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Development of a Single Animal Dynamic Airflow Whole-Body Exposure Apparatus

Michael S. Horsmon
Chelsea R. Boeri
Dennis B. Miller
Theodore S. Moran
Ronald A. Evans

RESEARCH AND TECHNOLOGY DIRECTORATE

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Commercially available whole-body (WB) aerosol exposure chambers are typically designed to house groups of small- to medium-sized animals or single large animals. These chambers range in size, generally from 250 to 1000 L; are constructed of stainless steel and glass and polycarbonate; and require dedicated laboratory space and ventilation for operation. The WB plethysmograph offered by several commercial manufacturers is often used as a small WB exposure chamber. A recent study in our laboratory required the use of a WB exposure system that would allow for easy observation of animal behaviors and have the capability to collect data from implanted telemetry devices and resistance to decontamination solutions. Commercially available products do not meet all of these requirements; therefore, a system was designed to meet them. Herein, we describe the design, construction, and optimization of a single animal WB exposure apparatus for small- to medium-sized animals.

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PREFACE

The work described in this report was authorized under project number CB10704. The work was started in March 2020 and completed in January 2021. This work was performed at the U.S. Army Combat Capabilities Development Command Chemical Biological Center (DEVCOM CBC; Aberdeen Proving Ground, MD).

The use of either trade or manufacturers' names in this report does not constitute an official endorsement of any commercial products. This report may not be cited for purposes of advertisement.

All animal use was conducted in accordance with an animal use protocol approved by the DEVCOM CBC, Institutional Animal Care and Use Committee and in accordance with the *Guide for the Care and Use of Laboratory Animals*.*

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This report has been approved for public release.

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Guide for the Care and Use of Laboratory Animals, 8th ed.; The National Academies Press: Washington, DC, 2011.

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1. INTRODUCTION

Commercially available whole-body (WB) aerosol exposure chambers are typically designed to house groups of small to medium sized animals or single large animals. These chambers range in size, generally from 250-1000L, are constructed of stainless steel and glass/polycarbonate and require dedicated laboratory space and ventilation for operation. The whole-body plethysmograph offered by several commercial manufacturers is often used as a small WB exposure chamber. A recent study in our lab required the use of a WB exposure system that would allow for easy observation of animal behaviors, the ability to collect data from implanted telemetry devices and resistance to chemicals used in decontamination solutions. Commercially available products do not meet all of these requirements therefore, a system was designed to fulfill these requirements. Herein we describe the design, construction and optimization of a single animal WB exposure apparatus for small to medium sized animals.

2. BACKGROUND

2.1 Brief Review of Available Systems

Inhalation toxicology studies can be designed around several routes of exposure depending on the specific goals of the research question under investigation. These routes can generally be divided into intra-tracheal instillation, nose-only inhalation, head-only inhalation, and whole-body inhalation. One of the most difficult, yet most realistic, methods is the whole-body exposure. This is especially important when attempting to research the effects of compounds that have the ability to penetrate the skin. Only whole-body exposure can accurately represent the pharmacokinetic properties and capture the pharmacodynamic responses for compounds that exhibit toxicity through inhalation and percutaneous exposure.

There are several types of whole-body exposure apparatus available on the commercial market. These can be broken down into two major groups. Large stainless steel and glass/polycarbonate chambers over 250L in design such as the Hazelton chamber; and small vertical acrylic/polycarbonate cylinder chambers under 10L. Both systems are reviewed by Wong[1].

Both of these designs have disadvantages that the horizontal whole-body chamber (HWBC) described here overcomes. The Hazelton style chamber is designed for group exposure or large animal exposure, has a large footprint in the laboratory and requires dedicated air handling systems which may not be available to smaller laboratories. These chambers are an excellent choice for exposing large animals or larger groups of small animals, but are unnecessary for studies involving small groups of small animals or single exposures of small to medium sized animals. Further, depending upon the amount of metal used in construction, the transmission of telemetry signals from animals with implants monitoring vital signs may be

degraded. Advantageously, these chambers can hold larger groups of small to medium sized animals, generally have good aerosol distribution characteristics, and can be decontaminated with harsh chemicals (bleach, alcoholic sodium hydroxide).

