25th IPST ANNIVERSARY

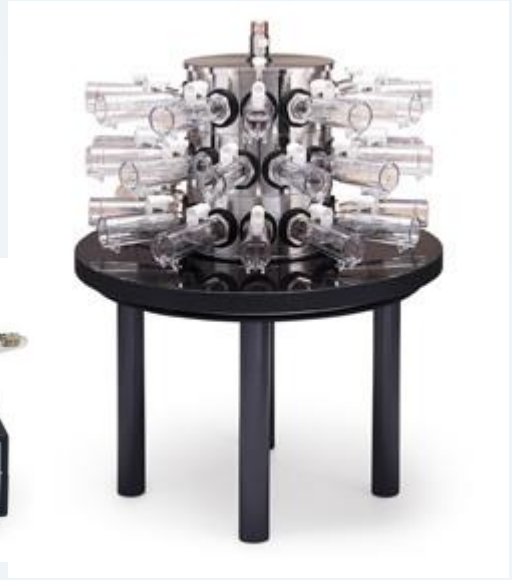
Inhalation studies at IPST

For additional Information:
Daniel J. Barabino, Ph.D.
Senior Director, Business Development
Cell : 631-269-4222
Tel. : 819-820-1515
dbarabino@ipstherapeutique.com

Inhalation studies at IPST

Three (3) steps to your efficacy study(ies):

1. Inhalation parameters and configuration (Technical trials)
2. Inhalation PK(/PD?)
3. Inhalation study (Efficacy)



Inhalation studies at IPST

<u>The heart of any inhalation facility:</u>

- **ONARES: Oro-nasal Respiratory exposure systems**
  Fits 12 animals per stack: IPST has 4 systems with 2 to 4 stacks, respectively

- **Whole-body exposure chambers**
  IPST has chambers for mice, rats, guinea pigs
  Allows concomitant monitoring of respiration, cough, and drug administration

Inhalation studies at IPST

Nose-only exposure systems

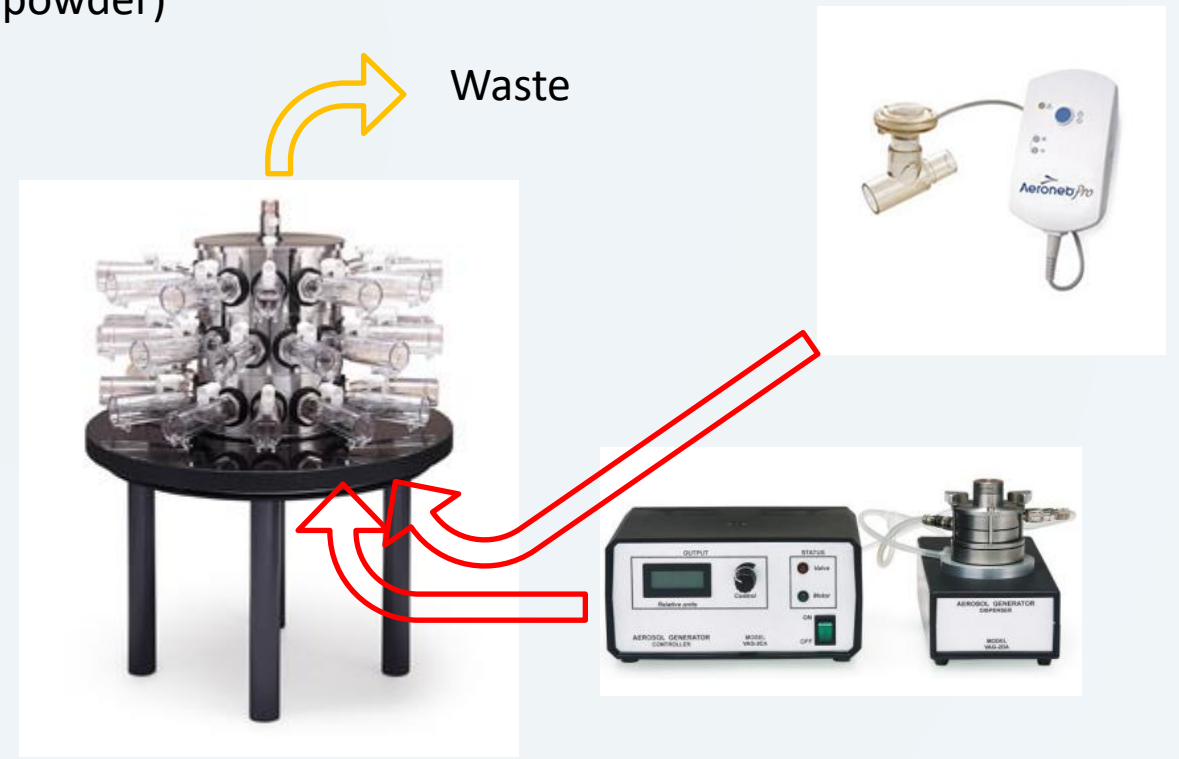
Animals are acclimated to the restraining tubes for a week prior to the experiment

