I. Overview (30 m)

a. Brief history, aerosol exposures

b. Equipment/animals

c. Class III cabinets

d. Procedural video

II. Aerosol generation (15 m)

a. Overview of generation technologies

b. Collison nebulizer

c. Viability

III. Sampling & characterization (15 m)

a. Methods of sampling (impinger, filter, etc.)

b. Particle sizing

c. Deposition and retention

IV. Dose (15 m)

a. Definition of dose

b. Calculation

c. Importance of the 'spray factor'

BREAK

V. Emerging Technology (30 m)

a. Genesis of the automated technology

b. Application

VI. Examples: aerosol exp. of animals (30 m)

a. *Yersinia pestis*

b. *Bacillus anthracis*

c. Staphyloccocal enterotoxin B

Meaning of Dose

- Inhalation toxicology
  - Term “dose” not used
  - Exposure concentration (Lct50)
- Medicine
  - “Lung dose” defined as mass delivered to lung
  - Only a fraction of “inhaled” mass
- Chemical defense
  - Mass of agent deposited per unit body weight
  - Dose response scales with weight
- Biological defense
  - “Presented dose” at stated size
  - Dose in mass per unit body weight for toxins (μg/kg)
  - Dose in colony or plaque forming units for bacterial or viral agents

Challenge "Dose"

Respiratory Function

Deposition Fraction

Aerosol Concentration

Challenge Dose

Exposure Duration

Discrete Respiration: $D = \sum_{n=1}^{m} R_n \times C_n \times f_n$

Rate Approximation: $D(t_{\exp}) = \int_0^{t_{\exp}} R(t)C(t)f(t)dt$

Meeting the Challenge

Assay

System Flow Rate

Nebulizer Function

Starting Solution Preparation

Agent Growth

Sampler Function

Aerosol Concentration

Plethysmography Function

Anesthetic

Animal Model

Time of Measurement

Animal Size

Respiratory Function

Chamber Temperature

Nebulizer Function

Animal Model

Chamber Humidity

Deposition Fraction

Chamber Volume

System Flow Rate

Concentration Decay

Exposure Duration

Challenge Dose

RIID Presented Dose Calculation

$$D(t_{\exp}) = \int_0^{t_{\exp}} R(t)C(t)f(t)dt$$

- *f(t)*: assumed 100 % deposition (presented dose)
- *R(t)*: assumed constant minute volume (MV)
  - Plethysmograph: point measurement of MV
  - Guyton's Formula: MV[ml] = 2.1 x $(Wt)^{0.75}$
- *C(t)*: assumed constant concentration
  - Aerosol sampling and assay
  - All glass impinger, impactor, filter, etc.
- t_{exp}: fixed at time of exposure

$$D = R \times C \times t_{\exp}$$

Presented Dose Calculation

$$D = R \times C \times t_{\exp}$$

- R = MV measurement or estimate
- C = integrated air sample determined concentration
  - – Eg: All glass impinger aerosol sampling

$$C = \frac{C_{agi} \times V_{agi}}{Q_{agi} \times t_{\exp}}$$

- Presented dose calculated:

$$D = MV \times \frac{C_{agi} \times V_{agi}}{Q_{agi} \times t_{\exp}} \times t_{\exp} = \left(C_{agi} \times V_{agi}\right) \times \frac{MV}{Q_{agi}}$$

Dose Calculation vs. Control

- Calculation
  - Retrospective
  - What dose was delivered?
  - Measurement of relevant parameters
  - Assay for aerosol concentration
  - Plethysmography for respiratory function
  - Length of exposure

- Control
  - Prospective
  - How to deliver a desired dose?
  - Measurement and control of relevant parameters
  - Real-time aerosol concentration
  - Real-time respiratory function
  - When to terminate exposure?

$$D(t_{\exp}) = \int_0^{t_{\exp}} R(t)C(t)\,dt$$

Constraints on Controlling Dose

$$D\left(t_{\exp}\right) = \int_0^{t_{\exp}} R(t)C(t)\,dt$$

- Respiratory function
  - Individual animal dependent (size, metabolism, anesthesia, age)
  - Beyond *control* of aerosol system
  - Can be *measured* in real-time during exposure
- Exposure time
  - Exquisite *control* and *measurement* during exposure
  - Constraints – anesthetic, nebulizer, sampling device
- Aerosol concentration
  - Equipment dependent (nebulizer, chamber, flow)
  - Starting concentration dependent
  - ***Limited control during exposure***
  - ***Limited real-time measurement during exposure***

