Inhalation Administration to Mini-Pigs

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Global Lead of Inhalation Sciences and Engineering

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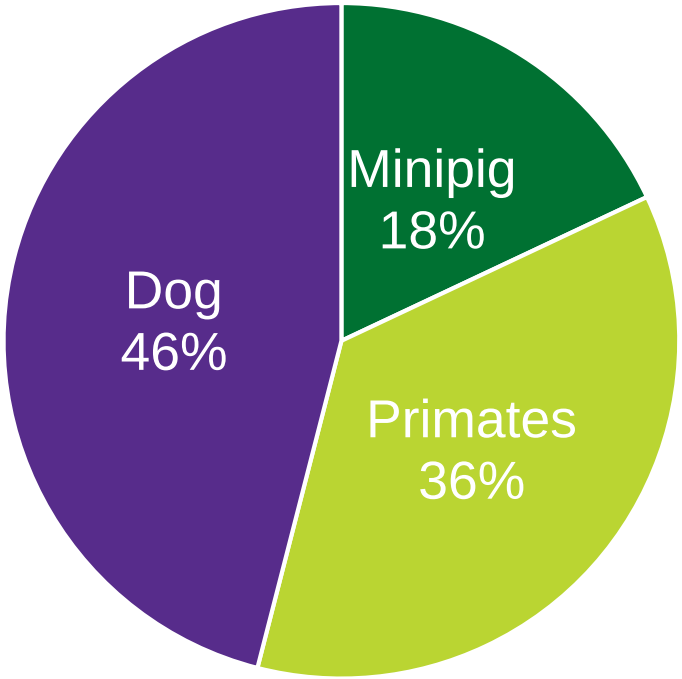
Large animal species selection – non-inhaled

Toxicology Studies by Species (Last 5 Yrs.)



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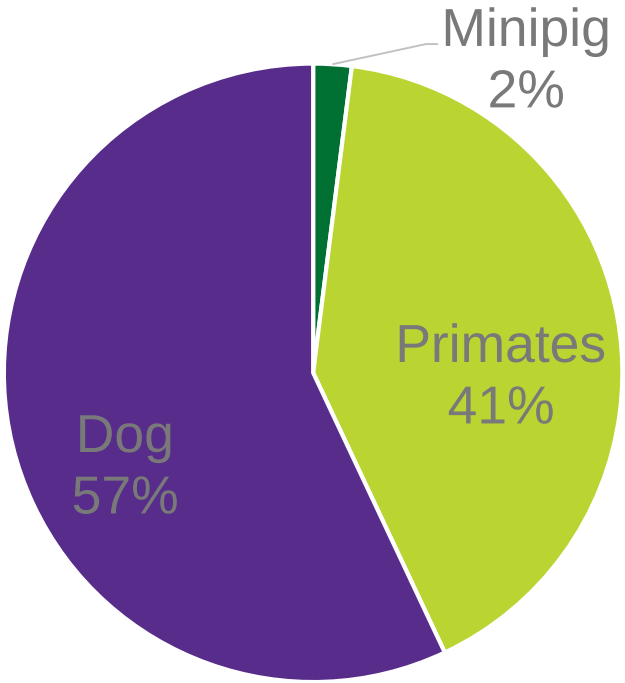
Large animal species selection – Inhalation specific

Inhalation Studies by Species (Last 5 Yrs.)



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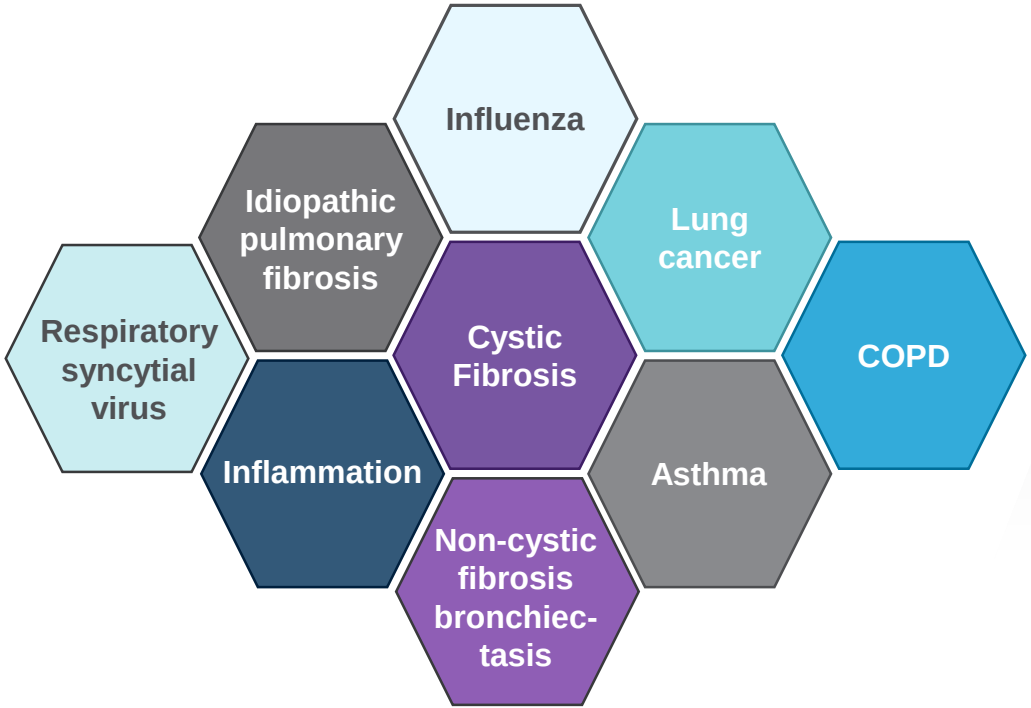
Respiratory Therapies



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Species selection

- Scientific
  - Background data of the respiratory tract
  - UK
    - “No other species is suitable or it is not practicable to obtain animals of another suitable species”
  - EU similar ethical approach to the UK
  - US
    - Most relevant species
- Legalisation
  - UK
    - Second species justification irrespective if they have capability
    - Animal (Scientific Procedures) act is not extended to minipigs or pigs
    - Ethical influence

