ACT American College of Toxicology

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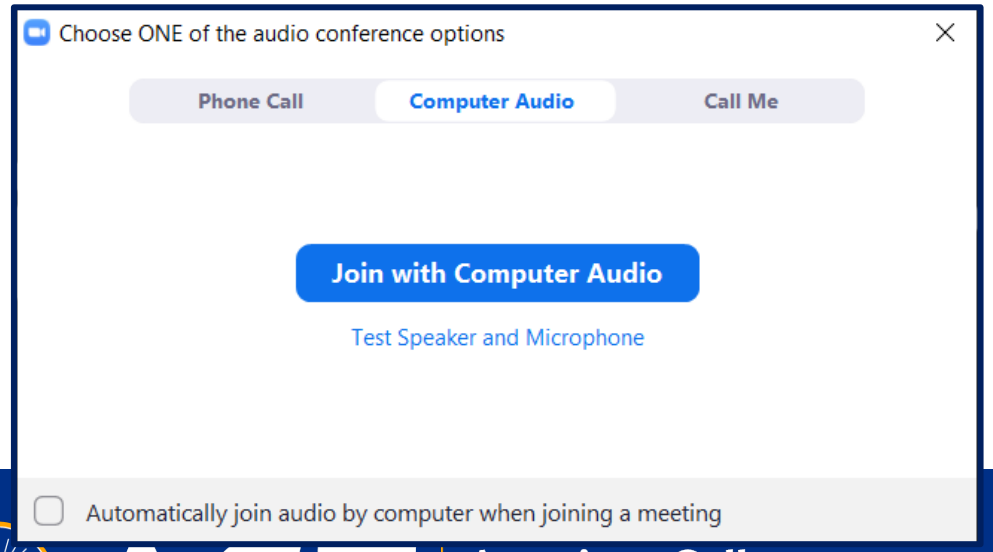

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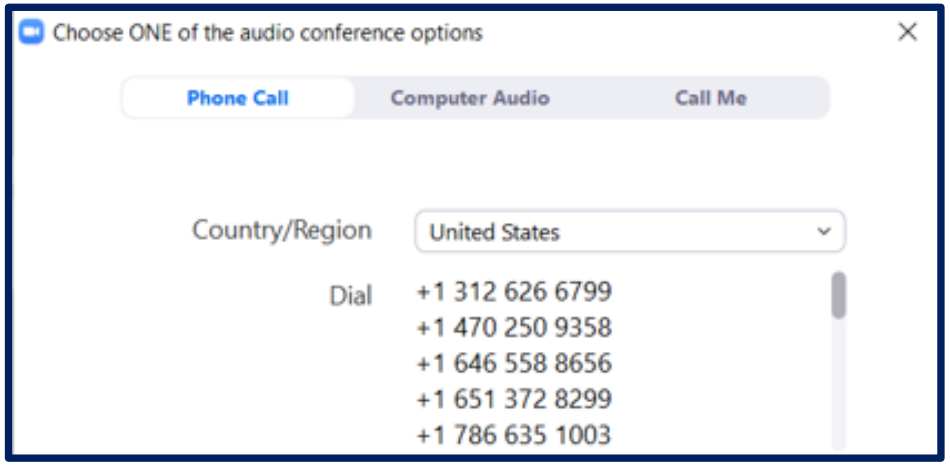

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Approach to Development of an Inhalation Excipient for Regulatory Approval: Case Studies with HFA-152a and DSPC

Speakers:

Jake McDonald, PhD

Philip J. Kuehl, PhD

Approach to Development of an Inhalation Excipient for Regulatory Approval; Two Case Studies with HFA-152A(1,1-Difluoroethane) and DSPC (1,2-Distearoyl-sn-glycero-3-phosphocholine)

Philip J. Kuehl, PhD and Jake McDonald, PhD

Overview

Types of Inhalation Formulations and Excipients

Unique Attributes to Conduct of Inhalation Studies

Regulatory Approaches for Development of Excipients

- *As a stand-alone excipient that is used as a platform*
- *As an excipient developed as a part of a therapeutic development*

Example-HFA Development

Example-DSPC Development

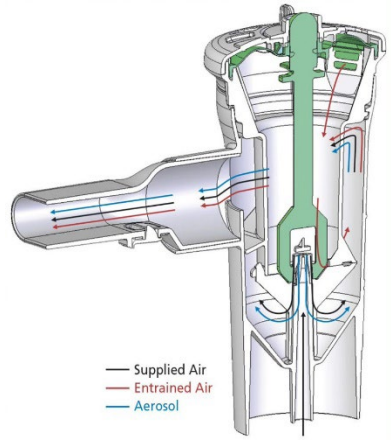
Take Home Message

Formulation for Inhalation Drug Delivery

- Formulation via three major delivery methods
  - Nebulizer, pMDI (pressurized meter dose inhaler), dry powder
  - Others exist but are specialized
- GRAS (generally recognized as safe) excipients for inhalation
  - There is an ‘unwritten’ list, albeit a short list
  - In vitro testing
  - Beyond chemical and physical stability, dose uniformity, etc.
- Inhalation specialty considerations
  - Dose calculation
  - Compound needs

