MINIPIG INHALATION

Göttingen Minipig® as Animal Model for Inhalation Toxicity Studies

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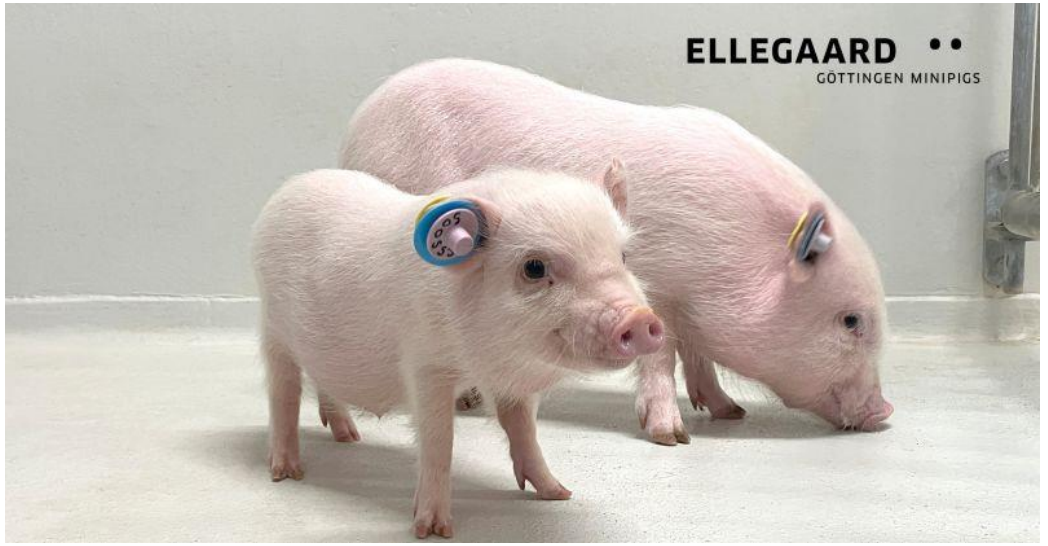
Webinar overview

- Introduction
- Why choose minipigs for inhalation studies?
- Scientific value of minipigs in inhalation
- Disease targets
- Targeting the respiratory system
- Minipig habituation, training and compliance
- Mask designs, inhalation systems
- Study designs
- Q/A



https://minipigs.dk

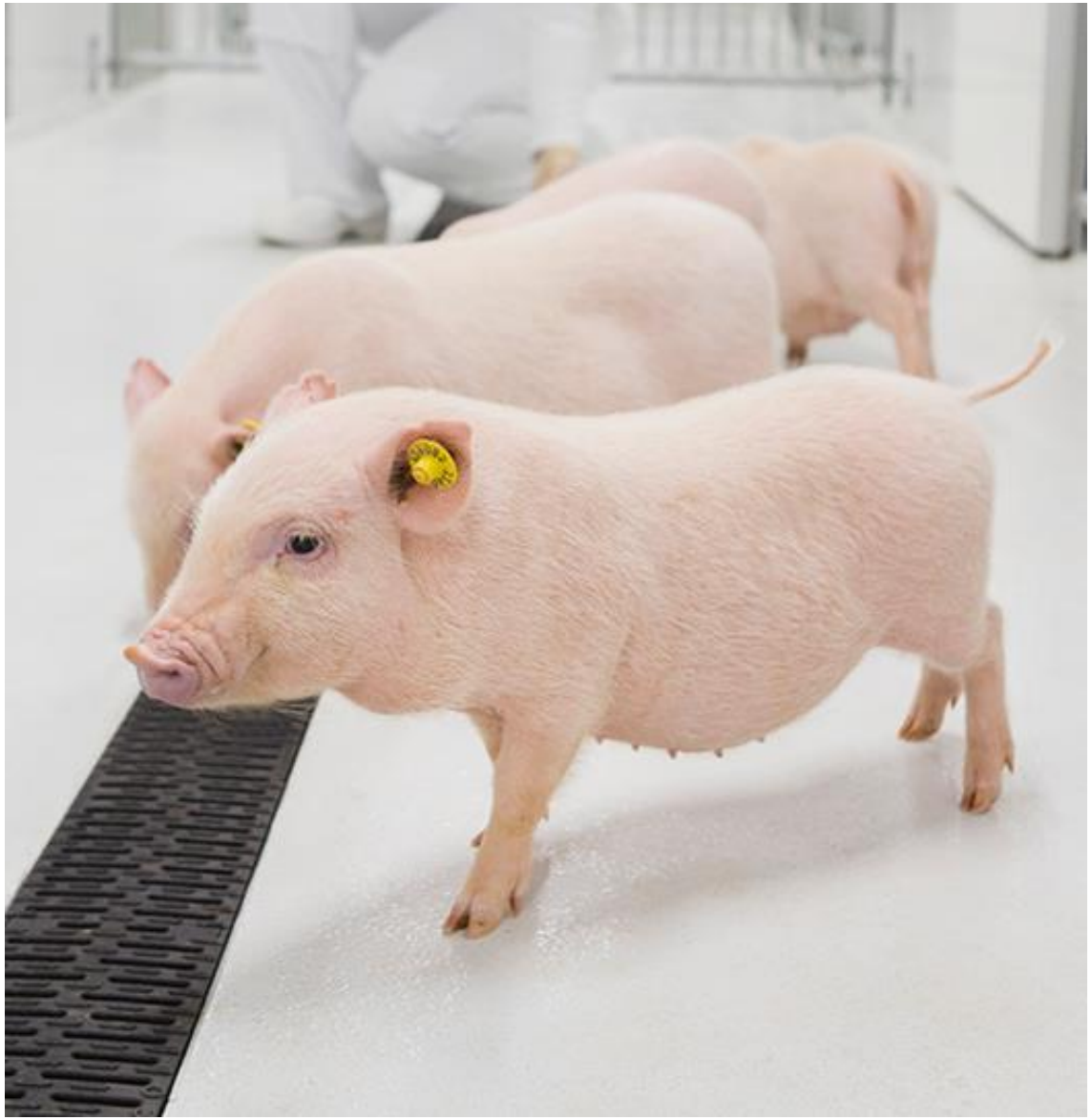
Why choose minipigs?

Ethical factors

- Being increasingly viewed as an alternative second species
- EU requires justification of why minipig not suitable before using cyno/dog
  - Greater general acceptance as the non-rodent preclinical model

Industry-related factors

- Growing global industry interest
  - UK/EU and U.S.