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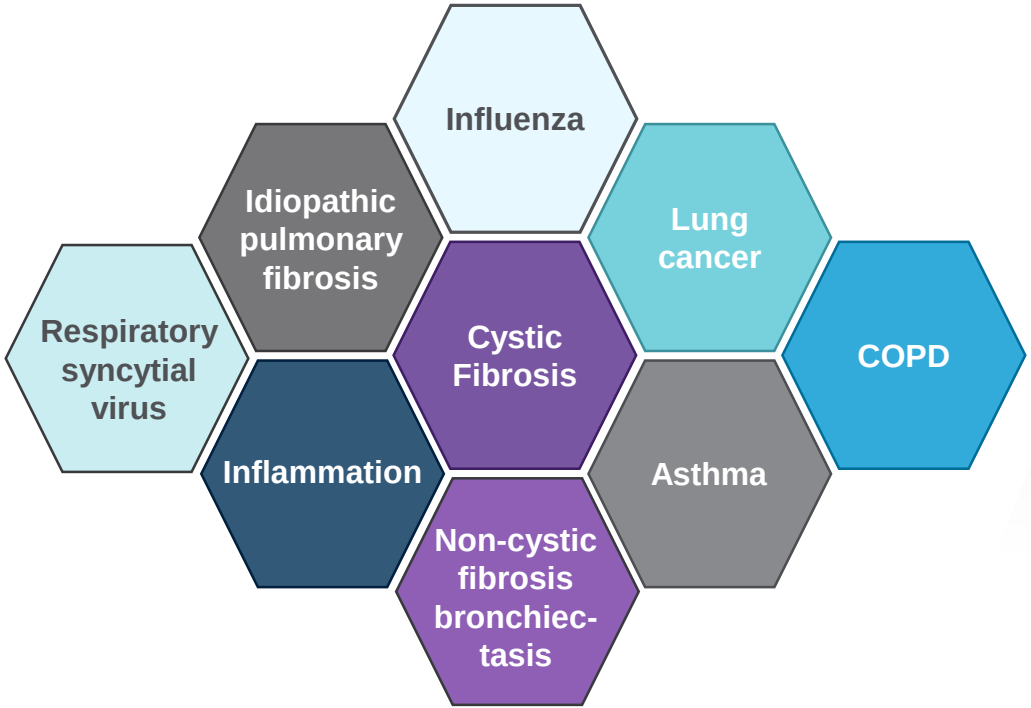
Respiratory Therapies



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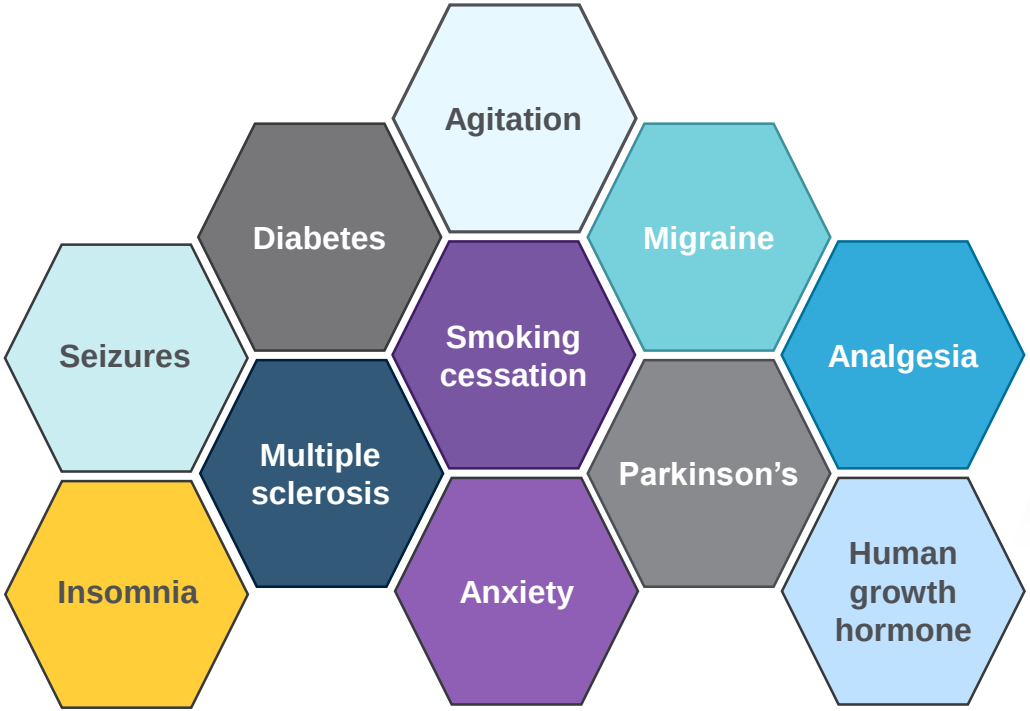
Systemic Therapies



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Case Study:

Inhalation methodology success and cardiovascular and respiratory effects of β2-adrenergic agonist

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Animal Transfer

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Whole-Body Exposure

- Up to 4 minipigs per chamber
- >12 air changes/hr
- Rubber mat over a Stainless Steel grating floor
- Clear retaining shield when opening and closing the chamber door

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Restraint Training

- Recommend a total of 10 consecutive days
- Day 1 – no mask
- Animals prefer to stand, unlike dogs

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Restraint Training

- Days 2 to 4 – mask attachment with air
- Animals prefer to stand

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Restraint Training

- Day >5
- Animals prefer to sit, lie and/or be stroked
- Edible treat

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Restraint Training Example

Day	Total Time of Restraint (minutes)	Mask Fitted Time (minutes)	Introduction of Air Flow Through Mask
1	15	0	NO
2	30	15	YES
3	30	30	YES
4	40	40	YES
5 - 8	60	60	YES
9 - 10	90	90	YES

- Increasing duration of total time of restraint and mask time
- Airflow per animal between 5 and 7 L/min