Types of Inhalation Formulations

- Pressurized Metered Dose Inhalers (pMDI)
- Dry Powder
- Nebulizer



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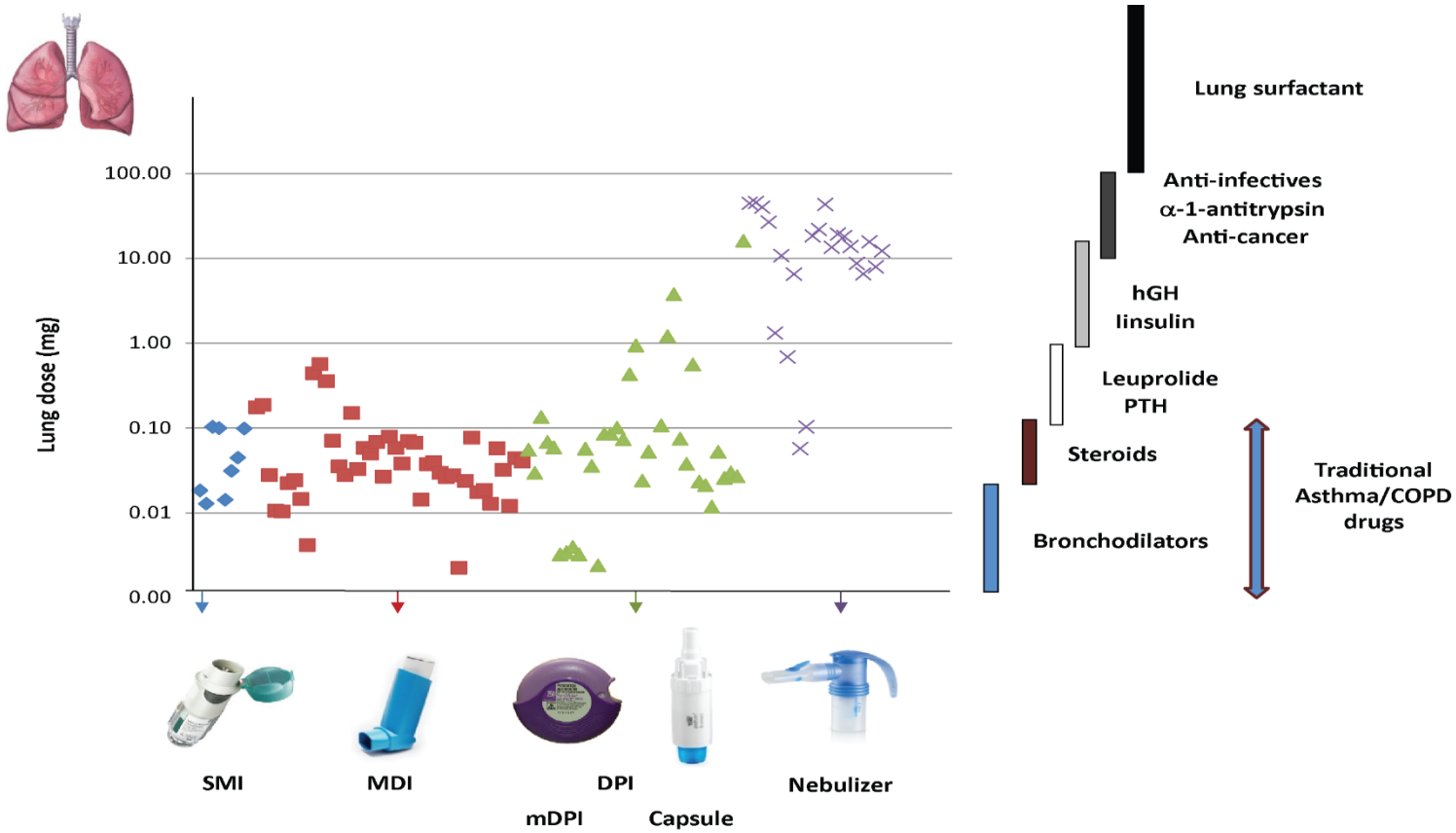
Formulation Development/Selection



DPI: Dry powder inhaler
mDPI: metered DPI
SMI: soft mist inhaler

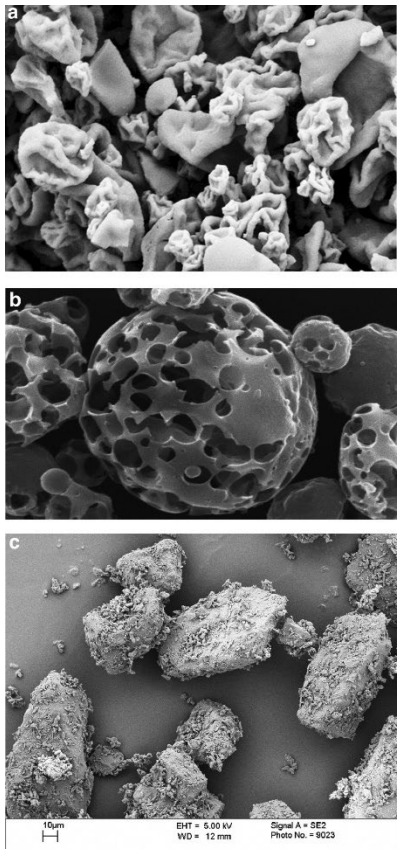
Published in: Hastedt, J.E., et al. AAPS Open 2, 1 (2016). https://doi.org/10.1186/s41120-015-0002-x

Nebulizers – Formulation Strategy

- Solution or suspension formulations
- Suspension
  - Surfactants (Tween 80)
  - Micronized API (active pharmaceutical ingredient)
    - Physical stability (high melting points)

Dry Powders

- API (active pharmaceutical ingredient) alone or blended
- Physical micronized, spray dried or other
- Excipients
  - Leucine, lung surfactants, Lactose
- Clinical and/or preclinical generation systems
- Support wide range of aerosol concentrations

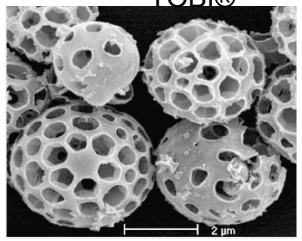


Published in: Stephen W. Stein and Charles G. Thiel.Journal of Aerosol Medicine and Pulmonary Drug Delivery.Feb 2017.20-41.http://doi.org/10.1089/jamp.2016.1297

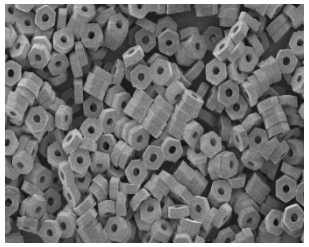
Dry Powders

- Multiple approaches (Both Spray Drying and Others)

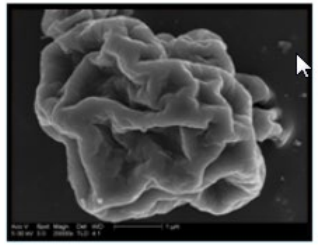
PulmoSphere™ (Novartis)
TOBI®



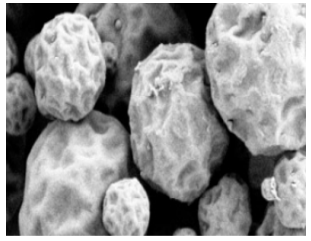
PRINT® (Liquidia)



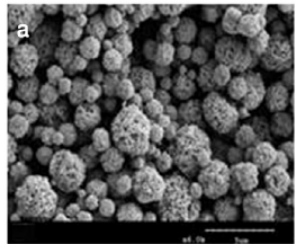
TechnoSpheres (MannKind)
AFREZZA®

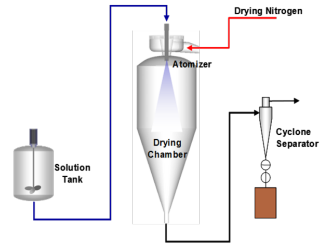


iSPERSE (Pulmatrix)



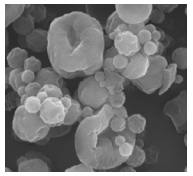
Emulsion free nanoporous/nanoparticulate microaprticles (NPMP's)

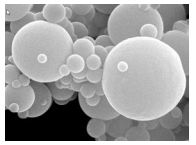


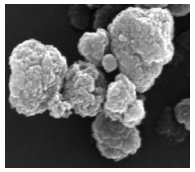


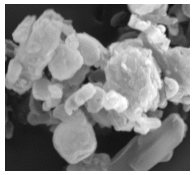
Engineered Dry Powder Particles

Neat API		Single Solvent Solution
Amorphous API/Excipient		Single Co-Solvent Solution
Crystalline API/Excipient		Single, Dual, or Variable Process Settings – Solution or Suspension
Mixed Approaches		Single, Dual, or Variable Process Settings – Solution or Suspension









