

Model Development of HD induced Lung Toxidrome in Swine Model

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Introduction

Sulfur Mustard (HD) is a vesicating chemical agent created during World War I that is still stored by many countries for use in chemical warfare. Inhalation of HD at high concentrations causes devastating pulmonary injury leading to the formation of airway obstructing fibrin casts, often leading to respiratory failure and death. No reliable therapeutic currently exists to treat the effects of HD injury.

In an effort to close this gap, MRIGlobal has developed an intratracheal HD vapor exposure model in Yorkshire Pigs. Because of the similar size and physiology of their lungs to humans, swine have been increasingly used as a model for studying human respiratory disease, including the effects caused by HD inhalation.

The goal of this study was to identify the HD dose that caused extensive lung injury in Yorkshire pigs and produced 70%-100% lethality over 48h. This was accomplished by using an up-down dose range finding study. In order to cause extensive lung injury, the dose needed to cause fibrin cast formation and severe clinical respiratory signs. Following exposure to HD, animals were monitored continuously for clinical signs and vitals, and once terminated, bronchoalveolar lavage fluid was collected from the right middle lobe to measure protein in the fluid which is indicative of damage. Following lavage, the rest of the lungs were fixed and dissected to identify cast formation. This effort resulted in a model for HD vapor exposure in Yorkshire pigs that reproducibly causes exposed animals to succumb to an HD challenge of 60 $\mu\text{g}/\text{kg}$ within 24 hours. Development of this model is essential to the success of future studies aimed at testing the efficacy of medical countermeasures against HD-induced pulmonary toxicity.

Methods

This study utilized 10 female Yorkshire Cross pigs (40-50 kg). Using an intratracheal vapor exposure model, pigs were challenged with HD vapor in a dose range finding fashion, ranging from 50 to 90 $\mu\text{g}/\text{kg}$.

Challenge: Animals were sedated with Propofol and Midazolam and intubated in preparation for HD vapor challenge. Animals were brought up to the neat chemical agent laboratory, and connected to a pneumotach pressure transducer for real time monitoring of respiratory parameters. The animal was connected to the exposure system and a target accumulated volume (L) was set based on the vapor concentration ($\mu\text{g}/\text{L}$) and the target dose ($\mu\text{g}/\text{kg}$). Once the animal respired the target accumulated volume, the animal was disconnected from the HD exposure system.

Vital Signs and Clinical Observations: Following challenge, pigs were monitored for symptoms of pulmonary distress and signs were scored on a 5 point scale. Observations included measurement of heart rate, respiratory rate, pulse oximetry, body temperature, level of consciousness, color, respiratory effort, cough, lung sounds, and general behavioral observations.

