PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

Development of a Novel *In Vitro* Aerosol Exposure System: the Independent Holistic Air-Liquid Aerosol Exposure System (InHALES)

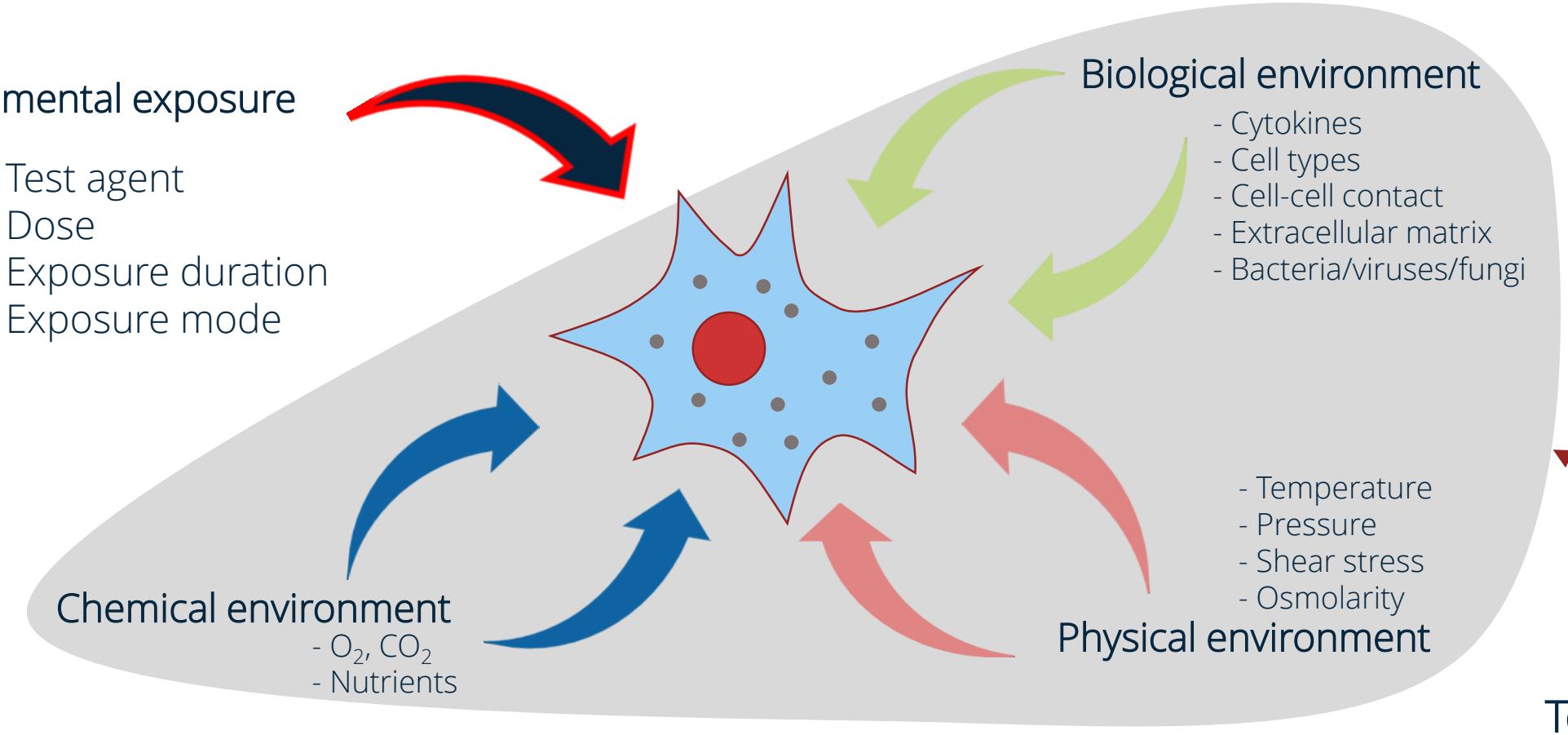
Sandro Steiner

Ecopa Symposium

November 2018

Paris

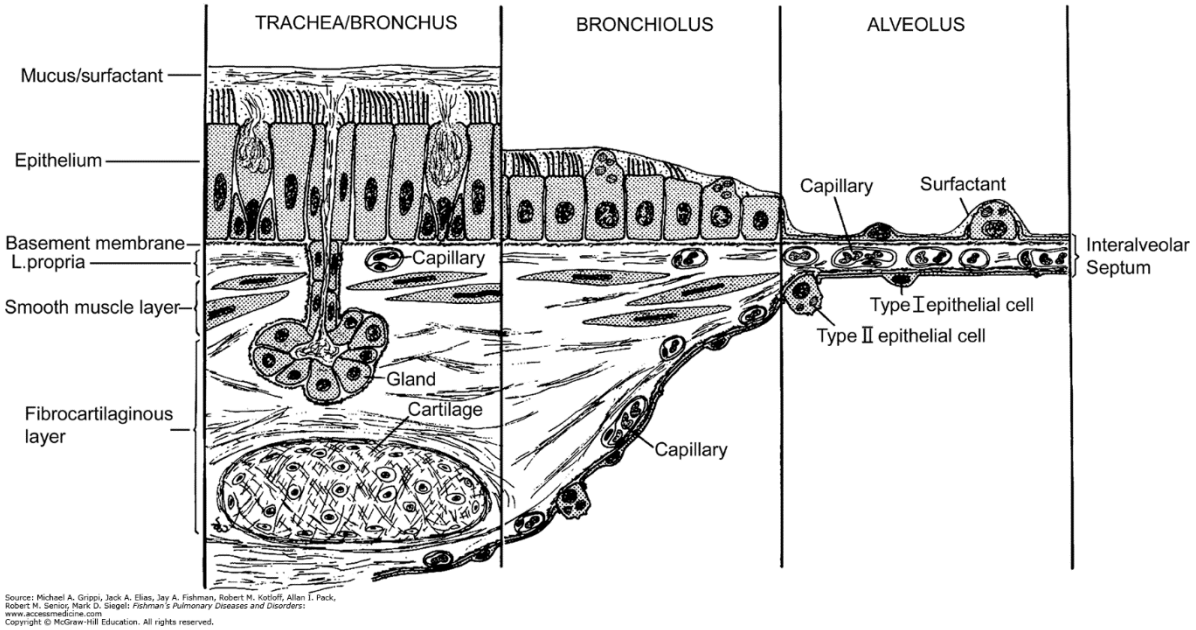
Parameters defining *in vitro* exposures



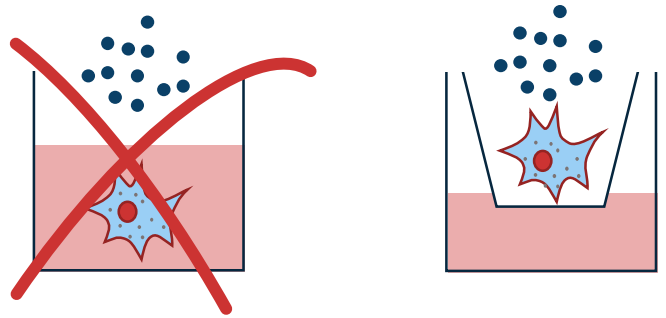
