PRECLINICAL DEVELOPMENT FOR INHALATION DRUGS

Paul Smith - Manager Inhalation Toxicology

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HICKORY GOLF IN GULLANE

Twilight on Gullane Hill

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RESPIRATORY DISEASE

A few numbers

Worldwide:

- 235 million with asthma
- > 200 million with COPD
- > 8 million develop TB annually
- Rates rising in developing countries

Deaths annually worldwide:

- > 4 million from ARS
- > 1.3 million from lung cancer
- > 1.3 million < 5 yr olds from pneumonia

Respiratory market estimated ca. $50 billion by 2022

INHALATION THERAPY

- Inhalation is the preferred treatment of respiratory disease
- Medication is inhaled
  - DPI's and pMDI's via mouth
  - Nebulisers via face mask
- Local therapy - at site of action
- Advantages
  - Avoids first pass metabolism / breakdown in GI tract
  - Therefore lower doses could be required which
  - Reduces risk of side effects
  - Onset may be more rapid
  - Suitable where oral bioavailability is poor
  - Painless (no needles) – improves compliance

REGULATORY OVERVIEW

COUNTRY	REGULATORY AGENCY
Europe (London base will change after Brexit)	European Medicines Agency – EU agency for evaluation of Medicinal Products (EMA) Centralised: pharma submit a single MA. Compulsory for medicinal products from biotechnology, orphan products, advanced therapy (such as gene therapy), new actives for various diseases such as diabetes, cancer, HIV/AIDS, degenerative diseases Decentralised: for those outside the scope of the centralised procedure based on mutual acceptance or recognition of national authority
	European Commission - Considers recommendations from EMA
	Several committees including Committee for Medicinal Products for Human Use (CHMP), Orphan Medicinal Products (COMP) and PDCO (paediatric)
Japan	Ministry of Health, Labour and Welfare (Kosei – lodo – sho) Regulates drugs and biological products
	Pharmaceutical and Food Safety Bureau Evaluates efficacy, safety and quality
United States	Food and Drug Administration (FDA) • Centre for Drug Evaluation and Research (CDER) • Centre for Biologics Evaluation and Research (CBER)

REGULATORY OVERVIEW

ICH = International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use

- Brings together authorities from Europe, Japan and USA and experts from pharmaceutical industries to expedite global drug development
- QSEM (Quality, Safety, Efficacy, Multidisciplinary) Guidelines
  - Q = CMC of drug + drug product
  - S = Safety, e.g. Carc, TK/PK, Immunotox, Safety Pharm, Biotech (S6)
  - E = Clinical trials
  - M = Cross-cutting topics, e.g. M3(R2), M4 (CTD), M8 (eCTD)

- CTD assembles all the QSE information into a common format and harmonises the drug approval process
- Is the mandatory process in Europe and Japan for MAA and also, since April 2017, in USA for a NDA

ICH REQUIREMENTS

CTD - 5 modules

1. Region specific, administration, prescribing information
2. Summaries (pharmacology, mode of action, proposed clinical use, non-clinical and clinical overview)
3. Quality topics: CMC, drug substance, drug product
4. Non-clinical study reports
5. Clinical study reports

REGULATORY COMPLIANCE

Standard approaches for the preclinical development of conventionally administered drugs also apply to most respiratory drugs (ICH M3(R2))

Modifications as necessary

- Inhalation
- Intranasal

Dosing device (drug product) - Pharmaceutical Quality Guideline, CHMP, 2006 [not non-clinical *per se*], e.g.

- Excipients and extractables
- Dose Uniformity
- Droplet size distribution
- Leak rate
- Actuator / mouthpiece deposition

REGULATORY COMPLIANCE

Small molecules

Preclinical safety evaluation (ICH M3(R2))

Genetic toxicology

- Mutagenicity
- Chromosomal damage

Safety pharmacology

- Respiratory
- CNS
- Cardiovascular

Animal toxicity studies

- Two species (rodent and non-rodent)
- Single dose (rarely done)
- Preliminary range finding
- 14/28 day repeat dose

Pharmacokinetics

REGULATORY COMPLIANCE

Biologics

Arguably less rigid than for small molecules

ICH S6 (R1)

- Product should be comparable as for initial clinical studies
  - due to risk of host cell contaminants e.g. bacteria, yeast, plant
- Careful selection of species
  - target sequence homology / in vitro assays / functional activity
  - 2 species (if appropriate) for 1 month studies
  - 1 species (rodent) for longer studies (6 months for chronic use)
- Use proposed clinical route
- Consider dose levels (dose frequency as clinical study)
  - PK/PD approaches to find high dose that gives max. pharmacological effect or 10-fold multiple of clinical dose

REGULATORY COMPLIANCE

Biologics

- Immunogenicity (ADA)
- Safety pharmacology
- Pharmacokinetics
- Reproductive studies – case by case
- Genotoxicity – not applicable
- Local tolerance – as part of repeat dose
- Carcinogenicity – assessed on case by case
- CPMP guidelines

LEAD CANDIDATE SELECTION

High Throughput Screening

- Binding assays
- Activity assays
- Cell based assays

Combinatorial chemistry

- Produce library of a large number of chemicals

Molecular modelling

- Computer based techniques for deriving and manipulating structures and reactions of molecules