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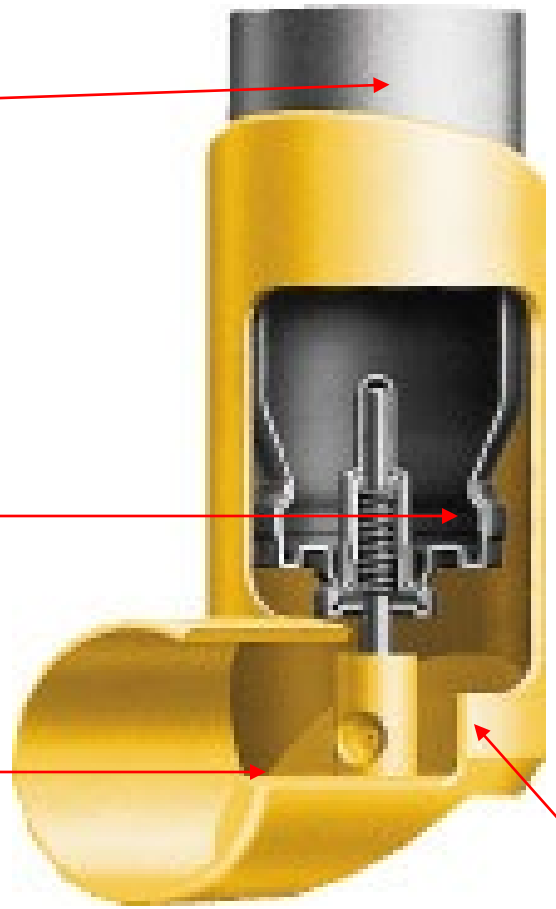
pMDI

Canister (contains formulation)

Metered Valve

Actuator Mouthpiece



Spray Orifice

- Liquified gas provides energy for atomization
- Drug can be in a solution or suspension
- Multi-dose
- Breath actuation available

Published in: Stephen W. Stein and Charles G. Thiel.Journal of Aerosol Medicine and Pulmonary Drug Delivery.Feb 2017.20-41.http://doi.org/10.1089/jamp.2016.1297

Formulation Strategy - pMDI

- Propellant
- Cosolvents
  - Ethanol – up to 10%
- Solution / Suspension
  - Surfactants
- Physical micronization or engineered particles

Published in: Stephen W. Stein and Charles G. Thiel.Journal of Aerosol Medicine and Pulmonary Drug Delivery.Feb 2017.20-41.http://doi.org/10.1089/jamp.2016.1297

Excipients for Inhalation-Goal

- If a company owns an Excipient DMF (Drug Master File) the DMF data are proprietary
  - Business model: Company provides access to DMF for a fee and/or royalty, which allows company to use that excipient without further testing

Regulatory Approaches for Excipients

- The International Pharmaceutical Excipients Council of the Americas Excipient Master File Guide provides guidance (FDA focused, but intended to expand) towards development of an Excipient DMF
  - Excipient DMF is an FDA submission that may contain information to support IND (investigational new drug), NDA (new drug approval), BLA (biological license application), another DMF or export
  - Excipient DMF follows ICH for overall nonclinical development
  - Excipient Margin of Safety must be >100-1000's (based on no observable adverse effect level; NOAEL)

https://ipecamericas.org/sites/default/files/ExcipientMasterfileGuide.pdf

Regulatory Approaches for Excipients

- This presentation below describes general attributes of an inhalation nonclinical program and provides two examples of programs that were successfully executed
  - The major requirements are nonclinical safety packages, CMC, and clinical safety (efficacy not required)

https://ipecamericas.org/sites/default/files/ExcipientMasterfileGuide.pdf

Nonclinical Inhalation Study Attributes

- Required to adapt device/formulation to specialized delivery systems
- Aerosol delivery uses specialized equipment for ‘exposure’ of animals or cells to replicate clinical aerosols
- Special characterization of test atmospheres are used in lieu of a dose formulation analysis
- Maximum achievable or feasible dose often used as top dose
- Description of dose requires a combination of data and calculations based on empirical available data

Typical Program Major Components (non-clinical)

- Aerosol Development
- Initial pilot – pharmacokinetics (PK) and Tolerance
- Rodent non GLP (good laboratory practice) safety
- Large animal (canine) non GLP safety
- Validation(s)
- Rodent GLP repeat dose
- Large Animal (canine) GLP repeat dose

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Aerosol Delivery

- Nose only, whole body, oral aspiration, intratracheal installation, intubated, anesthetized/awake
- Mice, rats, ferrets, rabbits, guinea pigs, rabbits, dogs, primates and humans
- Aerosol generation of aqueous, non-aqueous, dry powder and novel formulations
  - Nebulizer, dry powder, pMDI, etc.