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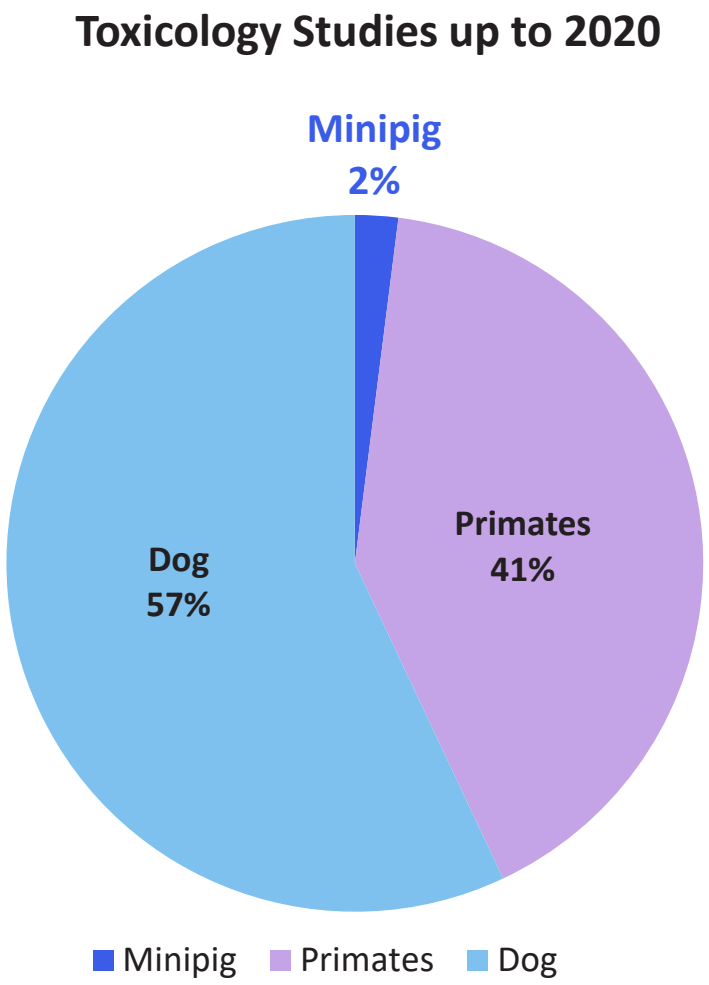
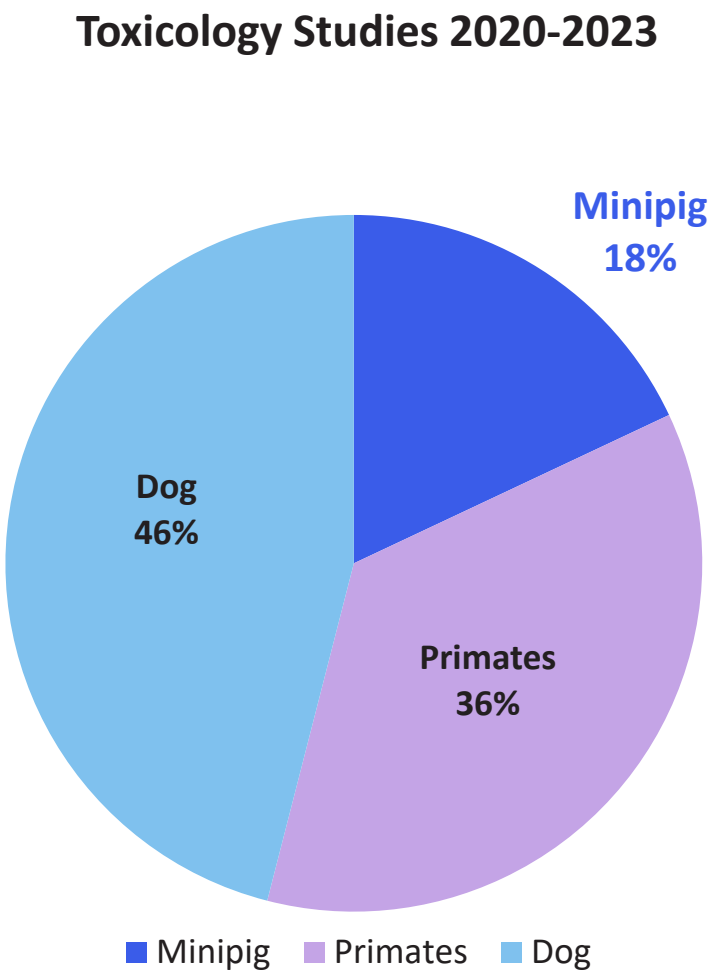
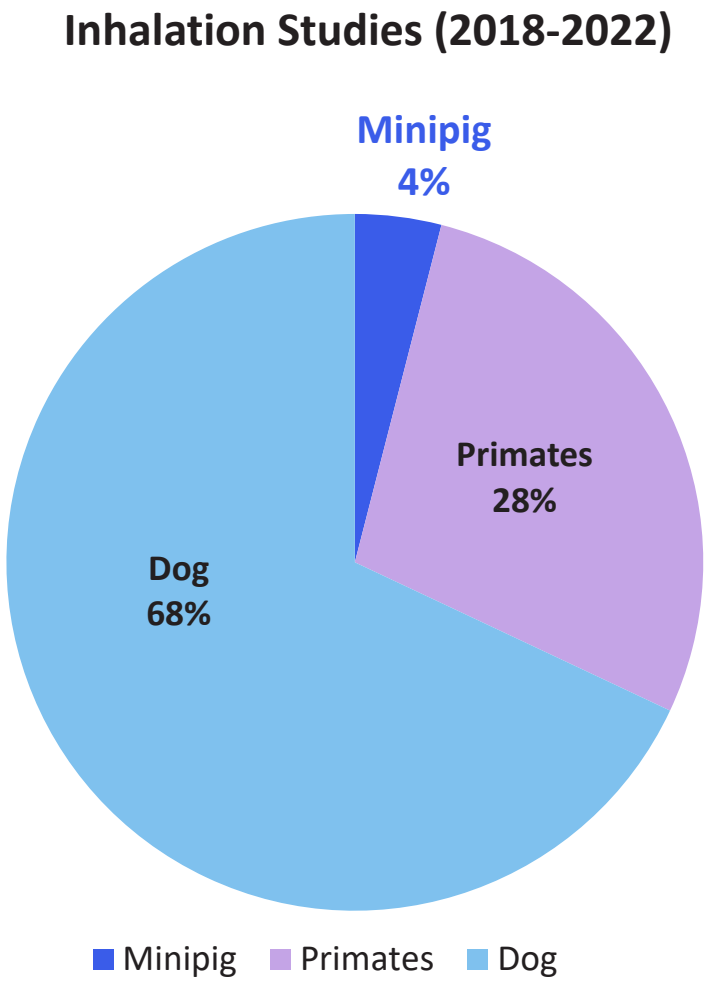
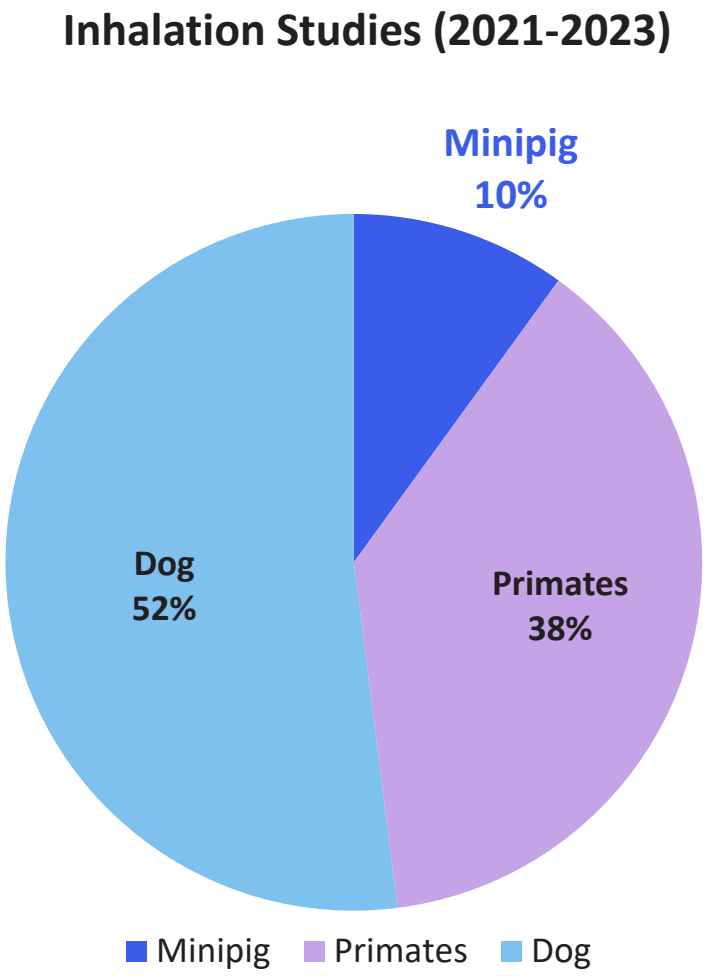
Large animal species selection: Non-inhalation studies





