SOUTHERN RESEARCH

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Characterization of a Nose-Only Inhalation Exposure System for *Bacillus anthracis* Murine Studies

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Abstract

A nose-only inhalation exposure system for use in murine inhalational anthrax studies was designed, assembled and characterized. The exposure system consisted of an aerosol generation, conditioning, and delivery line, a plenum, an aerosol characterization platform and an air handling station. It was operated at a negative pressure of approximately -0.5 inches water column inside a Class II Type A2 biosafety cabinet (The Baker Company, Sanford, ME). *Bacillus anthracis* Sterne challenge aerosols were generated using a Collison three-jet nebulizer (BGI, Inc., Waltham, MA), dried and mixed with dilution air from a radial flow in-line mixer, and directed through a delivery line into the 32-port radial plenum (In-Tox Products, LLC, Albuquerque, NM). The aerosol characterization platform consisted of an Aerodynamic Particle Sizer (TSI, Inc., Shoreview, MN), MicroDust Pro nephelometer (Casella CEL, Kempston Bedford, England), and glass and stainless steel impingers. Isoaxial sample collection ports were used to interface the aerosol characterization platform with the plenum. All vacuum and pressurized air flows were metered through mass flow and pressure controllers (Alicat Scientific, Inc., Tucson, AZ) or calibrated critical orifi. The inhalation exposure system was characterized by determining the target concentration, particle size distribution, time-to-concentration (T_{90}), and plenum uniformity. A target aerosol concentration of 8.5E4 cfu/L (100 Balb/C mouse LD_{50}) was achieved repeatedly with a nebulizer loading concentration of 5.0E9 cfu/mL. The particle size distribution (mass median aerodynamic diameter, geometric standard deviation) of the challenge aerosol was 1.28±0.03 µm, 1.26. At target concentration levels, plenum T_{90} was 2.3 minutes and spatial uniformity was 6.3%.

System Configuration

Figure 1. Inhalation Exposure System Schematic and Picture.

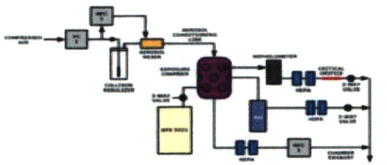
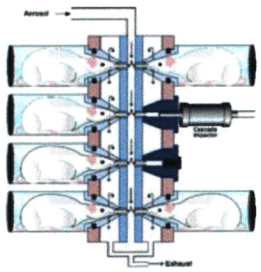
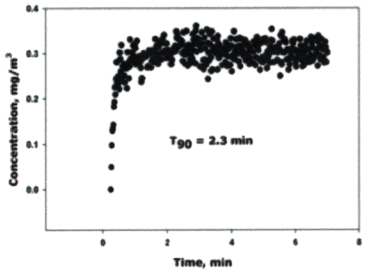


Figure 2. Inhalation Exposure Plenum with Positive Flow-By™ Rodent Restraint Tubes.



Time-To-Concentration

Figure 3. Aerosol Concentration Profile



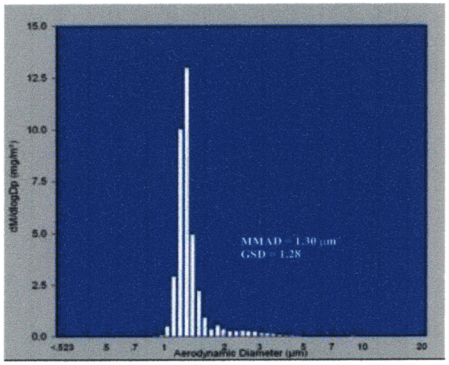
The time required to reach 90% of the target equilibrium concentration in the exposure plenum (T_{90}) was determined using nephelometer data. Particle concentration versus time data was entered into a spreadsheet and graphed. A non-linear regression was run using the modified single, one parameter exponential growth equation, $y = ae^{ax}$, to estimate a. The regression parameter and the time of maximum concentration, T_{max}, were then used to calculate T_{90} using the equation:

$$T_{90} = \frac{-0.1 + a \times T_{max}}{a}$$

Figure 3. is a representative set of time versus concentration data.