Administration of the test article can last up to 1.5 hour

Test articles can be nebulized (liquids) or vaporized (dry powder)

Aerosol concentration is controlled by:

- on-off cycle (nebulizer)
- frequency (dry powder)
- flow-through rate
- chemistry (% active ingredient in mixture)
- duration of administration
- particle size



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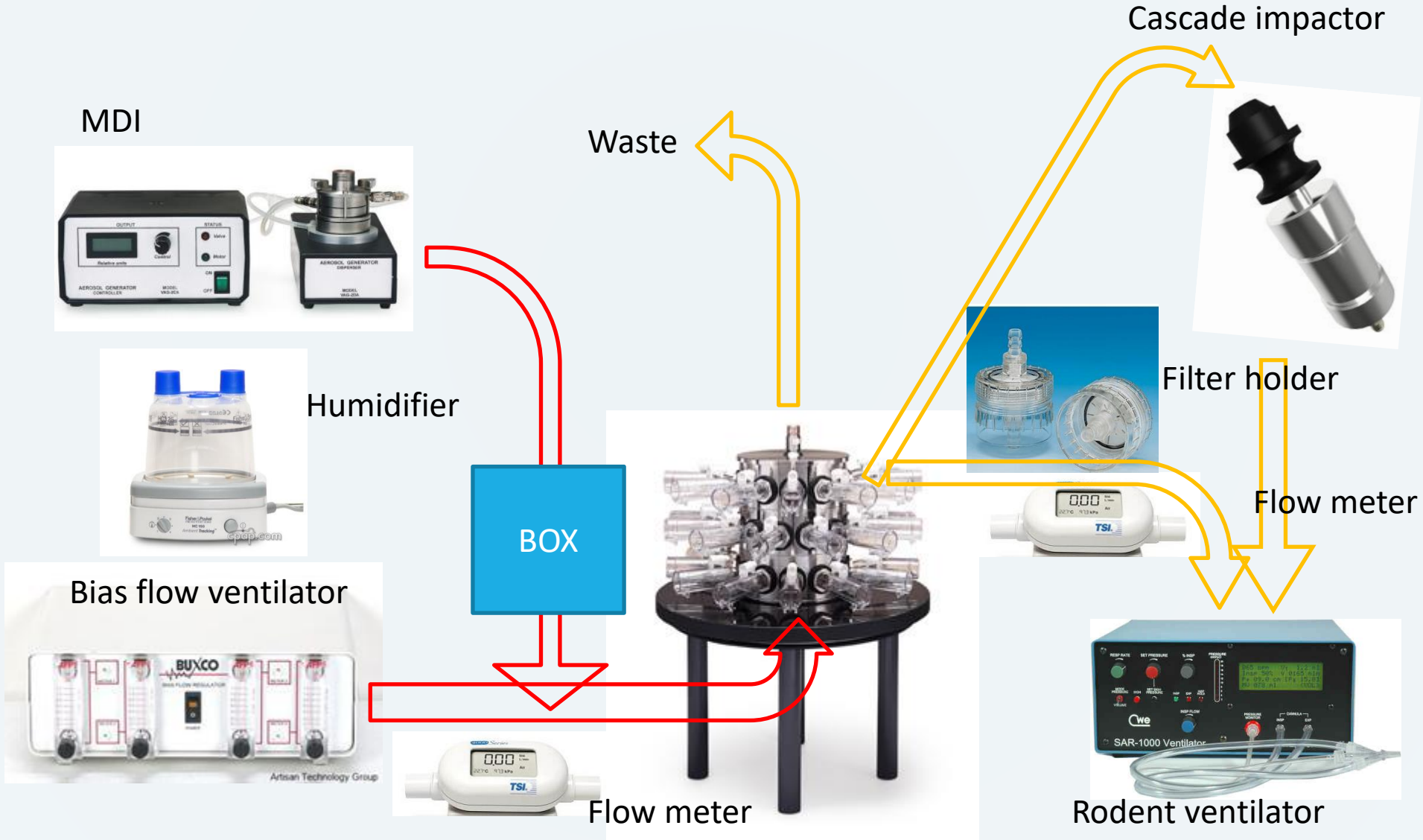
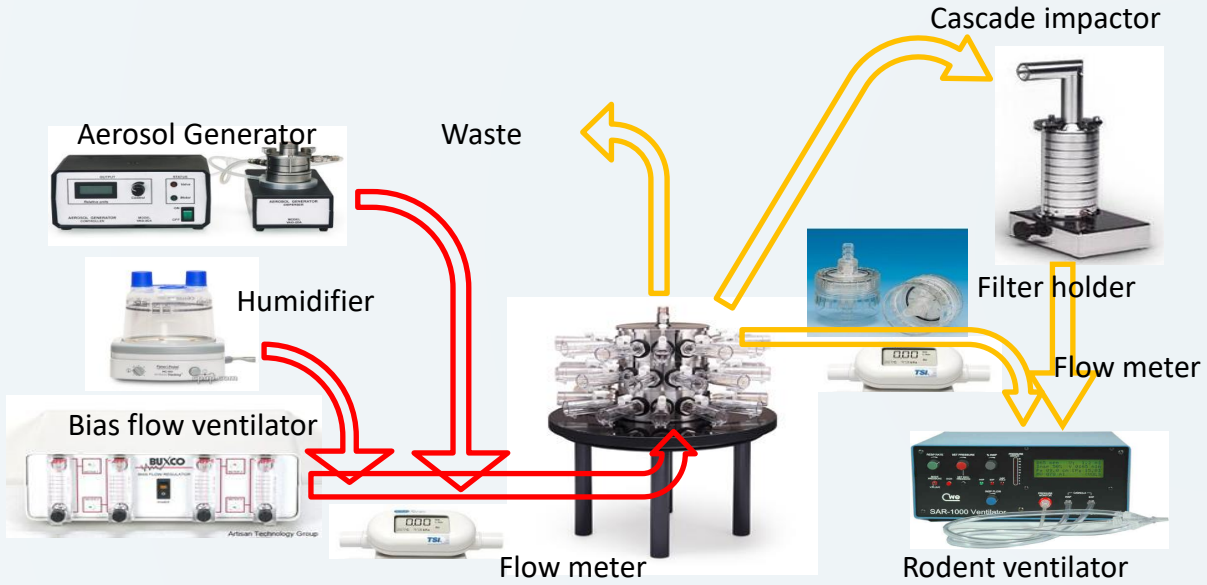
The actual inhalation system