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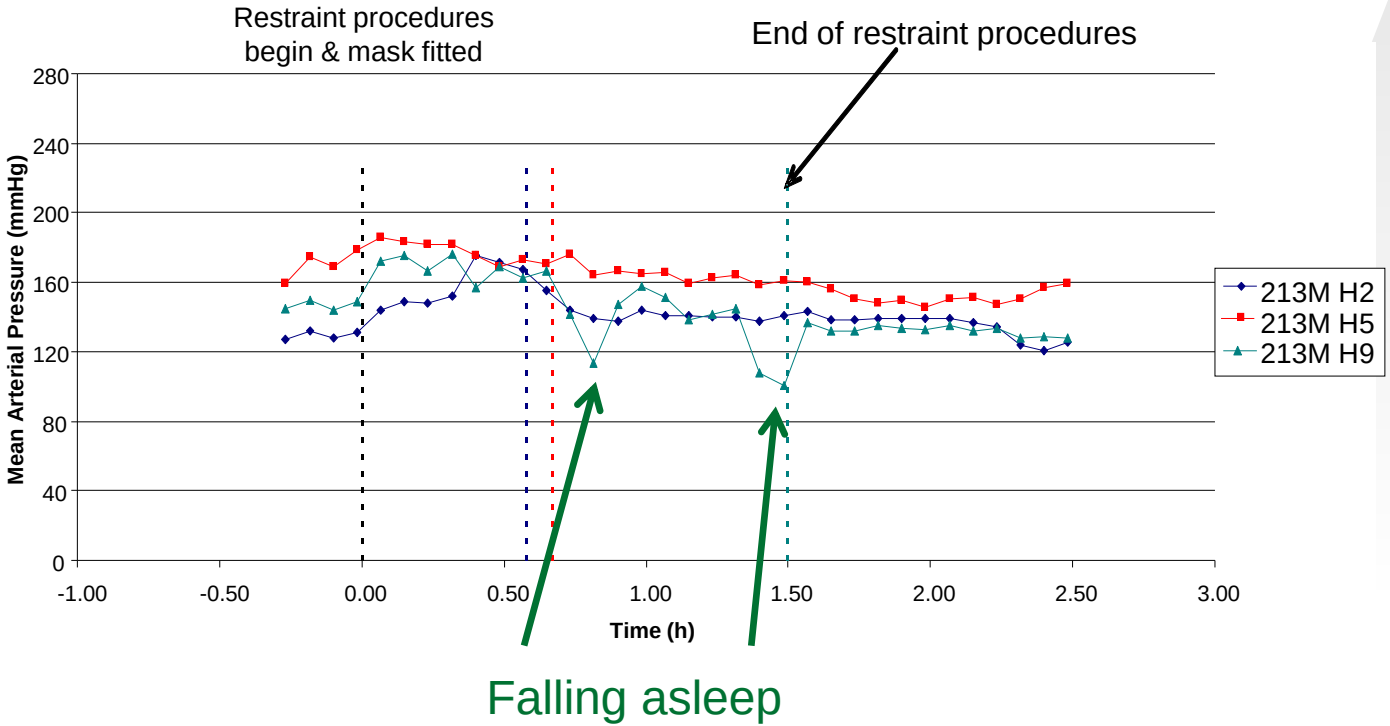
Restraint Training



Inhalation dosing

- Assess cardiovascular and respiratory effects of β2-adrenergic agonist, Albuterol (Salbutamol)
- Single 15 mins inhalation exposure
- Telemetered minipigs (approximately 20 to 24 kg)
- Arterial BP, HR and ECG Lead II (PR and QT intervals)
- Target inhaled dose of 0.35 mg/kg based on Covance equivalent dog data[1]

[1] Miyamoto, M. et al (2009). *Journal of Pharmacological and Toxicological Methods,* Vol. 60, Issue 2, Sept.-Oct., 252-253

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Inhalation dosing

- Pari LC Sprint nebulizer
- 50 mg/mL solution in water
- Water vehicle
- Aerosol Conc = 83.3 µg/L
- MMAD = 1.7 µms
- Bide RMV equation[1]
- Achieved inhalable dose = 0.35 mg/kg

[1] Bide, R.W. et al (2000).; *J. App. Tox.,* 20, 273-290

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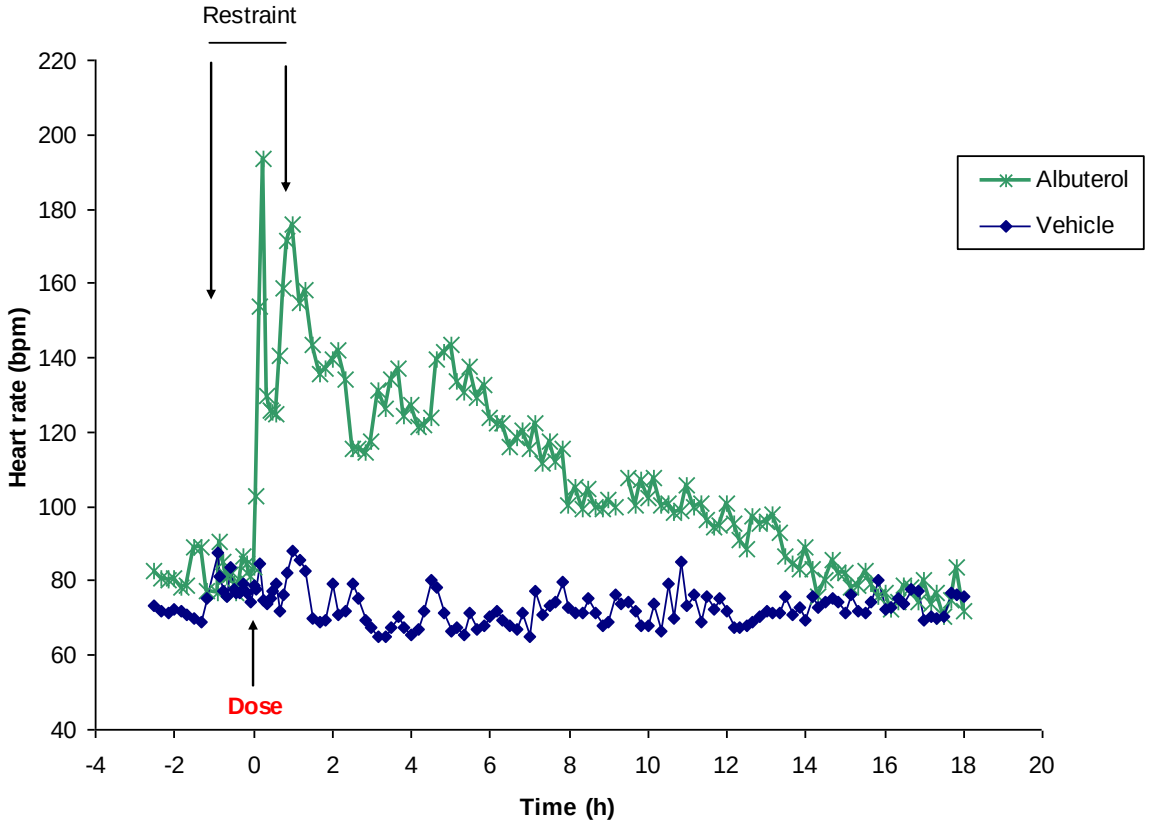
Heart rate



