

US EPA ARCHIVE DOCUMENT

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

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Outline.

- Objective and General Approach
- Starting Point:
 - LD₅₀ 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:

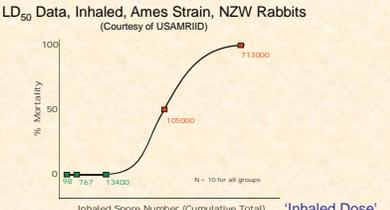
To further characterize the risk of inhalational anthrax in man, using animal data (rabbit), to obtain better scientifically-defensible remediation guidelines.

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

LD₅₀ Data and Mean Time to Death (MTD) in NZW Rabbits.

LD₅₀ Data, Inhaled, Ames Strain, NZW Rabbits
(Courtesy of USAMRIID)



Inhalation

Deposition

Transport Across Epithelium

Transport Through Lungs

Lymph Nodes

Blood

Populate Tissue (including lungs)

Exposure (~15 min.)

Time (Hours)

MTD

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 μm 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 (x10⁵) 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.

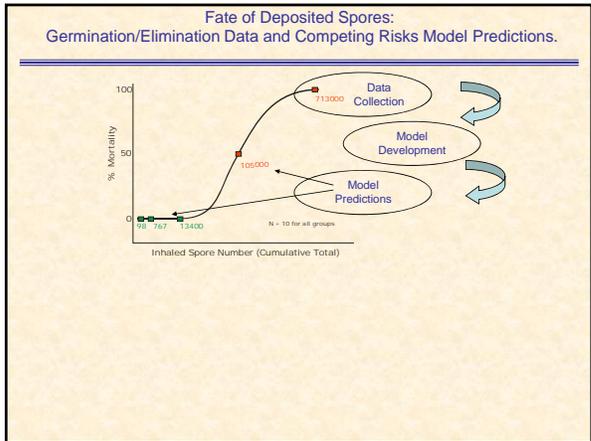
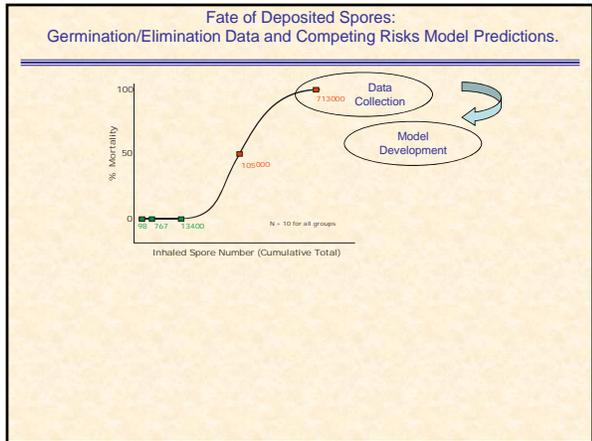
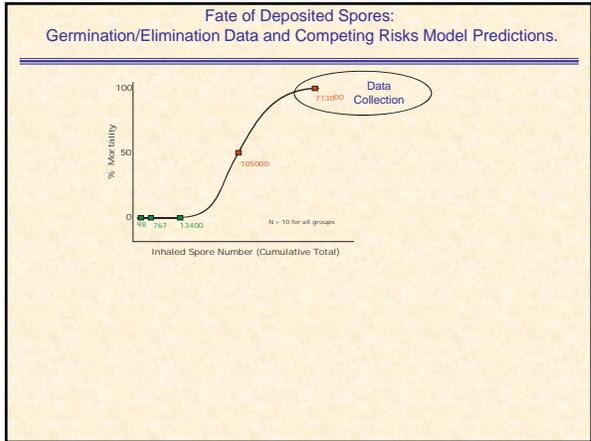
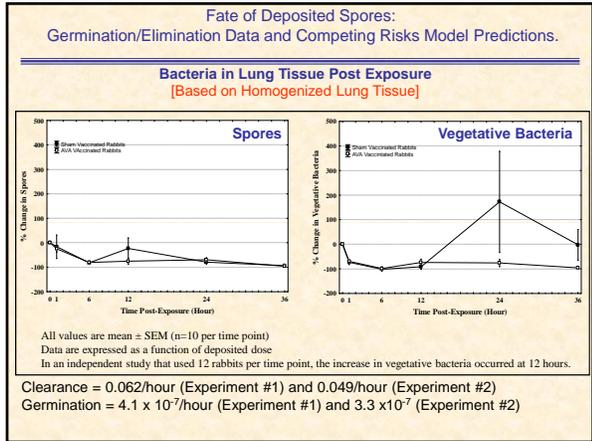
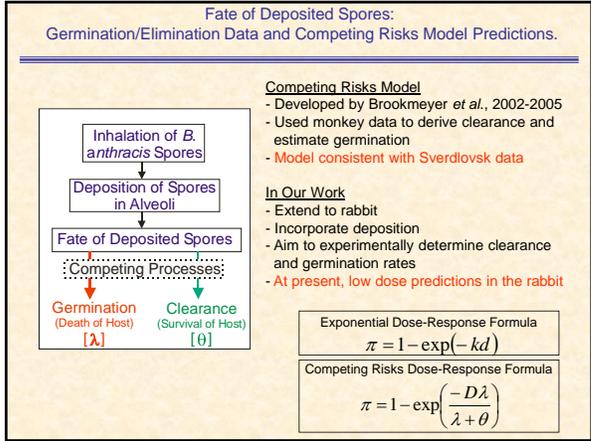
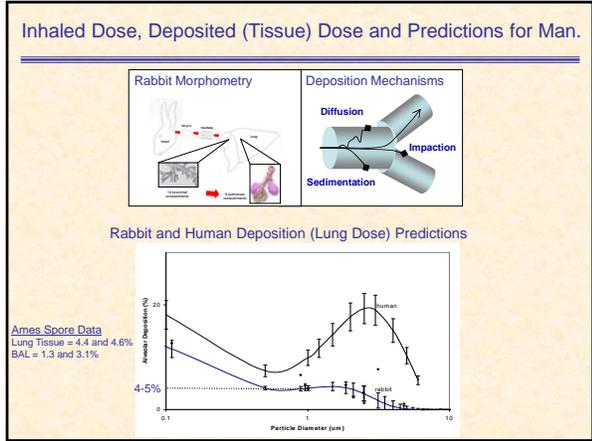
Deposited (Alveolar/Lung) Dose
[Based on Bronchoalveolar Lavage (BAL); 2 Washes]