INHALATION PLATFORM TYPES

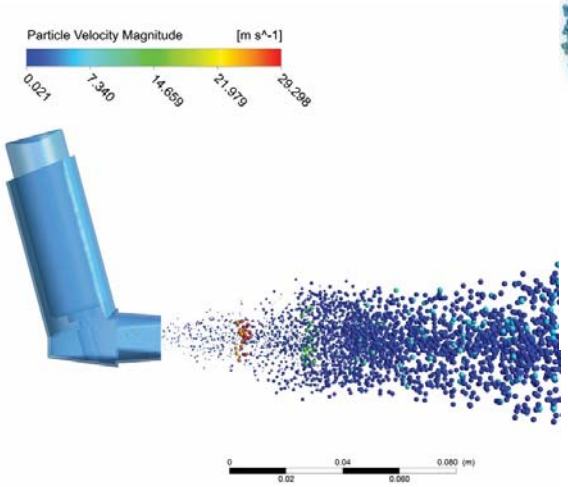
Inhalation Science (CRL-EDI) can work with any inhalation dosage form type. Each one brings its own challenges:

DRY POWDERS

Arguably the most complex platform, but with many advantages such as superior delivery characteristics (improved patient compliance, short dosing times and lung deposition) and shelf life stability

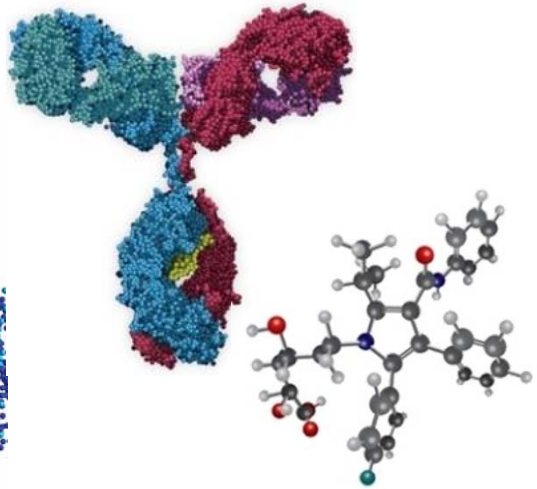
LIQUID AEROSOLS

Generally the easiest to formulate if aqueous solubility permits. Although electronic generation systems (compared to gas driven systems) have made them more portable, dosing times remain lengthy



METERED DOSE INHALERS

The most widely used inhalation platform, although the market is moving more and more towards DPIs. Montreal protocol has made reformulating challenging. Limits on phys-chem stability.



BIOLOGICS AND CHEMICAL

Broncho-pulmonary therapeutic area still dominated by classical chemical lead candidates, but an increasing shift towards biomolecules. Solid aerosol biologic therapies considered state of the art platform tech

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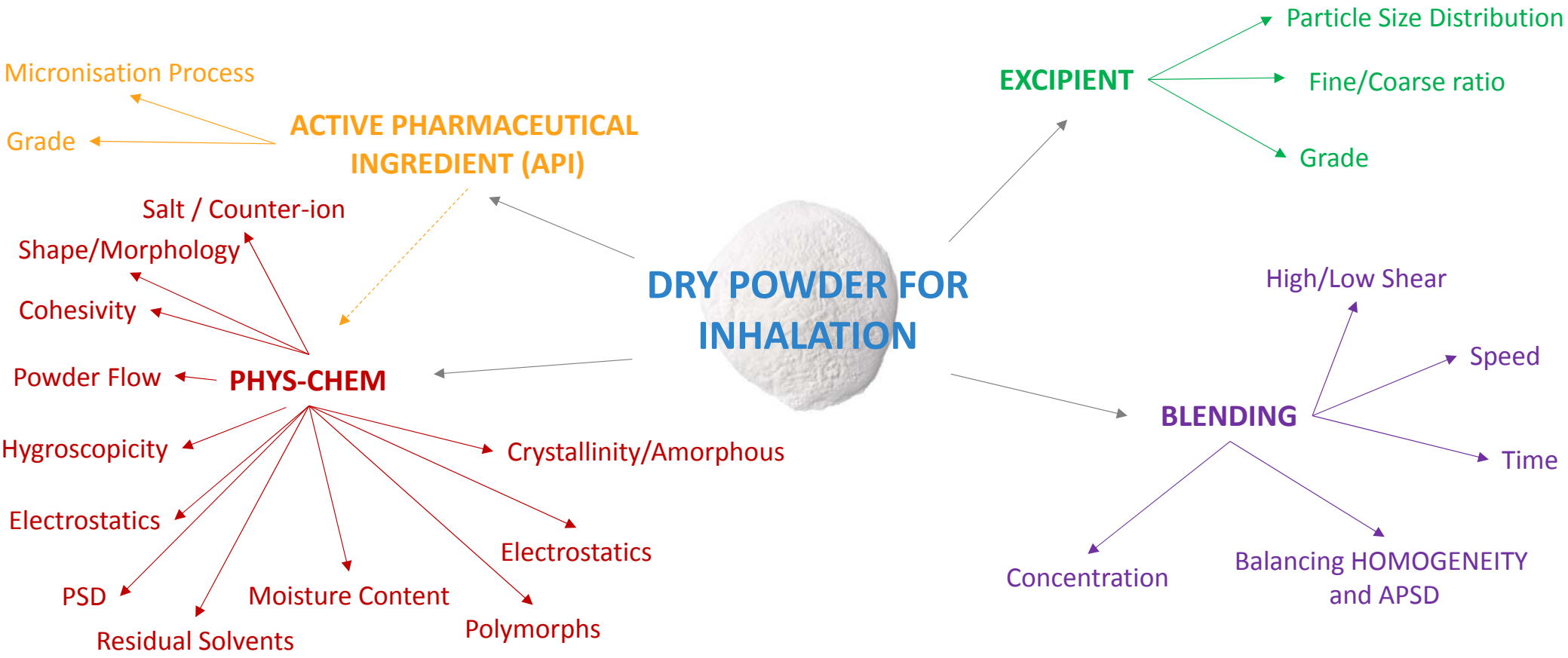
FORMULATION CONSIDERATIONS – DPI'S

DPI Formulation Considerations: Key Variables to Consider…

- This is a top level overview, but there are many more subtleties to consider...



