**Supplemental Material**

**Physiological responses to a single low-dose of *Bacillus anthracis* spores in the rabbit model of inhalational anthrax**

Sarah C. Taft1#, Tonya L. Nichols1, Stephanie A. Hines2, Roy E. Barnewall2, Gregory V. Stark2, Jason E. Comer3,4,5

*1U.S. Environmental Protection Agency. National Homeland Security Research Center, Cincinnati, OH.*

*2Battelle Memorial Institute, Columbus, OH.*

*3Institutional Office of Regulated Nonclinical Studies, University of Texas Medical Branch at Galveston, Galveston, TX.*

*4Department of Microbiology and Immunology, University of Texas Medical Branch at Galveston, Galveston, TX.*

*5Sealy Center for Vaccine Development, University of Texas Medical Branch at Galveston, Galveston, TX.*

Running title: Physiological responses to a single low-dose of *B anthracis*

Table S1. Group mean daily inhaled doses of aerosolized *B. anthracis* Ames strain spores. Mean group and individual animal doses, particle size and geometric standard deviation, mortality, and time to death data for the single inhaled low-dose challenge.

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| --- | --- | --- | --- | --- |
| **Group Mean Daily Inhaled Dose, CFU (Standard Deviation)** | **Animal** | **Inhaled Dose (CFU/animal)** | **Mass Median Aerodynamic Diameter, (µm) (Geometric Standard Deviation)** | **Time to Death from Challenge Day (day)** |
| 2.00  (4.58 x 10-1)  Irradiated Spores | L23220 | 0 | 0.96 (1.33) | Survived |
| L23216\* | 1.00 x 101 | Survived |
| L23218 | 0 | Survived |
| L23223 | 0 | Survived |
| L23222 | 0 | Survived |
| 2.86 x 102  (4.32 x 101) | L23215 | 3.22 x 102 | 0.82 (1.48) | Survived |
| L23206 | 2.98 x 102 | Survived |
| L23210 | 2.18 x 102 | Survived |
| L23219 | 3.21 x 102 | Survived |
| L23211 | 2.73 x 102 | Survived |
| 2.06 x 103  (3.42 x 102) | L23217 | 1.48 x 103 | 0.92 (1.57) | Survived |
| L23230 | 2.02 x 103 | Survived |
| L23228 | 2.23 x 103 | Survived |
| L23227 | 2.32 x 103 | Survived |
| L23229 | 2.24 x 103 | Survived |
| 2.54 x 104  (5.21 x 103) | L23235 | 1.76 x 104 | 0.87 (1.59) | 11 |
| L23205 | 2.73 x 104 | Survived |
| L23225 | 2.59 x 104 | 4 |
| L23231 | 2.41 x 104 | Survived |
| L23207 | 3.19 x 104 | Survived |
| 2.75 x 105  (7.41 x 104) | L23201 | 1.78 x 105 | 1.12 (1.33) | 4 |
| L23234 | 2.96 x 105 | 6 |
| L23212 | 3.29 x 105 | Survived |
| L23200 | 2.19 x 105 | 3 |
| L23214 | 3.54 x 105 | 6 |
| 8.27 x 106  (1.69 x 106) | L23204 | 5.95 x 106 | 1.12 (1.31) | 4 |
| L23203 | 8.86 x 106 | 5 |
| L23213 | 7.29 x 106 | 3 |
| L23221 | 8.88 x 106 | 2 |
| L23232 | 1.04 x 107 | 4 |

CFU – colony forming unit(s)