Aerosol Concentration

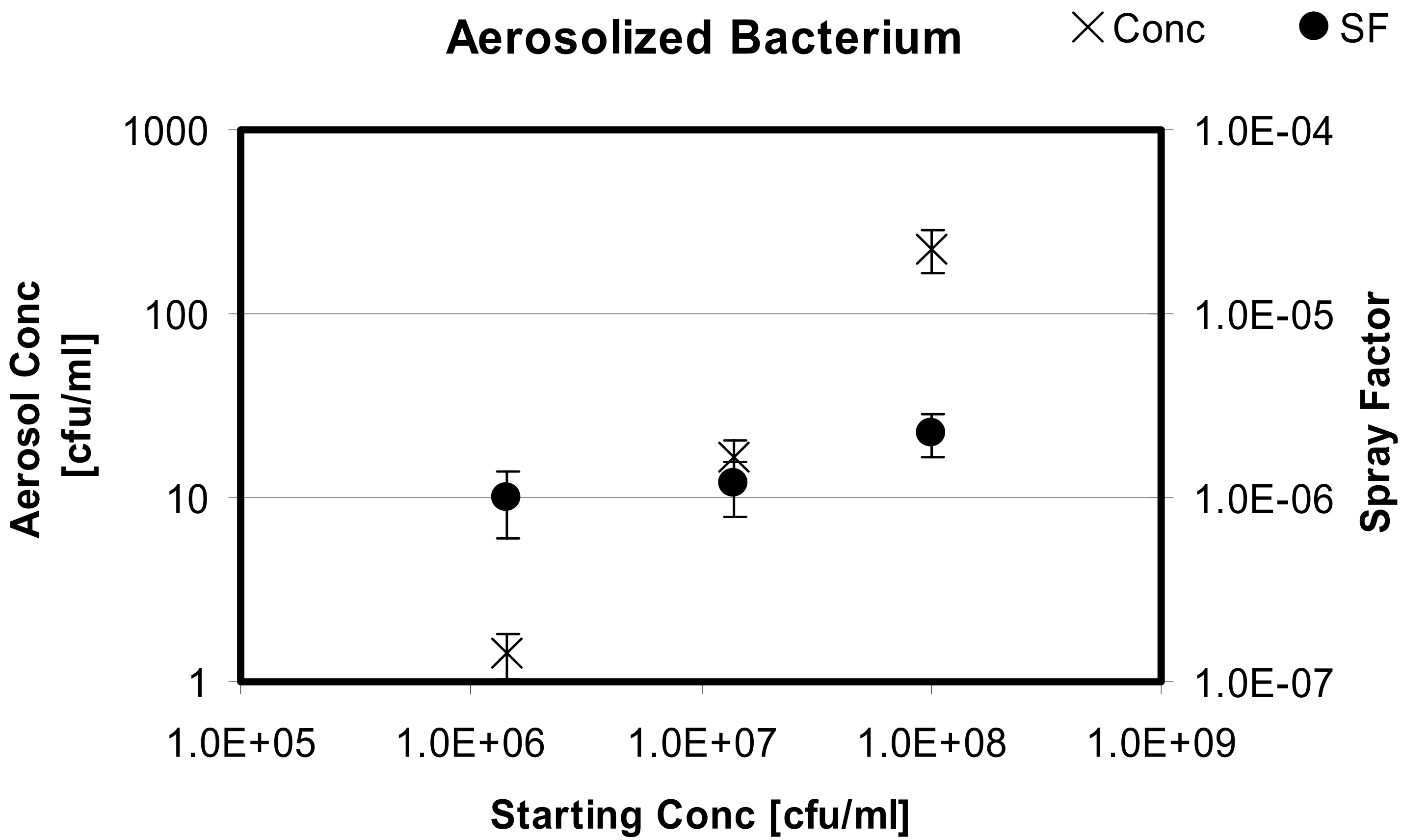
- ‘Spray Factor’ defined as ratio of aerosol concentration to starting concentration
- Used to predict aerosol concentration for a given starting solution
- Unitless
- Function of:
  - Nebulizer
  - Agent
  - System flow

Spray Factor Determination

- Run sham exposure with agent, strain, chamber, nebulizer, and flows used in aerosol challenge
- Use a range of starting concentrations
- Calculate SF

$$SF = \frac{C_{aero}}{C_{neb}} = \frac{\left\{\dfrac{C_{agi} \times V_{agi}}{Q_{agi} \times t_{exp}}\right\}}{C_{neb}}$$

Example of SF Determination



SF = 1.5e-6

Controlling Dose

$$D = R \times C \times t_{\mathrm{exp}}$$

- Use SF to project aerosol concentration
  - Determine starting concentration
  - Determine exposure time

$$D = MV \times \left(C_{neb} \times SF\right) \times t_{exp}$$

Controlling Dose

$$D = R \times C \times t_{\exp}$$

- Use SF to project aerosol concentration
  - Determine starting concentration
  - Determine exposure time

$$D = MV \times \left(C_{neb} \times SF\right) \times t_{exp}$$

- SF affects dose *control* but not dose *calculation*

$$D = \sum_{n=1}^{m} R_n \times C_n \qquad D\left(t_{exp}\right) = \int_0^{t_{exp}} R(t)C(t)dt$$

Controlling Dose

$$D = R \times C \times t_{\exp}$$

- Use SF to project aerosol concentration
  - Determine starting concentration
  - Determine exposure time

$$D = MV \times \left(C_{neb} \times SF\right) \times t_{exp}$$

- SF affects dose *control* but not dose *calculation*
- Can be used as an excellent quality management tool

Dose Conclusions

- Aerosol system must be well characterized by agent, strain, nebulizer, flow, etc.
- Precision and accuracy of dose calculation is different from precision and accuracy of dose control
- Dose *calculation* requires measurement and documentation of what *occurred* during exposure
- Dose *control* requires real-time measurement and control of what *is occurring* during exposure
- Goal is to achieve real-time measurement and control of all parameters to determine dose during exposure

$$D\left(t_{exp}\right) = \int_0^{t_{exp}} R(t)C(t)\,dt$$