Particle Size Distribution

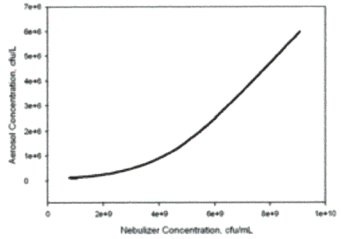
Figure 4. Aerosol Particle Size Distribution



Particle size distribution was determined by samples collected with a model 3321 Aerodynamic Particle Sizer. A typical distribution, presented as Mass Median Aerodynamic Diameter (MMAD) and Geometric Standard Deviation (GSD), is shown in Figure 4.

Target Aerosol Concentration

Figure 5. Aerosol Concentration Range-Finding.

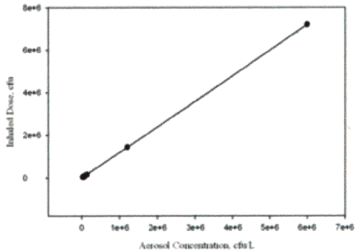


Bacillus anthracis Sterne aerosol challenge concentrations (cfu/L) were calculated from spore concentrations, (cfu/mL), collected from a custom designed stainless steel impinger liquid volume of the impinger sample (mL), impinger flow rate (L/min) and sample time (min) using the equation:

$$\text{cfu / L} = \frac{\text{cfu / mL x mL}}{\text{L / min x min}}$$

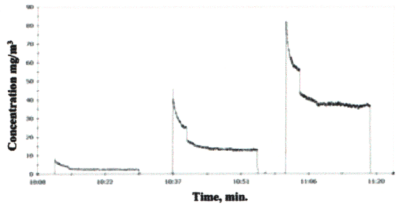
Aerosol concentration as a function of nebulizer loading concentration is shown in Figure 5.

Figure 6. Murine Inhaled Dose Estimates.



Inhaled dose was estimated as the product of the aerosol concentration, mouse minute volume and exposure duration. Challenge aerosol concentrations observed in the exposure system as a function nominal inhaled dose are shown in Figure 6.

Figure 7. Real-time Concentration Versus Time "Fingerprints".



Relative challenge aerosol concentration was monitored in "quasi" real-time. Traces from three tests with spore nebulizer concentrations of 1.0E9, 4.5E9, and 9.1E9 cfu/mL, 1-r respectively, are shown in Figure 7.

Uniformity

Figure 8. Plenum Uniformity Filter Sample Locations

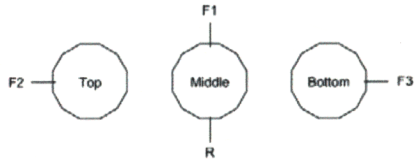


Table 1. Plenum Uniformity Filter Sample Data

	Total Variation	Temporal Variation
	Filter Weight (mg)	Filter Weight (mg)
	0.288	0.225
	0.225	0.216
	0.232	0.258
	0.258	
MEAN	0.251	0.233
STDEV	0.029	0.022
%CV	11.4	9.5

After the target aerosol concentration was established, uniformity of the challenge atmosphere was determined. Temporal variation ($CV_{temporal}$) was calculated from three filter samples that were collected sequentially from a reference sample port location, R. Total variation (CV_{total}) was calculated from three filter samples collected simultaneously from sample port locations, F1, F2, and F3, plus one reference sample. Sample port locations are given in Figure 8. Filter sample collection data is presented in Table 1. Spatial variation ($CV_{spatial}$) was 6.3% as calculated using the equation:

$$CV^2_{spatial}(\%) = CV^2_{total}(\%) - CV^2_{temporal}(\%)$$

References

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Acknowledgements

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