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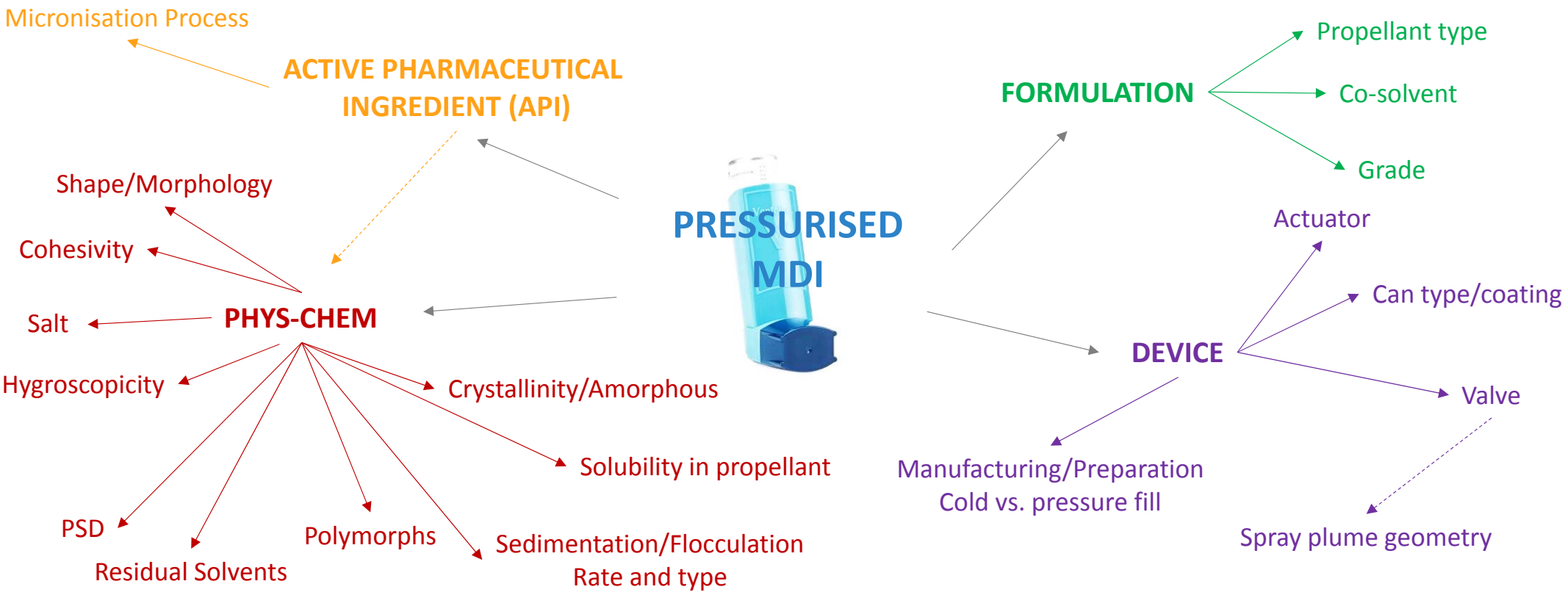
FORMULATION CONSIDERATIONS – PMDI'S

pMDI Formulation Considerations: Key Variables to Consider…

- Generally, these have much less variables the DPIs, but the device/formulation interaction, dominates performance.



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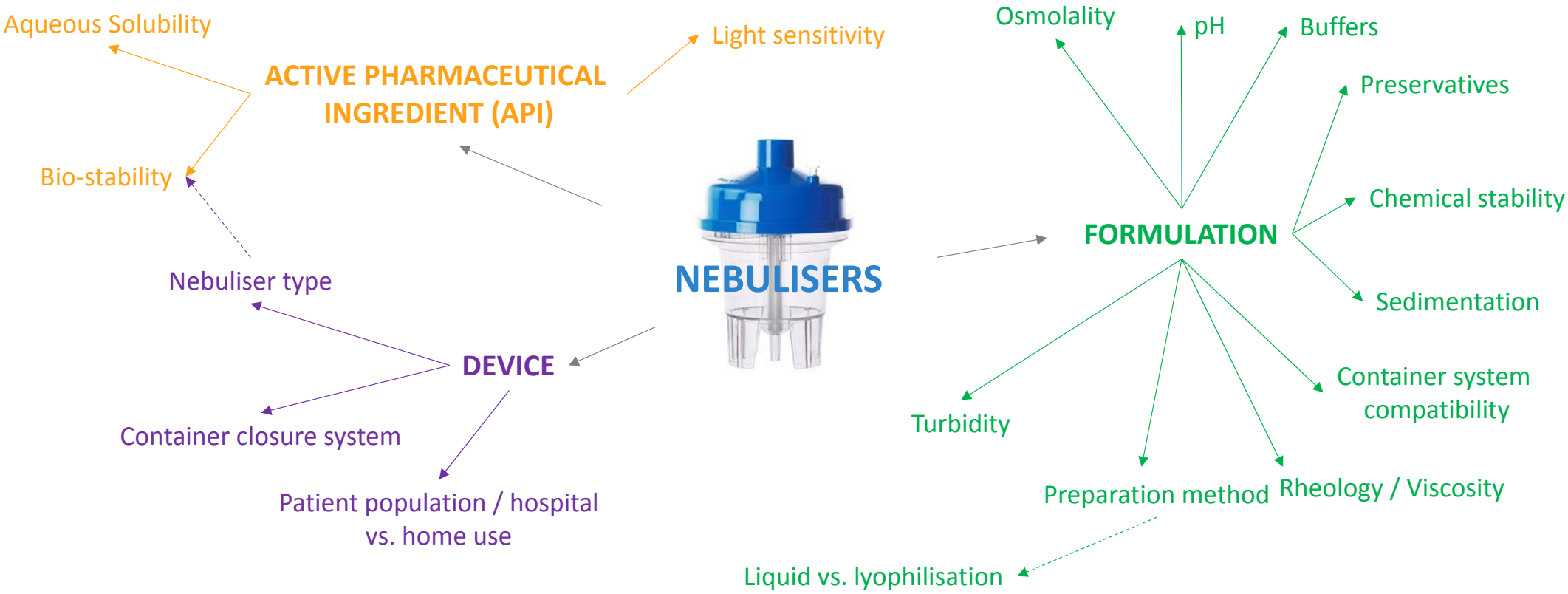
FORMULATION CONSIDERATIONS – NEBULISERS

Nebulizer Formulation Considerations: Key Variables to Consider…

- The simplest formulation to prepare but with several long stability and patient centricity issues...