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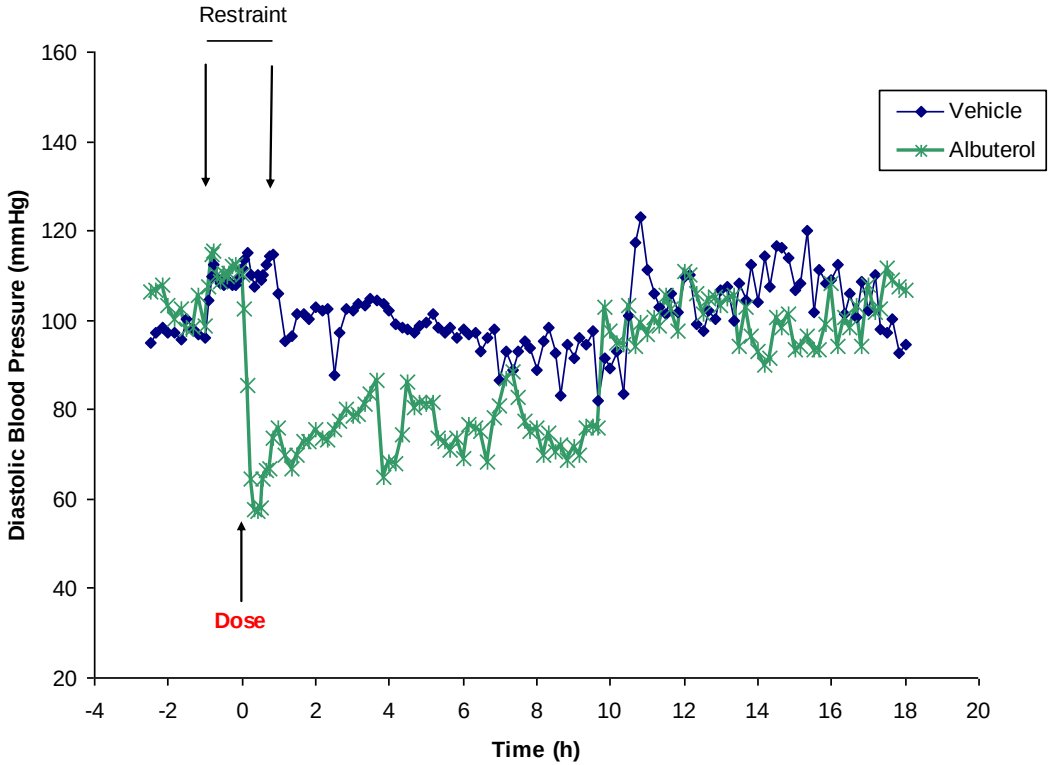
Diastolic arterial BP

- Systolic arterial BP was similar



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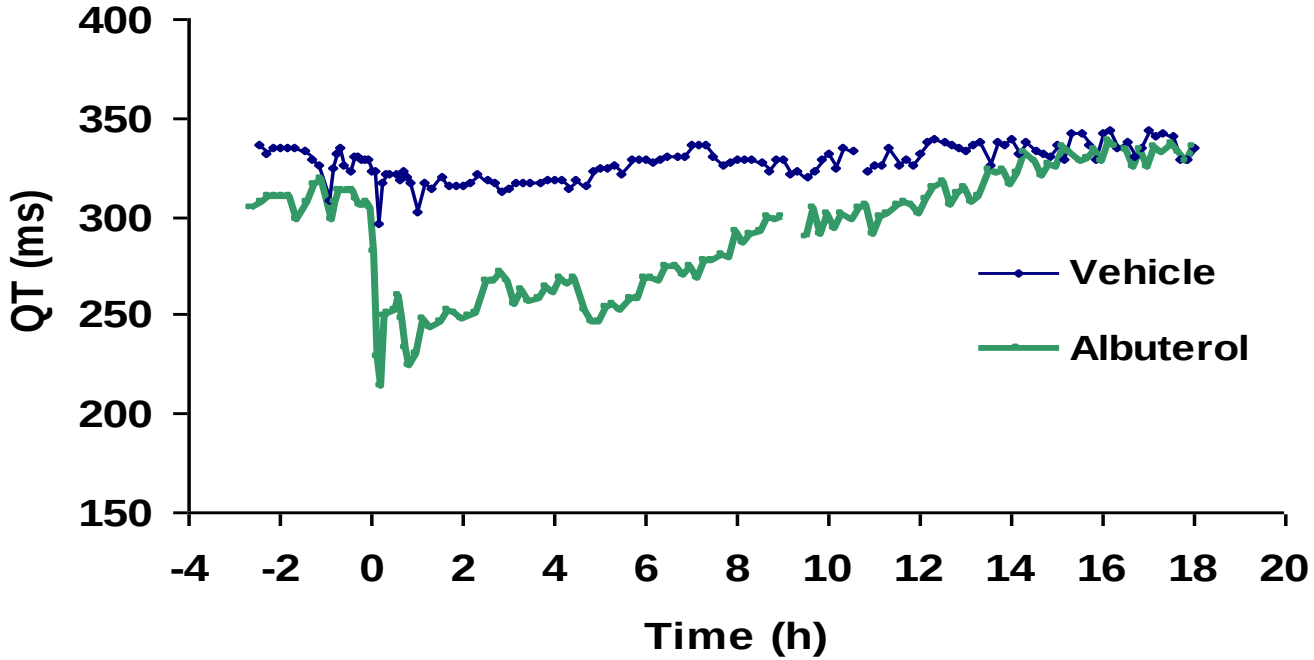
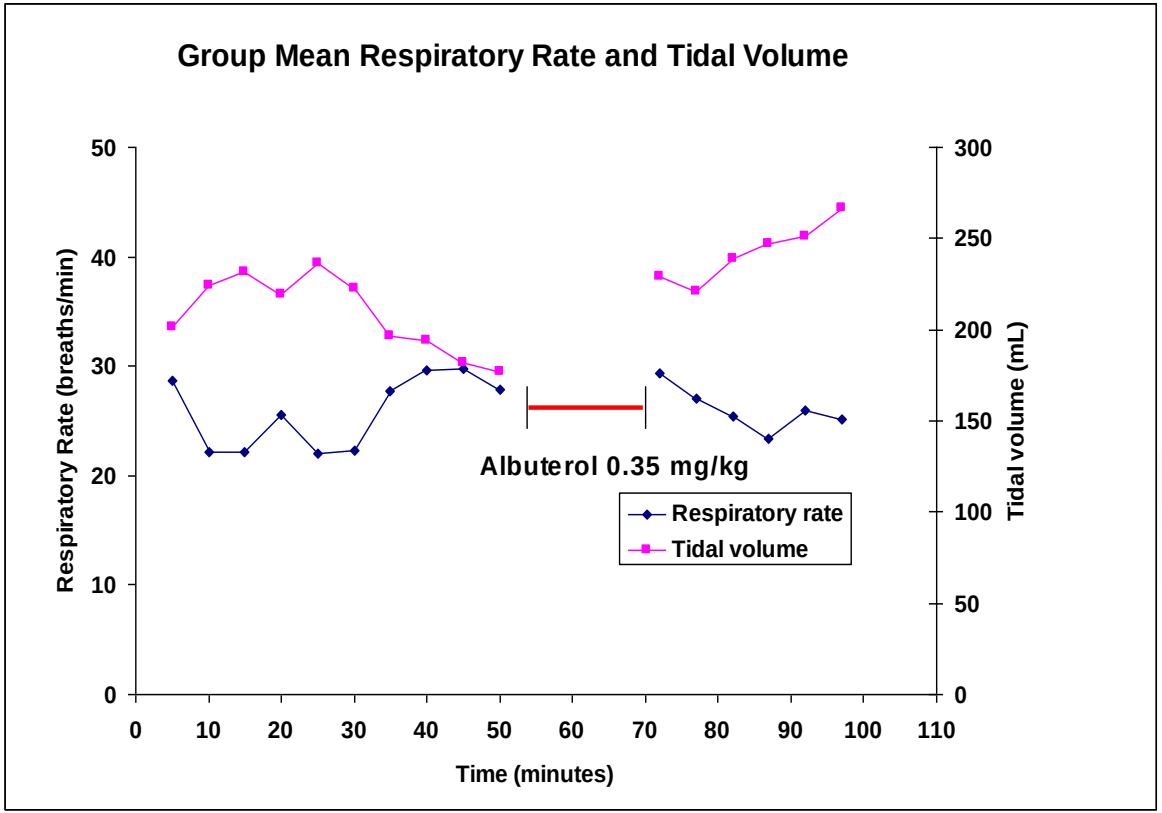
ECG lead II QT interval