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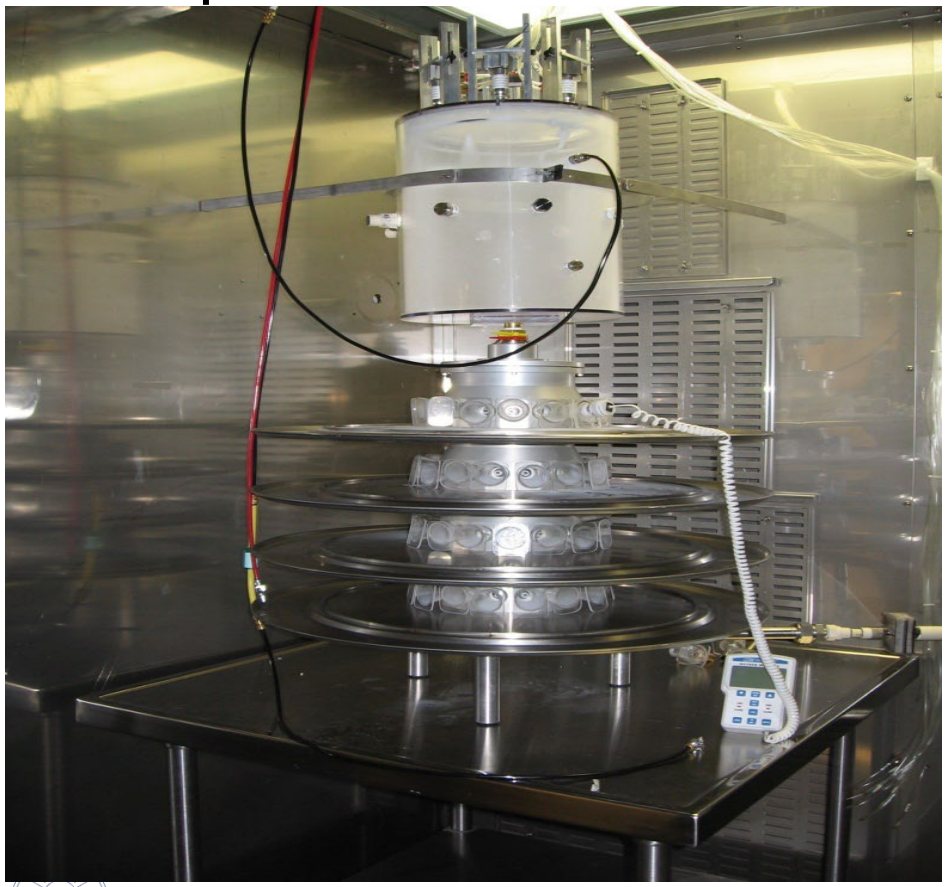
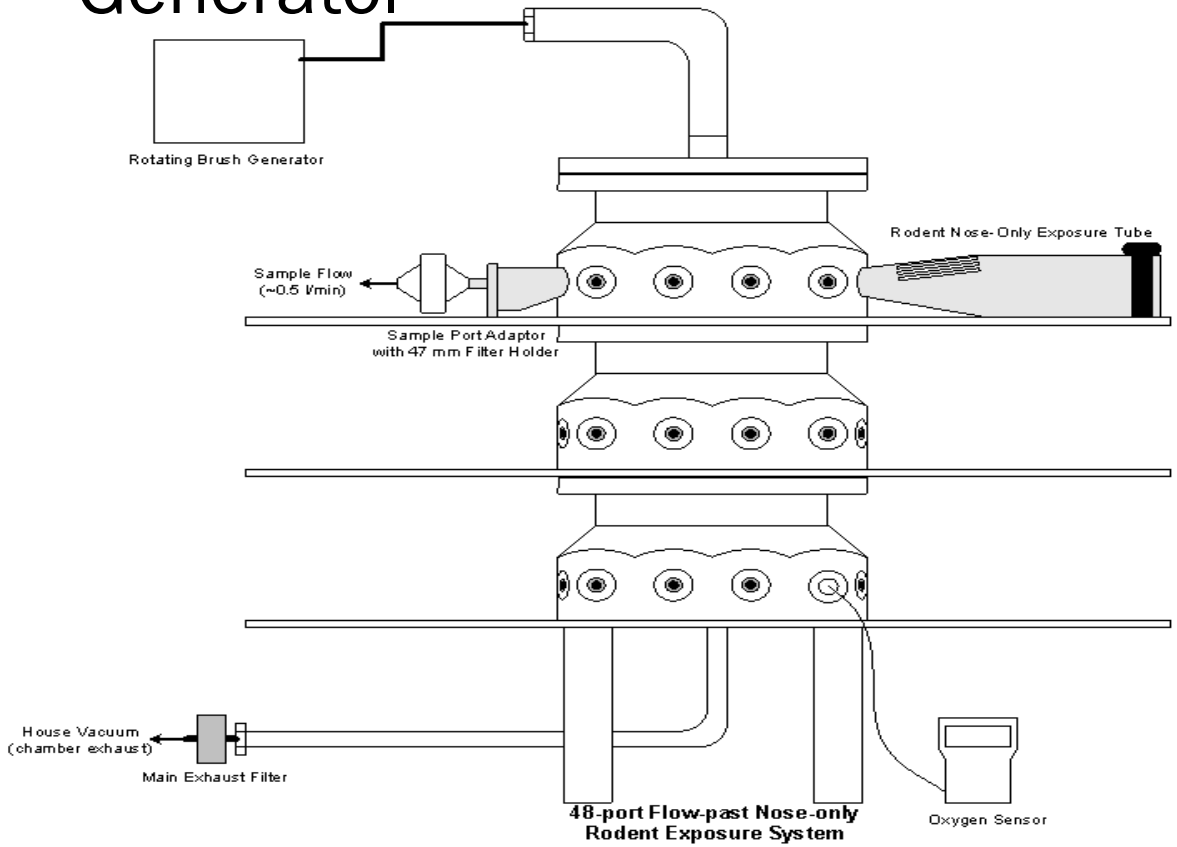
Rodent Exposure Systems

- pMDI Generation



- Dry Powder – Rotating Brush Generator



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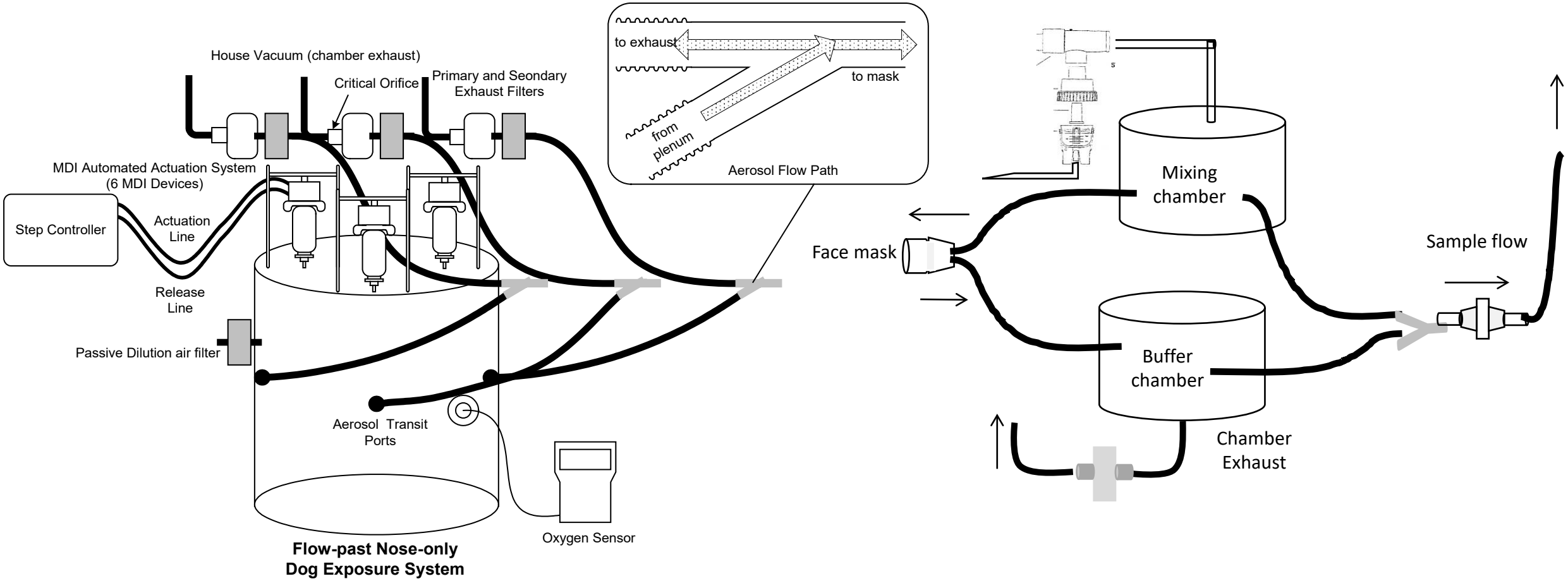
Large Animal Exposure Systems