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INITIATION OF PRECLINICAL TOXICITY STUDIES

Clinical nebulisers can be used in the preclinical environment, but may need to be modified

DPI's and pMDI's cannot

- Breath actuated
- Bolus delivery

Doses targeted in preclinical studies require

- Several multiples of the clinical dose
- Sustained delivery

Requires expertise in drug delivery to the animal model

DRUG REQUIREMENTS

Test item supply

- Inhalation administration requires more drug than other pre-clinical routes of administration, e.g. *10 mg/kg oral = 1.7g vs ca 50g inhalation = x30-fold higher*

Why is this?

- Need a dynamic air flow to meet breathing demand of animals
- Continuous supply of fresh aerosol to animals
- Significant losses in the aerosol generation and exposure system through deposition / sedimentation

LEAD-IN TIME FOR PRECLINICAL STUDIES

Test item supply

- Test item requirements for inhalation ca. x30 oral route

Analytical method development & validation

- 3-6 weeks to develop & validate method for filter and APSD analysis

Preliminary aerosol characterisation

- 6 weeks for aerosol technical trials, partly in parallel with validation

Time to start Maximum Tolerated Dose/Dose Range Finder

- 3-8 weeks from animal arrival to end of dosing

Up to 18 weeks for completion of development and dose range finding studies

PRECLINICAL INHALATION STUDIES

Methods of inhalation dosing for various species

Rats, mice, rabbits

- Nose only exposure in restraint tubes

Dogs

- Face mask +/- mouth tube

Primates

- Head only, face mask or helmet

INHALATION CHAMBERS

Rodents

Flow-past chamber

- Multiple tiers: up to 160+ animals
- Fresh aerosol to each port

Flow-through chamber

- Multiple tiers: up to 140 animals
- Flow through aerosol

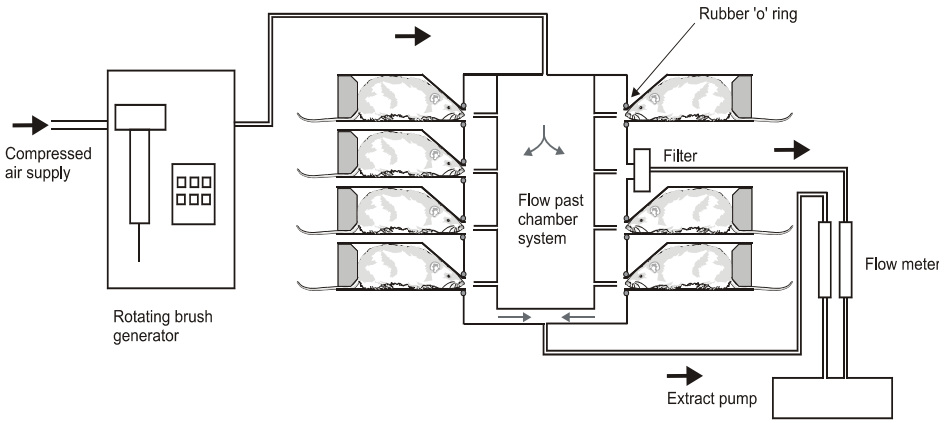
Tube restraint

- Polycarbonate tubes of different sizes with backstop

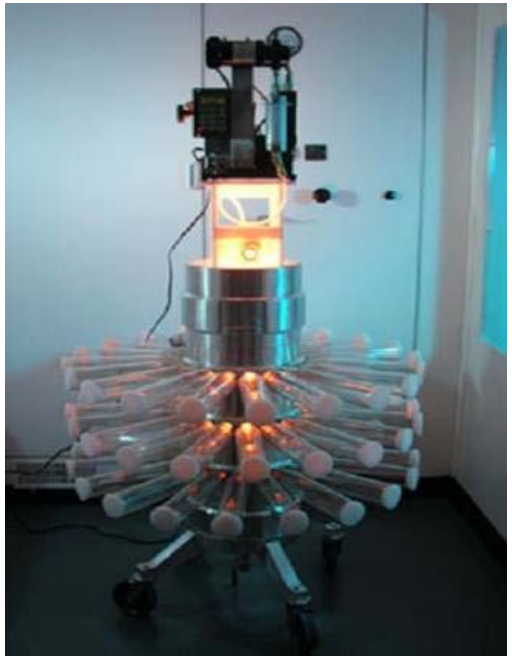
INHALATION EXPOSURE SYSTEM

Rodents

Flow-past chamber



Flow-through chamber



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INHALATION EXPOSURE SYSTEM

Dogs

Plenum type chamber

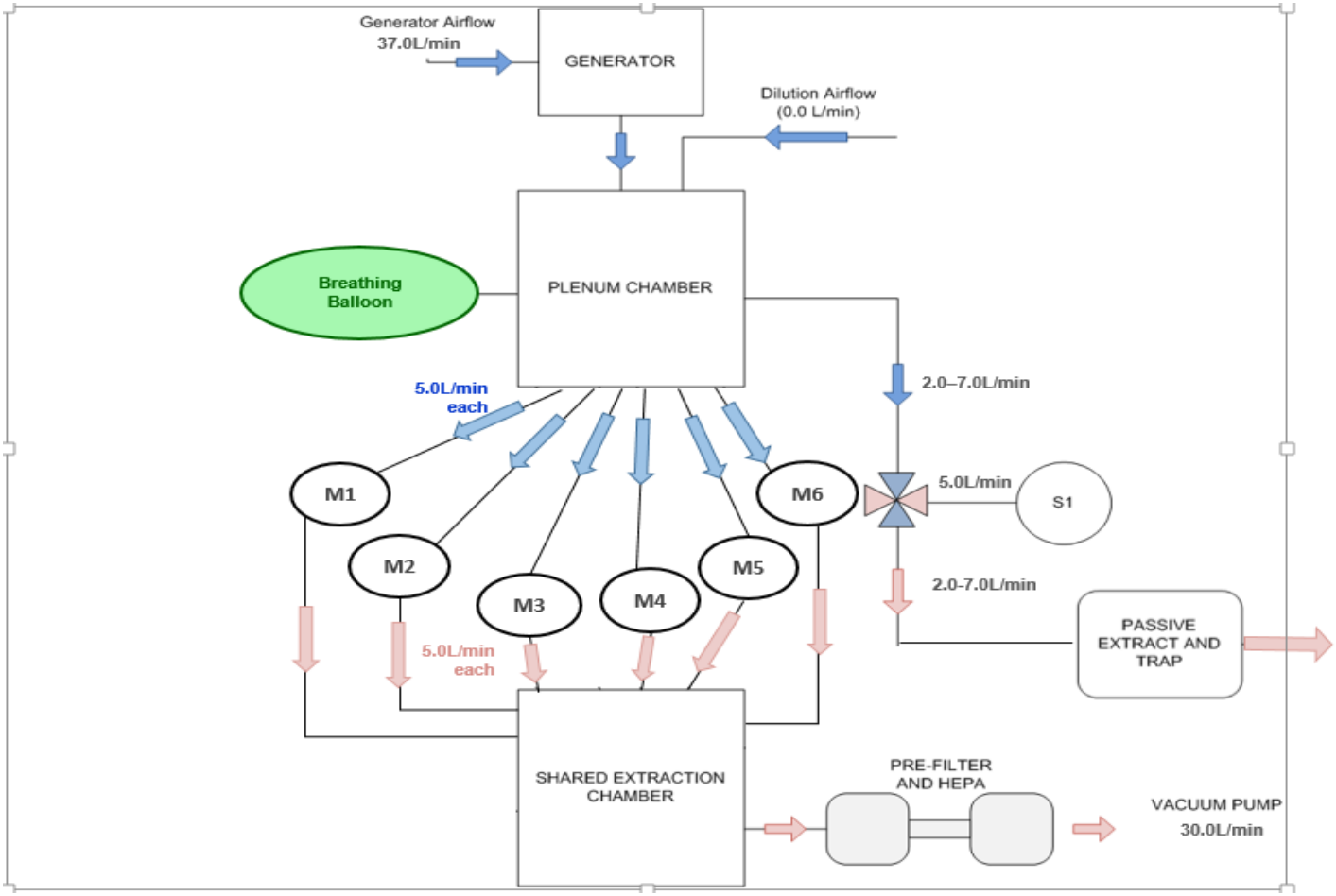
- Multiple masks attached
- Masks fitted with or without mouth tube

Sling restraint or platform

Extended or multiple exposures

10 days acclimatisation to dosing procedure

NON RODENT INHALATION SYSTEM



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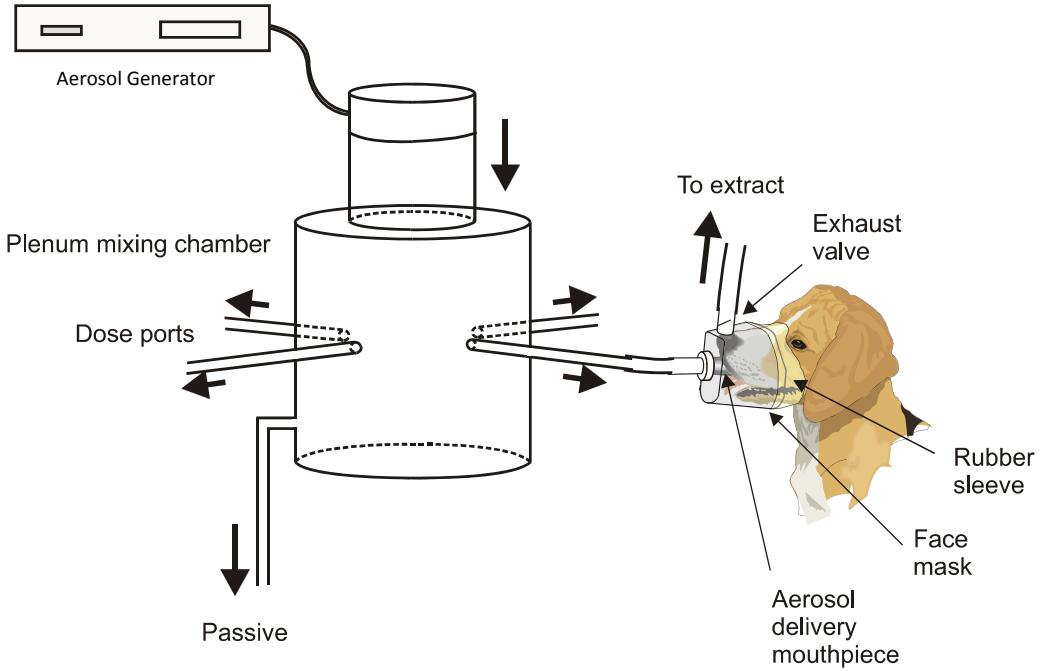
INHALATION EXPOSURE SYSTEM