The small acrylic/polycarbonate cylinder chambers are a good option for individual exposures of small animals, but are too small for ferret and rabbit sized animals to comfortably make normal postural changes. Their cylindrical shape, with a small point source inlet in the middle of the top can, theoretically, result in poor aerosol distribution characteristics especially with acute (2-5 minute) exposure durations which do not provide much time for homogenous mixing of the aerosol. Further, the choice of acrylic or polycarbonate for construction is not compatible with some harsh decontaminants, especially those containing alcohol (alcoholic sodium hydroxide). Anecdotal evidence from our lab has shown that when plethysmograph tubes constructed of extruded acrylic undergo multiple rounds of decontamination with alcoholic sodium hydroxide they begin to craze. At this time, it is unknown if this can progress to cracks. This is consistent with previously reported test methodologies using isopropyl alcohol as a crazing agent in the testing of cast acrylic aircraft parts[2]. Further, ethanol has been used as a stress-craze inducing agent in polycarbonate[3, 4]. With time and repeated decontamination cycles it is possible that these crazes could progress to cracks which would result in an unusable chamber.

Advantageously, these smaller chambers do not require dedicated air handling systems and can fit within a chemical fume hood. The plastic materials do not significantly degrade telemetry signals from implanted animals and, by design, they can be used as whole-body plethysmographs. This is beneficial when monitoring the respiratory physiology of exposed animals is desired.

The HWBC described here effectively combines the advantages of both of these systems resulting in a single animal WB exposure apparatus for small to medium sized animals (up to ~5 kg). By turning the cylinder on its side, increased surface area is provided for the animal to move and exhibit normal behaviors and postural changes. The horizontal orientation allows space to place an appropriate length of duct work ensuring a uniform aerosol distribution within the chamber. The use of radio frequency transparent materials resistant to chemical degradation throughout allows for maximum transmission of telemetry signals and decontamination with harsh chemicals.

3. MATERIALS AND METHODS

3.1 Generation System and Inlet Plumbing

The HWBC was designed to be operated at a pressure of -0.50” to -0.75” of water and ~30 L/min of airflow. The configuration presented here is set up to be able to generate particle aerosols, gas/vapor atmospheres or a combination of the two. Chemical aerosols were generated with a double needle atomization device operated at an airflow of 1.0 L/min. Specifics of the aerosol generation device and its operation are previously detailed[2,3]. Vapor/gas atmospheres were generated from tanked sources or vaporized with a Collison Nebulizer (CH

Technologies, Westwood NJ). Mixed atmospheres were combined at the point of aerosol generation to ensure even mixing. At the point of gas/vapor generation, a ¾" swage lock tee fitting was placed inline allowing for a supply of HEPA filtered makeup air to mix with atomized and/or gas/vapor material which then entered a 10" long stainless steel (SS) cone stepping up the diameter from 2" to 4". The cone is connected to a short mixing chamber consisting of two Stairmand disks contained at each end of a 6" long section of 4" diameter SS pipe. This expansion chamber is then connected to the inlet cone of the HWBC.

3.2 HWBC Specifications

The HWBC consists of three main components, the inlet cone, animal zone, and outlet cone (Figure 1). The inlet and outlet cones are constructed of DSM Somos 9120 Photo polymer (DSM Desotech Inc, Elgin IL), and provide a connection between the 4" SS inlet tubing and the 9.25" glass animal zone. A channel in the inlet and outlet cones is filled with urethane rubber gasket (PMC-121-30, Reynolds Advanced Materials, Allentown, PA) which forms a seal with the glass animal zone. A grate with Stairmand disk attached (constructed of DSM Somos 9120 Photo polymer) serves to keep animals from entering the inlet cone and creates a final point of turbulence for mixing of the atmosphere upon entry to the animal zone. The animal zone is constructed of tempered glass and measures 9.25" dia. x 14"L x ¼" thick. It contains a plastic grate placed in the bottom to support the animal and is itself supported on polylactic acid plastic legs which also house the bottom portion of the latching mechanism. The outlet cone is a mirror image of the inlet cone in design and shape but it also contains 8 sampling ports. The outlet cone also contains a grate to keep the animal from entering the outlet cone. The terminal end of the outlet cone is connected to a 4" SS plate with a ¾" swage lock fitting, this fitting is connected to ¾"plastic tubing which serves to supply vacuum that operates the chamber. The sampling ports allow for connection of gravimetric, optical and gas/vapor sampling devices for characterization of the atmosphere.