- QT similar to HR



Respiratory data



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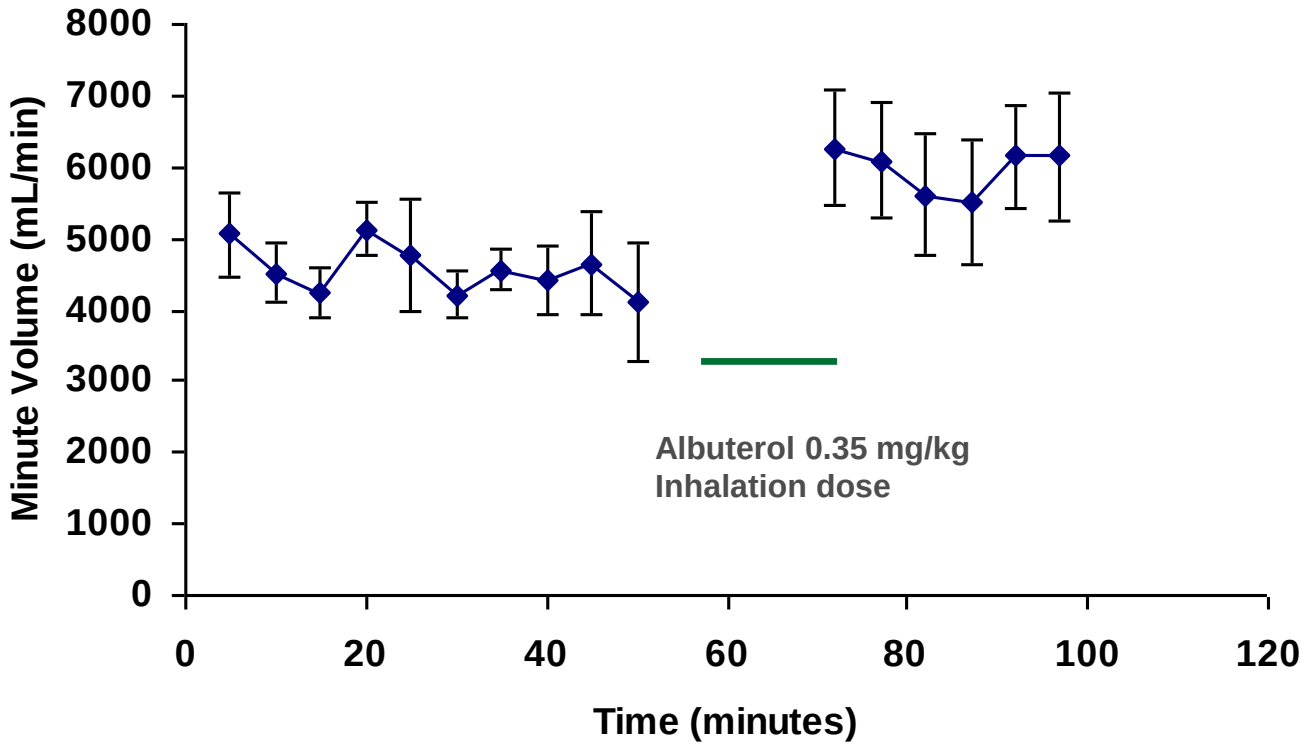
Respiratory Minute Volume data



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Respiratory Minute Volume data

- Difference in RMV based on known algorithms
  - No mini-pig data used
- Recommend RMV recording during study until a revised algorithm

Mean Bodyweight (kg)	Approx. respiratory minute volume pre-dose (L/min)	Alexander et al (L/min)[1]	Bide et al (L/min)[2]	McMahon et al (L/min)[3]
22.2	4.5	8.5	6.1	3.1

[1] Alexander D.J. et al (2008). *Inhal. Tox*., 20, 1179-1189.
[2] Bide, R.W. et al (2000).; *J. App. Tox.,* 20, 273-290.
[3] McMahon T.A. et al (1977). Species Differences in Aerosol Deposition. Inhaled Particles IV (edited by Walton, W.H.), Part 1: 23-32.