Dogs



Platform restraint used for dosing

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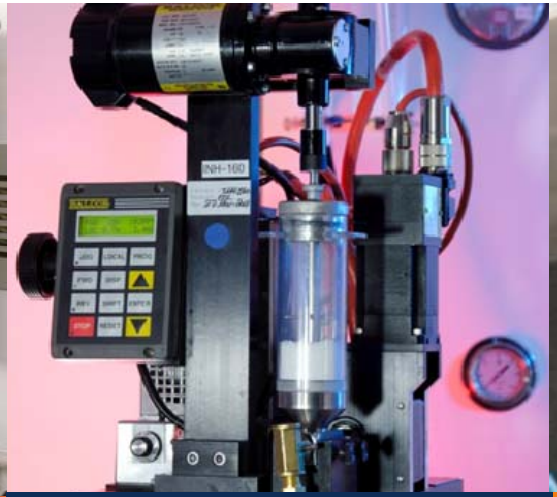
AEROSOL GENERATION

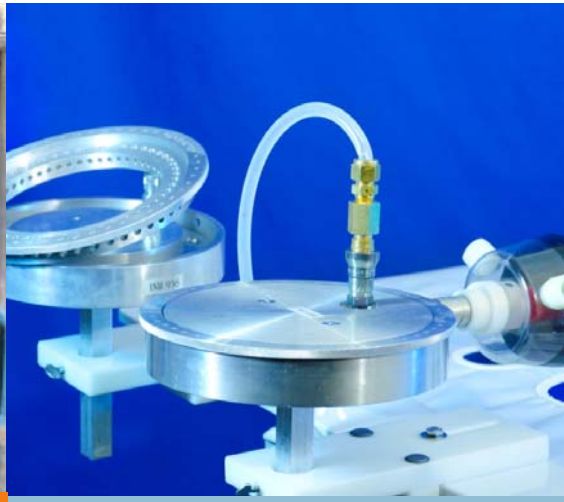
Powders

Rotating brush generator



EDPDS

Wright dust feed



MURS device

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AEROSOL GENERATION

Powders

	PALAS™ ROTATING BRUSH GENERATOR 1000	PALAS™ ROTATING BRUSH GENERATOR 2000	EMMS™ WRIGHT DUST FEED (WDF)
			
Technical Info*	**Volume Flow:** 0.5 – 5.0 $m^3 \cdot hr^{-1}$ **Mass Flow:** 0.04 – 430 $g \cdot hr^{-1}$ **Can sizes:** 7, 10, 14, 20, 28 mm	**Volume Flow:** 2.5 – 5.0 $m^3 \cdot hr^{-1}$ **Mass Flow:** 1.0 – 560 $g \cdot hr^{-1}$ **Can sizes:** 16, 20, 28, 32 mm	**Mass Flow:** 0.009 – 75.9 $g \cdot hr^{-1}$
Overview	The RBG is the primary workhorse in generating dry powder aerosols for inhalation toxicology studies. Lots of experience and its operation is well characterised	The larger version of the 1000, allowing for greater mass flows and aerosol concentrations to be generated	WDF is used for low aerosol concentration generation. It requires significant compaction to aid its operation, which is not suitable for all powders

AEROSOL GENERATION

Liquids

- Airjet nebuliser
- Electronic / mesh nebuliser
- Atomisers

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AEROSOL GENERATION

Liquids

Electronic / mesh nebuliser

- Software adapted
- Reservoir manufactured
- Side stream airflow to deliver aerosol
- Performance varies from mesh to mesh
- Output can vary with formulation strength
- Robust cleaning required

AEROSOL GENERATION

Metered dose inhaler

- Metered Dose Inhalers:
  - Automated Actuating Device
  - Number of actuations available per canister
  - Frequency of actuation

AEROSOL MONITORING

Pre-study aerosol characterisation

- 6 weeks prior to Day 1
- Determine most appropriate aerosol generator
- Most appropriate sampling media
- Conditions to achieve target aerosol concentration and respirable particle size
- System efficiency

Aerosol sampling

- Samples representative of animal breathing zone

Spatial & temporal variation

- Determined prior to initiation of dosing

AEROSOL MONITORING

Concentration

Aerosol monitoring equipment

- Filter sampling devices
- Liquid impingers
- Liquid traps/bubblers
- Real time aerosol monitors

Aerosol concentration

- Daily (gravimetric, at least once weekly analysis)
- +/- 10% of target
- <15% variation

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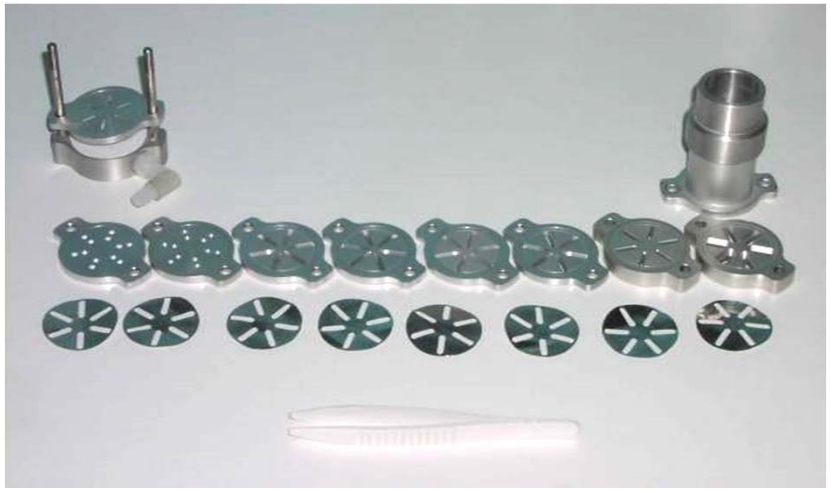
AEROSOL MONITORING