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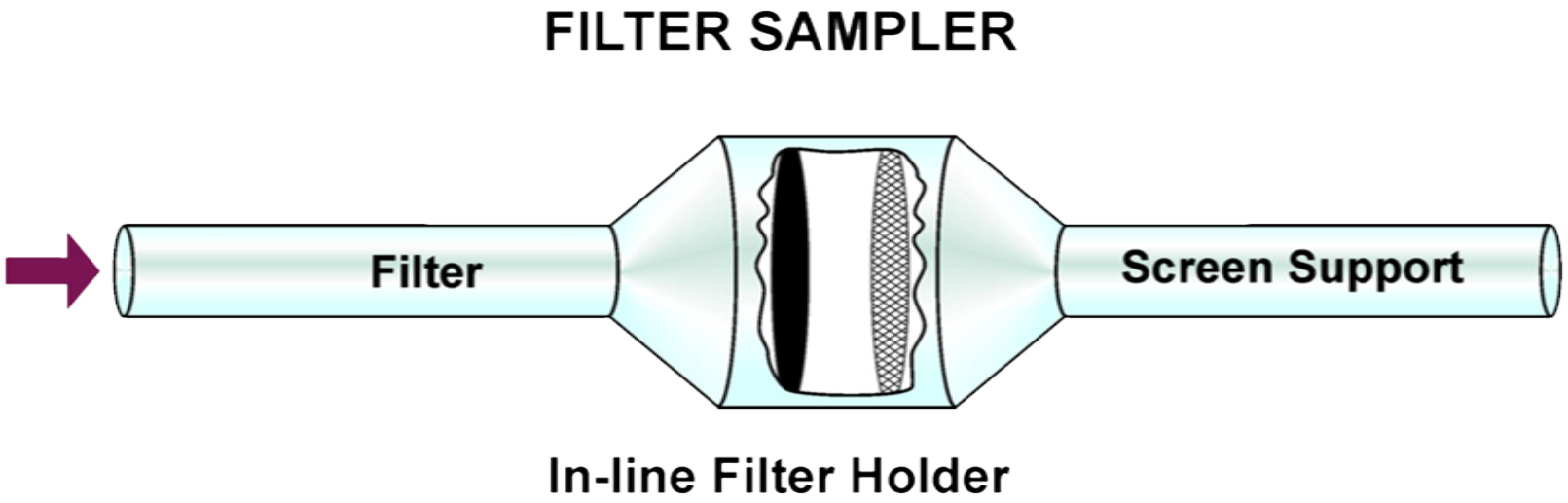
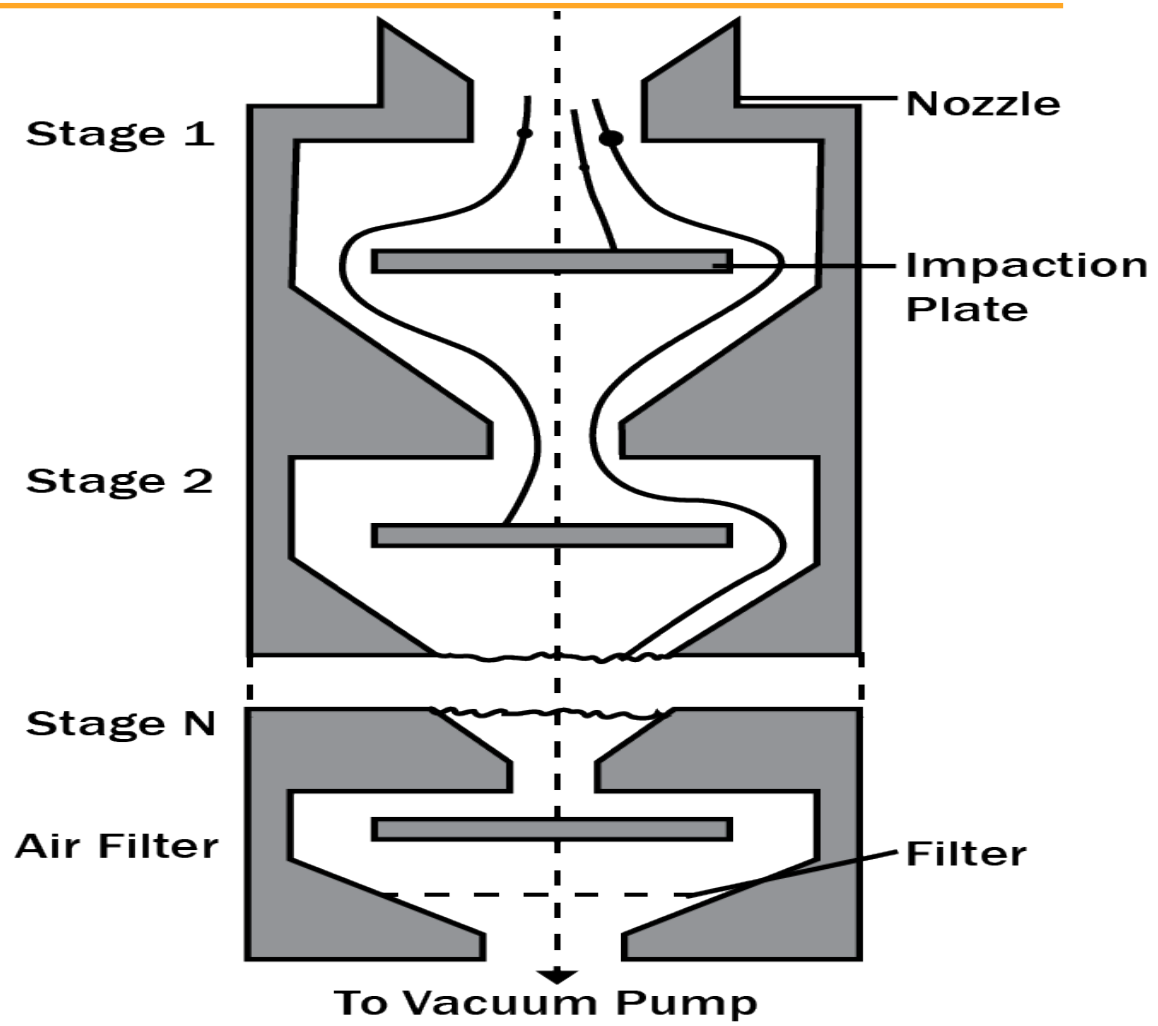
Aerosol Concentration

- Aerosol Concentration – mass concentration per volume of air (mg/L)
- Filter Sample
  - Differential weight – total material (test article and excipient)
  - Chemical analysis – compound specific



Particle Size Distribution

- Particle Size Distribution – mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD)
- Cascade Impactor
  - Inertial impactor to classify particles by size
- Time of Flight / Laser diffraction



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Inhalation Toxicology – Inhaled Dose

- "Dose" in an inhalation setting is often debated
  - Presented dose, deposited dose, pulmonary dose, etc.
- Why is dose not as straight forward and how to we calculate it?

$$Inhaled\ Dose = \frac{Aerosol\ Concentration * RMV * time}{Body\ Weight} * DF$$

- Aerosol concentration – measured real time
- RMV – respiratory minute volume can be measured (plethysmography) or calculated (Bide, 2000; AIT working group, 2008)
- Body weight – measured
- DF – Deposition fraction

Deposition Fraction to Define Dose

- All inputs into the calculation of inhaled dose can be measured directly
  - Except deposition fraction
- Presented Dose when DF = 100%
  - Why not just use this?
  - This assumption would be constant, inaccurate but consistent
- Safety Study Doses Default to mg/kg lung dose (Tepper, Kuehl 2016) for protocol design
  - 10% in rodents
  - 25% in large animals