3.3 Outlet Cascade

The outlet cascade of the HWBC begins with ¾" plastic tubing that is connected to the outlet cone of the chamber. From there the tubing connects to an inline HEPA filter backed up with an inline carbon filter. This is followed by passage through a Hastings LI-1D laminar flow element, connected to two 30 L/min mass flow controllers (Model 5158E Brooks Instrument, Hatfield, PA) and finally connected to a vacuum pump (Model 2567B, Welch Inc., Sheboygan, WI). The exhaust from the vacuum pump is them plumbed through a second carbon filter and back into the chemical fume hood.

3.4 Distribution Analysis

Two methods of distribution analysis were employed to characterize the aerosol distribution within the HWBC. The first method was a series of repeated measurements to determine aerosol concentrations in the middle of the chamber. For this protocol, an aerosol of dexmedetomidine hydrochloride (HCl) (USP standard CAS 145108-58-3, Sigma Aldrich Inc. St. Louis, MO) dissolved in chromatography grade ethyl alcohol (CAS 64-17-5, Sigma Aldrich Inc.

St. Louis, MO) at a concentration of 100mg/mL was generated and allowed to stabilize, as indicated by a DustTrak II Aerosol Monitor (Model 8530, TSI Inc., Shoreview, MN). Once the aerosol concentration stabilized, one glass fiber filter (25mm, Type AE, Pall Corp, Port Washington, NY) sample was collected simultaneously from each of three ¼" SS sampling tubes located at points E, G and H in Figure 1, in the center of the chamber and 2.5cm, 17.5cm and 33cm from the inlet screen respectively. This test was repeated on three different days using the same aerosolization solution and generator settings for a total of three samples per sampling location. Following collection of aerosol samples, the precise concentration of material at each sampling site was determined by analytical chemistry (see section 3.5).

The second method of distribution analysis was designed to detect the effect of the chamber edge on the aerosol concentration, which is designated here by the term edge-effect sampling. For this protocol, a stable aerosol was generated, as indicated by the DustTrakII aerosol monitor. Once the aerosol concentration stabilized, each of the three different length tubes, from the repeated measures test, were changed in orientation and the DustTrakII reading was monitored for two minutes. For example, the inlet sampling tube was rotated to nearly touch (~1/4" stand-off) the side of the animal chamber on the left, then rotated to nearly touch the side of the animal chamber on the right, then elevated to nearly touch the top of the animal chamber and lowered to nearly touch the floor of the chamber. The same procedure was performed for the other two sampling tubes and the results were manually tabulated.

3.5 Dexmedetomidine Analytical Analysis

Glass fiber filters were extracted by adding 1 mL of methanol to the sample and allowing it to extract for a minimum of 15 minutes. The extracts from the glass fiber were diluted as necessary with methanol in 2 mL glass autosampler vials (Agilent Technologies, Santa Clara, CA), spiked with isotopically labelled internal standards, mixed using a vortex mixer on a high setting for 30 seconds, and transferred to autosampler trays for analysis using LC/MS/MS technologies.

Samples were analyzed using an Agilent 1290 Infinity Series Binary Pump Ultra-High Performance Liquid Chromatograph Model G4220A interfaced with an Agilent 6490 Triple Quadrupole Mass Spectrometer (Agilent Technologies, Santa Clara, CA). Injections of 1 µL of extract were made using an acetonitrile solvent wash in the FlushPort for 10 seconds to prevent carryover. Injections were made with a constant flow rate of 0.2 mL/min through a BEH C_8 Column (Waters, Inc.) 2.1 mm x 50 mm, 1.7 µm df with the column compartment held at room temperature. The solvents were 0.1% Formic Acid/H_2O (A) and 0.1% Formic Acid/Methanol (B). The liquid chromatography gradient program was initially 2% B (2 min. hold) going to 90% B (2 min. gradient) and holding for 0.5 minute, then back to 2% B (1 minute gradient, 0.5 minute hold) to re-equilibrate the column for a run time of 6 minutes total.

