age groups will be a first step in

Downloaded by [US EPA Library] at 05:44 06 December 2011

quantifying the effects of age. Having age data such as presented in Marshall and Gerone (1961) for the older age groups as well would give a better picture of the age dependency of the dose-response relationship. Having these older age groups would complete the age dependency picture and provide more insight and likely a better improvement post modification; however, this was not available from Marshall and Gerone (1961). Also, considering the difficulties in using nonhuman primates in symptomatic disease progression animal models as mentioned previously, and the constraints of the quality controls, host species other than the ideal are needed for symptomatic disease progression animal models.

Purpose of Current Work

As already mentioned, the very elderly and the very young have a greater risk of mortality from smallpox when compared to the total population. Also, since the animal model study separated the animals into groups based by age (in hours), this work attempted to quantify the effect of age on the dose response. This analysis is the first known quantification of the dependency of age in microbial dose-response modeling.

Downloaded by [US EPA Library] at 05:44 06 December 2011

METHODS AND MATERIALS

Dose–Response Model Fitting

Maximum likelihood estimation (MLE) was used to fit dose-response models to the source data (Table 1) from Marshall and Gerone (1961). In this method the

Table 1. Dose-response data delineated by age of mouse exposed intraperitoneally.

Mouse age	Dose (PFU)	Number of mice	Positive responses (death of mouse)	Negative responses
0 to 24 hours	100000	9	9	0
	10000	9	9	0
	1000	9	4	5
	100	9	3	6
	10	8	1	7
24 to 48 hours	1000000	11	11	0
	100000	8	8	0
	10000	8	3	5
	1000	8	5	3
	100	7	1	6
48 to 120 hours	2000000	9	9	0
	200000	9	7	2
	20000	8	4	4
	2000	7	1	6
120 to 168 hours	5000000	9	8	1
	500000	9	6	3
	50000	8	0	8
	5000	8	0	8

predicted response (π_i), which is a function of the dose (d_i) and model parameter(s) (Θ) (Eq. 1), is compared directly to the observed response (π_i^o), which is a function of observed positive responses (p_i) and total number of subjects (n_i), from the dose-response data (Eq. 2).

$$\pi_i = P_I\,(d_i, \Theta) \tag{1}$$

$$\pi_i^o = p_i/n_i \tag{2}$$

The model parameter(s) Θ are adjusted to minimize the negative two log-likelihood ratio, herein termed the deviance (Haas *et al.* 1999). The deviance directly compares the predicted response using the models to the observed response from the data. This goodness-of-fit test statistic is minimized using the MLE function in the statistical analysis package **R**.

With the deviance minimized the goodness-of-fit must be investigated and shown statistically significant. In order to accomplish this, the minimized deviance is compared to the chi-squared (χ^2) distribution with degrees of freedom defined as the number of parameters (m) subtracted from the number of doses (n). The null hypothesis (the fit is satisfactory) is rejected if the minimized deviance is greater than the upper 5th percentile of the χ^2 distribution.

In this work two dose-response models were employed, namely the exponential and beta Poisson models, and their approximations can be seen in Eqs. (3) and (4), respectively (Haas *et al.* 1999). Both dose-response models establish the probability of a specific response (P(R)), and since the response defined in the data is death, these models describe the probability of death.

$$\text{Exponential}: \mathrm{P}\,(R) = 1 - \exp(-k \cdot \text{dose}) \tag{3}$$

$$\text{Beta Poisson}: \mathrm{P}\,(R) = 1 - \left[1 + \left(\text{dose}/N_{50}\right) \cdot \left(2^{1/\alpha} - 1\right)\right]^{-\alpha} \tag{4}$$

The best-fitting model must be ascertained from those that show a good fit to the data. When there are models with differing numbers of parameters, the inclusion of additional parameters must be justified as making a statistically significant improvement to the fit of the model. The exponential model (Eq. 3) has only one parameter, k, which is the probability of one organism causing a response, and the beta Poisson has two parameters, α and N_{50}, which defines the slope of the model and is the median infectious dose, respectively. The fit of the models were compared to each other to find the best-fitting dose-response model, again using the minimized deviance as the parameter of fit. The difference of the minimized deviances (Δ) is determined and using the null hypothesis that the simpler model (the model with fewer parameters) is the best fit, Δ is compared to the χ^2 distribution with one degree of freedom, again with the rejection region being the upper 5th percentile.

