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Study:	AEROHIPPUS[R]
Drug:	Beclomethasone dipropionate (BDP) MDI
Drug source:	QVAR (Riker/3M) – 80ug/puff
Delivery device:	AeroHippus[R], Trudell Medical International, London, ONT
Radiolabel:	^{99}mTc sodium pertechnate
Label source:	Massachusetts General Hospital Nuclear Medicine Pharmacy, Boston, MA 02114
Pharmacists:	Ron Callaghan PhD and Allegra Bruce R.Ph. M.S.
PI:	Andrew Hoffman, D.V.M., D.V.Sc., D.A.C.V.I.M.
Co-I:	Kathleen Hunt, B.Sc., C.N.M.T.
Transporter:	Phillip Surrette

Study design

Single exposure of radiolabled beclomethasone HFA defined as 10 actuations.

Timetable of studies

9:00-10:00	Extraction of 99mTc DPTA at MGH
10:00-12:00 AM	Radiolabel (99mTc DPTA) and BDP/propellant added to empty canister and crimped within glove bag at MGH
10:00-11:00	First 2 of 6 horses brought inside for acclimation
12:00-13:00	Transport of drug canister(s) to Tufts.
13:00-16:30	Delivery of drugs, scans (approximately 45 min each)
16:30-17:30	Radiation safety evaluation of experimental sites

Labeling procedure

Briefly, a solution of MEK and 1 ml technetium is placed in a purpose- built metal canister. After MEK is evaporated, a frozen (-70^{o}C) solution of BDP/propellant is poured into the technetium lined canister. The canister is then sealed with a metering valve using a custom collet and crimper (JG Machinery, NJ) and placed in an ultrasonic bath for five minutes for warming and pressurization before dispensation to the transporter.

Radioaerosol treatment of horses

All horses were unsedated, restrained only by halter and lead shank in the Pulmonary Function Testing Laboratory. They were positioned with their nose close to a HEPA filter device to reduce scatter of particles. They were administered 10 puffs of the radiolabled beclomethasone in this room. The canisters were shaken gently between actuations of the MDI into the AeroHippus. The AeroHippus device (1 per horse) was placed against the nose as recommended for each actuation. A 15-30 delay was introduced between puffs. If the horse detached the device during actuation or before 2 breaths were completed, that dose was repeated. This occurred in less than 5% of doses. Once 10 puffs were administered, the horses were sedated with detomidine (2 mg IV) for the gamma scintigraphy and walked immediately to the Nuclear Medicine room down the hall for measurements. The sedation was employed to maintain the steadiest positioning of the horse. The horse's noses were covered with a loose paper cloth to prevent snorting of radioaerosol. Using this method of treating horses in a different room from measurement, we completely avoided background counts in this experiment.

Measurement protocol:

(1) Dose counter for canister (pre-treatment)
(2) Background count in scan room just before measurements
(3) Sedated horses were walked into the scan room.
(4) The following areas were scanned (60 sec each):
  a. Left cranial lung
  b. Left caudal lung
  c. Right caudal lung
  d. Right cranial lung
  e. Nose
  f. Larynx/Pharynx and trachea
  g. Mask (AeroHippus) – all parts
(5) Background count was repeated after measurements; averaged with (2)
(6) Canister (post-treatment) for comparison with pre-treatment values

