PBBK Modeling of Low-Dose Infectivity for Inhalational Anthrax.

The Joint U.S. Environmental Protection Agency and Department of Homeland Security Conference on Real-World Applications and Solutions for Microbial Risk Assessment

April 9, 2008

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Funding by:
EPA: DW-17-92215501-0 and DTRA: BA06TAS022

Outline.

- Objective and General Approach
- Starting Point:
  - LD_{50} Data
  - Mean Time to Death
  - Disease Progression
- High (Lethal) Dose Data and Model Development
  - Inhaled Dose, Deposited (Alveolar/Lung) Dose and Model Predictions
  - Fate of Deposited Spores
  - Germination/Elimination (Lung) Data Model Predictions
  - PB/BK Systemic (Bacteremia) Model and Predictions
- Low (Non-Lethal) Dose Data:
  - Bacterial and Host Data
  - Non-Lethal Infection
  - Acquired Immunity
- Current Studies
- Future Studies

Objective and Approach.

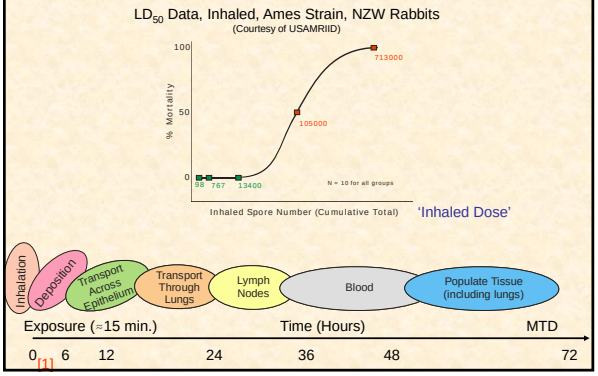
Objective:

To further characterize the risk of inhalational anthrax in man, using animal data (rabbit), to obtain better scientifically-defensible remediation guidelines.

Approach:

- Interdisciplinary team (biologists, mathematicians and modelers)
- Biological Model Development
  - development of relevant animal model(s) (rabbit, inhalation, Ames, AVA)
  - collect data on host and bacteria
  - quantify the infection; dose-response
  - methods development
- Mathematical Model Development
  - different models for use as independent stand-alone models or collectively
  - stochastic/probabilistic models of disease outcome
  - deterministic models of disease outcome and mechanisms of infection
  - PBBK models
  - deposition
  - multispecies
  - application to other diseases/agents

LD_{50} Data and Mean Time to Death (MTD) in NZW Rabbits.



[1]

Inhaled Dose, Deposited (Tissue) Dose and Predictions for Man.

Characterization of Inhaled Heat-Shocked Spore Dose

	Inhaled Dose ($x10^6$)
Total Viable Spores	44.28 ± 5.7
Heat Resistant Viable Spores	28.97 ± 3.4
Heat Sensitive Viable Spores	15.30 ± 3.5
Heat Resistant Viable Spores (%)	70.07 ± 2.9 %
Heat Sensitive Viable Spores (%)	29.93 ± 2.9 %

All values are mean ± SEM (n=60); data pooled from 6 separate experiments
MMAD = 1 um determined using TSI Aerosol Particle Sizer and TSI Scanning Mobility Sizer

Deposited (Alveolar/Lung) Dose
[Based on Homogenized Lung Tissue]

	$x10^6$	% of Inhaled Dose
Inhaled Dose (Total Viable Spores)	19.58 ± 2.5	-
Total Viable Bacteria Recovered in Homogenized Lung Tissue	0.93 ± 0.21	4.63 ± 1.1 %

Values are mean ± SEM (n=10)
In an independent study (14 months apart) that used 12 rabbits, 5.34 ± 0.3 ($x10^6$) Ames spores were inhaled and the percent recovered in homogenized lung tissue immediately following exposure was **4.40 ± 1.2%**

Inhaled Dose, Deposited (Tissue) Dose and Predictions for Man.