Aerosol exposures *in vitro*; challenges

1st challenge: alignment of exposure conditions with physiology

The strucutral and functional non-uniformity of the human respiratory tract has to be accounted for



Aerosol exposures need to be conducted at the air-liquid interface



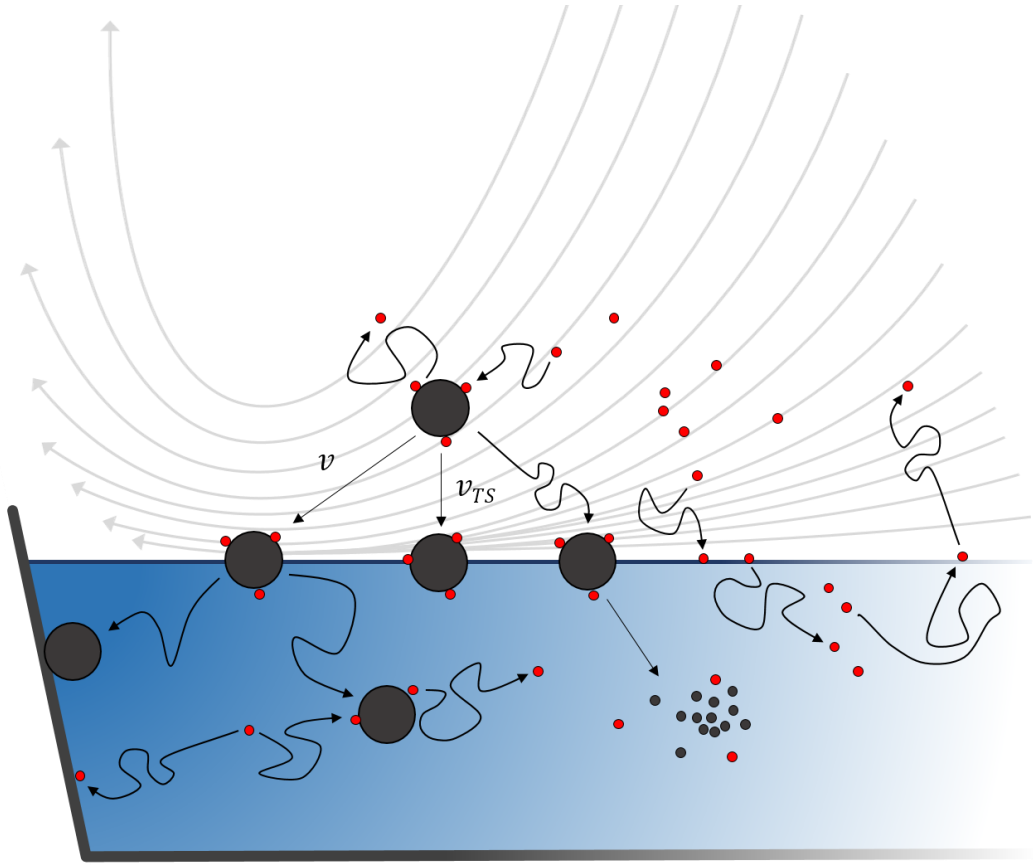
→ Controlling aerosol flow
→ Controlling aerosol dilution
→ Aerosol conditioning (humidity, temperature)

PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

Aerosol exposures *in vitro*; challenges

2nd challenge: the complexity of aerosol dynamics

- Aerosols are often complex mixtures:
  - Particles of different sizes and different composition
  - Gases and semi-volatile compounds with different physicochemical properties
- Particles and gases as well as particles of different sizes show different physical behavior
- Semi-volatile compounds are dynamically partitioned between the particulate and the gaseous phase

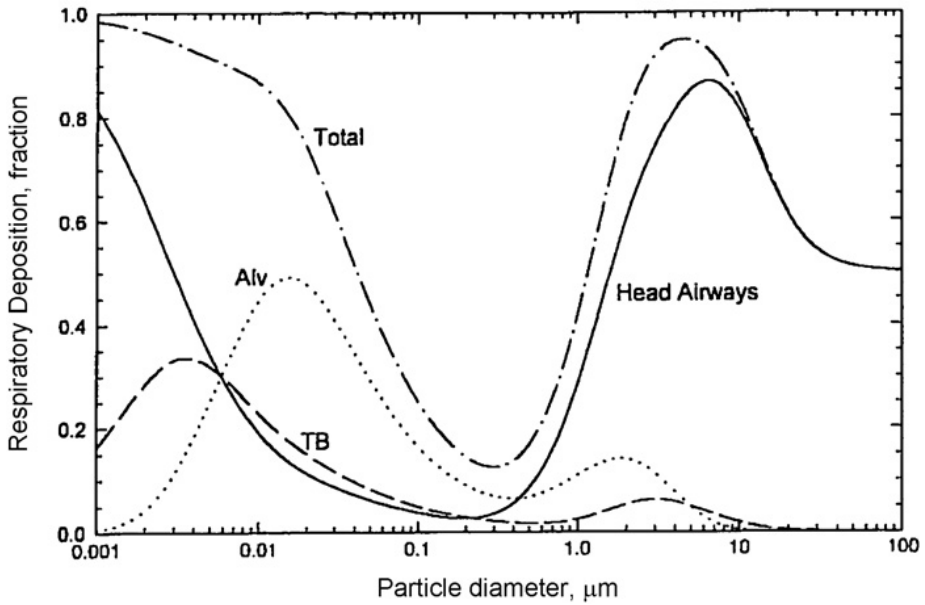


PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

Aerosol exposures *in vitro*; challenges

Consequences:

- Regiospecific deposition of aerosol particles in the respiratory tract *in vivo*
- Compound-specific absorption efficiency of volatile and semi-volatile aerosol constituents *in vivo*



Carvalho et al., International Journal of Pharmaceutics 406 (2011) 1–10

***In vivo* retention of cigarette smoke constituents in % as reported in various publications**

	1,3-Butadiene	2-Butanone	2-Pentanone	Acetone	Acroleine	Benzo[a]pyrene	Benzene	Butanal	Fluorene	Isoprene	Isovaleraldehyde	Naphthalene	Nicotine	NNK	NNN	Phenanthrene	Propionaldehyde	Propionitrile	Pyrene	Solanesol	Toluene
Moldoveanu et al. 2007				93	100			99									98				
Zhang et al. 2012	<5				98																
Baker et al. 2006		82	82	76	97		75			49	94		79					71			92
Dalhamn et al. 1968				86						99											96
Armitage et al. 2004													99							71	
Feng et al. 2007										50			>98	84	97						
McGrath et al. 2009													47								
Moldoveanu et al. 2008							94														94
Moldoveanu et al. 2008						71			98			97				95			93		
Moldoveanu et al. 2008																				66	
Average *in vivo* retention, normalized to nicotine	0.1	1.0	1.0	1.1	1.2	0.9	1.2	1.2	1.2	0.8	1.2	1.2	1	1.0	1.2	1.2	1.2	0.9	1.2	0.8	1.2

Steiner et al., Toxicology in Vitro 52 (2018) 384–398

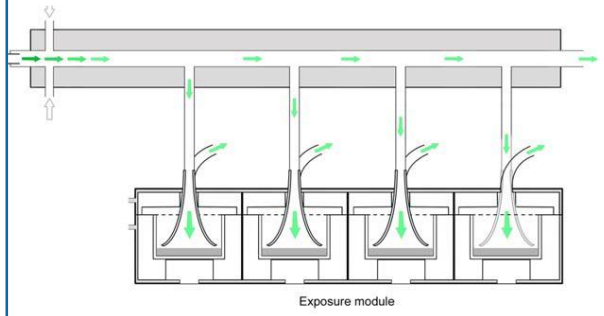
PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

Aerosol exposures *in vitro*; current situation

State of the art *in vitro* aerosol exposure systems:

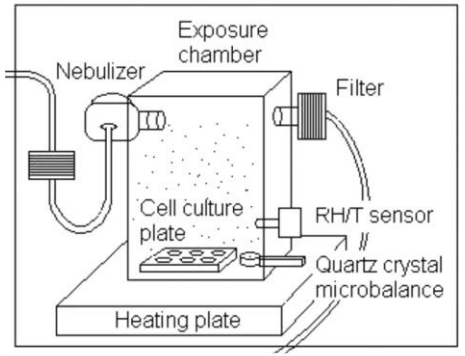
- ✓ Controlled aerosol conditioning
- ✓ Controlled aerosol dilution
- ✓ Controlled aerosol supply to cell cultures
- ✓ → **Feasible for exposures at the air-liquid interface**
- ✗ Limited ability to mimick dynamics of inhalation
- ✗ Limited ability to mimick physiology
  - Regiospecificity not captured
  - Relative delivery of different volatiles or of volatiles and particles not captured
- ✗ No control over aerosol evolution
- ✗ → **Potential introduction of experimental artifacts**

Stagnation flow/trumpet based systems



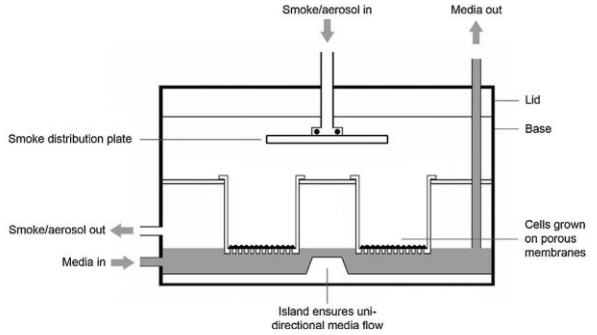
Thorne et al., Experimental and Toxicologic Pathology 65 (2013) 1183– 1193

Sedimentation based systems



Müller et al., Insciences J. 2011, 1(1), 30-64

Diffusion and sedimentation based systems



Thorne et al., Experimental and Toxicologic Pathology 65 (2013) 1183– 1193

PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

A promising new approach

In vitro aerosol exposure systems simulating relevant (macroscopic) structural and functional aspects of the human respiratory tract,

…as a high structural and functional similarity between an aerosol exposure system and the human respiratory tract is expected to result in a high degree of comaparability between *in vitro* and *in vivo* aerosol exposures

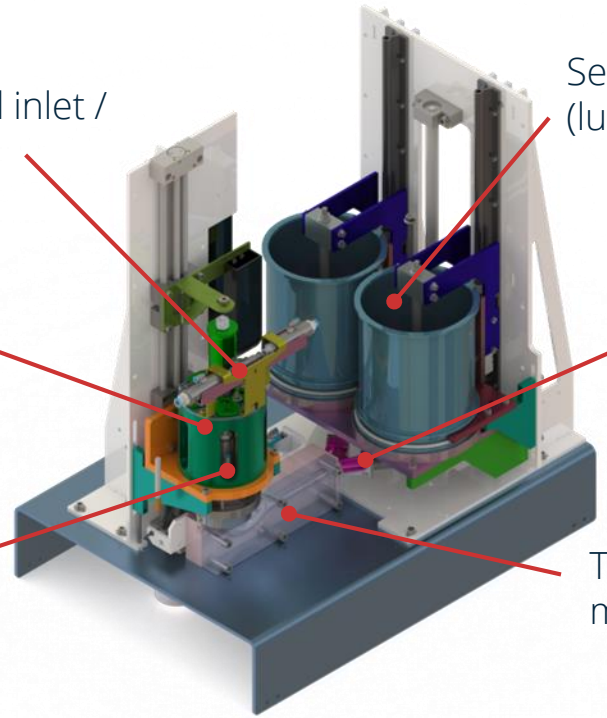
→ Physiologically realistic aerosol exposures *in vitro*

→ Determination of aerosol dosimetry in the human respiratory tract

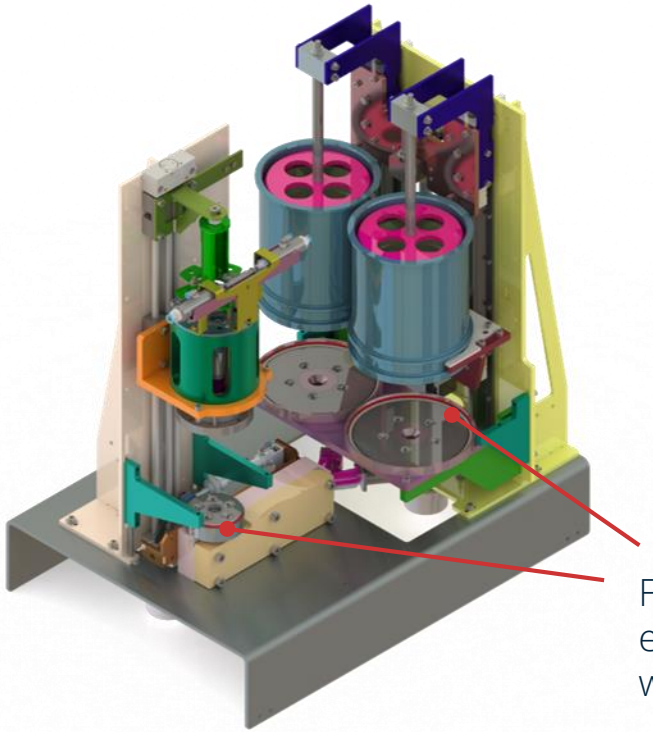
The InHALES prototype

A prototype of the 'Independent* Holistic** Air-Liquid Exposure System'