Computation of % depositions prior to attenuation correction

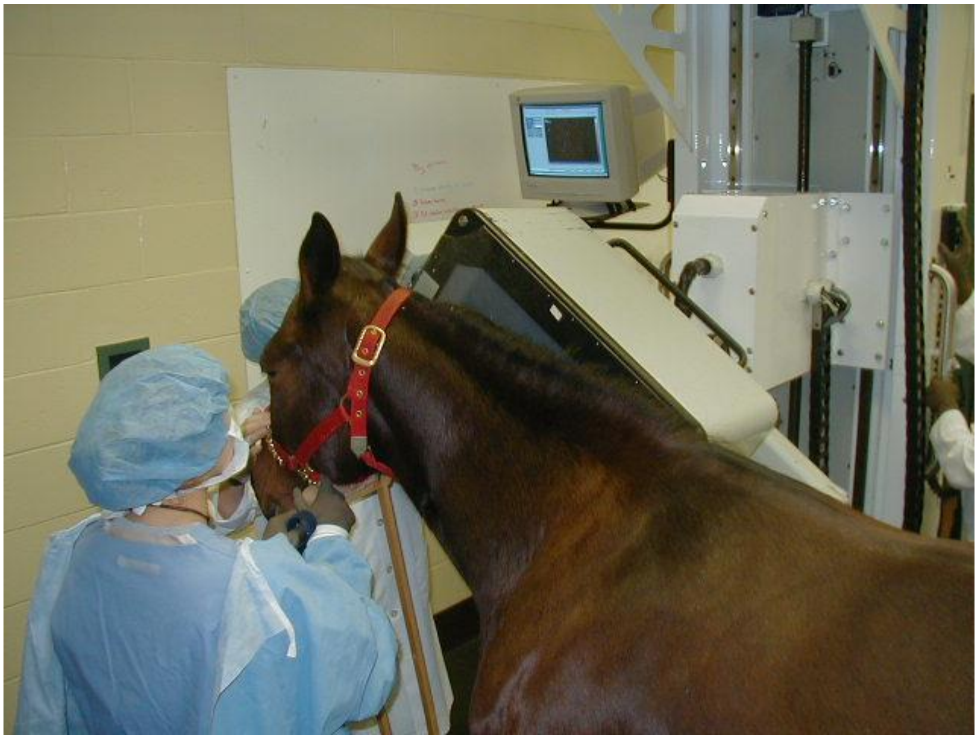
Raw counts from each area were first corrected for background by subtracting the average of the pre and post-measurement background counts. Each corrected count contributed to the denominator.

$\%X = 100 * X_{corr} / \Sigma a.\text{-}g._{corr}$

For example

- Total %lung deposition =

(Total counts from all 4 lung scans each corrected for background) ÷ (Total corrected lung scans + Larynx/tracheal + Nose + Mask)



Images acquired over larynx and tracheal region.

Images acquired of nasal region.

Images acquired over lung regions (example of R cranial).

<u>Attenuation correction of lung deposition data to obtain "actual" deposition:</u>

Two different techniques were employed to correct the raw deposition data for the effects of lung and chest wall attenuation of gamma rays.

1. Marlin et al (2003) measured the attenuation of ^{99m}Tc DPTA placed on the tip of an endoscope maneuvered into various regions of the lung. His findings suggest that none of the radiation from the contralateral lung is detected by the scanner. One scan picks up gamma radiation in a conical region (pointed towards the horse). The greatest activity arises from radioaerosol deposited peripherally where there is the least attenuation. Marlin (2003) estimated that a single scan (whole lung, one side of the chest) detected approximately ¼ of the total radiation deposited in the lung. In our case, we employed two scans on each side of the chest. One would therefore expect to pick up ½ of the radiation in the thorax. Hence we corrected our lung deposition data by multiplying each background corrected count by a factor of 2, but this may be an underestimation since much of the dose that settles in the large (central) airways may be too deep to detect, and the cranial lung is largely behind the shoulders and triceps which further attenuate the signal.

2. The traditional method for attenuation correction employs measurements of chest wall thickness and a formula to correct for attenuation by this layer.

corrected deposition = lung raw %* $e^{(0.153* CW)}$.

Where CW is the thickness (in cm) of the chest wall determined by ultrasound.

Chest wall thicknesses (cm) of horses participating in study (the average of these values within each horse was employed for their attenuation correction).