Deposited (Alveolar/Lung) Dose
[Based on Bronchoalveolar Lavage (BAL); 2 Washes)]

	$x10^6$	% of Inhaled Dose
Inhaled Dose (Total Viable Spores)	19.58 ± 2.5	-
Total Viable Bacteria in Sonicated BAL Fluid [2X Lavage]	0.25 ± 0.07	1.17 ± 0.3 %
Total Viable Bacteria in Sonicated BAL Fluid [Adjusted: x2.64]	-	3.08 ± 0.9 %

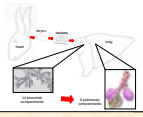
Values are mean ± SEM (n=10)
In an independent study that used 12 rabbits, 5.34 ± 0.3 ($x10^6$) Ames spores were delivered and the percent recovered in [adjusted] was 1.32 ± 0.2%

Lavage Number	Recovered Macrophages ($x10^6$)	Correction Factor
Experiment #1		
2X	8.02 ± 3.0	-
4X	13.66 ± 3.7	1.70
8X	21.22 ± 6.0	2.65
Experiment #2		
2X	13.34 ± 3.5	-
4X	18.65 ± 3.7	1.40
8X	26.71 ± 4.0	2.00

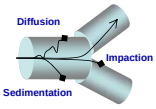
All values are mean ± SEM (n=5 and 4)

Inhaled Dose, Deposited (Tissue) Dose and Predictions for Man.

Rabbit Morphometry

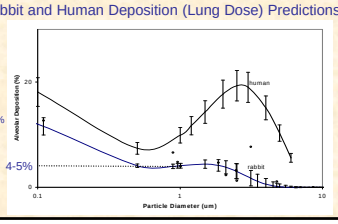


Deposition Mechanisms

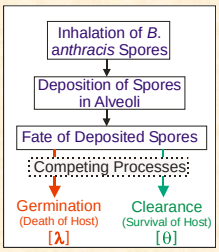


Rabbit and Human Deposition (Lung Dose) Predictions

Ames Spore Data
Lung Tissue = 4.4 and 4.6%
BAL = 1.3 and 3.1%



Fate of Deposited Spores: Germination/Elimination Data and Competing Risks Model Predictions.



Competing Risks Model
- Developed by Brookmeyer *et al.*, 2002-2005
- Used monkey data to derive clearance and estimate germination
- Model consistent with Sverdlovsk data

In Our Work
- Extend to rabbit
- Incorporate deposition
- Aim to experimentally determine clearance and germination rates
- At present, low dose predictions in the rabbit

Exponential Dose-Response Formula

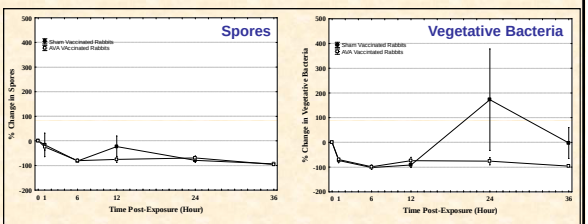
$$\pi = 1 - \exp(-kd)$$

Competing Risks Dose-Response Formula

$$\pi = 1 - \exp\left(\frac{-D\lambda}{\lambda + \theta}\right)$$

Fate of Deposited Spores: Germination/Elimination Data and Competing Risks Model Predictions.

Bacteria in Lung Tissue Post Exposure
[Based on Homogenized Lung Tissue]



All values are mean ± SEM (n=10 per time point)
Data are expressed as a function of deposited dose
In an independent study that used 12 rabbits per time point, the increase in vegetative bacteria occurred at 12 hours.