Detection was performed using positive ion electrospray ionization with Agilent Jet Stream followed by Multiple Reaction Monitoring (MRM) analysis and data was collected for MRM ion transitions for each compound and for the deuterated internal standard. The method utilized a drying gas temperature of 225ºC, drying gas flow of 15 L/min, nebulizer pressure of 20 psi, sheath gas heater set to 250ºC, sheath gas flow of 9 L/min, capillary voltage of 1500V, Vcharging set to 500V, and electron multiplier delta of +100V. Ion transitions were

collected using unit resolution with a fragmentor voltage of 380V, collision energy of 22V for each transition, cell accelerator voltage of 1V, and a dwell time of 50 ms/cycle. The ion transitions collected were dexmedetomidine 201.2 > 132.9, and D4-Dexmedetomidine 205.2 > 137.0. Example chromatograms can be found in Figure 5.

The calibration curves (0.5-1000 ng/mL) were constructed by plotting the ratio of analyte area to labeled internal standard area against the ratio of analyte mass on-column to internal standard mass on-column using Agilent MassHunter Quantitative Analysis software. The concentrations of unknown samples were determined using the slope and intercept calculated by 1/x weighted linear regression analysis of the calibration curves. Solvent spiked samples and calibration standards were analyzed in each group of samples to verify calibration and internal standard solution integrity.

3.6 Use as a Whole Body Plethysmograph

Briefly, the ability of the HWBC to function as a whole body plethysmograph was assessed. In one experiment, an animal was placed into the chamber and a DP-45 differential pressure transducer (Validyne Engineering, Northridge CA) was connected to one of the sampling ports. Changes in pressure due to the animals' respiration were acquired with the Ponemah Physiology Platform (Data Sciences International, Minneapolis MN) and processed to detect respiratory rate and volume.

4. RESULTS

4.1 Performance and Distribution Results

The performance and distribution characteristics of the HWBC were evaluated by generating a solid particle aerosol from a solution of dexmedetomidine HCl and ethyl alcohol. Repeated measurement experiments with sampling performed at three locations within the chamber showed atmospheric concentrations that ranged from 7.35 to 10.65 mg/m^3 (Figure 2). The average concentration over all three experiments was remarkably consistent ranging from 9.19 to 9.46 mg/m^3 (Figure 3). Finally, dust track recordings from all three locations over the 2-minute experiment indicate consistency between all three sampling locations (Figure 4).

Sampling from locations near the edges of the chamber and comparing this to measurements from the center of the chamber is a reasonable method to determine if there is heterogeneity in the aerosol due to edge effects of airflow through the chamber. The data from the edge effects experiment indicates homogeneity of the aerosol concentration, with a range of 0.69-0.75 mg/m^3 across all locations sampled via DustTrakII recordings during the experiment (Table 1). The maximum percent difference between all locations is 8.3%.

4.2 Performance as a Whole Body Plethysmograph

Unfortunately the results of the single experiment designed to assess performance of the HWBC as a whole body plethysmograph were unsuccessful in the current configuration. The amplitude of the signal from animal respiration was dampened by movement artifact and chamber flow to the point that individual respirations were not regularly detected.

5. DISCUSSION

The advantages of the HWBC are a function of its features. The triple Stairmand disk design element is incorporated to ensure the best possible chance of obtaining a uniform aerosol distribution in the chamber. Here a uniform distribution is defined as an average concentration difference between any two points of measurement less than 10%. The stainless steel, tempered glass and resin materials are all able to withstand at least 5 rounds (this is the total number of rounds of decontamination applied as of writing this report) of decontamination by immersion in alcoholic sodium hydroxide for 60 minutes. Previous work involving the use of telemetry devices in this chamber confirm that the tempered glass chamber allows for excellent telemetry signal transmission in addition to clear visibility of animal responses. The outlet cone design allows for aerosol sampling from a region that does not interfere with the animals' breathing/mobility zone.

The design of this chamber was based upon methods utilized to generate uniform aerosols within duct work for HEPA filter testing. When a point source (such as the coaxial needle atomizer) is used to generate an aerosol within duct work, the minimum length of ductwork required to achieve a uniform distribution is equal to 30 times the hydraulic duct diameter (30 d_h) in length[7]. One method to reduce this length is through the use of a device that generates additional turbulence in the airstream known as a Stairmand disk. A Stairmand disk is a plate with the same geometric shape as the duct (but proportionally smaller than the duct), suspended in the center of the duct. Air flowing past the disk creates turbulence and mixing of the aerosol. The required diameter of the Stairmand disk is calculated as the diameter of the duct divided by the square root of two[8]. The use of a Stairmand disk can reduce the total length of duct work required to achieve a homogenous aerosol distribution to 10 d_h[7]. Attempts at further reducing this distance are detailed in Loughborough, D. (1989). In summary the use of multiple generation points can result in a modest reduction in length, however the incorporation of a second Stairmand disk with 0.25 d_h between them can reduce the distance to 4 d_h. Assuming a linear relationship in the reduction of d_h, a third disk should reduce this to 1.6 d_h.