Horse	Weight (kg)	R cranial (cm)	R caudal (cm)	L cranial (cm)	L caudal (cm)
Mariah	632.0	2.7	3.0	3.2	3.2
Vermont	434.0	2.6	2.4	2.3	2.8
Storm	436.0	3.6	3.0	3.8	2.9
Toes	537.0	2.7	1.7	2.7	1.8
Sand	492.0	2.5	1.9	2.7	1.8
Kalypso	535.0	2.9	2.0	3.2	2.5

Results The thickness of the chest wall for each horse was measured by ultrasound using 3.5 and 5.0 Hz probes to obtain CW in the corrected attenuation formula. Shown below are the CW measurements in the 5 study horses. There was a linear correlation between body and CW (cm), although one outlier (Storm) was observed.

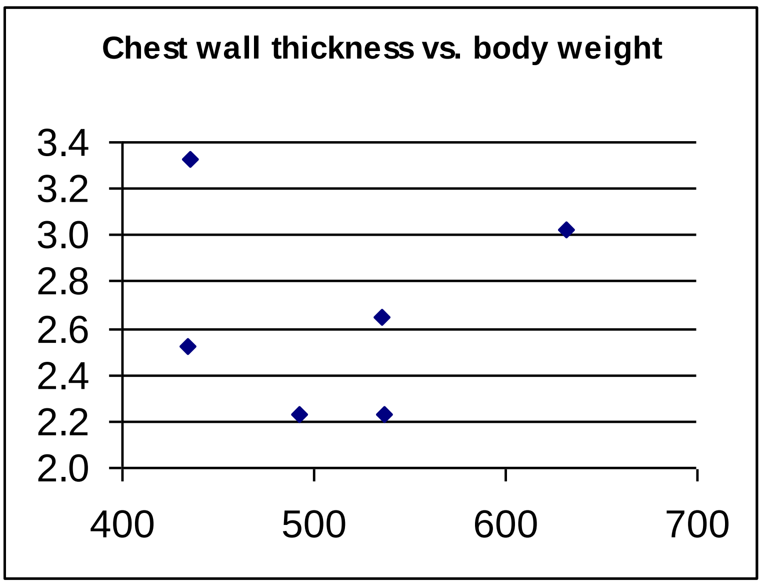
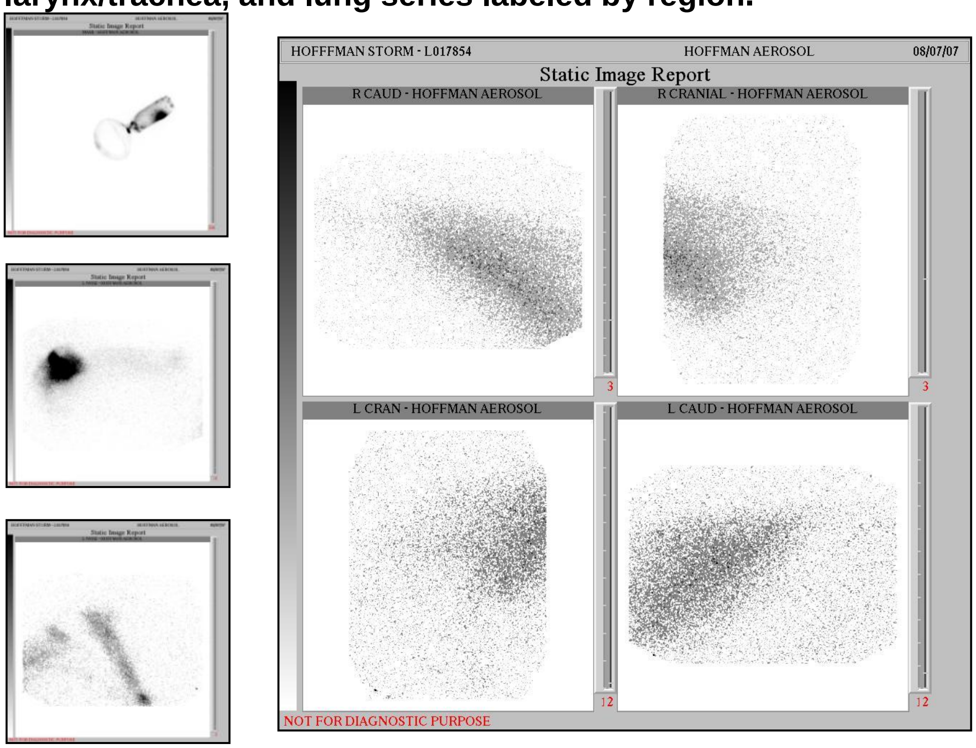


Table 1. % of radioaerosol deposited into the lung, corrected using methods 1 (Marlin) and 2 (attenuation coefficient).
Mean deposition was 18.2% of total radioisotope delivered from MDI.