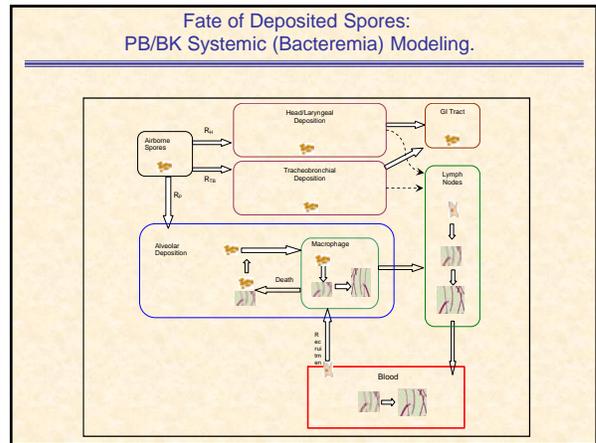
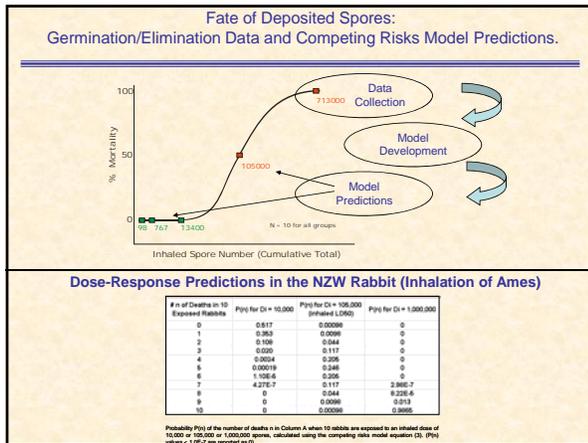
	x10 ⁶	% of Inhaled Dose
Inhaled Dose (Total Viable Spores)	19.58 ± 2.5	-
Total Viable Bacteria in Sonicated BAL Fluid [2X Lavage]	0.25 ± 0.07	1.17 ± 0.3 %
Total Viable Bacteria in Sonicated BAL Fluid [Adjusted: x2.64]	-	3.08 ± 0.9 %

Values are mean ± SEM (n=10)
In an independent study that used 12 rabbits, 5.34 ± 0.3 (x10⁵) 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	11.34 ± 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)