Large animal species selection – Inhalation specific





Scientific value of minipig in inhalation

- Göttingen minipig has defined biological status (Specific Pathogen Free - SPF)
- Larger size compared to dogs and nonhuman primates
  - Larger organs
- Smaller size compared to commercial swine
  - Easier to house/handle
- Similar anatomy and physiology to humans
- Suitable for infectious diseases (i.e., influenza) due to similar pathogenic susceptibility to humans

Translational value

- Broadly similar morphological structure and distribution of airways
  - Lobar and bronchial anatomy – similar number of bronchial generations
  - Highly lobulated lungs, well-defined pulmonary lobules, interlobular septae [incomplete in humans (collateral interlobular ventilation)/complete in pigs]
  - Abundant visceral pleural collagen (thicker pleural membranes than other species) – vascular supply from bronchial arteries in both species
  - In contrast to other animals (e.g., dogs), large numbers of submucosal glands – good large animal model of airway inflammatory disease and CF
- Publication of the pig genome: overcoming of limitation of porcine model compared with rodent models
  - Immunome analysis demonstrated a greater similarity between human and pig than between human and mouse

Table 2
Non-transmissible respiratory diseases pro and con of the porcine model.

		Pros	ConS
RESPIRATORY INFECTIONS	*B. pertussis*	-naturally infected	
	Mycobacterium sp	-naturally infected	
	S. aureus	-naturally infected	
	P. aeroginosa	-naturally infected	
	Influenzavirus	-natural sensitivity to human and swine strains with mild clinical signs	-no example of highly pathogenic infections
	Coronavirus	-natural porcine *αCoronavirus* (PrCoV) similar to the human seasonal HCoV-229E	-disputed sensitivity to SARS-CoV-2
	Orthopneumovirus	-natural porcine *Orthopneumovirus*?	-not sensitive to hRSV
VACCINATION	Delivery	-skin structure -upper respiratory tract structure	
	Adjuvant	-functional TLR8 -cDC1 interstitial and cDC2 intra-subepithelial locations -αGalSer response	-TLR3 expression on pDC not on cDC1
MICROBIOTA		-omnivorous species	-intestinal microbiota similar to herbivore - data paucity on porcine respiratory microbiota
RESPIRATORY ALLERGY AND ASTHMA	Induction	-cDC2 location -cDC2 expressing IgE receptor (FcεRIα)	-no stable chronic allergic asthma porcine model
		-IgE similar to human	
	Clinical signs	-lung smooth muscles porcine model -efficacy of tryptase inhibitors	
ACUTE AND CHRONIC INFLAMMATIONS		-model of early inflammatory ARDS responses -constitutive PIM	-no infectious model of ARDS induction -constitutive PIM
LUNG GRAFT		-size allowing similar surgical intervention -primary graft dysfunction model -*ex vivo* lung perfusion model	

SARS: Severe Acute Respiratory Syndrome; hRSV: human Respiratory Syncytial Virus; cDC: conventional Dendritic Cell; pDC: plasmacytoid Dendritic Cell; ARDS: Acute respiratory distress syndrome; PIM: Pulmonary Intravascular Lymphocyte. Bibliographic references are in the main text.

Bertho & Meurens (2021) The pig as a medical model for acquired respiratory diseases and dysfunctions: An immunological perspective, Molecular Immunology 135:254-267,ISSN 0161-5890

Disease targets – Respiratory

Approximately 800 diseases: respiratory + systemic

- Pulmonary arterial hypertension
- Respiratory syncytial virus
- Idiopathic pulmonary fibrosis
- Asthma
- Influenza
- Cystic fibrosis
- Non-cystic fibrosis bronchiectasis
- COVID
- Lung cancer
- Inflammation
- COPD

Disease targets – Systemic

Approximately 800 diseases: respiratory + systemic

- Diabetes
- Insomnia
- Seizures
- Multiple sclerosis
- Agitation
- Smoking cessation
- Human growth hormone
- Parkinson’s
- Migraine
- Analgesia
- Mental disorders – anxiety

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Classes of compounds

Historical domain

- β-agonist, corticosteroids, anti-muscarinics
- Other NCEs compound classes
  - Recently psychedelics

Growth of biopharmaceuticals

- Proteins, peptides
- Antibody therapeutics
  - Monoclonal antibodies
  - Antibody drug conjugates
  - Fragment antibodies
- Nucleotide based therapeutics

Considerations on compounds tested

- Many compounds have historically been tested in dogs – dogs still non-rodent species of choice
- Minipigs are used for development of new compounds with:
  - No background data
  - New modalities
- First compound tested on minipigs has recently been approved by FDA