Age Dependency Analysis

In the animal model study (Marshall and Gerone 1961), the suckling mice were separated into different dose groups based on the age of the mouse (Table 1). The models were first fitted to the data with all age groups pooled together to ascertain if an overall model could be fitted to all age groups. The data were then delineated

Downloaded by [US EPA Library] at 05:44 06 December 2011

in the same form as the original study report, and each of the separate age groups were used to determine if dose-response models would fit the individual age groups.

Once dose-response models with goodness-of-fit were established it was observed that there was a potential trend that the dose-response parameters took with age. In order to determine if there was indeed a trend, the Cochran-Armitage trend test (Haas *et al.* 1999) was performed for α, N_{50}, and k to age of the mice in hours. Once it was shown that there was a significant trend of the parameters with mouse age in hours, an exploratory data analysis (EDA) was used to hypothesize the functional forms that could describe this trend. The EDA was performed using SPSS.

Once a candidate model was chosen to describe the age dependency trend, this model needed to be incorporated into the dose-response models for a full maximum likelihood estimate. This results in the addition of age-dependent dose-response parameters, which take into account the effect age has on the parameters, to the overall dose-response model. Placing the age-dependent parameters into the dose-response models modifies the models to allow for the effects of age on the model. These modified dose-response models now must be optimized and the goodness-of-fit ascertained using the same methods described before.

Rather than comparing the two separate dose-response models to determine the best- fitting model, the modified model must be compared to the original unmodified form. Additional parameters have been placed into the modified models; therefore, the addition of these parameters must be justified statistically. The same procedure for comparing models with differing degrees of parameterization was used in an endeavor to determine if the inclusion of the additional parameters is justifiable.

Downloaded by [US EPA Library] at 05:44 06 December 2011

RESULTS

Fitting Dose–Response Models to Available Data

The two dose-response models were fitted to the data shown in Table 1, in an attempt to model the overall response for the entire data set. The first run was without separating out the different age groups, but rather pooling the age groups together as one large data set. The goodness-of-fit analysis performed on the pooled age groups it is shown in Table 2. As can be seen, when data from all ages pooled together neither of the unmodified dose-response models showed a good fit to the data. Once the data are separated into the same age groups as reported in the original study, acceptable dose-response models can now be fitted to the data. In Table 3 is shown that when the data are delineated by age as was reported (Marshall and Gerone 1961), both dose-response models exhibit a good fit to the data.

Table 2. Goodness of fit for all ages pooled.

Number of subjects	Model	Minimized deviance	Degrees of freedom	$\chi^2_{1,n-m}$ critical	Conclusions
18	Exponential	285.048	17	27.587	Neither model is a good fit.
	Beta Poisson	69.958	16	26.296	

Table 3. Goodness of fit for data separated by age group.

Group	Number of subjects	Model	Minimized deviance	Degrees of freedom	$\chi^2_{1,n-m}$ critical	Conclusions
0 to 23 hours	5	Exponential	8.457	4	9.488	Both models show a good fit to the data.
		Beta Poisson	5.767	3	7.815	
24 to 48 hours	4	Exponential	18.674	3	7.815	
		Beta Poisson	8.814	2	5.992	
48 to 120 hours	4	Exponential	6.604	3	7.815	
		Beta Poisson	1.085	2	5.992	
120 to 168 hours	4	Exponential	6.978	3	7.815	
		Beta Poisson	2.675	2	5.992	

Knowing that there are dose-response models that demonstrate a good fit to the data the best-fitting model must be determined. In Table 4, it can be seen that all but the youngest of mice have a dose-response relationship best described by the beta Poisson model, rather than the exponential model. The hypothesis that the optimized parameters follow a significant trend with age of the host must be examined.