* No active aerosol supply required, the system is able of sampling aerosols by itself
** The complete human respiratory tract is simulated in one system



An array of piston pumps connected by a model of the conducting airways

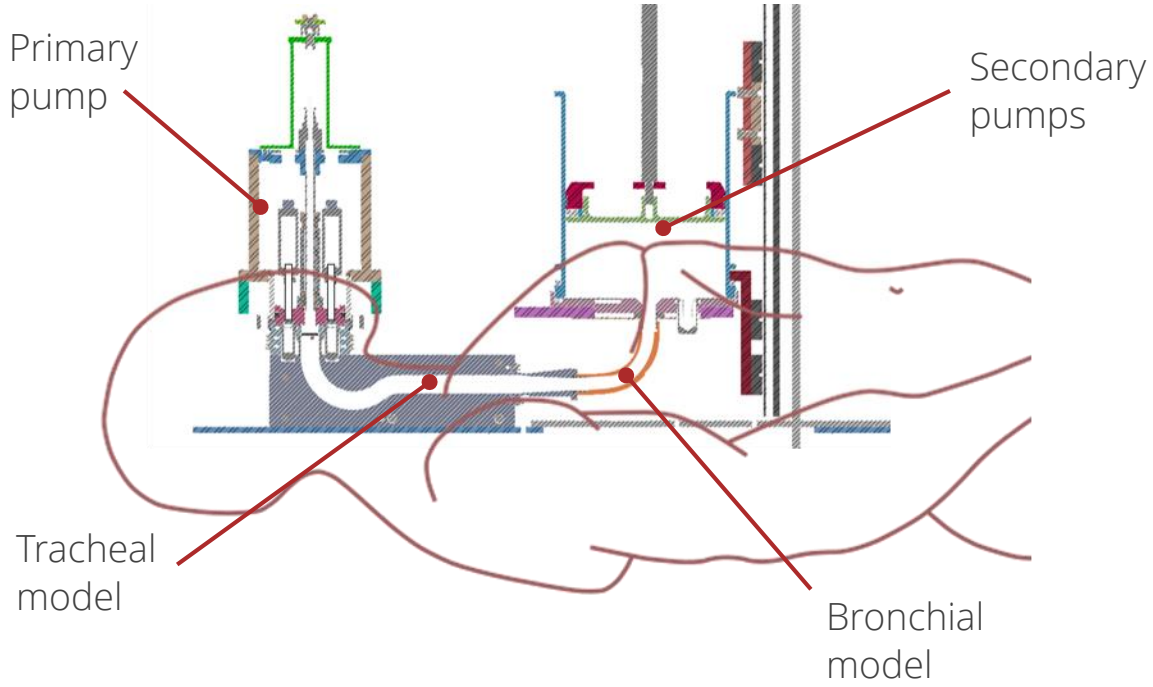


Five positions for cell culture exposures in each pump (24-well format transwell inserts)

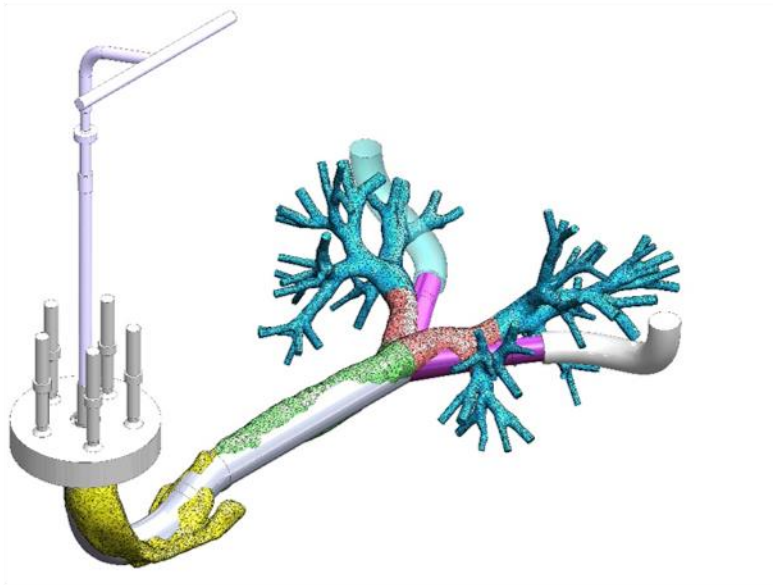
Cell culture positions in the tracheal and brochial model are not included in the prototoype

The InHALES prototype

Mimicking human physiology



Pump dimensions and relative positions reflect the overall human respiratory tract