In the present situation, our point source is generated into 2" diameter duct of 6" in length after which the aerosol enters a cone which gradually increases the diameter to 4" over the course of 10" of length. It is here that the first Stairmand disk is placed, 6" downstream a second Stairmand disk is placed at the inlet of a cone that further increases the diameter to 9". At the end of this inlet cone a third Stairmand disk is placed to provide thorough mixing of the aerosol as it enters the animal zone. While theoretically it should take approximately an additional 14.4" of length to achieve a uniform aerosol distribution, the data from the distribution analysis indicate there is a uniform distribution within 2.5 cm of the inlet screen. The most

plausible explanation for this is that the aerosol was already homogenously distributed before encountering the third disk. However, the system was not tested without the third disk, therefore it cannot be definitively said that homogenous distribution before the third disk occurred. There also exist interrelationships between the aerosol generation flow, ductwork flow and spacing between Stairmand disks that were not the focus of this study. We can only attest that under the circumstances described a homogenous aerosol was achieved. It may be possible to further reduce the length of ductwork and achieve a similar result.

Finally, the ability of this chamber to function as a whole body plethysmograph was briefly investigated. In the current configuration, this chamber did not work well as a whole body plethysmograph. There was not enough differential pressure amplitude to effectively calculate respiratory volume under the present operating conditions. Further attempts at optimization for this purpose were not pursued since animals would be implanted with telemetry devices to monitor respiratory parameters, however it may be possible to optimize for this endpoint if necessary. Overall, the HWBC represents a solution to several problems associated with performing WB inhalation toxicology studies with highly toxic chemical agents in small to medium sized animal models.

6. FIGURES & TABLES

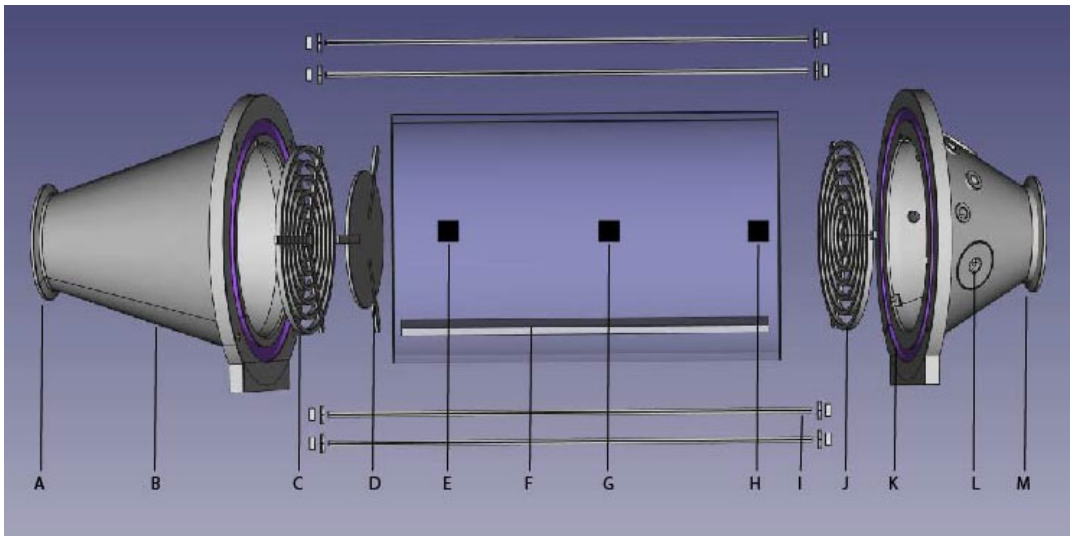

Figure 1. Schematic of the HWBC.

This representation of the chamber excludes the inlet and outlet plumbing because any number of plumbing fixtures may be adapted to the chamber. A. Chamber inlet fitting and location of second Stairmand disk, B (first disk is in the upstream duct work). Chamber inlet cone. C. Inlet screen. D. Second Stairmand disk. E. Inlet sampling site. F. Perforated animal support platform. G. Middle sampling site. H. Outlet sampling site. I. Threaded rod and assembly hardware. J. Outlet screen. K. Sealing surface. L. Sampling ports. M. Chamber outlet fitting.