Tepper, Kuehl, et al. Int J Tox. 2016

Reporting Dose/Margin Using Multiple Approaches (Example HFA-152a)

Excipient X Inhalation Species Dose Comparisons							
Species	Study Type	Drug X Dose (mg drug/kg body weight/day; 100% deposition fraction all species)	Animal / Man Safety Factor	Drug X Dose (mg drug/kg body weight/day; 100% man; 10% rat and mouse; 25% dog deposition fraction)	Animal/Man Safety Factor	Drug X Dose (mg Drug/g lung weight/day)	Animal/ Man Safety Factor
Man	Phase I Clinical Maximum Dose	2.0 mg/kg/day	1	2.0 mg/kg/day	1	0.11 mg/gram	1
Rat	14-Day Inhalation Study	24,000 mg/kg/day	12,000	2,400 mg/kg/day	1,200	3,996 mg/gram	36,327
Mouse	14-Day Inhalation Study	37,000 mg/kg/day	3,500	3,700 mg/kg/day	1,850	3,481 mg/gram	31,645
Dog	14-Day Inhalation Study	3,000 mg/kg/day	1,500	750 mg/kg/day	375	224 mg/gram	2,036

Example: HFA-152a

- HFA-152a has been developed as a novel pMDI excipient due to its lower greenhouse gas potential compared to existing excipients

Non-Clinical Program of HFA-152a

- Review of HFA-134a and HFA-227 consortium program and submission package
- Constant communication with global regulatory agencies to understand the studies required for the propellant vs. the drug product
- Ultimate goal is an IND to allow clinical studies and a subsequent DMF on HFA-152a

IND (Investigational New Drug) Program Highlights

- Analytical Assay
  - Gas chromatography-Flame ionization detector-headspace auto sampler
  - Gas collection in foil bags
- Assay was developed to compliment the real time FID (Flame ionization Detector)
- Full GLP validation to standard analytical assay criteria

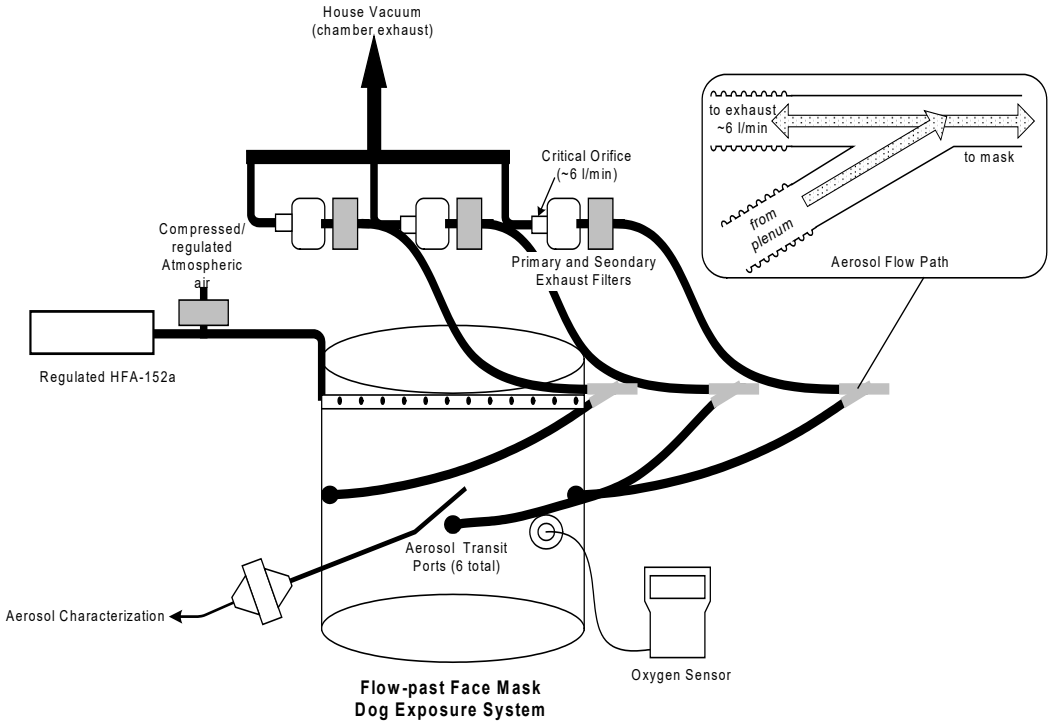
IND Program Highlights

- Aerosols developed and evaluated for generation about a wide range of species
- Evaluated maximum feasibility based on displacement of oxygen (as low as 15.5 %) to attain safety factor in the 1000's
- Manage 'flammability' aspect
  - Grounding lab coats, chambers, equipment
  - Required real-time monitoring to ensure concentration below flammable limits

IND Program Highlights

- Aerosols developed and validated for:
  - In vitro
  - Mouse
  - Rat
  - Rabbit
  - Dog
- 25,000 to 350,000 ppm aerosol concentrations required for the range of species and study designs.

MDI Delivery System



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HFA-152a IND Package (Example)

Species	Details
Canine	Cardiac sensitization
Guinea Pig	Respiratory sensitization
Canine	Respiratory sensitization
NA	Analytical Validation
Canine	Bioanalytical validation
Rat	Bioanalytical Validation
Mouse	Bioanalytical validation
Human	Bioanalytical validation
Rabbit	Bioanalytical validation
In vitro	Bioanalytical validation
Canine	Aerosol validation
Rat	Aerosol validation
In vitro	Aerosol Validation
Rabbit	Aerosol validation

HFA-152a IND Package

Species	Details
Rat	In vivo micronucleus
In vitro	Micronucleus
In vitro	AMES
Mouse	MTD
Mouse	14 Day GLP Tox
Rat	MTD
Rat	14 Day GLP Tox
Rat	6 month GLP tox
Rat	2 year carc
Canine	MTD
Canine	14 day GLP tox
Canine	180 day GLP tox
Rat	Repro pilot
Rat	Repro MTD
Rat	Repro tox
Rat	Repro Tox
Rabbit	Repro tox
Rabbit	Repro Tox
Rabbit	Repro tox
Rabbit	Repro tox

IND Program Highlights

- Rat Studies
- Dose range finding
- 14 day GLP tox
- 180 day GLP inhalation tox
- 2 year GLP carcinogenicity
- Repro toxicology

- Clinical observations during exposures (200,000 ppm exposure level) that recover nearly immediately post exposure
- No and / or incidence histopathology findings only

IND Program Highlights

- Canine Studies
- First maximum tolerated dose study conducted in canines
  - Primary toxicology end point anesthetic effect caused by reduction of oxygen as the HFA-152a replaced ambient air
- Dose range finding
- 14 day GLP tox
- 180 day GLP inhalation tox
- 270 day GLP inhalation tox

- Transient clinical signs across high dose (125,000 to 150,000 ppm) groups recovery nearly immediately post exposure

IND Program Highlights

- Bioanalytical Assay
  - Gas chromatography-Flame ionization detector-headspace auto sampler
  - Collect blood in K_3EDTA tubes, analytical transfer plasma to gas tight head space vials
- Full Blood GLP Validation:
  - Mouse, rat, guinea pig, rabbit, canine and human
- Stability issues with repeat injections
  - ISR determined not to be possible
- Human assay 0.216 to 216 mg/L range