Conclusions

- Demonstrated effective habituation
- Procedures well tolerated for up to 90 mins
- Demonstrated the sensitivity to cardiovascular and respiratory effects of the β2-agonist
  - With HR, BP and ECG responses similar in magnitude to those of the dog.
- Mean RMV was 4.5 L/min compared with up to 8.5 L/min for known algorithms

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Case Study:

Assess maximum exposure duration, lactose delivery and Respiratory minute volume assessment

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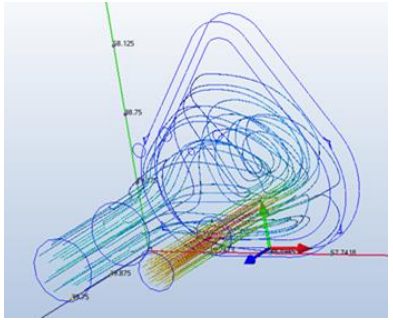
Mask Design and Sizes

- AutoCAD software merges 3D scanned images
- Computational fluid dynamics
- Industrial 3d ProJet MJP2500 printer
  - Stereolithography
  - 32 micron resolution
- Better mask fitting designs for large animals
  - “Personalised” mask fitting
  - Shape and depth
- Improved animal welfare
- Entry and exit ports
  - Ensures no CO_2 accumulation
- Silicone malleable seal
- Bench top assessment



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Restraint Training Regime

Day	Total Time of Restraint (minutes)	
	Slow	Fast
1	Harness only	Harness only
2-4	15	30
5-7	30	60
8-10	60	120
11-13	120	240
14-16	180	
17-19	240	

- No difference in behaviour
- Airflow per animal 8 L/min as 25-30kg

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Lactose LH201 Delivery

- Animals dosed for 1 hr
- 4 hrs of restraint
- Lactose was accepted
- Aerosol conc = 0.323 mg/L
- MMAD = 4.5 µms
- 7 days was scheduled but stopped after 2 days as animals were compliant

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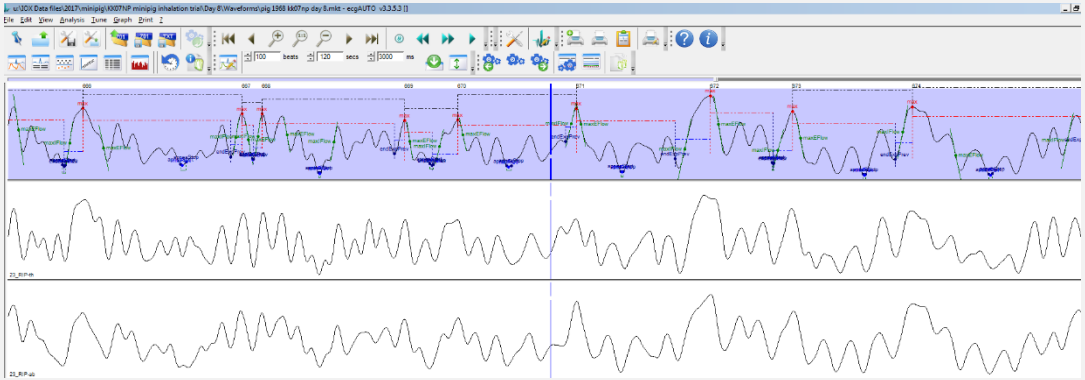
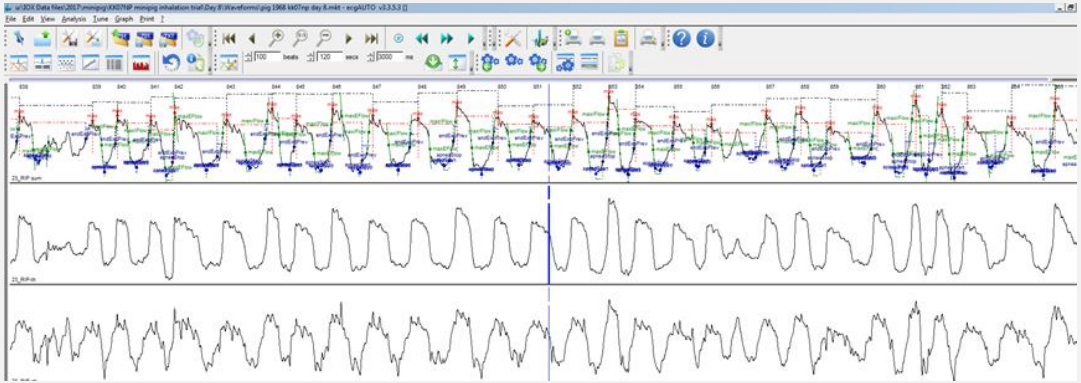
Measurable vs Non-Measurable RMV Data