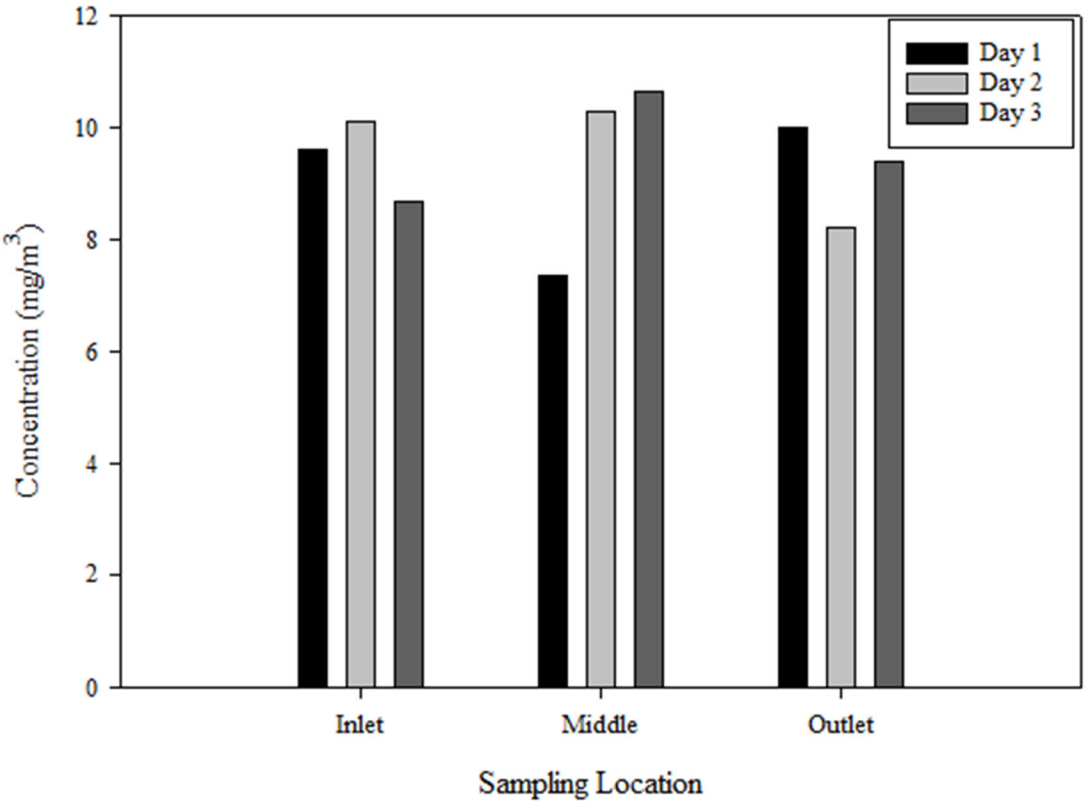

Figure 2. Daily Repeated Measurements.
Daily aerosol concentrations from each of three length sampling tubes located at the inlet screen, middle of chamber, and outlet screen.