Age Dependency Analysis

In order to determine if there is a statistically significant trend that the dose-response parameters follow, the Cochran-Armitage trend test was used. In Table 5 it is shown that from the outcome of the Cochran-Armitage trend test there exists a statistically significant trend for all parameters with respect to the age of the host. As such, a method of quantifying the trend that the parameters are following with respect to the age of the host is necessary.

Using the curve estimation tool in SPSS, multiple trends were examined. The results of the curve estimation are shown in Table 6; the adjusted-R^2 was the statistic used to determine the most suitable trend (fitting criteria being the log of the sums of the squared errors). These trends for α, N_{50}, and k can also be seen in Figures 1 through 3, respectively, with the most appropriate trend lines superimposed.

Once the most appropriate trend is known for the dose-response models, the process of modifying the underlying dose-response model for MLE estimation was

Table 4. Determination of best fitting dose-response model for each age group.

Group	Number of subjects	Model	Minimized deviance	Δ	$\chi^2_{1,0.05}$ critical	Conclusions
0 to 23 hours	5	**Exponential**	**8.457**	2.690	3.843	Exponential model is best fit.
		Beta Poisson	5.767			
24 to 48 hours	4	Exponential	18.674	9.860	3.843	Beta Poisson model is best fit.
		Beta Poisson	**8.814**			
48 to 120 hours	4	Exponential	6.604	5.519	3.843	Beta Poisson model is best fit.
		Beta Poisson	**1.085**			
120 to 168 hours	4	Exponential	6.978	4.303	3.843	Beta Poisson model is best fit.
		Beta Poisson	**2.675**			

Downloaded by [US EPA Library] at 05:44 06 December 2011

Table 5. Cochran-Armitage trend test results for the age dependency analysis.

Parameters compared	$\chi^2_{1,0.05}$value	Degrees of freedom	$\chi^2_{1,0.05}$critical	*p*-value	Conclusions
Age to α	2.178	1	3.843	0.140	Trend exists
Age to N_{50}	2.286	1	3.843	0.131	Trend exists
Age to k	1.595	1	3.843	0.207	Trend exists

Table 6. Curve estimation results showing a linear trend for α and exponential trends for N_{50} and k.

Dose-response model	Parameter	Estimated curve model	Adjusted R^2	R^2	R	Conclusions
Beta Poisson	α	Linear	0.519	0.679	0.824	Linear curve model is description of relationship.
		Logarithmic	0.180	0.453	0.673	
		Inverse	0.147	0.235	0.485	
		Exponential	0.431	0.620	0.788	
Beta Poisson	N_{50}	Linear	0.485	0.657	0.810	Exponential curve model is description of relationship.
		Logarithmic	0.214	0.476	0.690	
		Inverse	–0.30	0.313	0.560	
		Exponential	0.983	0.989	0.994	
Exponential	k	Linear	0.337	0.558	0.747	Exponential curve model is description of relationship.
		Logarithmic	0.637	0.758	0.871	
		Inverse	0.890	0.963	0.927	
		Exponential	0.967	0.978	0.989	