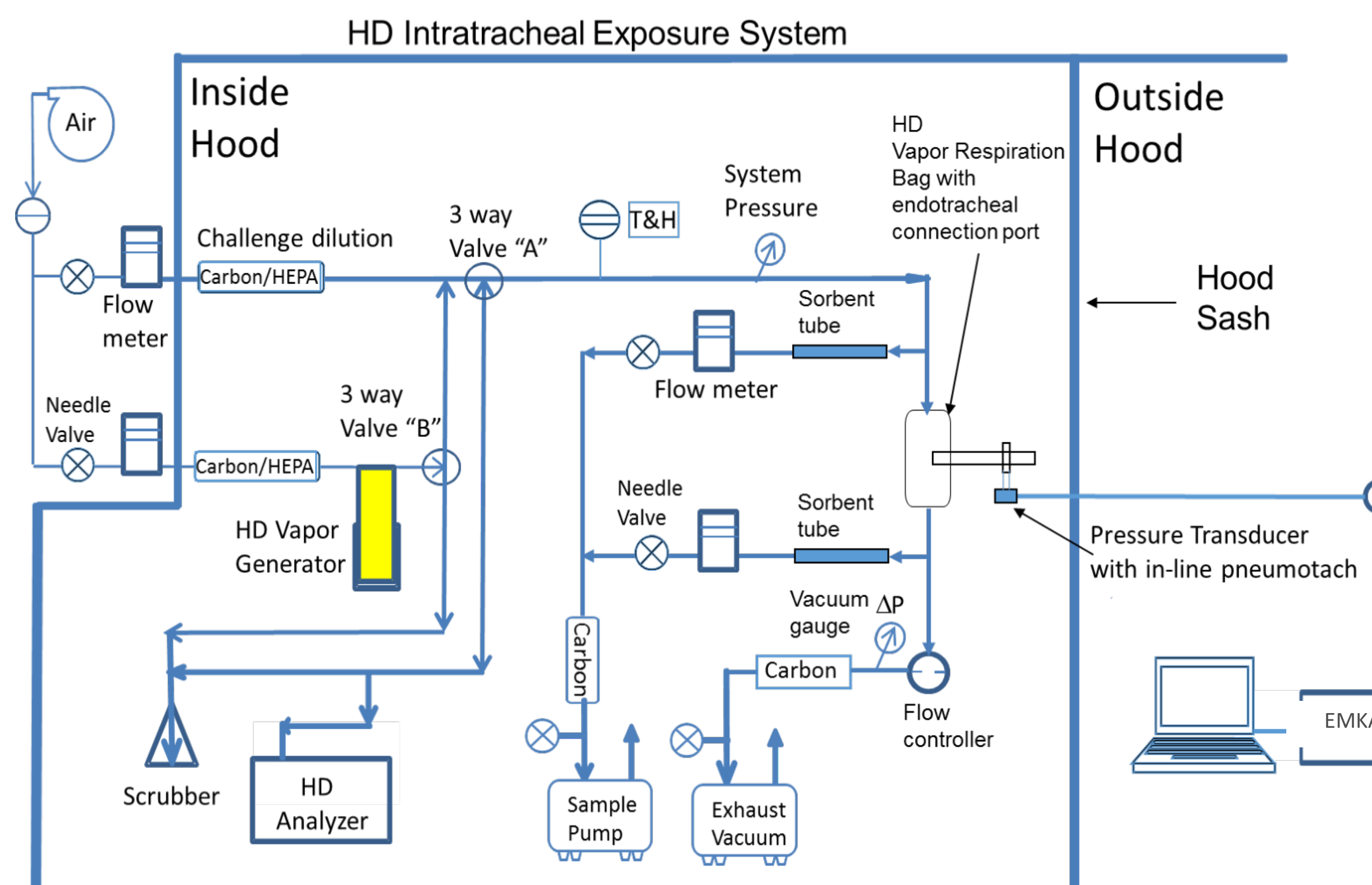
Endpoints: At the time of euthanasia, blood was collected for processing to plasma and measurement of blood gasses. Lungs were harvested and bronchoalveolar lavage fluid was collected from the middle lobe of the right lung. Differential cell counts and protein quantification was performed on the collected bronchoalveolar lavage fluid.