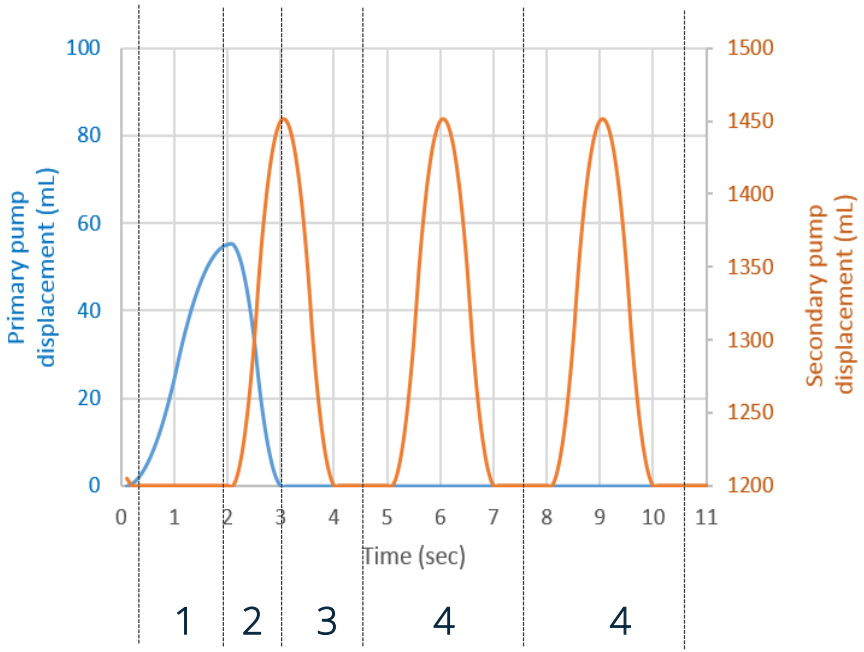
Dimensions and shape of the airways are based on *in vivo* data*, in the protoype version of the system only realized to G1 (main bronchi)

*Kleinstreuer et al. Journal of Aerosol Science 46 (2012) 34-52 , Fishman's Pulmonary Diseases and Disorders, 5th ed. 2015. ISBN-13: 978-0071807289. ISBN-10: 0071807284)

The InHALES prototype

Mimicking functional aspects of the human respiratory tract

Example: cigarette smoking



1) A puff is taken from a cigarette by the action of the primary pump

2) During a short period of time, the puff is kept in the 'oral cavity' (mouth-hold duration)

3) By the action of the secondary pumps, the puff is inhaled through the tracheal and bronchial model, along with a larger volume of clean air. The primary pump is thereby completely flushed with air passing through the dilution air inlets located in the piston plate of the pump

4) The secondary pumps take several 'breaths' of clean air with short intervals of rest in between

Any puff volume and duration, including accepted smoking protocols (e.g. Health Canada) can be programmed

As the dimensions of the system reflect human physiology, smoke dilution and travelling velocity are by default adjusted to the regiospecific, physiological conditions in the human respiratory tract

Any physiologically relevant residual air volume in the lung pumps, any relevant volume of inhaled air as well as any relevant timing can be programmed

Inhaled, diluted smoke is exhaled again. The tracheal model and the primary pump are thereby again exposed, but to depleted, aged smoke