Aerodynamic Particle Size Distribution

Aerodynamic particle size distribution measurements

- Aerosol within respirable range for test species
- MMAD / GSD
- 1-3 µm rat, slightly larger for dog/NHP
- Cascade impactor
  - Marple, Andersen, NGI, Dekati, Mercer
- Gravimetric and/or analytical assessments
- Weekly to begin with lower frequency thereafter



AEROSOL MONITORING

Aerodynamic particle size distribution

	ANDERSEN CASCADE IMPACTOR (ACI)		MARPLE PERSONAL CASCADE IMPACTOR 296 (MPCI)	
				
No. of Stages	8 plus filter		6 to 8 plus filter	
Particle Size Range	0.25µm – 9.00µm		0.52µm – 21.3µm	
Airflow (L·min^{-1})	28.3		1.0 – 5.0	
Main Features	☑Stacked system ideal for limited space. ☑Easy to use ☑Readily accepted by MHRA / FDA	⊗Calibrated for a single flow rate	☑Stacked system ideal for limited space. ☑Easy to use	⊗Only available for low flow rates ⊗Small stage volume

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AEROSOL MONITORING

Aerodynamic particle size distribution

	DEKATI LOW PRESSURE IMPACTOR (LPI)		NEXT GENERATION IMPACTOR (NGI)	
				
No. of Stages	12 plus filter		7 plus filter	
Particle Size Range	0.03µm – 10.00µm		0.24µm – 11.7µm	
Airflow (L·min^{-1})	10.0 – 30.0		15.0 – 100.0	
Main Features	☑ Stacked system ideal for limited space. ☑ Enhanced granularity ☑ Able to analyse fine particles/droplets	⊗ Lots more stages – more resource and cost associated with analysis	☑ Specifically designed for inhaled drug products. ☑ Time-saving equipment readily available ☑ Readily accepted by MHRA / FDA.	⊗ Very bulky design and more difficult to use on study

SUPPORT SERVICES

Analytical chemistry

Analytical method development & validation

- Validated sponsor method can be used, however need to assess
  - Suitability of sampling media
  - Recovery efficiency from media
  - Stability on media and in extraction solution
  - Verify/develop chromatographic conditions
  - Determine LOQ
  - Establish range of detector linearity
  - Assay specificity
  - Check assay accuracy and precision
  - Formulation accuracy (if applicable)

SUPPORT SERVICES

Analytical Chemistry

Blending - powders

- Powder formulation prepared for GLP studies
- Uniformity of blend and concentration (% w/w)
- Stability

Formulation – liquids

- Concentration
- Stability

Filter analysis for aerosol concentration and particle size

- Active ingredient extracted from various filter types
- Liquid chromatography-tandem mass spectroscopy (LC-MS)
- HPLC & UPLC

ESTIMATION OF DELIVERED DOSE

Dosimetry – use standard formula

$$\textbf{Dose (mg/kg/day)} = \frac{\text{AC (mg/L) x RMV (L/min) x D (min)}}{\text{BW (kg)}}$$

AC = Aerosol Concentration of API

RMV = Respiratory Minute Volume (respiratory rate x tidal volume)

- Calculated based on algorithm using BW
- Can be measured by HOP or Pneumotach

D = Duration of exposure to the aerosol

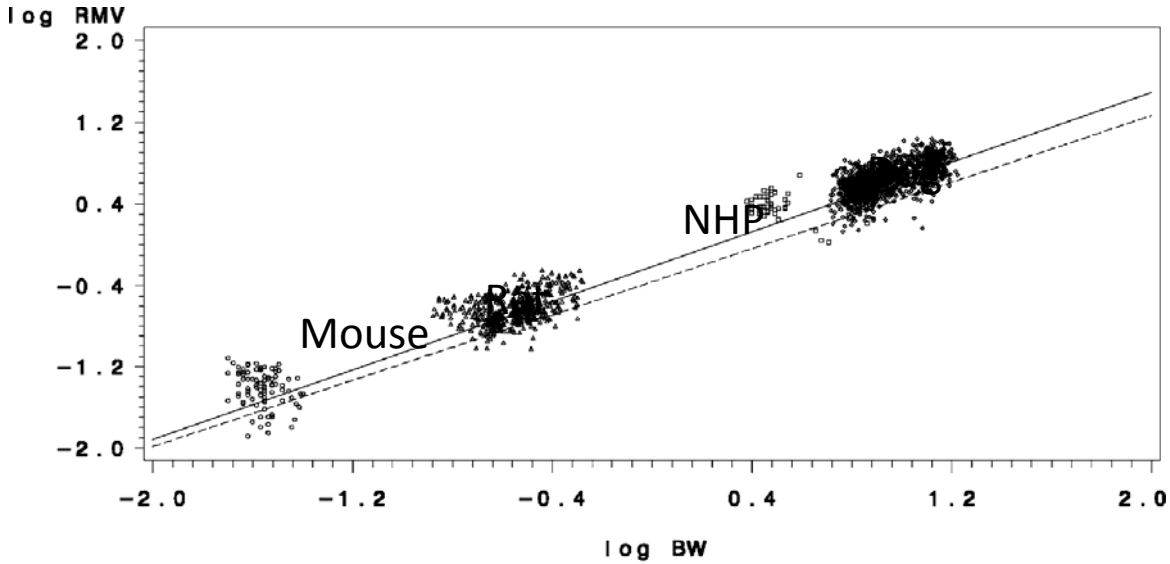
BW = Group Mean Body Weight

Can estimate pulmonary dose if required – by use of a deposition factor

FDA 10% for rodents, 25% for non-rodents, depends on MMAD

FACTORS AFFECTING DELIVERED DOSE

Respiratory Minute Volume (RMV)