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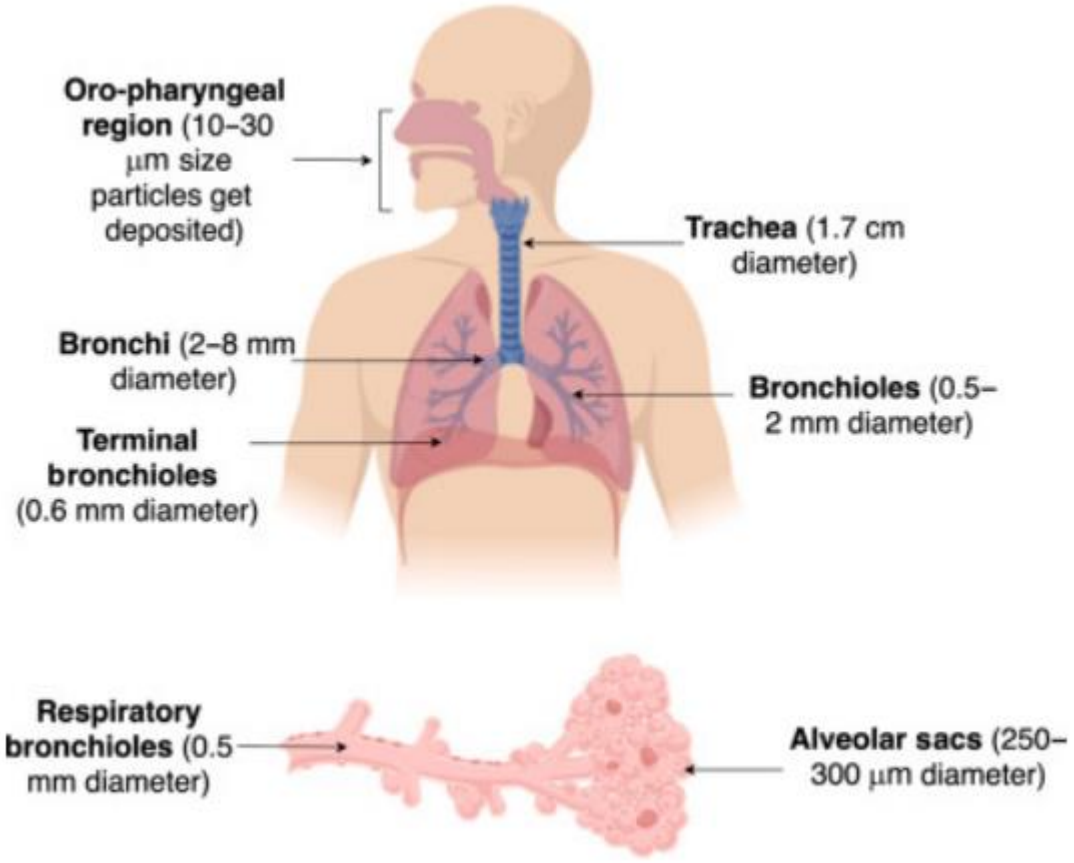
Targeting the respiratory system



https://www.sciencedirect.com/topics/pharmacology-toxicology-and-pharmaceutical-science/particle-deposition

Respiratory dosing comprises an umbrella of methodologies and techniques aimed at delivering compounds to the respiratory system

Administration to the respiratory system can be:

- Intranasal
  - Systemic and local effects
  - Highly vascularized and high membrane permeability
  - Easy and non-invasive self-administration (clinic)
  - Must be aware of volume, congestion, and local effects
  - Influenced by nasal anatomy and physiology
  - Device selection is dependent on formulation and species
- Intratracheal
  - Systemic and local effects
  - Anesthetized intubation limits toxicology assessments but provides POC, PK, PD data
  - Intratracheal instillation is a less physiological means for particle delivery, it allows for precise dosing of particles into the lungs at a specified time
- Inhalation (delivery of airborne particles to respiratory tract)
  - Conscious animal
  - Diffuse distribution to respiratory tract
  - Lung delivery
  - Repeated doses (regulatory studies)
  - Influenced by breathing patterns
  - Requires training and expertise

Targeting the respiratory system

	Intratracheal	Intranasal	Inhalation
Benefits	Good for very early screening studies	Good for upper airway or nasal disease targets	Clinical route of administration
Compound requirements	Small quantities	Small quantities	Large quantities
Deposition	Localized upper airways Variable respiratory tissue distribution Limited distribution to periphery	Localized upper airways Variable respiratory tissue distribution Limited distribution to periphery	Diffuse throughout lung airways and parenchyma
Efficiency of delivery	100%	~40-60% (remaining swallowed) depending on volume	~10-20% of total animal dose deposits in lung tissue (dependent on particle size)
Formulation	Solution/fine suspension (and powder)	Solution/fine suspension (and powder)	Any including dry powder
Pre-dosing aerosol characterisation requirement	None, relative to any other dose route	None, relative to any other dose route	Generation device Chamber/mask selection Aerosol concentration Particle size

Why inhalation as a route of administration?

Why?	Why not?
Local delivery to the respiratory tract	Animal compliance and cooperation?
Rapid adsorption due to high surface area and good vascularisation for respiratory and systemic therapies	Local irritancy of the respiratory tract
Avoidance of degradation in GI tract and first pass effect	Special apparatus is required
No real toxicology/minimal side effects	Higher cost of development
Patent extension when incorporated into a clinical device – “Evergreening”	High barrier to entry
Drugs that are not absorbed orally can be delivered via the lung	Particle size control
Easy methodology – painless, convenient and less intrusive and comfortable for the patient	Limited number of approved excipients
Improved stability compared to parenterals	
Misconception limited number of target diseases and compound classes	
Storage and transportation options may be simpler	

Minipig compliance to procedure is essential

- Dosing conscious pigs is essential
- 1 plus-hour repeat dose inhalation exposure
- Large numbers of pigs
- Acclimatisation
- Habituation/training

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Acclimatisation

- Consistent interaction with minipigs from arrival
- Habituate to staff
  - Hand feeding fruit/treats
  - Actimel via syringe
- Socialisation with staff in pens
- “Getting to know” individual preferences and inter-individual differences

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Equipment

- Harnesses
- Masks
- Inhalation suites
  - Minipig inhalation benches
    - On floor
    - Padded up with soft and comfortable bedding (vet bed, comfort bolsters)
  - Quiet environment (away from house pen)
  - Dim lighting
- Inhalation system
  - Set up and ready for sham dosing
- Lots of treats!

https://www.ezydog.co.uk/crosscheck-harness

Habituation/training

- Positive reinforcement
- Harness and lead
- Walking to inhalation suite (voluntary)
- Walking on inhalation bench (voluntary)
- Bench restraint
- Mask training
- Sham dosing
- Gradual increments in session durations

A successful training is a CONSISTENT training

Staff

Techniques

Location
(i.e., dosing suite)

Animals
(i.e., always in same groups)

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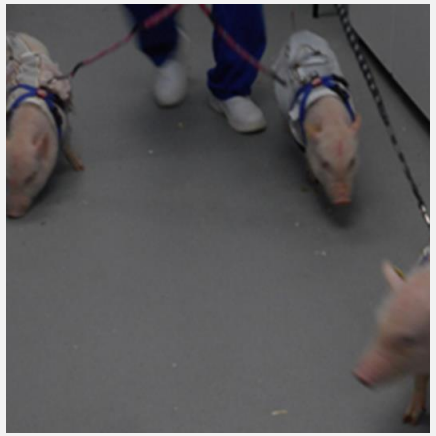
Habituation/training – Positive reinforcement



Harness and lead

Walking to inhalation suite (voluntary)

Bench restraint

Gradual increments in session durations

Walking on inhalation bench (voluntary)

Mask training

Gradual increments in session durations

Images: Labcorp

Restraint training example

- Driving principles
- Increasing duration of total time of restraint and mask time
- Airflow per animal depending on age/size
- Aim to achieve consistent behaviour observed