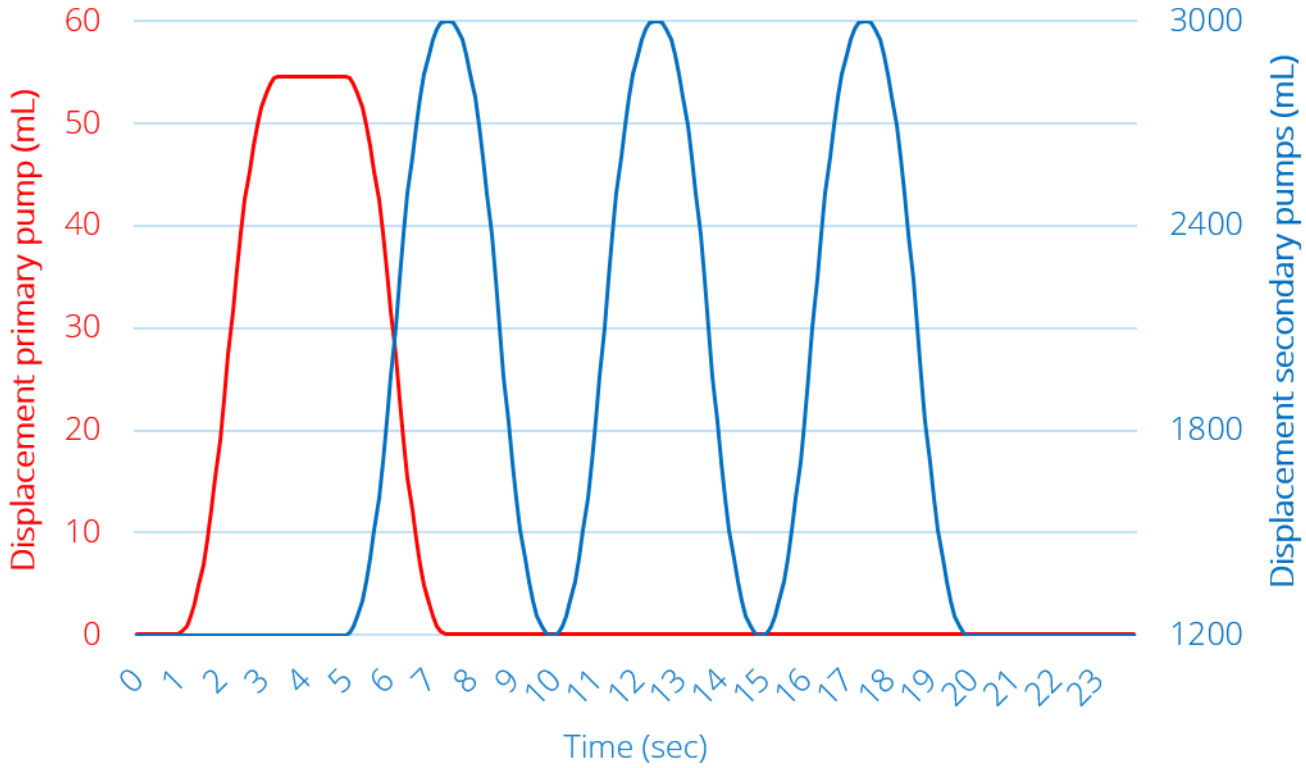
Prototype testing – proof of concept

Cell free exposure to:

- Smoke generated from 3R4F reference cigarettes (University of Kentucky)
- Disodium fluorescein labelled glycerol/propylene glycol aerosol

Test Cycle:

- A 55 mL puff is taken within 2 seconds
- 2 seconds 'mouth-hold period'
- The puff is inhaled along with 1800 mL clean air during 2 seconds
- The inhaled mixture of smoke and air is exhaled during 2 second
- Two times inhalation of 1800 mL clean air
- The residual volume in the lung pumps is set to 1200 mL
- 20 cycles are executed per test exposure



Prototype testing – proof of concept

Determination of aerosol deposition in the system:

1. Exposure of phosphate buffer saline (PBS) in cell culture inserts (in the pumps) or tape-patches (in the tracheal model)

2. Collection of exposed PBS, washing of exposed tape patches in PBS

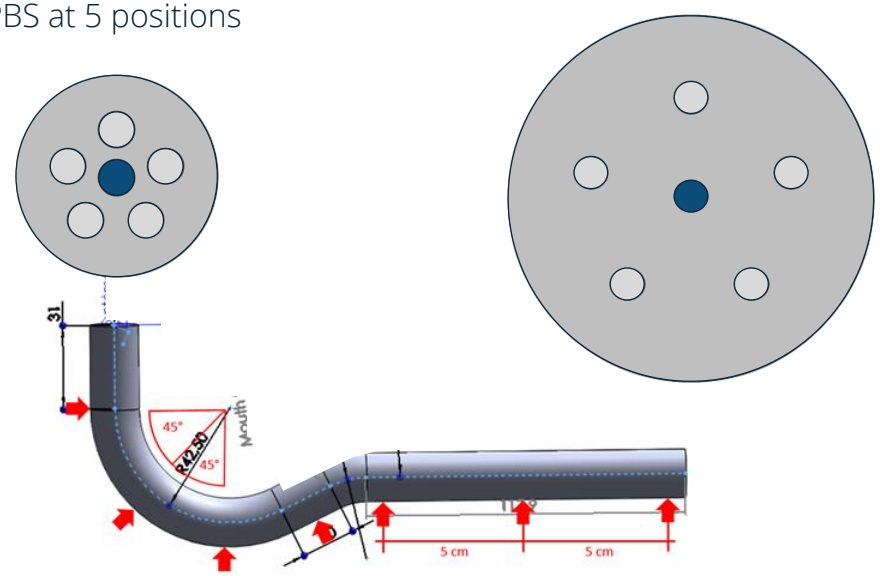
3. Quantification of deposited aerosol material in the PBS samples
   - 3R4F smoke: nicotine and 8 carbonyl compounds (by LC-MS)
   - Glycerol/propylene glyol aerosol: disodium fluosecein (by fluorometry)

Aim: Proof of concept

- Demonstrate uniformity of aerosol deposition across replica positions and repeatability of exposures

Primary pump:
Cell culture inserts with PBS at 5 positions

Secondary pumps:
Cell culture inserts with PBS at 5 positions in each pump



Tracheal model:
Tape patches placed at 7 positions (red arrows. This was only performed using glycerol/propylene glycol aerosol)