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Exposure System

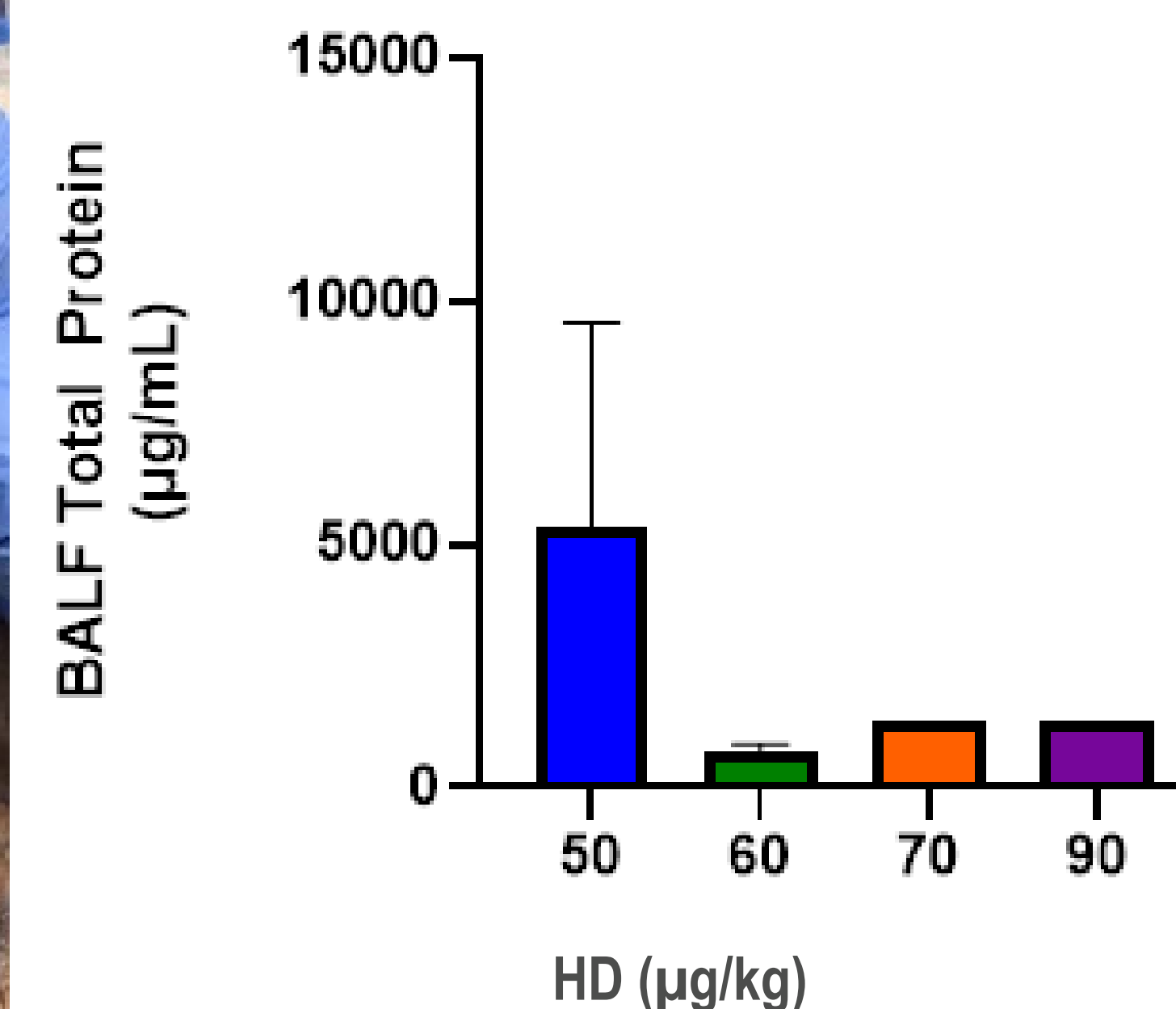


The exposure system was equipped with a heat controlled HD vapor generator with control and monitor flow meters to control system flow rates, while a dynamic push/pull system maintained system pressure at ambient conditions.