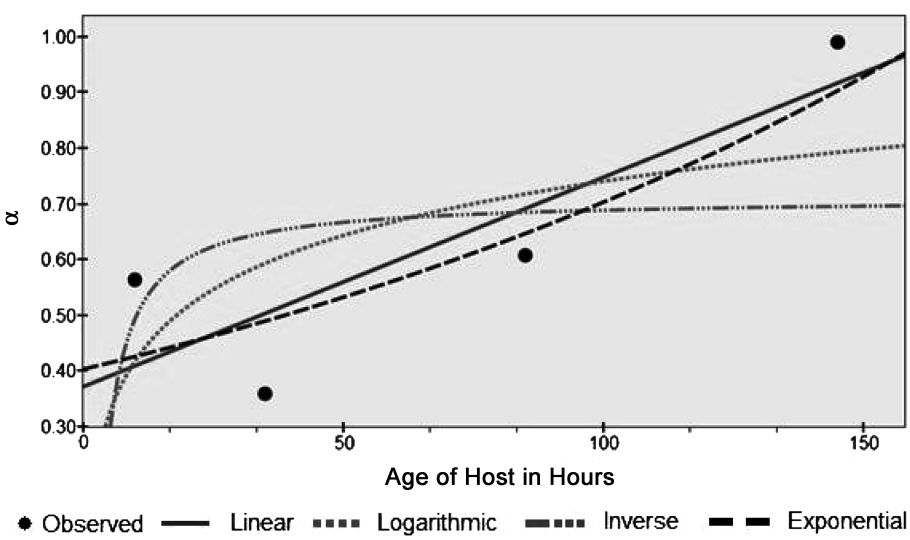

Figure 1. Beta Poisson model α parameter exhibits a trend with respect to age of the host.

Downloaded by [US EPA Library] at 05:44 06 December 2011

Downloaded by [US EPA Library] at 05:44 06 December 2011

Table 7. Improvement in fit from modified dose-response models, justifying the additional parameters.

Group	Number of doses	Model	Minimized deviance	Δ	Difference in degrees freedom for of modified model	$\chi_{0.05,\mathrm{ddof}}$	Conclusion
Age dependency	18	Exponential	262.77	22.27	1	3.84	Including age dependency allows better fit
No age dependency	18	Exponential	285.05				
Age dependency	18	Beta Poisson	19.26	50.70	2	5.99	Including age dependency allows better fit
No age dependency	18	Beta Poisson	69.96				

Table 8. Age dependent dose-response parameters.

Model	Age-dependent parameter
Exponential	$k_0 = 0.0000151$
	$k_0 = 0.00000101$
Beta Poisson	$\alpha_0 = 0.377$
	$\alpha_1 = 0.00264$
	$N_{50_0} = 184.683$
	$N_{50_1} = 0.0501$

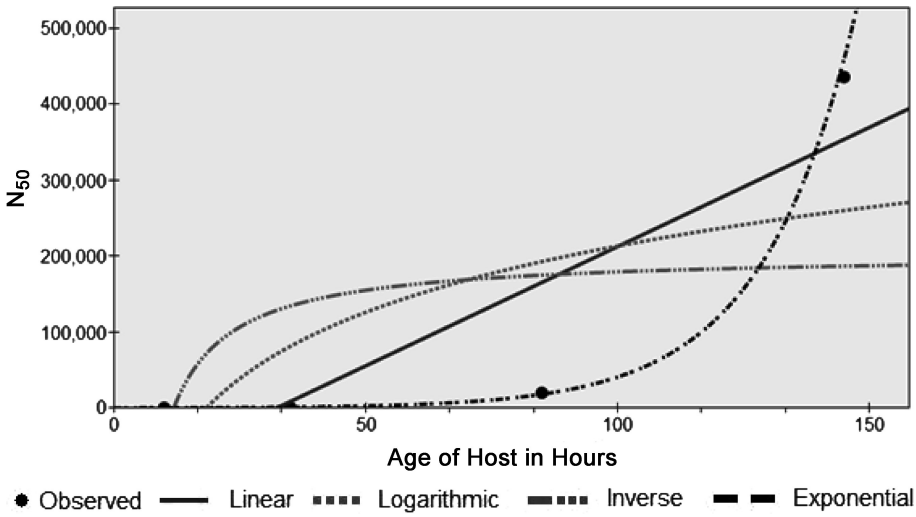

Figure 2. Beta Poisson model N_{50} parameter exhibits a trend with respect to age of the host.

the next step. The parameter k for the exponential model (Eq. 3) was changed using an exponential age dependency trend as in Eq. (5). The exponential model is modified using k^*, which has two parameters k_0 and k_1, thereby changing the exponential model from a one parameter model to a two parameter model.

$$k* = k_0 \cdot \exp\left(k_1 \cdot \text{age}\right) \tag{5}$$

$$\text{Modified Exponential P}\,(R) = 1 - \exp\left(-k* \cdot \text{dose}\right) \tag{6}$$

The age dependency for the parameters of the beta Poisson model was then examined. The unmodified beta Poisson model has two parameters, α, which exhibited