Clearance = 0.062/hour (Experiment #1) and 0.049/hour (Experiment #2)
Germination = 4.1 x 10^{-7}/hour (Experiment #1) and 3.3 x10^{-7} (Experiment #2)

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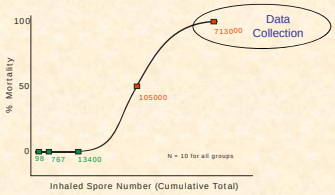

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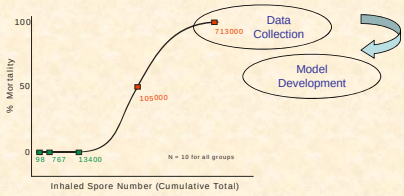

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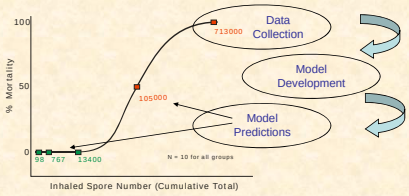

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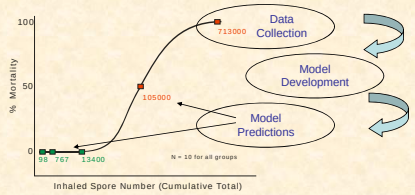
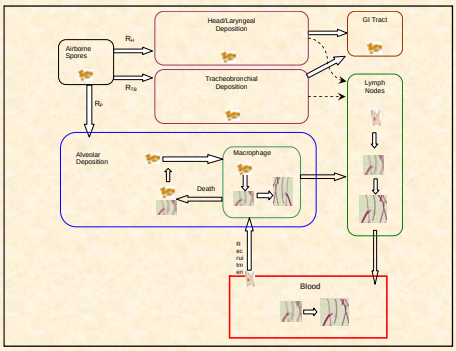


Dose-Response Predictions in the NZW Rabbit (Inhalation of Ames)

# n of Deaths in 10 Exposed Rabbits	P(n) for DI = 10,000	P(n) for DI = 105,000 (Inhaled LD50)	P(n) for DI = 1,000,000
0	0.617	0.00099	0
1	0.353	0.0096	0
2	0.109	0.044	0
3	0.020	0.117	0
4	0.0034	0.205	0
5	0.00019	0.246	0
6	1.10E-5	0.205	0
7	4.27E-7	0.117	2.96E-7
8	0	0.044	8.22E-5
9	0	0.0096	0.013
10	0	0.00099	0.9865

Probability P(n) of the number of deaths n in Column A when 10 rabbits are exposed to an inhaled dose of 10,000 or 105,000 or 1,000,000 spores, calculated using the competing risks model equation (3). (P(n) values < 1.0E-7 are reported as 0)

Fate of Deposited Spores: PB/BK Systemic (Bacteremia) Modeling.



Fate of Deposited Spores: PB/BK Systemic (Bacteremia) Modeling.

Lymph Nodes

Vaccine Group and Time Post-Exposure	n	Spores ($x10^6$)	Vegetative Bacteria
1 Hour			
Sham-Vaccinated[b]	4	0.0 ± 0.0	0.0 ± 0.0
AVA-Vaccinated[b]	4	0.0 ± 0.0	0.0 ± 0.0
6 Hour			
Sham-Vaccinated	5	0.0 ± 0.0	0.0 ± 0.0
AVA-Vaccinated	5	0.0 ± 0.0	0.0 ± 0.0
12 Hour			
Sham-Vaccinated	5	0.0 ± 0.0	43 ± 19
AVA-Vaccinated	5	0.0 ± 0.0	0.0 ± 0.0
24 Hour Experiment #1			
Sham-Vaccinated	5	0.0 ± 0.0	2204 ± 2204
AVA-Vaccinated	5	0.0 ± 0.0	1315 ± 1139
24 Hour Experiment #2			
Sham-Vaccinated	5	0.0 ± 0.0	50285 ± 20760
AVA-Vaccinated	5	55.0 ± 47.0	0.0 ± 0.0
36 Hour Experiment #1			
Sham-Vaccinated (2 died)	3	0.0 ± 0.0	$7 \times 10^6 \pm 3 \times 10^6$
AVA-Vaccinated	5	0.0 ± 0.0	0.0 ± 0.0
36 Hour Experiment #2			
Sham-Vaccinated	5	0.0 ± 0.0	$15 \times 10^6 \pm 13 \times 10^6$
AVA-Vaccinated	5	0.0 ± 0.0	0.0 ± 0.0