Note: for raw data, see 'Radioaerosol AeroHippus 2008.xls'

Horse Name	Device	Order of test	Date	Lung total%	% dep Marlin corr	Chest wall (cm)	Atten corr factor	%dep Atten Corrected	AVG
Mariah1 (omitted)									
Storm	AeroHippus2	2	8/7/2007	13.909	27.82	3.30	1.66	23.04	
Vermont	AeroHippus3	3	8/7/2007	18.496	36.99	2.50	1.47	27.11	
Toews	AeroHippus4	4	8/7/2007	4.954	9.91	2.20	1.40	6.94	
Sand	AeroHippus5	5	8/7/2007	5.765	11.53	2.20	1.40	8.07	
Kalypso	AeroHippus6	6	8/7/2007	7.851	15.70	2.70	1.51	11.87	
Mariah2	AeroHIppus7	7	8/7/2007	11.042	22.08	3.00	1.58	17.47	
			AVG.	**10.34**	**20.67**	**2.65**	**1.50**	**15.75**	**18.2**

Scan from Storm, showing from top left (clockwise) mask, nose, larynx/trachea, and lung series labeled by region.



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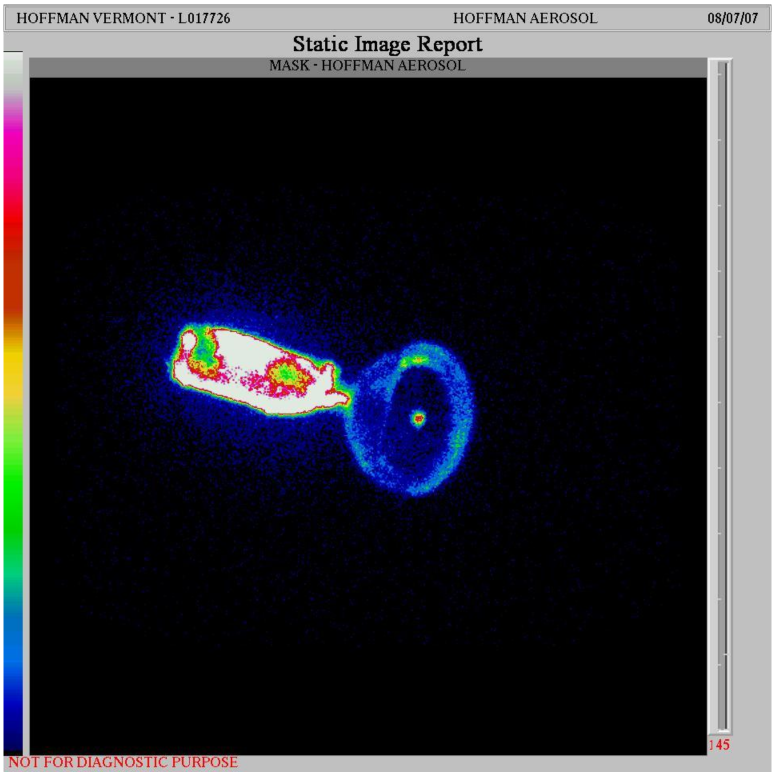
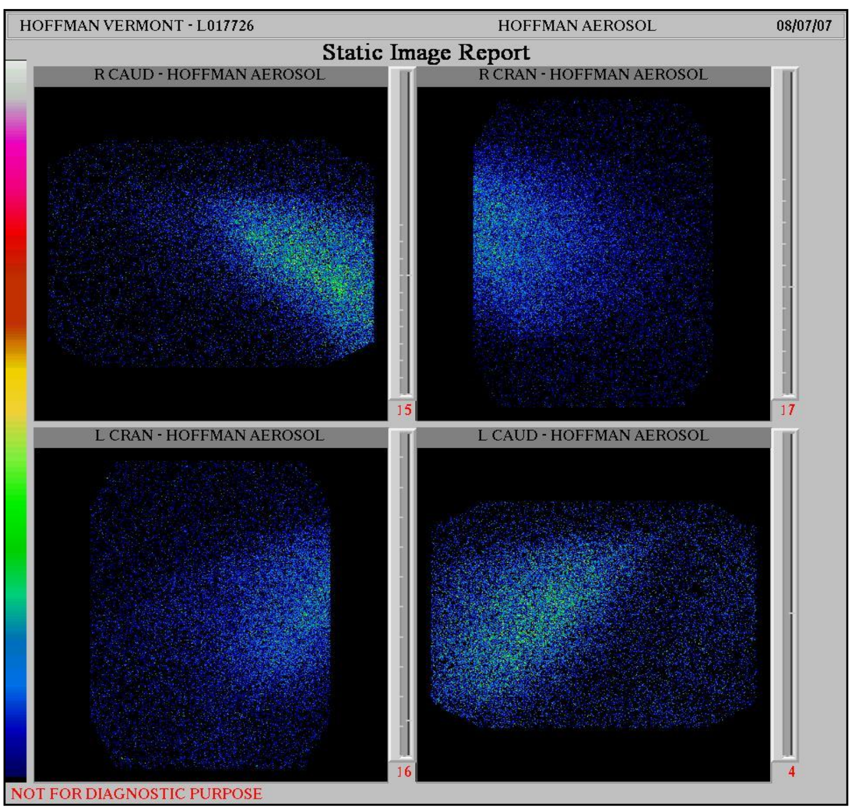
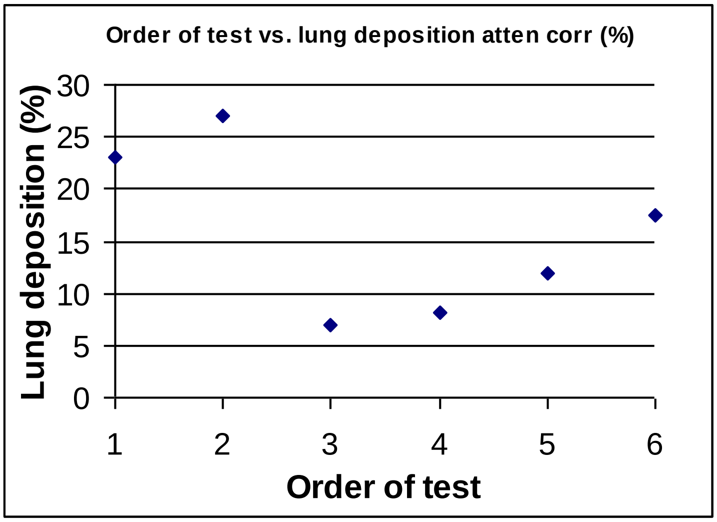
Scans from Vermont (mask and lung regions)

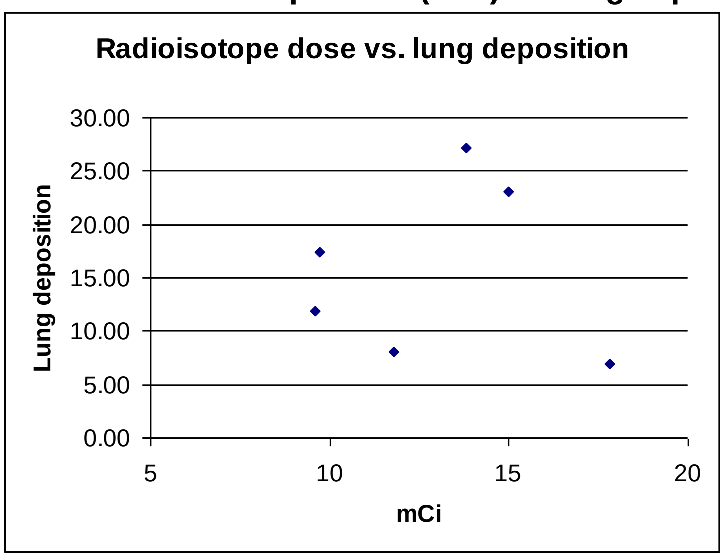




Effect of order of test on lung deposition (attenuation corrected).