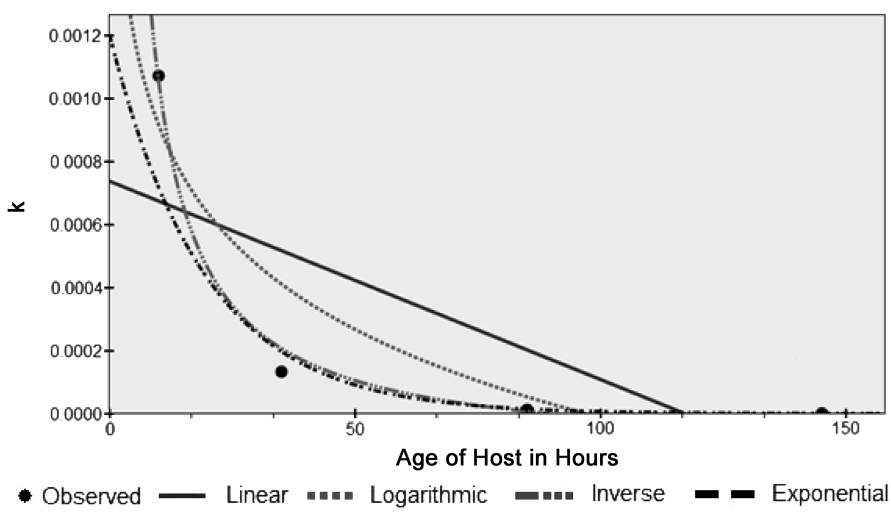

Figure 3. Exponential model k parameter exhibits a trend with respect to age of the host.

Downloaded by [US EPA Library] at 05:44 06 December 2011

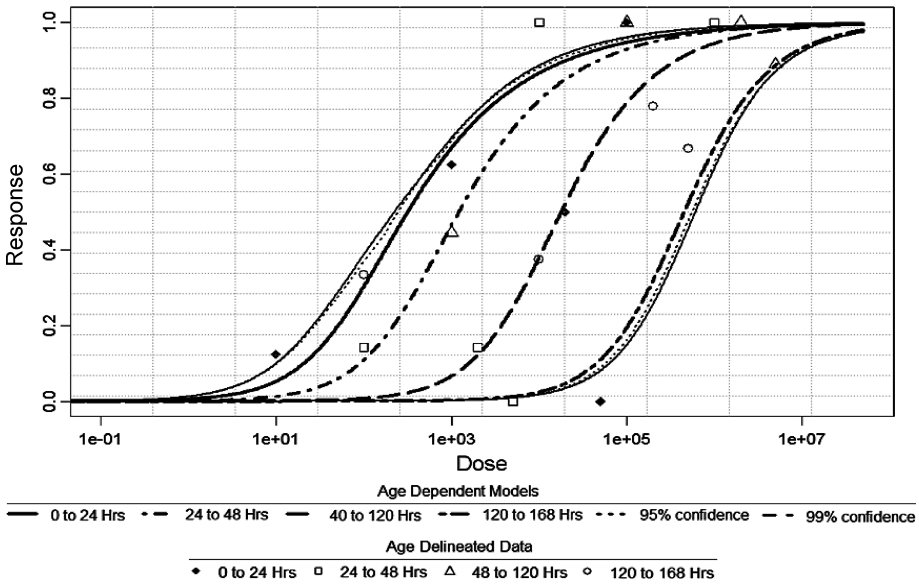

Figure 4. Modified beta Poisson dose-response models for each age group, encapsulated in overall confidence bounds for the modified model.

a linear age dependency (Eq. 7) and an exponential age dependency trend for N_{50} (Eq. 8). Placing these age-dependent parameters into Eq. (4) results in Eq. (9), which is the modified beta Poisson model. The modified beta Poisson model has now been changed from a two-parameter model to a four- parameter model.