IND Program Highlights

- NMR Metabolites
  - 19F NMR
  - Sealed tubes, assayed day of collection
  - Split samples for GC-NMR
  - External standard $CFCl_3$ in $CDCl_3$
- Metabolites expected between 90 and 115 ppm
- Rat plasma/urine
- Human urine

Clinical Study-Unique Aspect of Excipients is that Clinical Programs Only Focus on Safety/PK Example HFA

- Phase I clinical trial - Healthy male volunteers
- Assess safety
- Assess taste
- Quantify exposure
- Study performed under GCP with LSR (Lovelace Biomedical clinical trials group) under approved IRB
- HFA-152a (Koura, UK) pre-filled pMDI canisters
  - 50 µL metering valve

Key Endpoints

- Safety
  - Pulmonary Function Testing
  - Vital Signs
    - Heart rate, blood pressure, respiratory rate, SpO2
  - Clinical chemistry, hematology, urine analysis
- Taste
  - Bad, good, bitter, sweet, salty, metallic and cold
- HFA-152a Pharmacokinetics in blood
- Screen urine metabolites

Clinical Study

		Pre-Test	0	Post Dose	10	20	30	45	60	120	240	360
Procedures	Blood Collection PK Samples	X		X	X	X	X	X	X	X	X	X
	Urine Collection	X							X			
	Pulmonary Function Testing	X							X			
	Direct Physical Effects of MDI Use			X			X					
	Direct Taste Effects of MDI Use			X			X					
	Adverse Events/Vital Signs		X	X	X	X	X	X	X	X	X	X

Dose Delivery

- Uniform delivery key for all end points
- Prior to enrollment each subject individually trained on dose delivery
- Calm and complete exhalation
- 3 second calm inhalation
  - Timing actuation with the start of inhalation
- 10 second breath hold
- Four actuations in six minutes
  - Time 0 Immediately post last dose

Dose Delivery – what does this mean?

- Publications and personal experience range widely how many patients use pMDI's correctly
  - They all agree the number isn't as high as we would like it to be
- For this study all subjects utilized the pMDI correctly.
  - The date / study allows interpretation of the endpoints

Clinical Study – Adverse Events

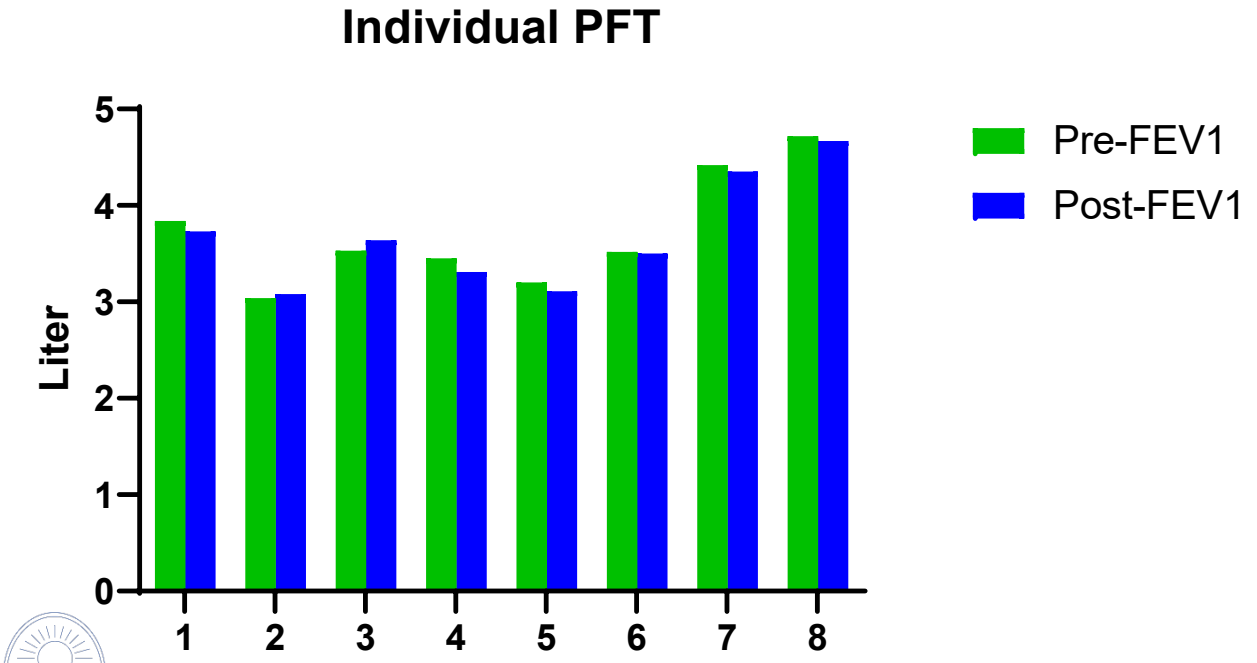
- No adverse events noted in any subject at any point
- All subjects completed the study

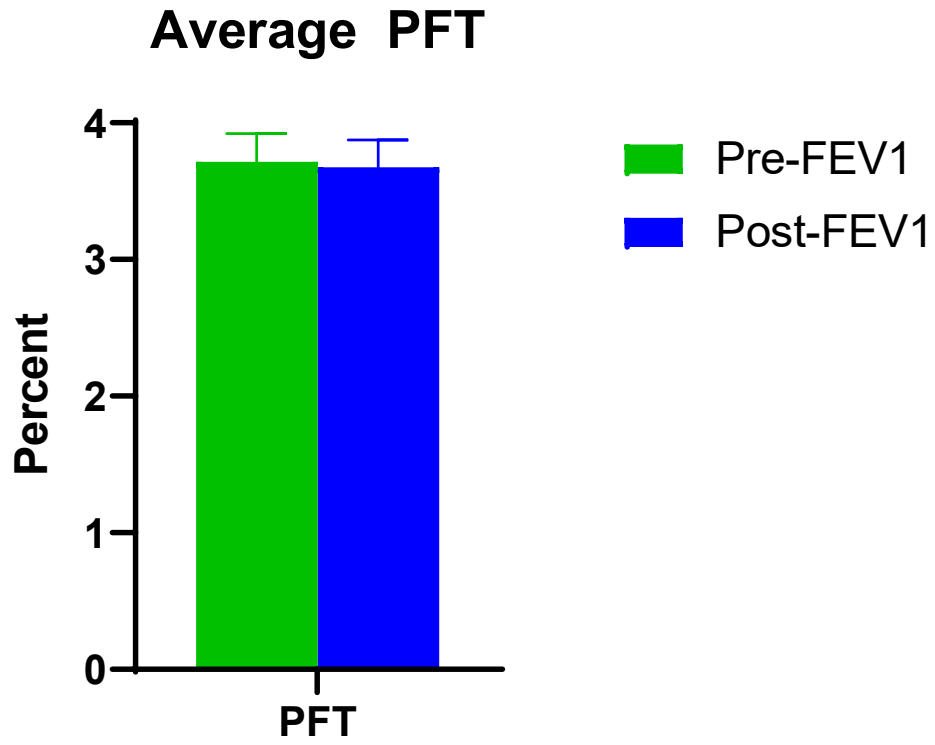
Vital Signs, Clinical Chemistry, Hematology and Urinalysis

- All Vital signs within standard ranges at all timepoints
  - HR (heart rate), temperature, Respiratory rate, blood pressure, oxygen saturation
- Clinical chemistry, Hematology, and urinalysis
  - Evaluated by physician
  - Compared against baseline
  - No HFA-152a related trends found in any end point

Safety Assessment

- All measured endpoints showed now changes pre/post dosing
- HFA-152a delivered by oral inhalation is safe (pulmonary function data shown here)





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HFA-152a Blood Measurements

- Blood collected in K_3EDTA tubes
- Immediate (0), 10, 20, 30, 45, 60, 120, 240 and 360 minutes post the last dose
- Samples assayed within stability windows
- Assayed with validated head space gas chromatographic assay.