Effect of radioisotope dose (mCi) on lung deposition.



Summary of potential confounders:

1. The order by which horses were treated with radioaerosol and subsequently tested was randomized, and showed no effect on lung deposition.

2. The dose of radioisotope delivered to each horse (mean±SD = 12.95±3.21 mCi) was highly consistent and had no effect on lung deposition.

Critique of study and Conclusions

Lung deposition of radioaerosol delivered by AeroHippus[R]

Lung deposition computed using 2 different methods of attenuation correction were similar. The average lung deposition in 6 horses was 20.7% (method 1) vs. 15.8% (method 2), for a mean lung deposition of 18.2%. In sum, 18.2% of the radioaerosol released from the MDI was found in the lung. This is an extremely efficient device. Factors that may have contributed to errors in these deposition values are discussed below. In general, we believe that the values are underestimations of deposition. To improve deposition, there needs to be a reduction in mask or nasal deposition which were on average 57% and 30% of the raw counts, respectively. In particular, greater release of the particles from the spacer (moreso than interface or valve) would substantially increase lung deposition. The degree of deposition in the mask may have been increased due to the high humidity (60-70%) and temperature (27oC) of the PFT lab, despite the use of air conditioning in that space.

Factors that may have contributed to *underestimation* of lung deposition

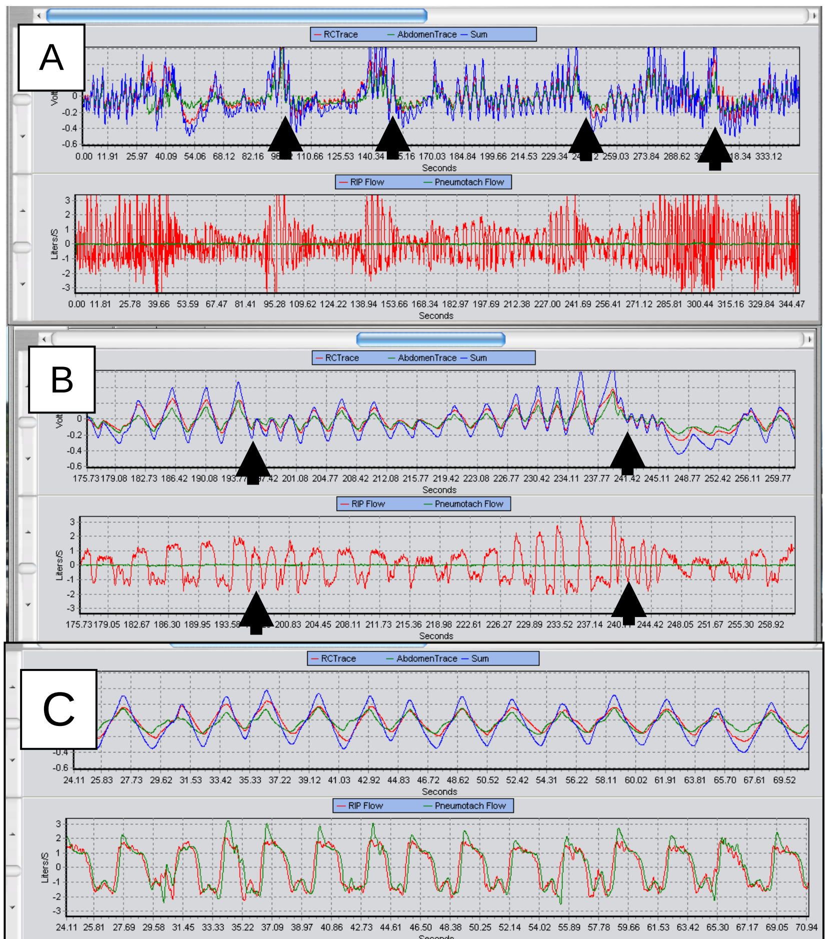
The attenuation correction factor was derived from ultrasound measurements which due to compression of soft tissues from the probe, likely underestimate chest wall thickness. Four of the six horses were obese, making this problem worse. Storm and Vermont were thinner than the other horses and exhibited higher raw lung counts. In the future non-obese subjects should be used. The fact that subjects received no prior training or exposure to the device and were not sedated likely reduced the total deposition achievable with this device. It would be interesting to repeat the study after some training. This was evidenced in part by irregularities in breathing pattern following each treatment (see Appendix 1). Rare instances where the device was inadvertently disconnected immediately after actuation may have contributed to greater mask deposition. The measurement of background was made without the horse in front of the camera; with the horse in front of the camera the actual contribution of background may be lower due to attenuation, therefore background counts may have been overestimated. Finally, we believe that the radioaerosol canister should be sonicated in the future as this will improve mixing of the drug with pertechnate and deposition, and this might increase deposition measurements.