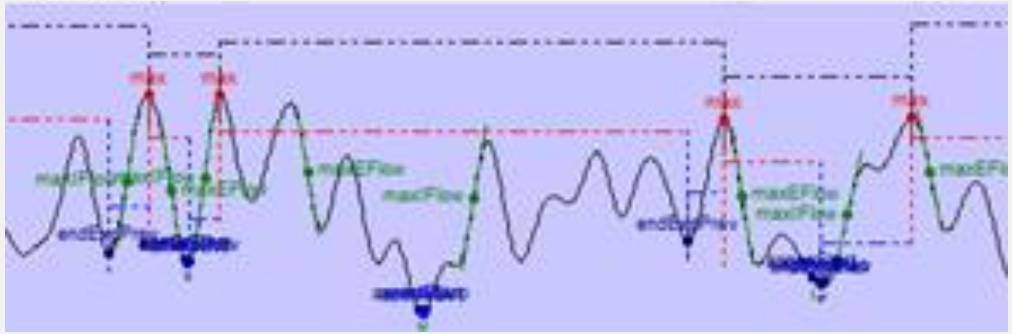


Non-measurable data

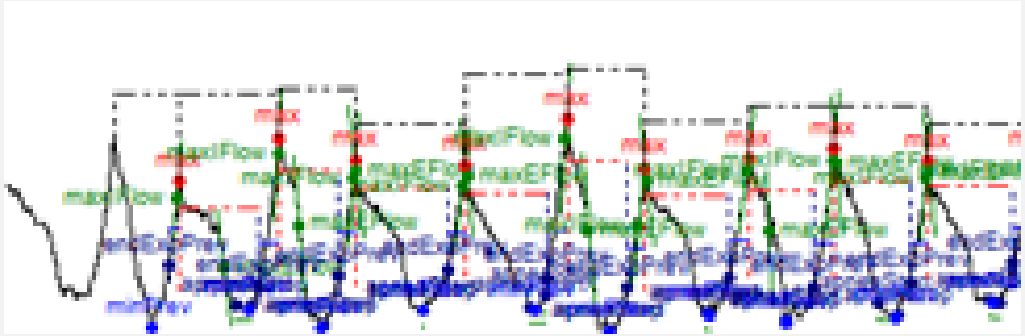


Measurable

Ideal vs Non-Measurable RMV Data

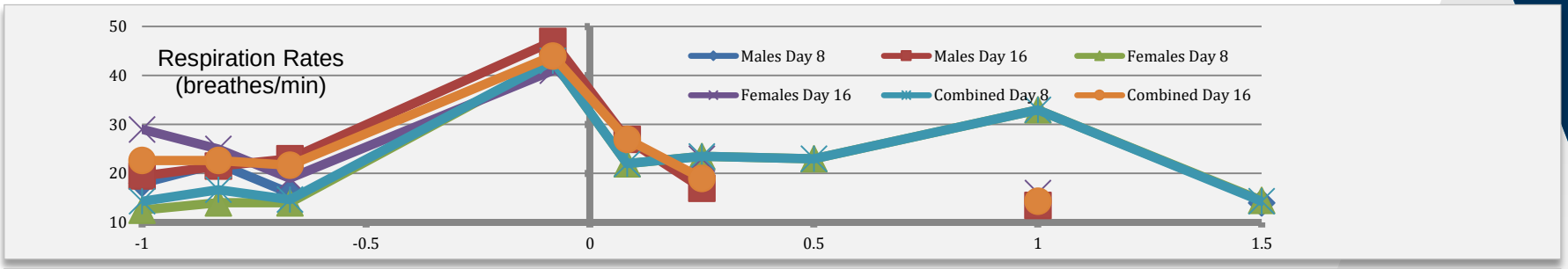


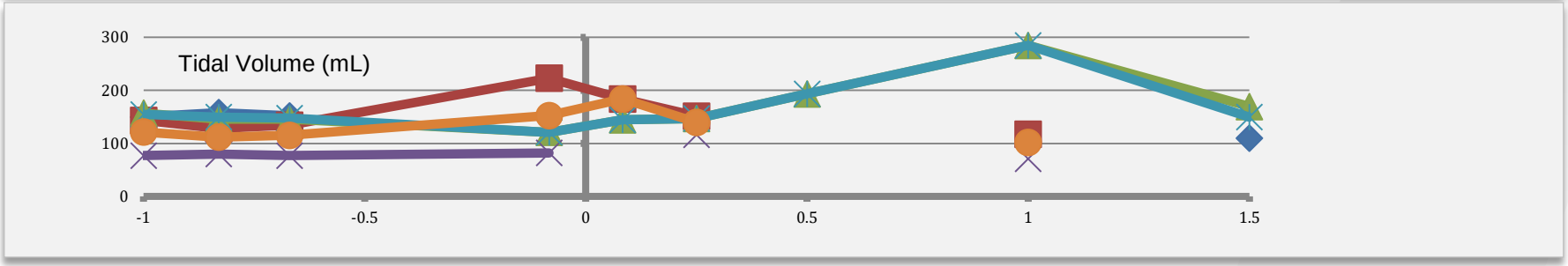
Non-measurable data

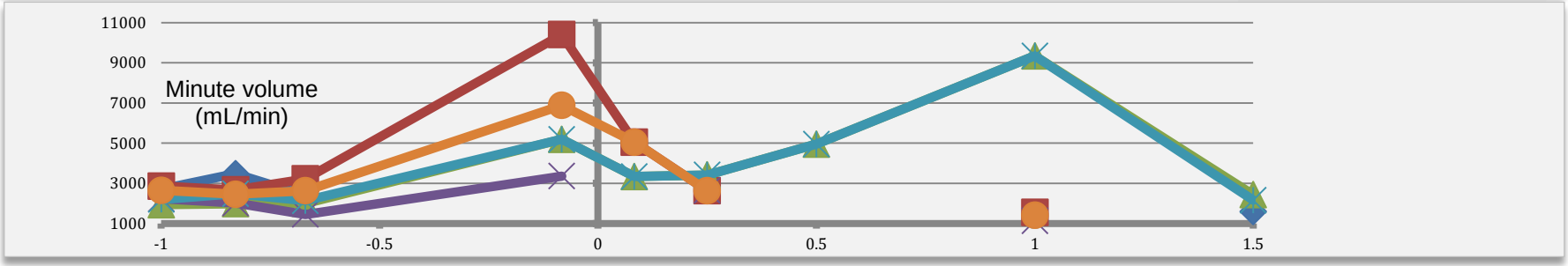


Ideal

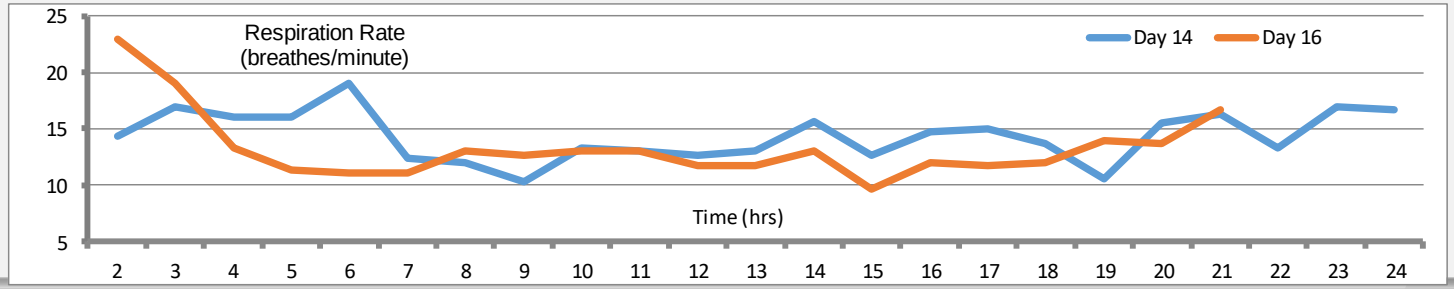
Pre- and During Restraint

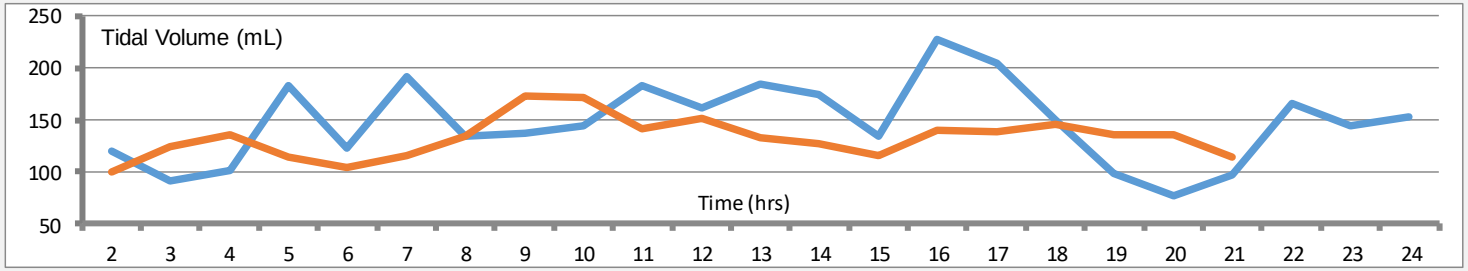


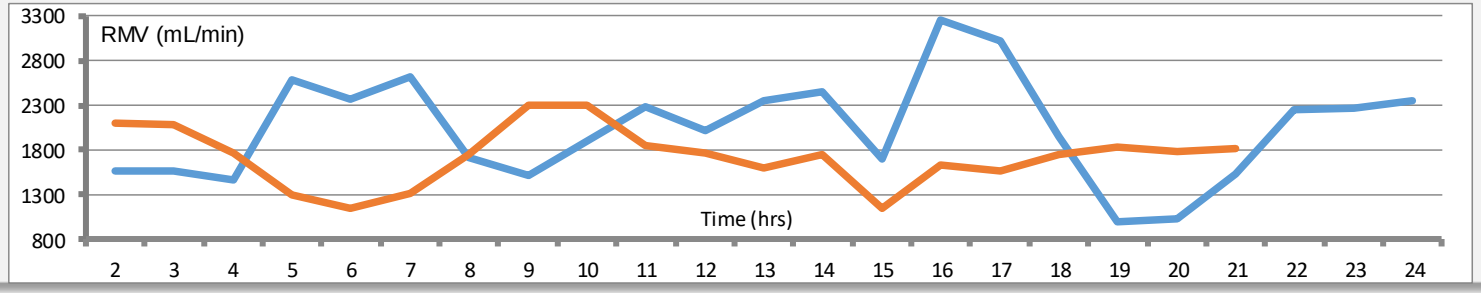




Post-Restraint







Respiration Data

Case Study	Mean Bodyweight (kg)	Time of Recording	Approx. Respiratory Minute Volume Pre-Dose (L/min)
β-agonist [1]	22.2	Pre-dose (but still restrained)	4.5
Exposure duration	27.5	During exposure	3.6

- Recommend RMV recording during study until a revised algorithm

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Animal Dosing Observations

- Learn quicker than dogs
- Wider variety of temperament
  - Sit or lie quietly
  - Stroked
  - But equally vocal on handling
- Relatively easy to handle and habituate
- Naturally quiet disposition
- No toilet control
- Not prone to vomiting
- Toys or not

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Toxicokinetic Sampling

- Usual sampling site – ear vein
- During exposure is challenging
- Animal response
  - Vocalisation
- Insufficient sample