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 ^a	4	0.0 ± 0.0	0.0 ± 0.0
AVA-Vaccinated ^a	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	5028 ± 20760
AVA-Vaccinated	5	5.5 ± 4.7	0.0 ± 0.0
36 Hour Experiment #1			
Sham-Vaccinated (2 died)	3	0.0 ± 0.0	7 x 10 ⁵ ± 3 x 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 x 10 ⁵ ± 13 x 10 ⁶
AVA-Vaccinated	5	0.0 ± 0.0	0.0 ± 0.0

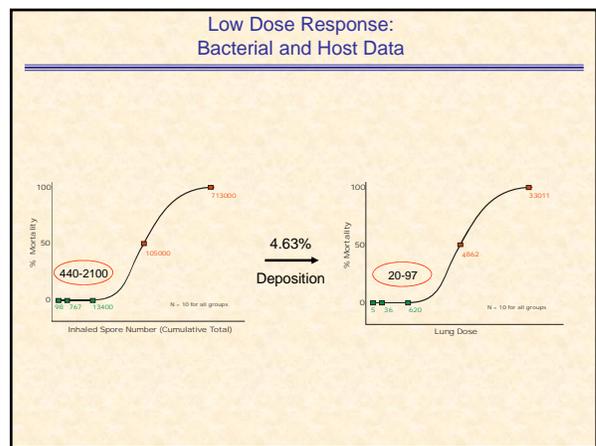
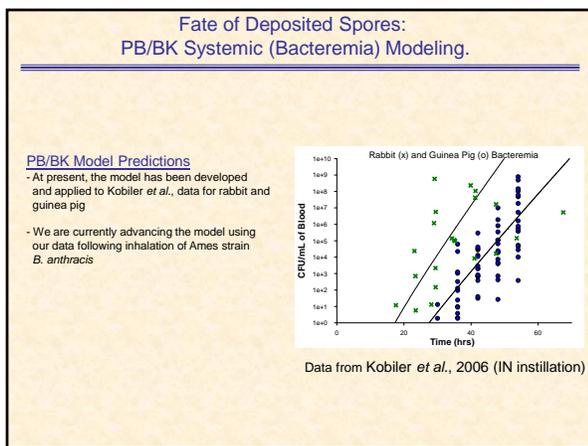
Values are mean ± SEM (two data points were removed due to contamination at the 1 hour time point) from TBLN