Technical trials



Step 1: Setting up the inhalation parameters

Concentration of TA in inflow air:

- Chemistry: % of active material vs. Inactive material in micronized mixture
- Aerosol generator frequency / nebulizer on/off cycle
- Particle count measured by inline laser at outlet of aerosol generator

Dose received by the animal

- A paper filter replaces one animal on the tower
- The rodent ventilator reproduces the breathing of an animal at one port of the nose-only system
- Test article quantity measured on the filter following a given administration duration
- Aerosol mean diameter is measured in the cascade impactor: smaller particles go deeper into the lungs, larger particles tend to land in trachea and larger bronchi

Technical trials

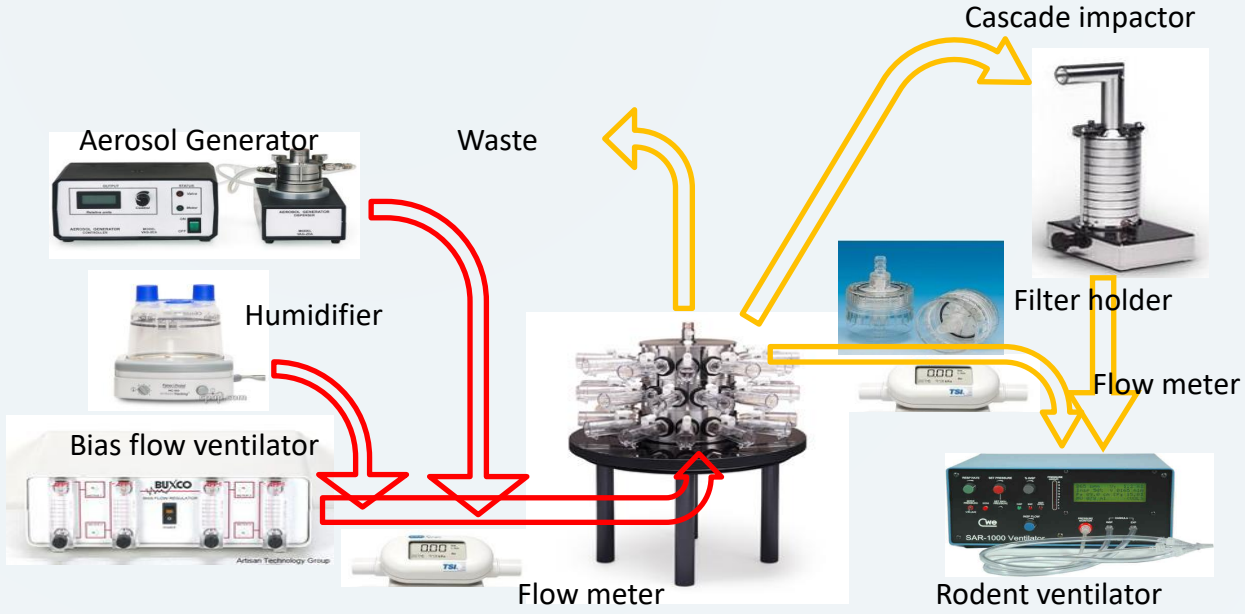
Step 1: Setting up the inhalation parameters ***Continued***

Typical starting conditions:

- VAG settings: 1300 mg/m^3
- 10, 20, 30 minutes of inhalation time
- Bias in-flow: 7L per minute
- 30% humidification of in-flow air
- 10 ports plugged; 1 port connected to filter holder; 1 port connected to impactor
- Rodent ventilator pulls through filter at 85 breaths/min, 0.25s inhalation time
- 0.5 L out-flow through impactor

At the end of the experiment:

Measure filter contents
Measure impactor contents
Adjust VAG frequency
Adjust inhalation time
Repeat trial



Step 2 : Animal pharmacokinetics / Pharmacodynamics

Run #1 of PK trial

- Plan for 30-minute run: load VAG with necessary powder
- Insert 11 animals into nose-only cones
- Attach filter holder to port #12
- Aerosolize material for 30 minutes continuously
- Withdraw 4 animals after 10 minutes, another 4 after 20 minutes, last 3 after 30 minutes.
- Draw blood from each animal upon exiting nose-cone; spin to plasma
- Harvest lungs in sub-group of animals to measure deposition IPD and distribution
- Draw blood from animals at pre-set times: suggest 1h, 2h, 4/6h and 24h post-inhalation
- Withdraw filter after 10 or 20 minutes; weigh contents to correlate with step 1
- Weigh VAG chamber after 30 minutes to have total amount of material aerosolized

At the end of the experiment:

Ship plasma to sponsor for contents analysis of test article: determine residence time in lungs and blood of the animals.

- Cmax
- Tmax
- Half-life
- Elimination
- AUC
- ...

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Step 2 : Animal pharmacokinetics / <u>Pharmacodynamics</u>

Run #2 of PK trial

- Adjust inhalation conditions based on PK run #1
- Repeat PK, with adjusted inhalation times, etc.
- Draw blood from each animal upon exiting nose-cone; spin to plasma
- Draw blood from each animal at pre-set times based on PK Run #1 results

Pharmacodynamics monitoring:

- Based on PK analysis of plasma samples
- Functional endpoints depend on efficacious mechanism for test article
- Animals are anesthetized one at a time, at selected post-inhalation times
- Animals are instrumented functional endpoint measurements (may be terminal)
- Animals are generally challenged acutely to reproduce the disease, with the test article efficacy being quantified at different times post-inhalation
- Compile up to 6 different post-inhalation challenge times

Step 3 : Actual efficacy study with inhalation-administration of test article

Varies with the indication

- Induce disease in suitable model
- Treat animals according to anticipated clinical schedule
- Measure functional endpoints
- Harvest organs and blood as required for biomarkers quantification

Some indications for which IPST has developed inhalation programs already

- Allergies
- Hypertension
- Cardiac infarct
- Pulmonary arterial hypertension
- Diabetes (hyperglycemia)
- Pulmonary fibrosis
- Cancer
- ...