  - 5min IAD probably first sample (unless microsampling)
- V-frame used for later samples

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Conclusions

- Procedures well tolerated for up to 240 mins
- Dosing lactose is practical
- Mean RMV was confirmed that the values are considerably lower than known algorithms
- Caution when using JET RIP during pre and post dose may need manual data review
- TK sampling post exposure was successful but is a challenge during exposure

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Test article requirement

- Larger body weight growth relative to dogs or primates
- The same or lower amount than dogs due to the indirect method of dose calculation
- Methods for reducing the requirements
  - Minimal airflows
  - Experience with similar formulations
  - Minimizing compound requirement overages
  - Minimizing prelim based on experience
  - Compound recovery
  - Internally modified equipment

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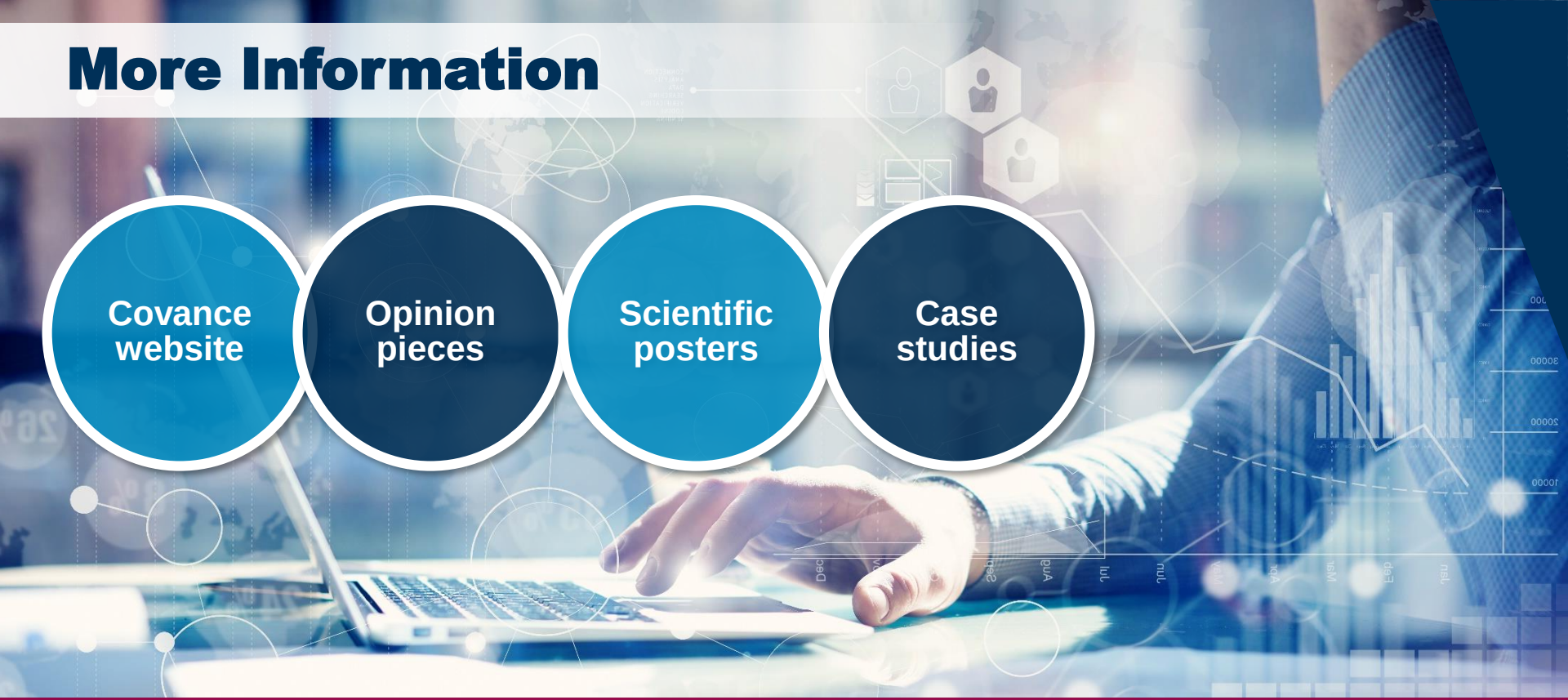
More Information



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Acknowledgements

- Somerset
  - Emily Ressegiue
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- Huntingdon
  - Helen Palmer
  - Antony Grasiewicz
  - Stephen Jordan

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