Day	Total Time of Restraint (Mins)	Regime
1	15	Restraint only
2	15	Restraint only
3	15	Restraint only
4	15	Restraint only
5	30	Restraint only
6	45	Restraint only
7	60	Restraint only
8	60	Mask with air
9	60	Mask with air
10	60	Mask with air
11	60	Mask with air
12	60	Mask with air
13	60	Mask with air
14	60	Mask with air

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Considerations on training

- Wide variety of temperament
  - Sit or lie quietly
  - Stroked
  - Vocal on handling
- When training starts early and is consistent
  - Can learn quicker than dog
  - Easy to handle and habituate

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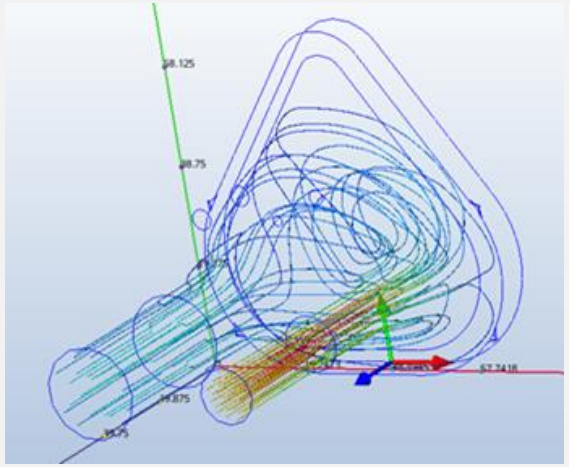
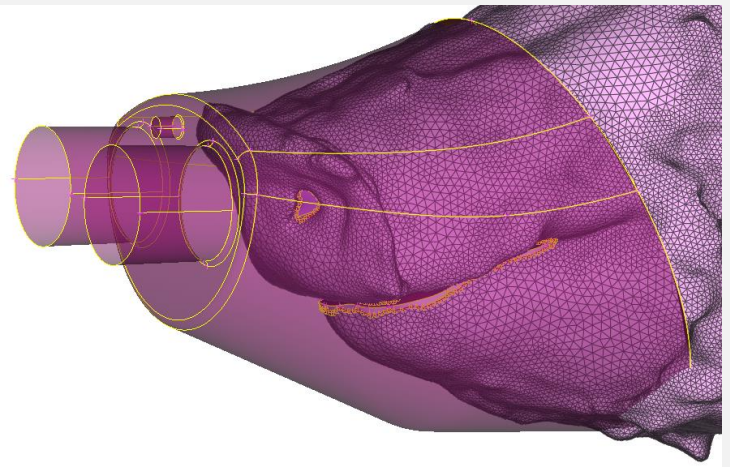
Mask design and sizes

- AutoCAD® software merges 3D scanned images
- Masks are 3D scanned, designed and printed
- Better mask fitting designs for large animals
  - “Personalised” mask fitting
  - Shape and depth
  - Entry and exit ports
- Ensures no CO_2 accumulation
  - Malleable seal
  - Improved compliance

Images: Labcorp





Continuous refinement cycles
Through CAD 3D modelling and Computational Fluid Dynamics