$$\alpha* = \alpha_0 + \alpha_1 \left(\text{age}\right) \tag{7}$$

$$N_{50}^{*} = N_{50_0} \cdot \exp\left(N_{50_1} \cdot \text{age}\right) \tag{8}$$

$$\text{Modified Beta Poisson P}\left(R\right) = 1 - \left[1 + \left({}^{dose}/_{N_{50}^{*}}\right) \cdot \left(2^{1}/_{\alpha^{*}} - 1\right)\right]^{-\alpha^{*}} \tag{9}$$

The modified models show an improvement in the fit of the dose-response models for the data. In Table 7 it can be seen that both the exponential and beta Poisson

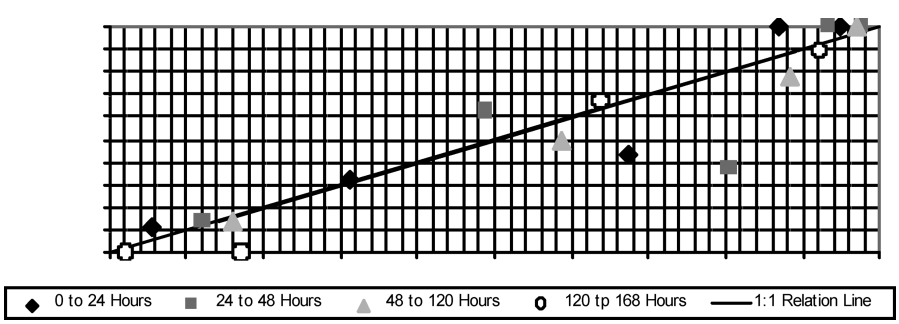

Figure 5. Comparison of the predicted probability of response from the modified beta Poisson model, to the observed probability of response.

Downloaded by [US EPA Library] at 05:44 06 December 2011

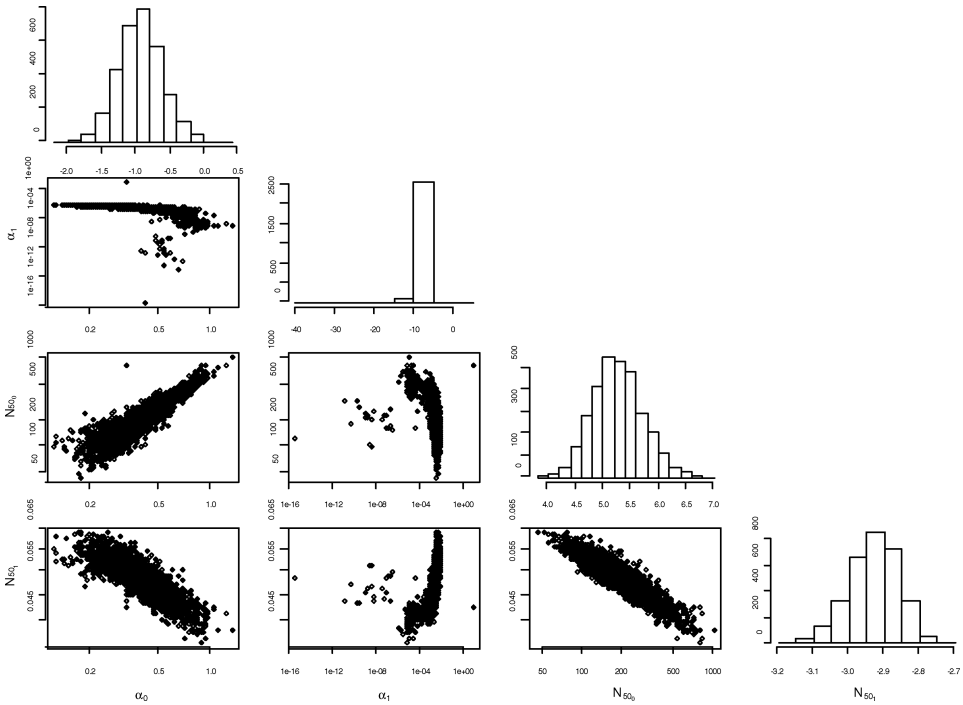

Figure 6. Comparison of bootstrapped age dependent parameters after 10,000 iterations.

models showed an improvement in fit. Overall the beta Poisson showed the greatest improvement in fit, with the deviance being reduced by 72%. The exponential model did not show as impressive an improvement, on the order of a 7% decrease in the deviance. The age dependent parameters in the modified the dose-response models are given in Table 8.