HFA-152a Blood Measurements

- NCA (non-compartmental analysis) only performed on values within the range of the assay
- T_{max}, C_{max}, and AUC (area under the curve) – standard
  - AUC only when three timepoints above LLOQ
- Quantification of clearance
  - Half life didn't add value based on rapid apparent terminal half life
  - Mean residence time used
    - Average residence time a molecule resides in the body

Treatment	T_{max} (min)	C_{max} (mg/L)	AUC (min*mg/L)	MRT (min)
Average	3.75	0.69	7.14	8.95

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Plasma Non-compartmental Analysis

- All subjects T_{max} at immediate or 10 minute time points (first two sample collection timepoints)

- HFA-152a is absorbed systemically
- HFA-152a is cleared rapidly

Summary

- HFA-152a has appropriate non-clinical safety/tolerance to support an IND
- Following oral inhalation from a pMDI HFA-152a is safe, well tolerated, and is rapidly cleared in humans.
- HFA-152a provides an alternative propellant for pMDI's with a lower global warming potential

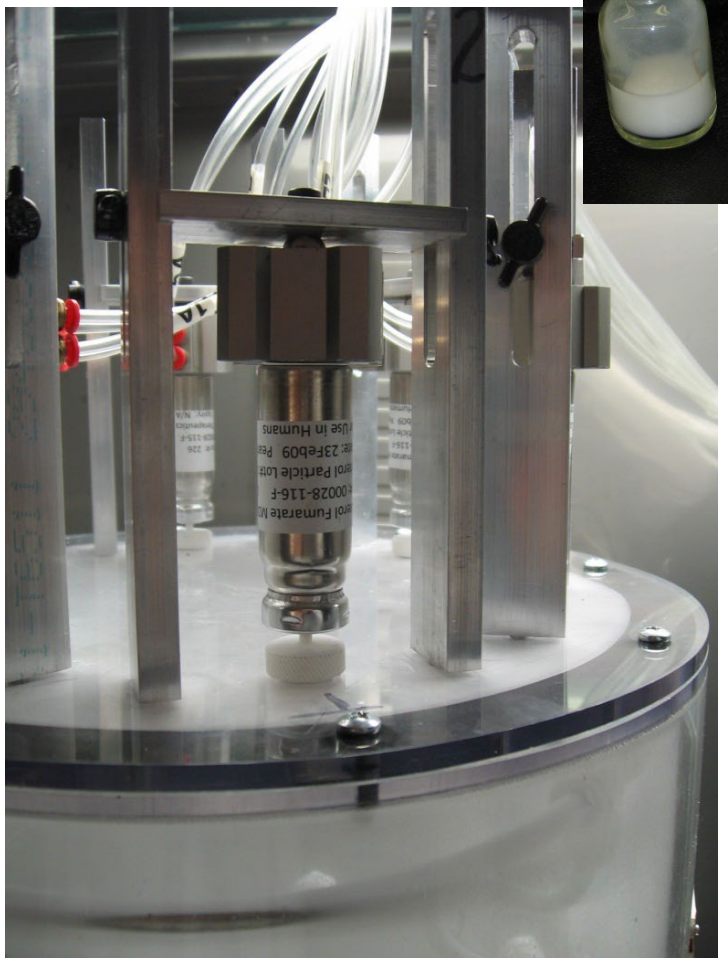
Example DSPC

- DSPC was developed as an excipient for dry powders due to it being GRAS and having good properties in the production of porous dry powders (Ohgoda and Robinson, 2020)

DSPC Example

- DSPC is a natural member of cell membranes which account for a major fraction of pulmonary surfactants.
  - Because of this DSPC and other lipids are considered GRAS
  - DSPC and other lipids are in several approved IV formulations
- DSPC/CaCl2 mixture were previously approved as a component of TOBI® Podhaler®
- DSPC was a vehicle control for the commercial products Bevespi (glycopyrrolate/formoterol fumerate) and Breztri (budesonide/glycopyrrolate/formoterol fumarate)
  - As a result: A dose-response was not included. All studies with DSPC were performed at the same dose as they were in the test article atmosphere for that respective study (~1-20 mg/kg/day)

MDI Delivery System



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DSPC IND Package (Example)

Study	Species
Analytical Validation	NA
Bioanalytical validation	Canine
Bioanalytical Validation	Rat
Bioanalytical validation	Mouse
Bioanalytical validation	Human
Bioanalytical validation	Rabbit
Bioanalytical validation	In vitro
Aerosol validation	Canine
Aerosol validation	Rat
Aerosol Validation	In vitro
Aerosol validation	Rabbit

DSSPC IND Package

Study	Species
In vivo micronucleus	Rat
Micronucleus	In vitro
AMES	In vitro
MTD	Mouse
14 Day GLP Tox	Mouse
MTD	Rat
14 Day GLP Tox	Rat
6 month GLP tox	Rat
2 year carc	Rat
MTD	Canine
14 day GLP tox	Canine
180 day GLP tox	Canine
Repro pilot (Oral Dose)	Rat
Repro MTD (Oral Dose)	Rat
Repro tox (Oral Dose)	Rat
Repro Tox (Oral Dose)	Rat
Repro tox (Oral Dose)	Rabbit
Repro Tox (Oral Dose)	Rabbit
Repro tox (Oral Dose)	Rabbit
Repro tox (Oral Dose)	Rabbit

Unique Attributes of DSPC Program

- Reproductive studies were not performed by inhalation based on PK/Disposition analysis
- Genotoxicity were not performed by aerosol delivery
- Overall no findings with the exception of minimal histopathology findings in the larynx (laryngeal squamous metaplasia) and minimal to mild hyaline degeneration in respiratory and olfactory epithelium
  - These are common findings in laboratory animals exposed to repeated aerosols and are considered an adaptive response and non-adverse

Summary

- Excipients can be approved through ICH guidance directed studies.
  - Programs are guided by a combination of ‘science’ and regulation
  - Goal is to achieve defined safety margins >100 and mitigate risk
- A number of unique aspects related to aerosol formulation and programs create some unique study and program designs
- If a DMF is approved that excipient can be licensed.

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  - Named and countless others too numerous to name

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