Improved animal welfare
Improved compliance
Study conduct
Study Integrity

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Masks in use

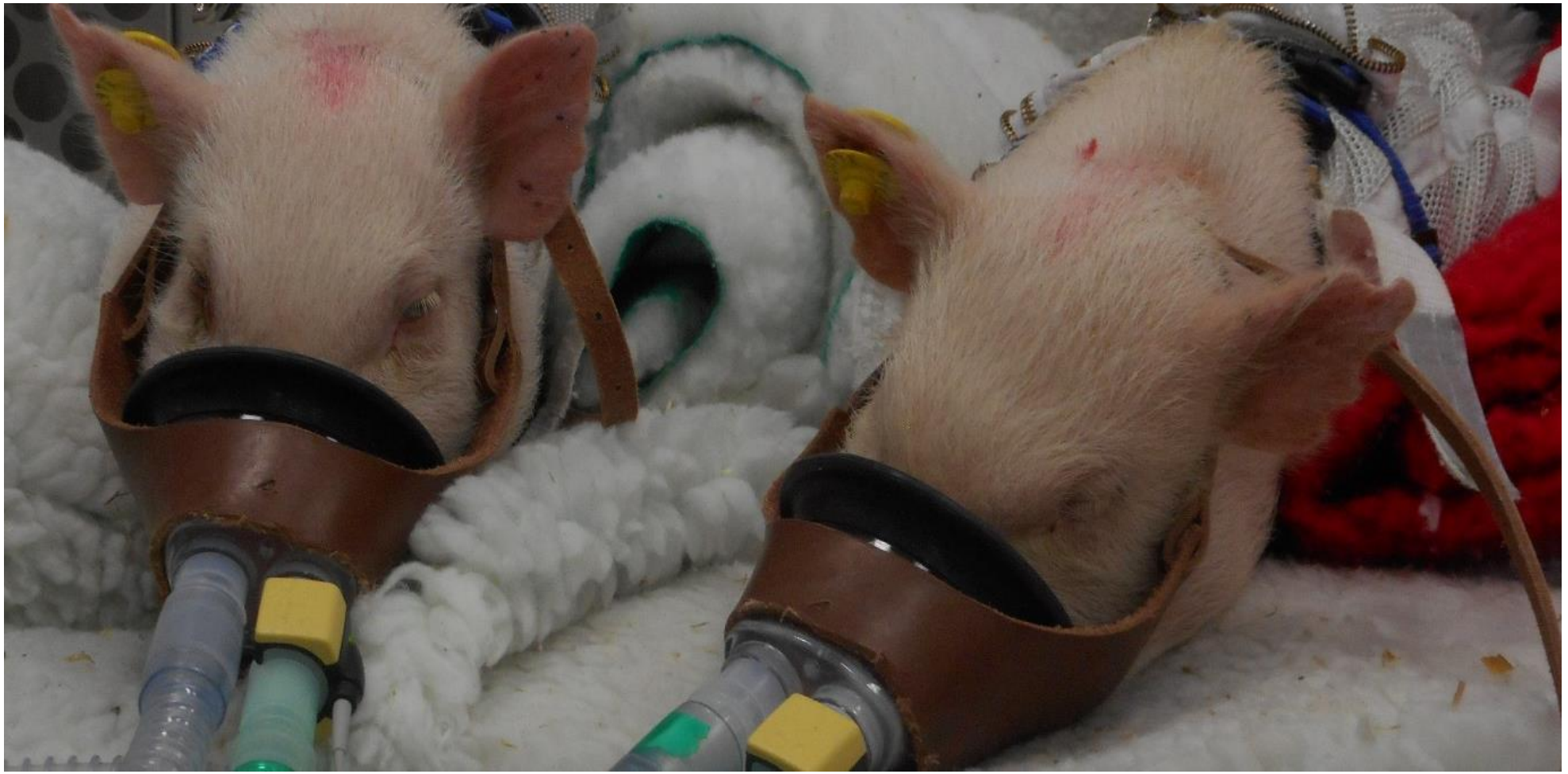
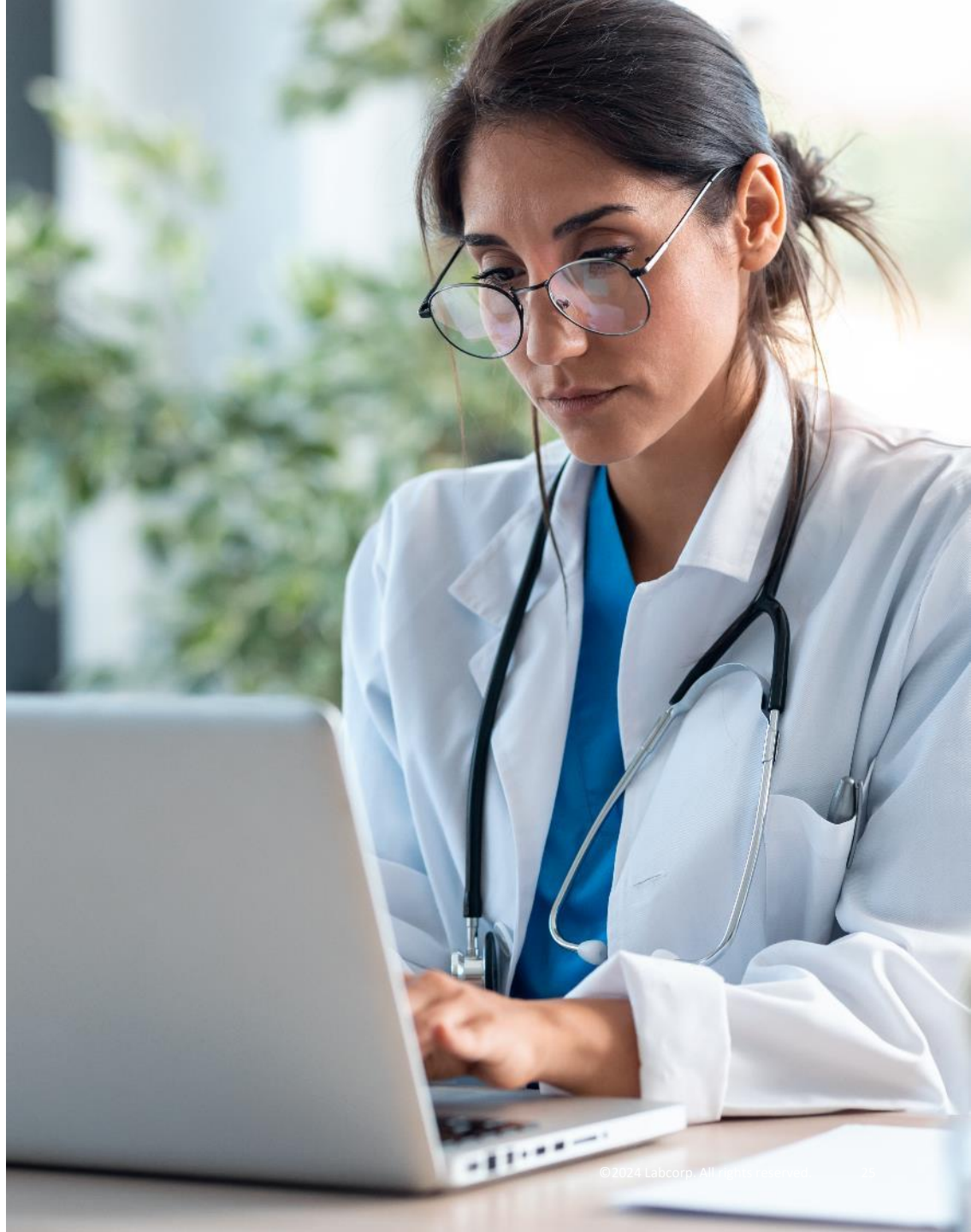


Image: Labcorp

Safety monitoring

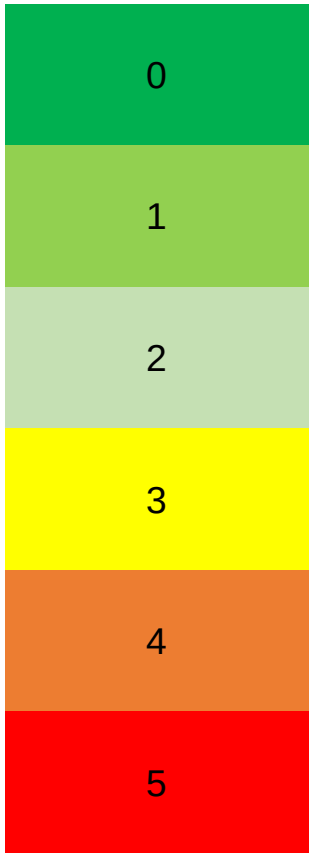
- Constant supervision
- Behavioural scoring
- Capnography



Constant supervision

- Staff continuously present
- High staff/minipig ratio:
  - 1-to-1 staff-to-minipig ratio during training and until animal settles
  - Then a max of 1 to 2 staff-to-minipig ratio

Behavioural scoring



No observation of aversive behaviours, e.g., trying to remove mask or leave bench

Occasional observation of aversive behaviours, e.g., trying to remove mask or leave bench

Occasional to intermittent observation of aversive behaviours, e.g., trying to remove mask or leave bench

Intermittent observation of aversive behaviours, e.g., trying to remove mask or leave bench

