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Performance of the LRRI Real-time Rabbit Plethysmography System

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Abstract

Background: Select agent studies using rabbits typically determine dose delivered to the animal by measuring chamber concentration of the aerosolized agent and estimating respiratory minute volume (MV) of the animal using Guyton's MV formula, cc/min = 2.1 (weight, g)$^{0.75}$. Lovelace Respiratory Research Institute (LRRI) employs a system that measures actual inhaled volume using real-time whole-body plethysmography. The LRRI rabbit plethysmography system is designed to contain an unanesthetized rabbit in a uniquely designed chamber for nose-only exposures. The plethysmograph system is composed of the plethysmograph chamber, screen pneumotachograph, pressure transducer, Buxco MAXII preamplifier, and BioSystem XA for Windows software.

Methods: The plethysmograph chamber is calibrated prior to each use by injecting a 20-mL bolus of air into the sealed chamber. A pressure drop is generated across a series of fine mesh screens (pneumotachograph) that are connected to the chamber. The pressure drop is detected by a transducer and a signal is sent to a preamplifier. The BioSystems XA software correlates the signal to flow rate. The rabbit is placed in the chamber and movement of air caused by the rise and fall of the thoracic cavity is detected by the pneumotachograph and frequency (F), tidal volume (TV), and MV are calculated by the software. The chamber is connected to a nose-only exposure system after which select agent aerosol is delivered. Concentration of the agent in the generator suspension is calculated to deliver the target inhaled dose in a predetermined inhaled volume.

Results: 104 rabbits weighing between 2.6 and 4.1 kg were exposed to a select agent with dose determined by chamber aerosol concentration and a target total inhaled volume of 10 L or 20 L. Average F was 181 ± 73 bpm, TV was 11 ± 5 mL, and average MV was 1707 ± 727 mL/min.

Conclusions: The LRRI rabbit plethysmography system demonstrates that inhaled volume can be quantified accurately. Results show that the F, TV, and MV of similar weight animals can differ significantly.

Introduction

Select agent inhalation exposure studies using rabbits normally require that the test animals be housed in closed chambers, which most systems employ, and exposed to the agent through a nose port. This requires that inhaled dose is estimated. Typically, select agent studies using rabbits estimate inhaled volume using Guyton's formula (MV, cc/min = 2.1*[body weight, g]$^{0.75}$, Guyton, 1927) or the Bide estimate (MV, cc/min = 499*[body weight, kg]$^{0.809}$, Bide, 2000). However, respiratory functions (F, TV, MV) of rabbits can vary over wide ranges during short-term exposures (<30 min), which affect the actual challenge dose. A whole-body exposure chamber designed at LRRI allows for collection of real-time plethysmography measurements to more accurately estimate the individual test animal's total inhaled volume.

Plethysmograph Chamber Design: The LRRI exposure system uses a modified chamber used as a whole-body plethysmography chamber. Major features of the chamber are shown in Figure 1. The top and back of the chamber are removable for ease of access when loading/unloading the test animal. The head section narrows and a cone attached to the inlet restricts head movement while providing a seal between the snout and the inlet. An adjustable butt plate (not shown) is adjusted to accommodate different sized animals and allows the animal to be fitted tightly in the box. A pneumotach that employs a set of 300 mesh screens is located in the top door. As the animal breathes, the pneumotach generates a signal that is measured by a pressure transducer. The transducer's electrical signal is detected by the Buxco Max II preamplifier that in turn is converted by an A/D module. The BioSystems XA software correlates the signal from the A/D module to flow rate (Figure 2). The software converts flow rate to respiratory functions, including F, TV, and MV.

Introduction (Continued)

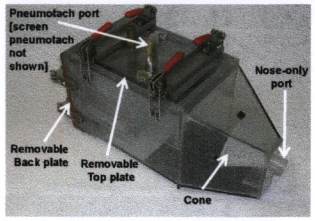

Figure 1. LRRI rabbit whole-body plethysmography chamber

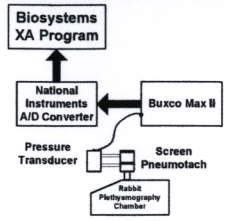

Figure 2. Schematic of pneumotach signal pathway

Methods

- Two rabbit plethysmography chambers are incorporated in the LRRI rabbit exposure system (Figure 3).
- Prior to placing rabbits in the plethysmograph chambers, each chamber is calibrated by injecting a 20-mL bolus of air into the sealed chamber. The BioSystems XA software correlates the signal to the flow rate.
- Concentration of the agent in the generator suspension is calculated to deliver the target inhaled dose in a predetermined inhaled volume (i.e., 5 L) or a set time (i.e., 10–20 min).
- The rabbits are placed in the chambers prior to exposure start and each chamber is connected to the nose-only exposure system. The operator verifies that the BioSystems XA software is displaying signals from each chamber (Figure 4).

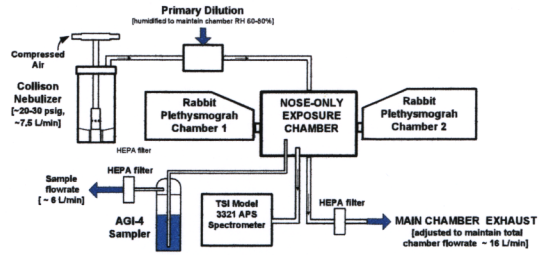

Figure 3. LRRI rabbit exposure system

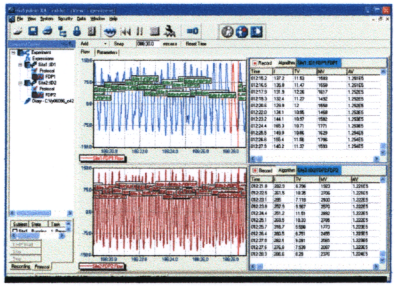

Figure 4. Comparison of F, TV, and MV between two rabbits

Results

- 104 rabbits weighing between 2.6 and 4.1kg were exposed to a select agent with dose determined by chamber aerosol concentration and total inhaled volume as measured by real-time plethysmography.
- MV measurements ranged from 280 to 3568 mL/min.
- F measurements ranged from 47 to 400 bpm.
- TV measurements ranged from 1 to 28 mL.

Discussion

- Using the Guyton and Bide equations, MV estimates for the 104 rabbits in these studies range from 773–1072 mL/min and 1088–1543 mL/min, respectively (Figure 5).
- Both Guyton and Bide estimates are much lower overall than real-time plethysmography.
- Guyton and Bide estimates are up to 74% lower than plethysmography.
- Plethysmography indicates much more variability in MV than the Guyton and Bide equations (Figure 5).

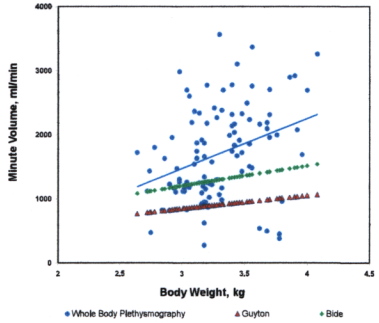

Figure 5. Minute volume vs. body weight

Conclusions

- Results show that the F, TV, and MV of similar weight animals can differ significantly.
- Overall, the Guyton and Bide equations underestimate MV and do not account for individual animal differences and variability.
- The LRRI rabbit plethysmography system demonstrates that inhaled volume can be quantified accurately. This is a critical parameter in accurately estimating the dose to individual animals.

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