The modified beta Poisson models for each age group can be seen in Figure 4. It can be seen that there is a clear difference between each age group that the modified beta Poisson model, as shown before, can quantitatively incorporate to improve the fit. As can also be seen in Figure 4 the youngest age group shows the highest risk of death, as is expected from what is known clinically in the very young population. In Figure 5 it can be seen that comparing the predicted probability of response determined using the modified beta Poisson model and the observed probability of response shows good agreement for each of the age groups. Shown in Figure 6 are the scatter plots and histograms of the bootstrapped parameters for the modified beta Poisson model after 10,000 iterations of the MLE. It can clearly be seen in Figure 6 that the parameters have a correlation to each other, which would be expected, with α_1 having the narrowest confidence bands.

CONCLUSION

The modified dose-response models that incorporated the age of the host age were capable of providing a better fit to the intraperitoneal exposure of suckling

Downloaded by [US EPA Library] at 05:44 06 December 2011

mice to *V. major*. This represents the first quantitative incorporation of the host into microbial dose-response models. These modifications are for the youngest age groups exposed to *V. major*. However, the approach demonstrated is a generalized case, therefore, application of this approach to other pathogens and age groups will be possible. The modifications made to the dose-response models show the potential for current dose-response models to be adapted in order to integrate more mechanistic and physiological factors. These further advancements will enable dose-response models to better describe the true dose-response relationship.

Although suckling mice are not the best option for depicting the human dose-response relationship, this is a first step to expanding current dose-response models to include quantitative factors. This work described how age dependency can modify dose-response models for *V. major*. However, other factors such as host heterogeneity or aerosol size can be within the spectrum of potential factors to be included in modifying current dose-response models. This work (among the other potential quantitative changes) allows exciting new developments that can advance the understanding of the dose-response relationship for *V. major* and other pathogens.

Downloaded by [US EPA Library] at 05:44 06 December 2011

ACKNOWLEDGMENTS

This research has been partially funded through the Graduate Assistantship in Areas of National Need (GAANN)—Educating Renaissance Engineers. This research has been partially funded through the U.S. Environmental Protection Agency, and U.S. Department of Homeland Security, Science to Achieve Results (STAR) grant program grant number: R83236201.

REFERENCES

Haas CN, Rose JB, and Gerba CP. 1999. Quantitative Microbial Risk Assessment, p 406. John Wiley and Sons, New York, NY, USA

Koplow D. 2003. Smallpox: The Fight to Eradicate a Global Scourge. University of California Press, Berkeley and Los Angeles, CA, USA

Marshall RG and Gerone PJ. 1961. Susceptibility of suckling mice to variola virus. J Bacteriology. 82(1):15–9

Massung RF, Liu LI, Knight JC, *et al.* 1994. Analysis of the complete genome of smallpox variola major virus strain Bangledesh—1975. Virology 201:215–40

Rabonivitz E and Bernkopf H. 1952. Susceptibility of suckling mice to infection with vaccinia and variola viruses. Bull Res Council Israel 2:207

Wehrle PF, Posch J, Richler KH, Henderson DA. 1970. An airborne outbreak of smallpox in a German hospital and its significance to other recent outbreaks in Europe. Bull World Health Organization 43:669–79

WHO. 1968. World Health Organization Technical Report Series, Smallpox Eradication, pp 1–52. Geneva, Switzerland

WHO. 2005a. Report by the Secretariat, Smallpox: Global Smallpox Vaccine Reserve. WHO A58/9. Geneva, Switzerland

WHO. 2005b. Report by the Secretariat, Smallpox: Destruction of Variola Virus Stocks. WHO A58/10, Geneva, Switzerland

WHO. Undated. WHO Smallpox Fact Sheet. Available at http:/www.who.int/mediacentre/factsheet/smallpox/en