Values are mean ± SEM (two data points were removed due to contamination at the 1 hour time point) from TBLN

Fate of Deposited Spores: PB/BK Systemic (Bacteremia) Modeling.

Blood

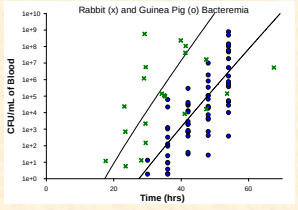
Vaccine Group and Time Post-Exposure	n	Spores	Vegetative Bacteria
12 Hour			
Sham-Vaccinated	6	0.0 ± 0.0	0.0 ± 0.0
AVA-Vaccinated	6	0.0 ± 0.0	0.0 ± 0.0
24 Hour (Experiment #1)			
Sham-Vaccinated	5	0.0 ± 0.0	$0.5 \times 10^6 \pm 0.3 \times 10^6$
AVA-Vaccinated	5	0.0 ± 0.0	0.0 ± 0.0
24 Hour (Experiment #2)			
Sham-Vaccinated	5	0.0 ± 0.0	$0.3 \times 10^6 \pm 0.2 \times 10^6$
AVA-Vaccinated	5	0.0 ± 0.0	5600 ± 3541
36 Hour (Experiment #1)			
Sham-Vaccinated (2 died)	3	0.0 ± 0.0	$0.3 \times 10^6 \pm 0.1 \times 10^6$
AVA-Vaccinated	5	0.0 ± 0.0	0.0 ± 0.0
36 Hour (Experiment #2)			
Sham-Vaccinated[b]	5	0.0 ± 0.0	$18.7 \times 10^6 \pm 16.4 \times 10^6$
AVA-Vaccinated	5	0.0 ± 0.0	0.0 ± 0.0

Values are mean ± SEM
Used 56 ml/kg blood volume and 3kg weight for rabbit

Fate of Deposited Spores: PB/BK Systemic (Bacteremia) Modeling.

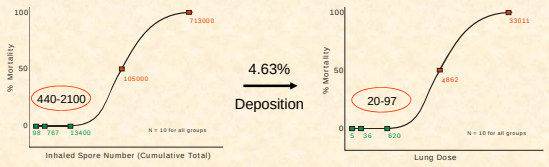
PB/BK Model Predictions

- At present, the model has been developed and applied to Kobiler *et al*., data for rabbit and guinea pig
- We are currently advancing the model using our data following inhalation of Ames strain *B. anthracis*



Data from Kobiler *et al*., 2006 (IN instillation)

Low Dose Response: Bacterial and Host Data

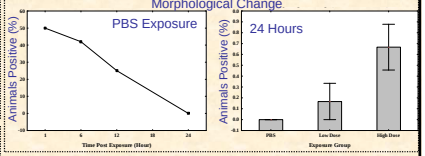


Low Dose Response: Bacterial and Host Data

PBS Aerosol
- Mixed (histiocytic and heterophilic) focal inflammation (Foci)
- lung discoloration immediately following exposure

Low Dose Response: Bacterial and Host Data

Morphological Change



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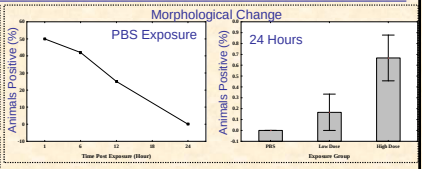