Factors that may have contributed to *overestimation* of lung deposition

Some radioaerosol was lost into the atmosphere and collected by the HEPA filter device stationed immediately adjacent to the nose of the horses during treatment. This amount of ‘exhausted’ radioaerosol was not included in the denominator of the lung deposition calculation.

Appendix I

The following are examples of respiratory patterns acquired with respiratory inductance plethysmography (RIP) in 2 horses (Storm and Sand) during the treatments. RIP is a technique that acquires volume data from inductance bands placed on the chest (blue) and abdomen (red). The hardware/software was XA Biosystem v 2.7 (Buxco Electronics). This technique has been well described in the horse (Hoffman AM, Kuehn H, Riedelberger K, Kupcinskas R, Miscovic MB. Flowmetric comparison of respiratory inductance plethysmography and pneumotachography in horses. *J Appl Physiol*, 2001;91:2767-2775). In each of the figures below (A and B) the top stripchart is a collection of volumetric measurements in Liters (RIB, ABD, and their SUM), and the lower stripchart is flow (Liters/sec). The Rib and Abd both contribute to tidal volume – their sum is equivalent to tidal volume. The response to actuation was a lowering (about 50%) of tidal volume and peak flows, with gradual return to baseline in 15-20 sec. The baseline volume (end-expiratory lung volume) shifted (up or down) as a response. There was no obvious change in abdominal-rib synchrony so the magnitude rather than the character of the breathing pattern was altered.



Breath Num	Start (s)	End (s)	Dosage	Rate (BPM)	Tidal Vol (l)	Min Vent	Peak Insp (l/	Peak Exp (l/	Insp Time (s)	Exp Time (s)	Delta Flow (l/s)	Theta	sRaw	Delay
27	90.45	93.42	Baseline	20.21	1.19	24.15	1.68	2.24	1.45	1.61	2.31	28.63	117.86	73.89
26	86.94	90.45	Baseline	17.07	2.38	40.63	2.31	1.81	1.83	1.70	1.87	12.13	108.03	52.80
25	83.34	86.94	Baseline	16.70	1.91	31.84	2.16	1.55	1.69	1.91	1.22	4.71	108.03	37.57
24	79.72	83.34	Baseline	16.55	1.66	27.53	1.62	1.27	1.75	1.89	1.09	5.54	98.21	40.34
23	75.84	79.72	Baseline	15.48	1.68	26.07	1.62	1.26	1.80	2.03	1.33	5.08	127.68	36.29
22	72.25	75.84	Baseline	16.70	1.76	29.43	1.70	1.36	1.75	1.88	1.22	2.79	108.03	37.57
21	68.70	72.25	Baseline	16.92	1.65	27.84	1.55	1.51	1.72	1.83	1.75	22.04	108.03	41.23