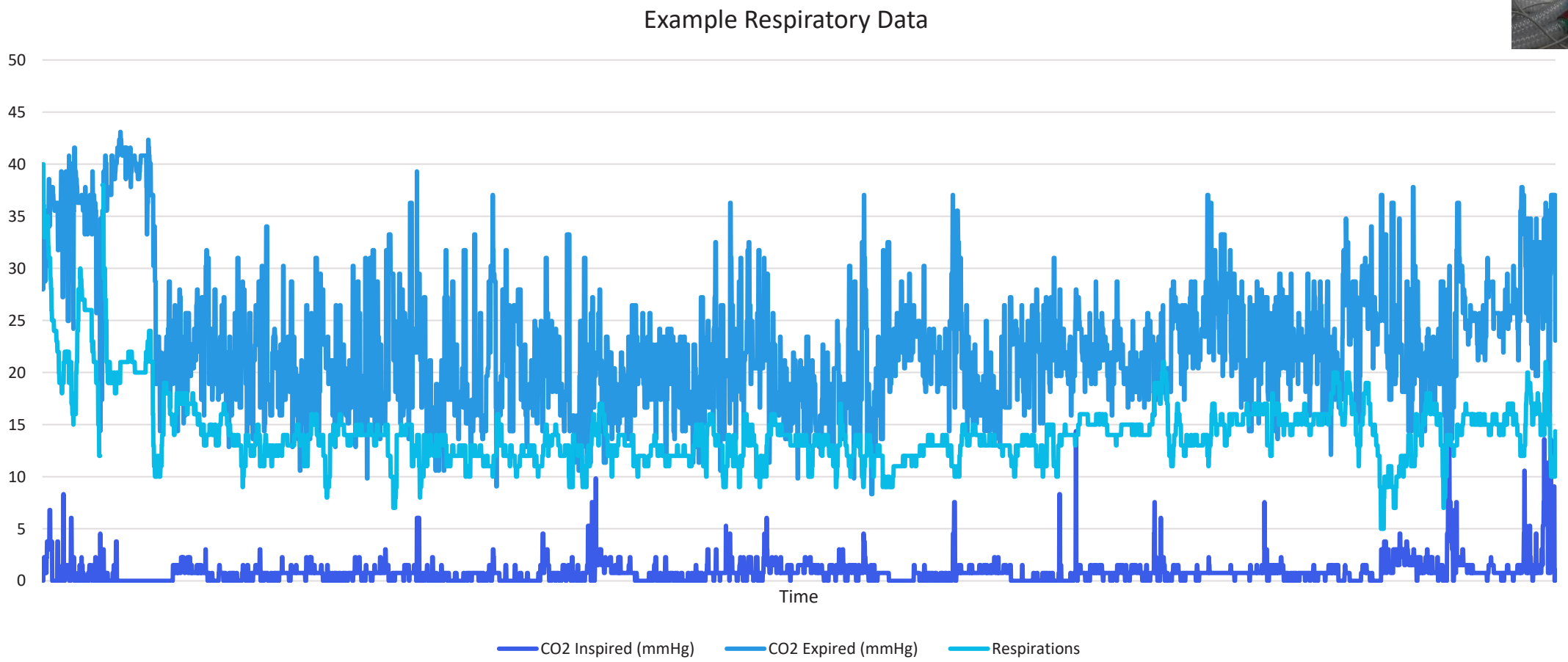
Intermittent to repeated observation of aversive behaviours, e.g., trying to remove mask or leave bench

Repeated observation of aversive behaviours, e.g., trying to remove mask or leave bench

Capnography provides real-time data about respiratory rate, depth and quality

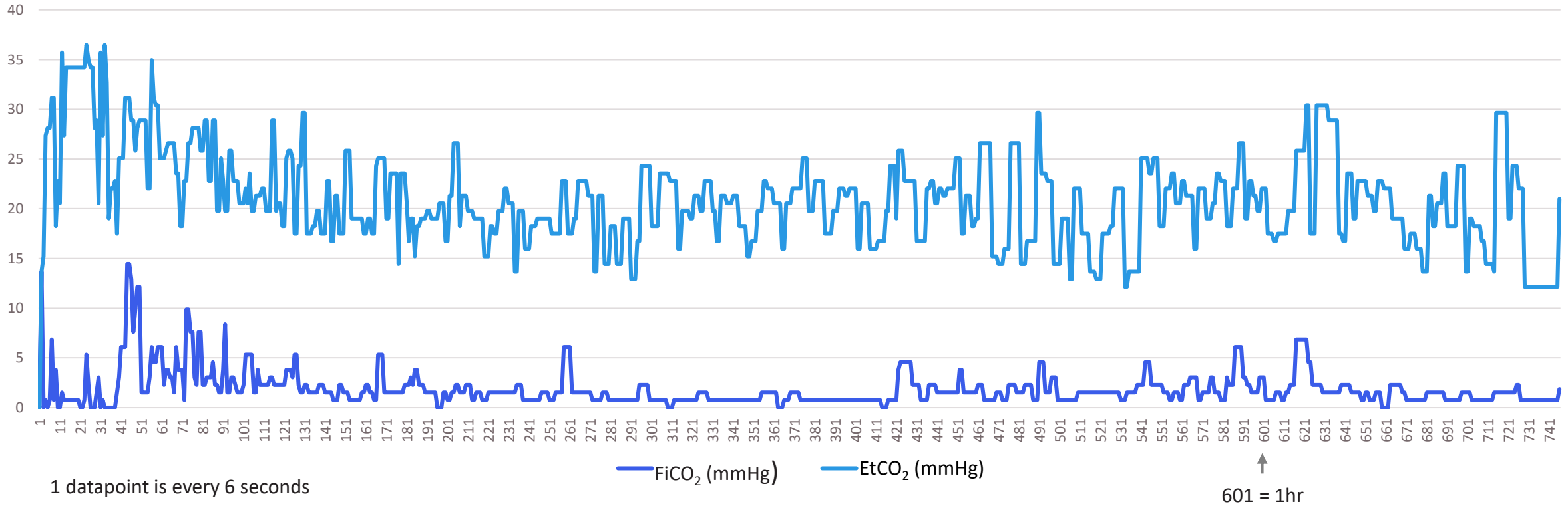
Image: Labcorp

Capnography



Capnography – Mainstream data

Real-time data about respiration rate, depth and quality



1 datapoint is every 6 seconds

Training highlights

- Start as early as possible
- Aim at training over consecutive days
- Gradual approach
  - Harness → walking to inhalation suite → walk on to the bench → bench restraint → mask → sham dosing (air)
  - Harness acceptance more difficult than mask acceptance!
- Continuous safety monitoring
  - Constant supervision
  - Behavioural scoring
  - Capnography
- Positive reinforcement (lots of treats)
- Consistent staff and groups of animals
- Account for inter-individual differences
  - Most (but not all) animals start with standing position (first few days) then prefer to sit/lie down and be stroked
- Account for target exposure duration (longer exposures/long studies may need longer training)
- Be vigilant on detecting signs of stress/tiredness

Inhalation system checks

- Performed by specialised and dedicated scientific and technical staff
  - Equipment
  - Expertise
    - Species-specific
    - Specific to inhalation route of administration
  - GLP and regulatory
- Failure to perform checks could result in:
  - Overdosing
  - Underdosing
  - Animal welfare incidents/issues
  - Reputation

Physiology to consider for calculating delivered dose

Respiratory Tidal Volume or Tidal Volume (TV) = Volume of air exchange in a single breath cycle (mL/Kg)
Influenced by: size of animal, age, sex, cardiovascular fitness, lung size-to-body weight ratio, length of respiratory tract, pathological changes

Respiratory Minute Volume (RMV) = TV x Respiratory Rate (RR) (L/min)
Influenced by levels of activity, stress

Habitual nasal breathers (breathe primarily through nose but can breathe through mouth if required)
Small lung size compared to body weight

Species	Lung Size as Percentage of Body Mass (%)		Reference
	Male	Female	
Göttingen Minipig (8 weeks)	0.951	0.911	Background Data | Ellegaard Göttingen Minipigs
Göttingen Minipig (6 months)	0.570	0.535	
Beagle Dog (6 months)	1.02	0.94	Choi et al., 2011
SD Rat (6 months)	0.43	0.53	Piao, Y., Liu, Y. and Xie, X., 2013
Cynomolgus Macaque (infant)	1.4 (males and females)		Amato et al., 2022
Cynomolgus Macaque (adult)	0.7% (males and females)		
Human (adult)	1.62	1.34	Mubbunu et al., 2018