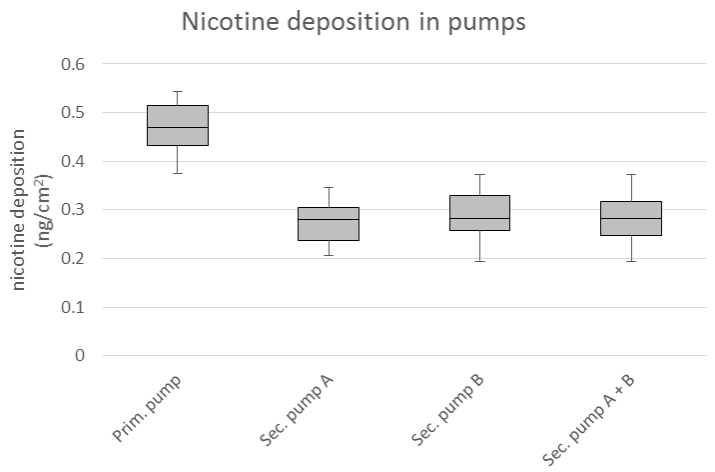
Prototype testing – proof of concept

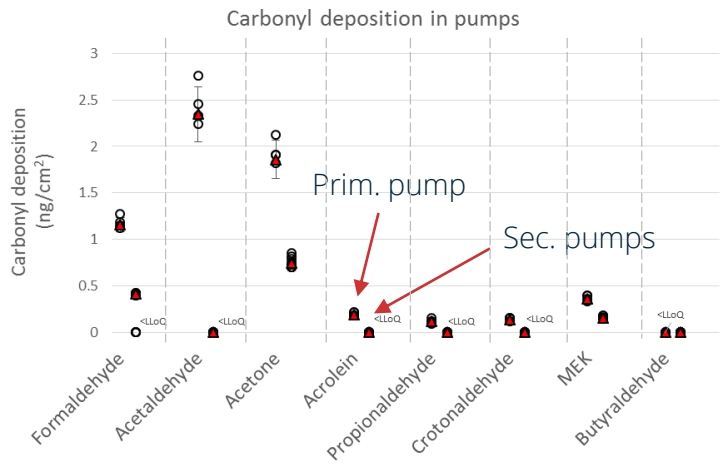
Aerosol deposition:

- High deposition uniformity in pumps
- Repeatabilitiy of exposures is provided
- Different deposition patterns for different aerosol types are indicative for a high sensitivity towards aerosol properties

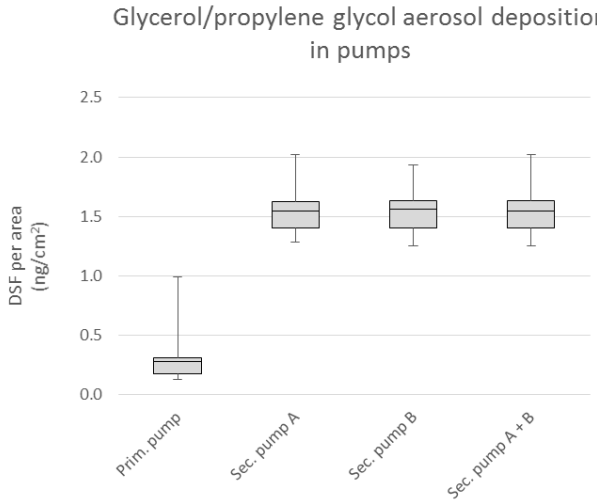
(Data generated in 5 independent experimental repetitions)

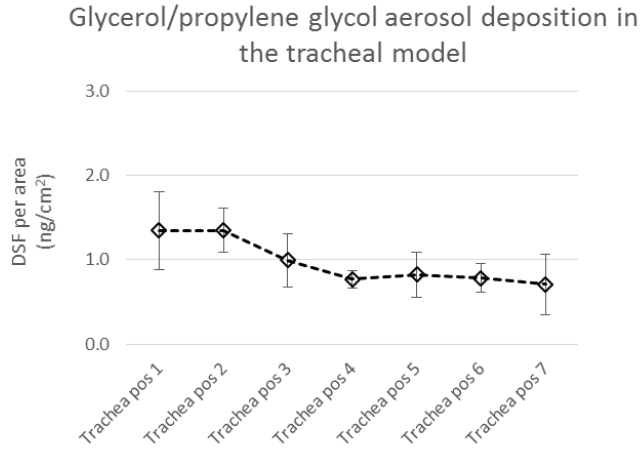
3R4F smoke





Glycerol/propylene glycol aerosol





PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

Conclusions and Outlook

- A prototype of a novel *in vitro* aerosol exposure system was developed
  - The system aims at simulating structural and functional aspects of the complete human respiratory tract
  - This is expected to render *in vitro* exposures highly representative for the *in vivo* situation
- As a proof of concept, cell free test exposures were conducted in the prototype system
  - The results indicate that the system allow conducting controlled and repeatable exposures
- The system is currently further developed
  - The complexity of the the airway model will be increased with respect to 3D shape and number of bifurcation generations, which is expected to render the aerosol evolution inside the system more realistic. A detailed comaprison of aerosol dynamics in the system with the ones *in vivo* will follow
  - Positions for cell culture exposures will be included into the tracheal and bronchial models
- The utimate proof of concept requires survival of cell cultures within the system during exposures
  - The according experiments are currently being conducted

PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

Thank you

Acknowledgements

PMI engineering and development team
- Pierre Hervé
- Shoaib Majeed
- Arkadiusz Kuczaj
- Julia Hoeng

PMI analytical chemistry team
- Quentin Dutertre
- Arno Knorr

LRP AG, Switzerland
- Markus Widmer and his team of engineers