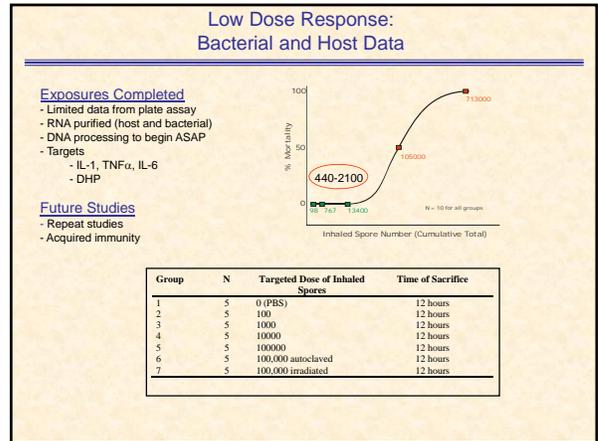
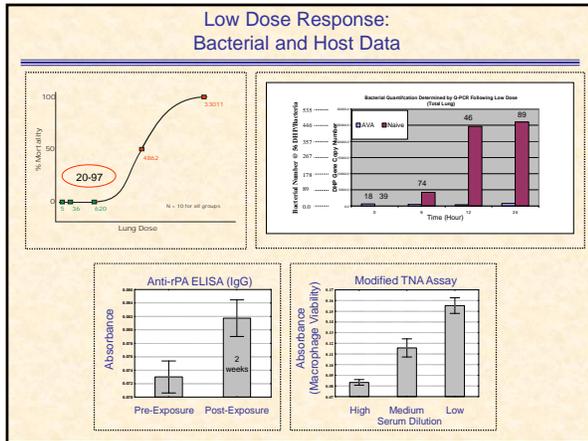
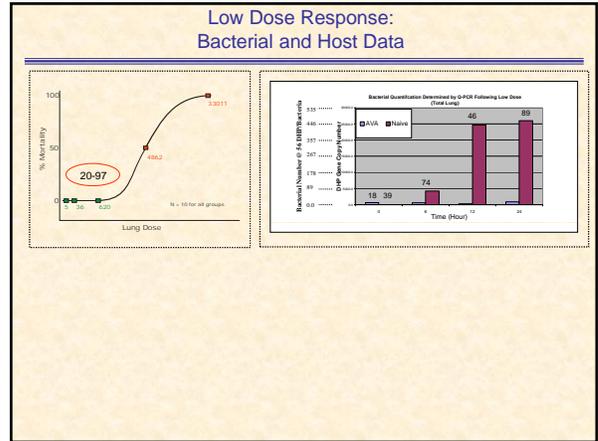
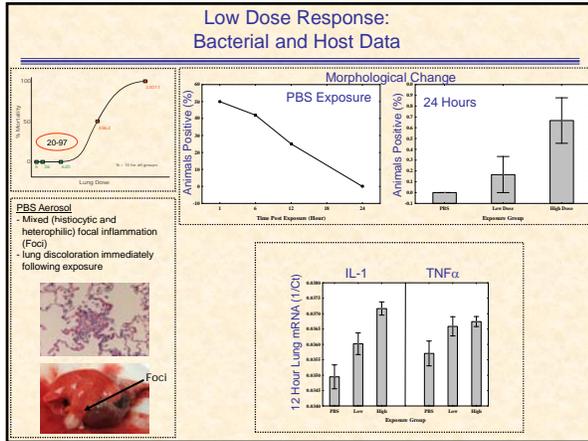
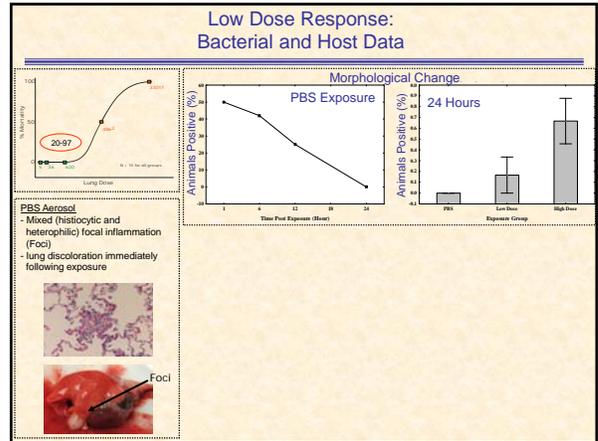
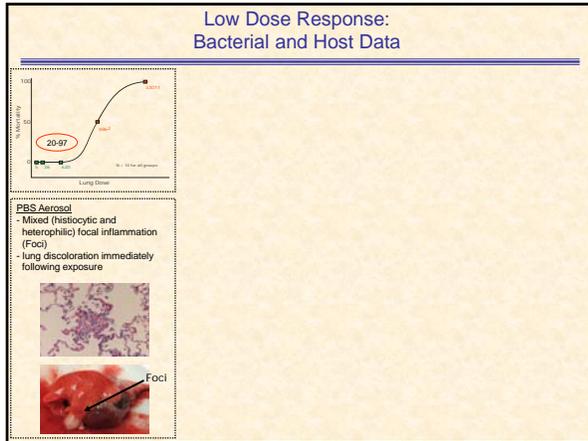
Fate of Deposited Spores: PB/BK Systemic (Bacteremia) Modeling.

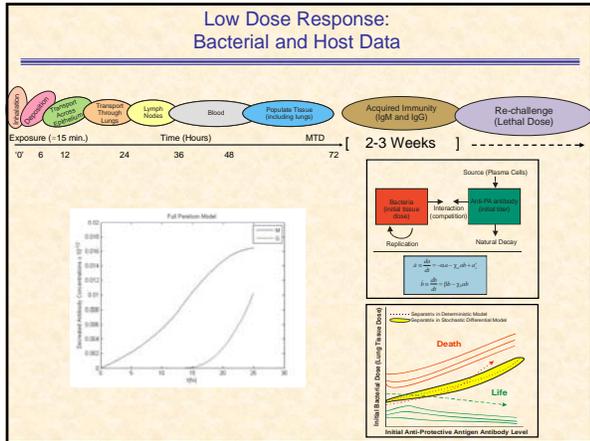
Blood

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 x 10 ⁵ ± 0.3 x 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 x 10 ⁵ ± 0.2 x 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 x 10 ⁵ ± 0.1 x 10 ⁶
AVA-Vaccinated	5	0.0 ± 0.0	0.0 ± 0.0
36 Hour (Experiment #2)			
Sham-Vaccinated ^a	5	0.0 ± 0.0	18.7 x 10 ⁵ ± 16.4 x 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







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Alveolar Macrophage
15.05 μm

Spore
1.40 μm

Red Blood Cell
5.59 μm

RAW 264.7 Macrophage
Vollum 1B *B. anthracis*