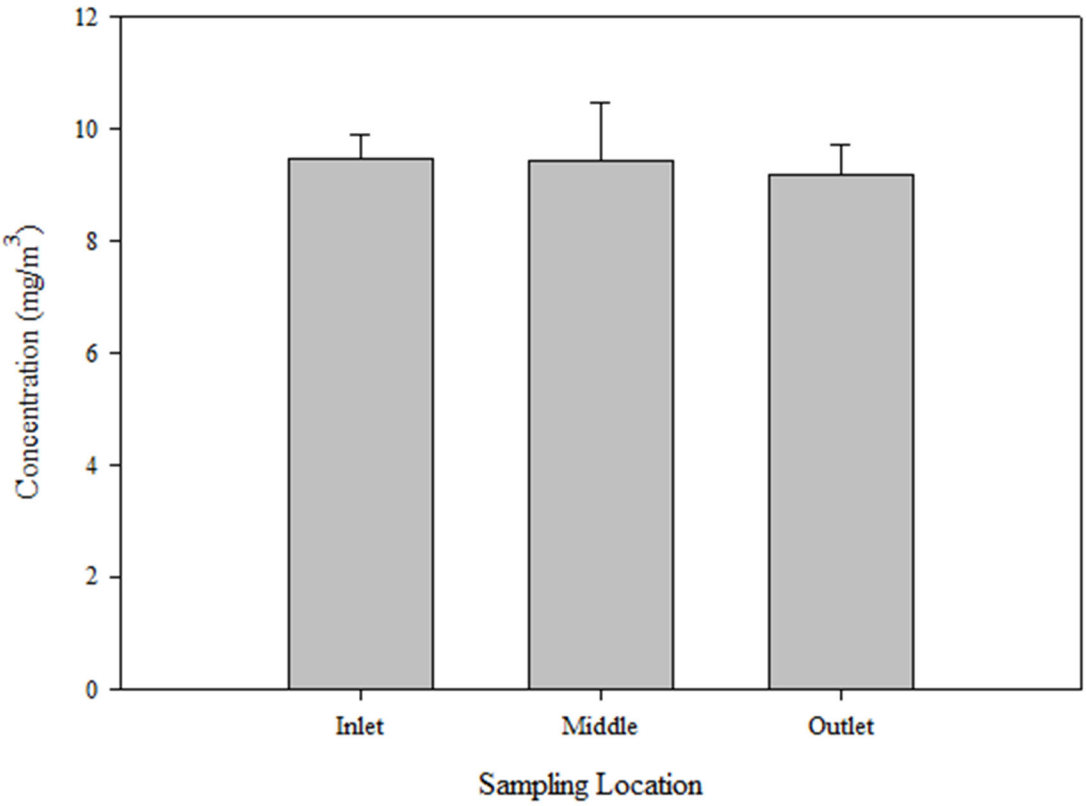

Figure 3. Mean Repeated Measurements.
Mean aerosol concentrations over three separate experiments from the inlet screen, middle of chamber, and outlet screen. Error bars represent the SEM.