RMV and delivered dose

- Alexander Equation (Alexander et al., 2008)
  RMV (L/min) = 0.608 x BW (kg)$^{0.852}$
- Estimate for anaesthetised minipig ~ 6-8L/min

Is this accurate for minipigs?

$$DD\ (mg/kg) = \frac{C\ (mg/L) \times RMV\ (L/min) \times D\ (mins)}{BW\ (kg)}$$

DD = Delivered Dose; C = aerosol Concentration; RMV = Respiratory Minute Volume; D = exposure Duration; BW = Body Weight

Due to low lung-to-body weight ratio, mean RMV values are considerably lower than known algorithms

Inhalation study design

- Same design as other routes of administration but:
  - Inhalation-specific habituation and training
  - Inhalation-specific equipment considerations and setup
    - Test item formulation (liquid, powder, gas)
    - Adequate monitoring equipment (i.e., capnography)
  - Standard study size is achievable

- Considerations for minipig inhalation studies:
  - Propensity for snuffling with regards to breathing pattern and RMV assessment
    - Need to prevent excessive dose inhaled per single breath
  - Compound requirements is less of a concern compared to other routes due to the non-linear BW relationship with dose
  - Elevated HR post-feeding
    - Dose prior to feeding

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Compound requirements considerations

- Larger body weight growth relative to dogs or primates
- No proportional increase as with other routes of administration
- The same or lower amount than dogs due to the indirect method of dose calculation (based on RMV)
- Increase due to higher airflow needed due to greater non-compliance at lower airflows
  - Only 20-30% increase even with a BW of a 14kg dog and 20kg minipig
- 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

https://www.technologynetworks.com/drug-discovery/news/ai-speeds-up-synthesis-of-important-chemical-building-blocks-367102

Toxicokinetic sampling

Standard sampling

- Sampling site: ++jugular vein
- During exposure is challenging
- Animal response
  - Vocalisation
- Insufficient sample
- 5min IAD probably first sample (unless microsampling or vascular catheters)
- V-frame or sling used for later samples
- Continuous refinements in sampling techniques

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Conclusions

- Multiple reasons for choosing minipigs (ethical, industry-related, scientific)
- Increased interest in minipig as non-rodent species in toxicology
- High translational value due to similarities to humans
- Multiple disease targets both respiratory and systemic
- Inhalation valid route of administration when targeting the respiratory system
- Acclimatisation, habituation, training crucial for successful study conduct
- Specific delivery system and monitoring required
- Study design similar to other routes of administration but inhalation- and species-specific dose calculations/compound requirements
- Considerations for blood sampling timepoints

Overall, a robust model for non-rodent regulatory inhalation work

Thank you for listening!

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Q&A

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Question	Answer from presenter
If dosing is only required on a weekly basis, do you see reversal of habituation/training?	Whenever dosing is not on a daily basis, non-dosing days are used to continue the training, to avoid reversal of training.
Can you please throw some light on Re-use of animals (in case the animals are not sacrificed, and the diagnosis is made with only respiratory function tests as per study protocol) and the required wash out period between subsequent studies	Usually, anatomic pathology and histology are included in regulatory toxicology studies, so re-use would not apply. Safety pharmacology studies would not necessarily include post-mortem endpoints. In these cases (and providing that the Project licence allows for re-use of animal) we would allow plenty of time for washout (likely a minimum of 4 weeks), but specific considerations are made on a case-by-case basis
How much in advance (to study start) do you bring the animals onsite, considering training involved and also the animals might take longer time for training/compliance?	Minimum 1 month before study start, but often we account for longer times depending on study requirements, number and age of animals in a batch
And according to your experience, is there low animal to animal variation compared to dog?	We would expect one or two animals per batch to require tailored training. Generally speaking, this would not significantly differ from what is expected in dogs. Within each group of animals, interindividual variation has to be considered but, similarly to dogs, animals tend to emulate each other (albeit we always account for exceptions)
Please could you comment on background pathology in the respiratory tract, and comparison to other species?	We have attached 3 scientific papers that provide information on background pathology in minipigs. We also have our own internal HCD (ed. Historical Control Data).
Is there anything which could confound interpretation of TI effects?	Like any other route of administration, spontaneous diseases and environmental factors, stressful events could confound TI effects. However, we tend to minimise any external factors

Question	Answer from presenter
Is there any problems related to salivation during the inhalation study? And if it happens, how do you solve that problem?	Salivation can happen during dosing. Close monitoring of the animals is essential. When it is felt that the salivation might interfere with the efficiency of delivery, we can quickly remove the mask and dry out. Should there be excessive salivation, animals might be removed for dosing
How does the habituation/acclimatisation in Minipigs compare to Dogs/NHP? Are there more animals that were not able to complete habituation/put on study?	There are some differences due to different inhalation systems and restraint methods typical of each of the 3 species, for example with dog inhalation benches being elevated from floor. This call for species-specific steps in the training. On average the number of animals resistant to training is low and comparable among the 3 species
What is the maximum duration (months) of studies you have completed?	We have successfully completed 1 months and do not perceive it as an issue going beyond that duration
Due you perceive it feasible to run 13w and 39w studies or would minipigs studies be suitable only for short term tox studies (4 weeks) i.e. the clinical indication needs to be considered?	We do not see as an issue running chronic studies
Can you describe the minipig immunome in a few more words?	Increasing knowledge of the pig immunome can lead to a multitude of application clinically relevant for humans. Here a couple of papers as examples: https://bmcgenomics.biomedcentral.com/articles/10.1186/1471-2164-14-332 https://www.stemcell.com/establishing-the-pig-as-a-large-animal-model-for-vaccine-development-against-human-cancer.html

Question	Answer from presenter
It is really cool that you design and print your own masks. Can others buy them? (since they might fit more Göttingen Minipigs)	We would be happy to sell masks to a company providing there was no conflict of interest
Are the minipigs sniffing when they have the mask on? My experience is that they are sniffing when they root, but not in general when breathing.	This depends on factors such as animals getting excited or tasting the compound. I would not say it is on a continuous basis, but it can be observed. We have taken RMV assessments during dosing and we have not had to exclude data as a result of sniffing. The animals preferentially sniff when they are in their pens (and rooting for food) and not during the inhalation dosing.
Would it be possible to put in a catheter e.g. ear vein catheter before study start? This way you could have continues blood sampling during dosing.	Yes, it is possible. In that case considerations must be made on number of allowed anaesthetic occasions during the study and possible disruptions in dosing days (i.e. suspending dosing on the days when the catheter are placed). From Ellegaard Göttingen Minipigs: or place the catheter before study start and maintain it during the study.

Referred scientific papers:

Spontaneous Background Pathology in Göttingen Minipigs: https://journals.sagepub.com/doi/10.1177/0192623314538344

Background Pathological Changes in Minipigs: A Comparison of the Incidence and Nature among Different Breeds and Populations of Minipigs: https://journals.sagepub.com/doi/10.1177/0192623315611762

International Harmonization of Nomenclature and Diagnostic Criteria (INHAND): Nonproliferative and Proliferative Lesions of the Minipig: https://journals.sagepub.com/doi/10.1177/0192623320975373