Results



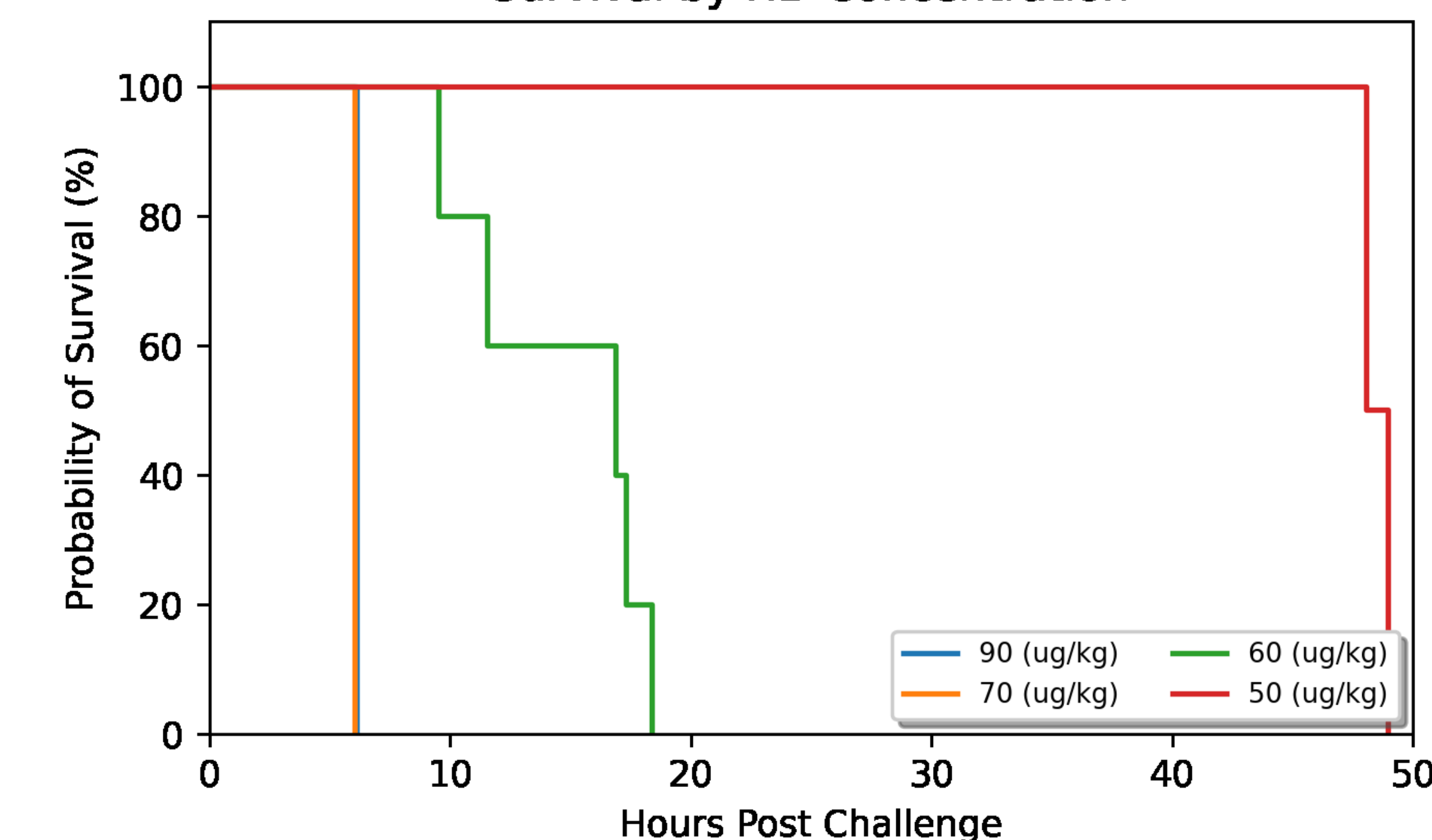
Fibrin cast development in lungs of Yorkshire pig challenged with 60 $\mu\text{g}/\text{kg}$ HD (black arrows)



Total protein from bronchoalveolar lavage fluid collected at the time of euthanasia