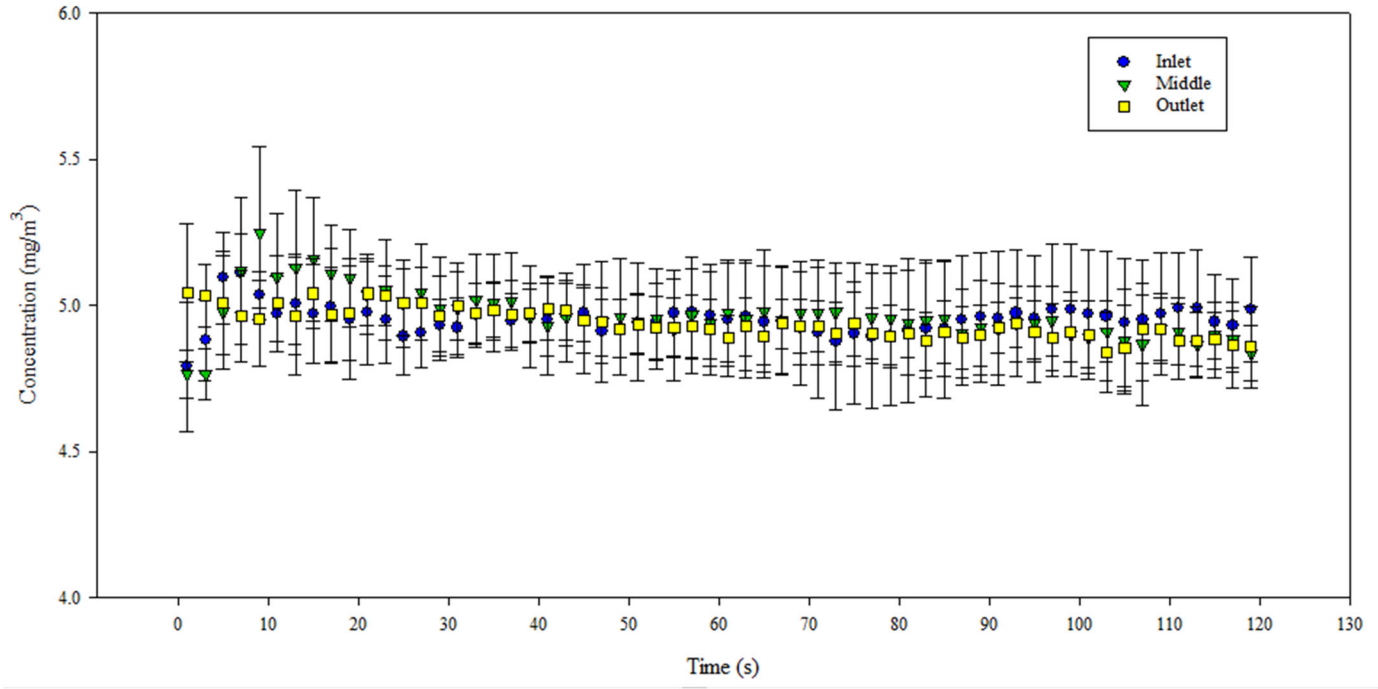

Figure 4. Average DustTrak Recordings.

The average DustTrak recordings, sampled every 2 seconds, over the duration of three, 2-minute sampling periods for the inlet screen, middle of chamber, and outlet screen sampling locations. Error bars represent the SEM.

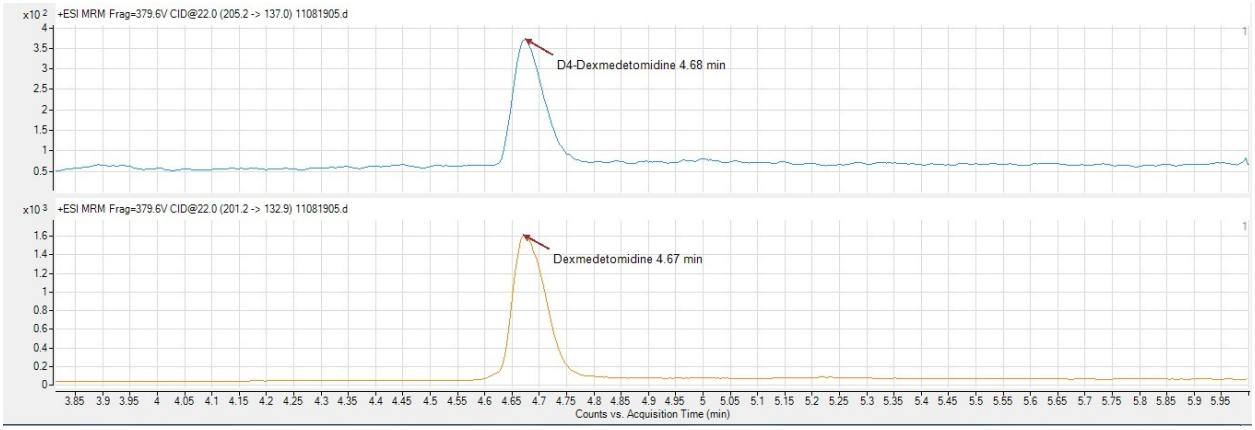

Figure 5. Example Chromatogram.

Multiple Reaction Monitoring Chromatograms of Dexmedetomidine and D4-Dexmeditimidine with each component injected at a concentration of 200 ng/mL. The isotopically labeled internal standard serves as a retention time reference and allows for calculated concentrations using peak area ratio determinations.

Table 1. Average DustTrakII measurements from a 2-minute sample at each orientation.

	Top (SEM)	Bottom (SEM)	Left (SEM)	Right (SEM)	Center (SEM)
Inlet Tube (mg/m^3)	0.73 (0.006)	0.73 (0.012)	0.69 (0.004)	0.71 (0.010)	0.72 (0.011)
Middle Tube (mg/m^3)	0.72 (0.008)	0.69 (0.008)	0.71 (0.009	0.69 (0.020)	0.72 (0.021)
Outlet Tube (mg/m^3)	0.71 (0.029)	0.75 (0.023)	0.74 (0.013)	0.75 (0.017)	0.73 (0.021)

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ACRONYMS AND ABBREVIATIONS

df	Film thickness of column
d_h	Hydraulic diameter
HCl	Hydrochloride
HEPA	High-efficiency particulate absorbing
HWBC	Horizontal whole-body chamber
Kg	Kilogram/s
L	Liters
mg/m^3	Milligrams per cubic meter
min	Minute
mL	Milliliter
MRM	Multiple reaction monitoring
ms	Millisecond/s
psi	Pounds per square inch
SS	Stainless Steel
V	Volts/Voltage
WB	Whole-body

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