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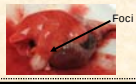
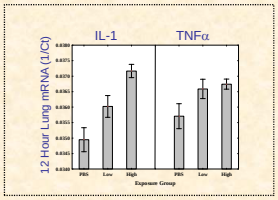
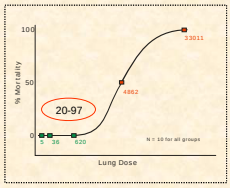
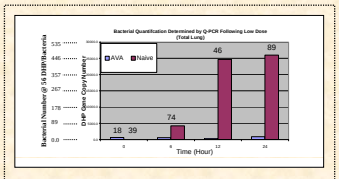


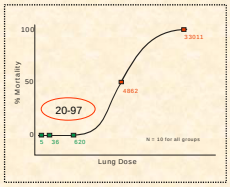


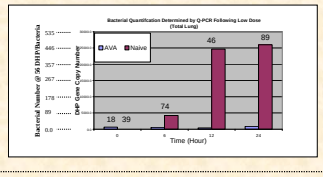
Low Dose Response: Bacterial and Host Data

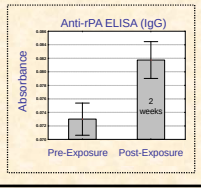


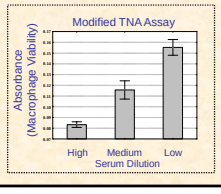


Low Dose Response: Bacterial and Host Data





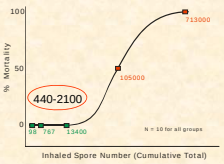




Low Dose Response: Bacterial and Host Data

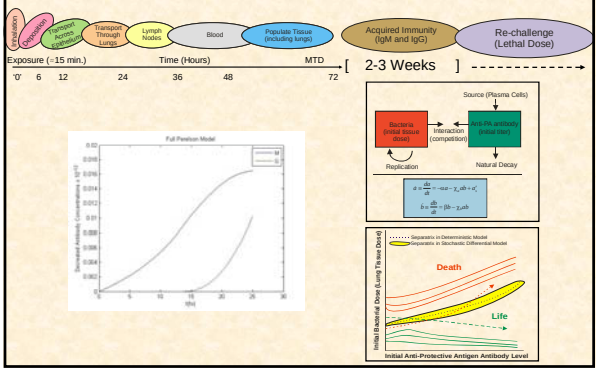
Exposures Completed
- Limited data from plate assay
- RNA purified (host and bacterial)
- DNA processing to begin ASAP
- Targets
  - IL-1, TNFα, IL-6
  - DHP

Future Studies
- Repeat studies
- Acquired immunity



Group	N	Targeted Dose of Inhaled Spores	Time of Sacrifice
1	5	0 (PBS)	12 hours
2	5	100	12 hours
3	5	1000	12 hours
4	5	10000	12 hours
5	5	100000	12 hours
6	5	100,000 autoclaved	12 hours
7	5	100,000 irradiated	12 hours

Low Dose Response: Bacterial and Host Data



Acknowledgements

NSWCDD
Monique Allen
George Andrews, PhD
Alan Berger, PhD
Ryan Mackie
Derrell McPherson, PhD
Dan Shegogue, PhD
Lindsay Sobota, MS
Brent Watson

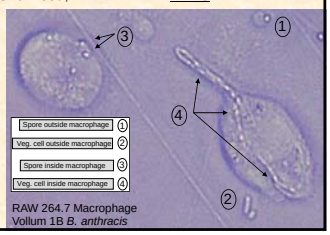
AFRL/HEBP (WPAFB)
Jeff Gearhart, PhD, DABT
Eric Hack

LRRI
Ed Barr
Trevor Brasel, PhD
Katie Overhiem, PhD
Robert Sherwood, PhD

EPA
Irv Baumel, PhD
Tonya Nichols, PhD

DTRA
Steve Channel, DVM

USAMRIID
NMRC



RAW 264.7 Macrophage
Vollum 1B *B. anthracis*