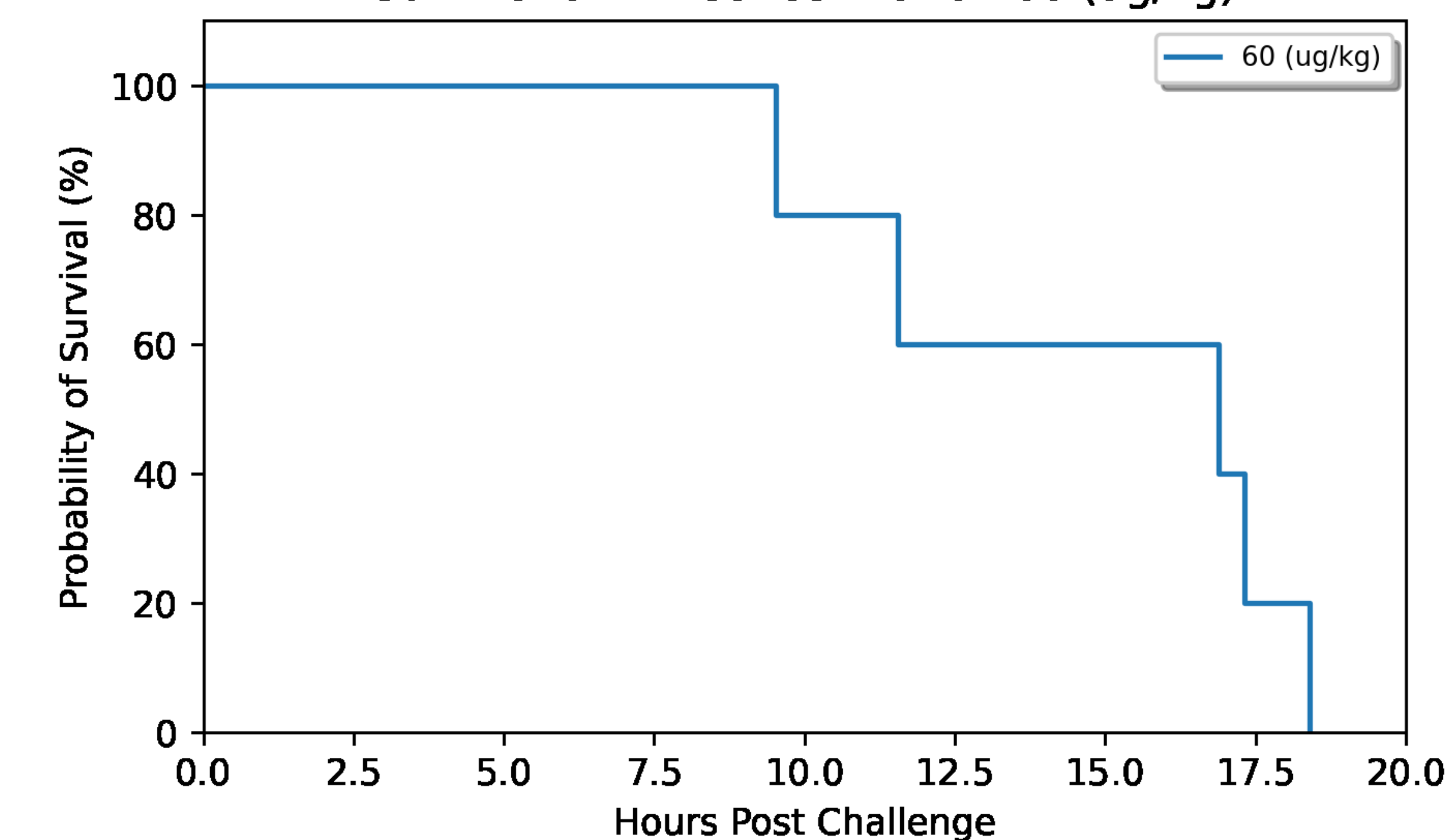
Survival

Survival by HD Concentration



48 hour survival curve of pigs exposed to 50 $\mu\text{g}/\text{kg}$ -90 $\mu\text{g}/\text{kg}$ HD vapor.

Survival of HD Concentration 60 ($\mu\text{g}/\text{kg}$)



Survival curve of pigs exposed to 60 $\mu\text{g}/\text{kg}$ HD vapor. Average time to death is 14.7 hours post exposure.

One animal omitted from data due to early death not caused by HD exposure

Conclusion

Conclusions: This study effectively showed that female Yorkshire Cross pigs exposed to Sulfur Mustard vapor at 60 $\mu\text{g}/\text{kg}$ using an intratracheal exposure model reproducibly succumb to challenge at an average of 14 hours following exposure. Protein levels within bronchoalveolar lavage fluid and recovered fibrin casts showed injury consistent with HD exposure.

Future Directions: This established model will be utilized by MRIGlobal to test medical countermeasures against HD-induced pulmonary injury and mortality

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