Association of Inhalation Toxicologists (AIT) Working Party Recommendation for Standard Delivered Dose Calculation and Expression in Non-Clinical Aerosol Inhalation Toxicology Studies with Pharmaceuticals. Alexander et al (2008)

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FACTORS AFFECTING DELIVERED DOSE

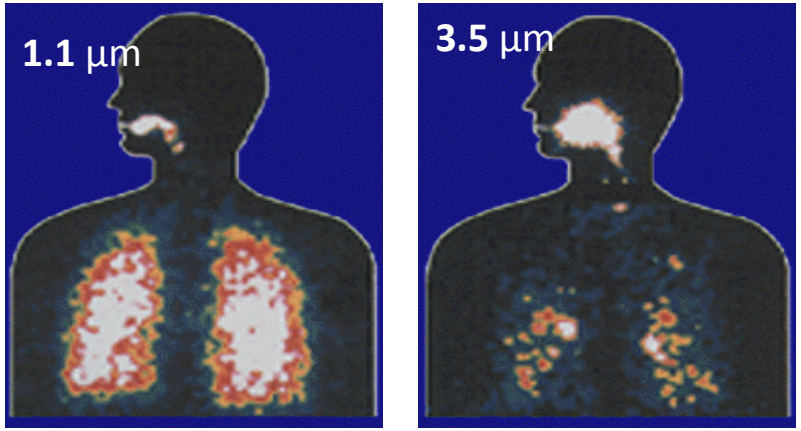
Daily dosing duration

Typical Dosing Duration (min) by Inhalation		
Species	**Minimum**	**Maximum**
Rodent - Rat/Mouse	10	300-360
Non-rodent - Dog	10	180-240
Non-rodent - Nonhuman primate (mask) (head-only)	 10 20	 30-60* 120
* If longer duration required, extend to 2 dose sessions		

FACTORS AFFECTING DELIVERED DOSE

Aerodynamic particle size distribution

- Gamma scintillation image following inhalation of radiolabelled drug
- Drug particles with smaller particle size distribution provide greatest lung deposition



http://www.chestjournal.org/cgi/reprint/122/2/510.pdf

- Accepted size distribution

Mouse:	1 - 2 µm
Rat:	1 - 3 µm
Primate:	1 - 5 µm
Dog:	1 - 5 µm

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PRECLINICAL STUDY DESIGNS

Range finding study design

- Single or 3-day escalating doses and repeat dose at MTD
- Repeat Dose for 7 or 14 days
  - Monitor aerosol concentration daily
  - Particle size distribution weekly
- In life observations
  - Signs, body weight, food consumption
  - Clinical pathology at termination
  - Ophthalmoscopy /ECG /Respiratory (not always)
- Toxicokinetics at intervals during study
- Pathology
  - Special emphasis on respiratory tract

PRECLINICAL STUDY DESIGNS

Repeat dose study design

- Control + 3 dose groups (Recovery in controls and high dose)
- Dosed daily by inhalation up to 9 months (12 months)
  - Monitor aerosol concentration daily
  - Particle size distribution weekly/monthly
- In life observations
  - Signs, body weight, food consumption
  - Clinical pathology at intervals and termination
  - Ophthalmoscopy /ECG /Respiratory
- Toxicokinetics at intervals during study
- Pathology
  - Special emphasis on respiratory tract

PRECLINICAL STUDY DESIGNS

Carcinogenicity study design (2 rodent species)

- Control (x2?) + 3 dose groups (50m+50f)
- Dosed daily by inhalation up to 24 months
  - Monitor aerosol concentration daily
  - Particle size distribution monthly
- In life observations
  - Signs, mass palpations, body weight, food consumption
  - Clinical pathology at intervals and termination
  - Ophthalmoscopy
- Toxicokinetics at intervals during study
- Pathology
  - Emphasis on respiratory tract
  - Tumour incidence

SPECIALISED STUDIES

Intranasal

Micropipettes (rodents) and nasal pump (large animals)

Dose volumes:

- Mice (4 to 10 µL/nostril)
- Rats (4 to 40 µL/nostril)
- Rabbits (50 µL/nostril)
- Dogs (0.1 to 0.5 mL/nostril)
- Non Human Primates (50 µL/nostril)

In order to obtain the target dose, multiple dosing can be performed daily:

- Rats: 40 µL/nostril x 2 sprays/nostril x 8 occasions/day
- Dogs: 150 µL/nostril x 2 sprays/nostril x 16 occasions/day

SPECIALISED STUDIES

Safety pharmacology

Rats

- CNS
- Respiratory
- Renal

Dogs

- Cardiovascular, telemetered
- Respiratory

SPECIALISED STUDIES

Reproductive

- All ICH guideline studies including fertility (ICH-1), embryo-fetal development (ICH-3) and pre and postnatal (ICH-2) designs can be performed by nose-only inhalation or intranasal routes
- Performed in rats and rabbits
- Exposures of up to 4 hours on a daily basis

SPECIALISED STUDIES

Neonatal and juvenile

- Inhalation neonatal and juvenile studies in rats and dogs for paediatric drugs can also be performed:
  - Rat pups from day 4 post partum
  - Dog pups from day 10 post partum
- Routine evaluation and specialised evaluation:
  - Behaviour function
  - Lung function
  - Immune status
  - Skeletal growth

SUMMARY & CONCLUSION

- The challenge in performing preclinical inhalation studies is to ensure good pulmonary delivery and multiples of the clinical dose
- Exposure of animals to the inhaled drugs requires specialised equipment to generate the atmosphere and restrain the animals
- These systems need to provide reproducible results on a daily basis with studies ranging from single dose to carcinogenicity

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