Table S2. Summary of individual gross and microscopic observations

| **Dose Group** | **Animal Number/**  **Death Status** | **Gross Findings** | **Microscopic Findings** |
| --- | --- | --- | --- |
| 100 LD50 (Irradiated) | L23220/FS |  | Lung: Unremarkable |
| L23216/FS |  | Lung: Unremarkable |
| L23218/FS |  | Lung: Multi-nucleated giant cells, minimal |
| L23223/FS |  | Lung: Unremarkable |
| L23222/FS |  | Lung: Multi-nucleated giant cells, minimal |
| 100 CFU | L23215/FS |  | Lung: Inflammation, suppurative, minimal  Lung: Multi-nucleated giant cells, minimal |
| L23206/FS |  | Lung: Inflammation, suppurative, minimal |
| L23210/FS |  | Lung: Unremarkable |
| L23219/FS |  | Lung: Inflammation, suppurative, minimal  Lung: Multi-nucleated giant cells, minimal |
| L23211/FS |  | Lung: Unremarkable |
| 1,000 CFU | L23217/FS |  | Lung: Inflammation, suppurative, moderate Lung: Multi-nucleated giant cells, moderate  Lung: Perivascular eosinophils, moderate |
| L23230/FS |  | Lung: Inflammation, suppurative, minimal |
| L23228/FS |  | Lung: Inflammation, suppurative, minimal  Lung: Multi-nucleated giant cells, minimal |
| L23227/FS |  | Lung: Bacteria (bacilli), minimal |
| L23229/FS |  | Lung: Inflammation, suppurative, minimal |
| 10,000  LD50 | L23235/FD |  | Lung: Inflammation, suppurative, minimal |
| L2320)/FS |  | Lung: Inflammation, nonsuppurative, minimal  Lung: Perivascular eosinophils, minimal |
| L23225/FD | Cavity, abdominal: Fluid, red, ~30 mL  Cavity, thoracic: Fluid, red, ~15 mL | Lung: Inflammation, suppurative, mild  Lung: Bacteria (bacilli), moderate |
| L23231/FS | Skin: Crust(s), hindlimb, red, left hind limb,  20 mm x 20 mm | Lung: Inflammation, nonsuppurative, minimal  Skin: Necrosis, moderate |
| L23207/FS |  | Lung: Multi-nucleated giant cells, mild  Lung: Bacteria (bacilli), minimal  Lung: Inflammation, nonsuppurative, minimal |
| 100,000  LD50 | L23201/FD | Cavity, thoracic: Fluid, red, ~15 mL | Lung: Inflammation, suppurative, mild  Lung: Multi-nucleated giant cells, mild  Lung: Bacteria (bacilli), mild |
| L23234/FD | Cavity, thoracic: Fluid, red, ~10 mL  Skin: Crust(s), hindlimb, dark, left hindlimb, 30 mm 5 mm  Skin: Crust(s), dark, back, 5 mm 5 mm  Skin: Fluid, abdominal, clear, ventral abdomen, ~8 mL  Thymus: Fluid, clear, ~8 mL | Lung: Inflammation, suppurative, moderate  Lung: Multi-nucleated giant cells, mild  Lung: Bacteria (bacilli), moderate  Skin: Necrosis, mild  Skin: Bacteria (bacilli), moderate  Skin: Inflammation, suppurative, mild  Skin: Hemorrhage, mild  Thymus: Atrophy, lymphoid, moderate  Thymus: Edema, mild  Thymus: Bacteria, mild |
| L23212/FS |  | Lung: Inflammation, suppurative, minimal |

|  |  |  |  |
| --- | --- | --- | --- |
| **Group Number** | **Animal Number/**  **Death Status** | **Gross Findings** | **Microscopic Findings** |
| 100,000  LD50 | L23200/FD | Cavity, thoracic: Fluid, red, ~20 mL | Lung: Inflammation, suppurative, minimal  Lung: Multi-nucleated giant cells, minimal |
| L23214/FD |  | Lung: Inflammation, suppurative, mild  Lung: Multi-nucleated giant cells, mild  Lung: Bacteria (bacilli), mild |
| 100 LD50 | L23204/FD | Cavity, thoracic: Fluid, red, ~15 mL | Lung: Inflammation, suppurative, mild  Lung: Multi-nucleated giant cells, mild  Lung: Bacteria (bacilli), mild |
| L23203/FD |  | Lung: Inflammation, suppurative, mild  Lung: Multi-nucleated giant cells, mild  Lung: Bacteria (bacilli), mild |
| L23232/FD | Brain: Discoloration(s), meninges, diffuse, red, affects all lobes  Lymph node, bronchial: Enlarged, dark, 3x  Lymph node, mediastinal: Enlarged, dark, 3x | Brain: Inflammation, suppurative, moderate  Brain: Hemorrhage, mild  Brain: Bacteria, minimal  Lung: Inflammation, suppurative, moderate  Lung: Bacteria (bacilli), minimal  Lymph node, bronchial:  Fibrin, minimal  Hemorrhage, mild  Necrosis, lymphoid, mild  Bacteria (bacilli), minimal  Lymph node, mediastinal:  Fibrin, minimal  Histiocytosis, mild  Necrosis, lymphoid, mild  Necrosis, vascular, mild  Bacteria (bacilli), minimal  Thymus: Atrophy, lymphoid, mild  Thymus: Edema, mild |
| L23221/FD | Cavity, abdominal: Fluid, red, ~60 mL  Intestine, small: Fluid, jejunum, green, ~50 mL Abdomen was distended | Intestine, small:  Inflammation, suppurative, mild  Bacteria (bacilli), mild  Lung: Inflammation, suppurative, mild  Lung: Bacteria (bacilli), moderate |
| L23213/FD | Lymph node, mediastinal: Enlarged, dark, 3x | Lung: Inflammation, suppurative, mild  Lung: Bacteria (bacilli), minimal  Lymph node, mediastinal:  Fibrin, moderate  Hemorrhage, moderate  Necrosis, lymphoid, marked  Bacteria (bacilli), marked |

FD = Found dead

FS = Final phase sacrifice