Development of a novel *in vitro* aerosol exposure system: the Independent Holistic Air Liquid Exposure System (InHALES)

S. Steiner, P. Hervé, S. Majeed, C. Pak, A. Kuczaj, S. Frentzel, J. Hoeng

PMI R&D, Philip Morris Products S.A., Quai Jeanrenaud 5, CH-2000 Neuchâtel, Switzerland

Background

- The delivery kinetics of volatile and particulate aerosol constituents as well as of particles of different sizes vary between different regions of the human respiratory tract *[1, 2, 3]*.
- Inhaled aerosols evolve in terms of particle size distribution and chemical composition when passing from the site of their generation through the respiratory tract to the alveolar spaces and back *[1, 2, 3]*.
- Available *in vitro* aerosol exposure systems are not able to capture this complexity *[4, 5]*. The aerosol fractions they deliver to cell cultures are therefore not or only partly representative for the *in vivo* situation.
- This may decrease the relevance of *in vitro* aerosol exposure experiment, especially when using complex cell cultures that are able to respond to physical and chemical stimuli in a highly differentiated manner.
- We developed an *in vitro* aerosol exposure system that mimics structural and functional aspects of the human respiratory tract: the *In*dependent *H*olistic *A*ir *L*iquid *E*xposure *S*ystem (InHALES) *[6]*.
  - Independent: Capable of actively inhaling aerosols, smoking cigarettes, or operating inhalers without the need for special aerosol generators or diluters. In particular, it is capable of closely simulating human smoking behavior, including the dilution of the concentrated puffs with fresh air during smoke inhalation.
  - Holistic: Consists of modules representing the relevant regions of the respiratory tract in the existing prototype the oral cavity, laryngopahrynx, trachea, main bronchi, and the lung lumen.
  - Air-liquid exposure system: Specifically designed for aerosol exposures at the air-liquid interface.
- A prototype of the system was built recently (**Figure 1**), and initial (cell culture-free) functional system characterization was performed as proof-of-concept.

The InHALES system

Mouth pump

- Capable of generating puffs of 0–130 mL in volume
- Free choice of puff duration and profile
- Entirely made of stainless steel, heat-sterilizable
- Aerosol inlet centrally located in the piston shaft
- Dilution air inlet symmetrically arranged in the piston plate
- Outlet and connection to the tracheal model centrally located in the base plate
- Five positions where transwell inserts (24-well format) with cell cultures can be placed **(Figure 1.1)**
- Leak tight butterfly valve at inlet to tracheal model
- If puffing is not required, the pump can be bypassed by deactivation

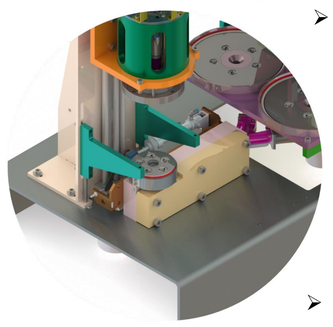

Figure 1.1: The mouth pump in opened position.

Trachea and main bronchi

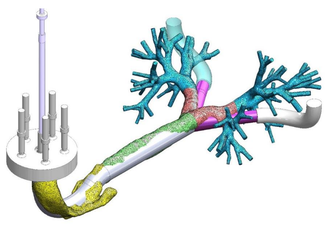

Figure 1.3: Superposition of human airways and the tracheal/bronchial model.

- In size and geometry based on human physiology **(Figure 1.3)**
  - Brno lung cast model *[7]*
  - Weibel's model *[8]*
- Entirely made of stainless steel, heat-sterilizable
- Currently no slots for cell cultures available (prototype version of the system)

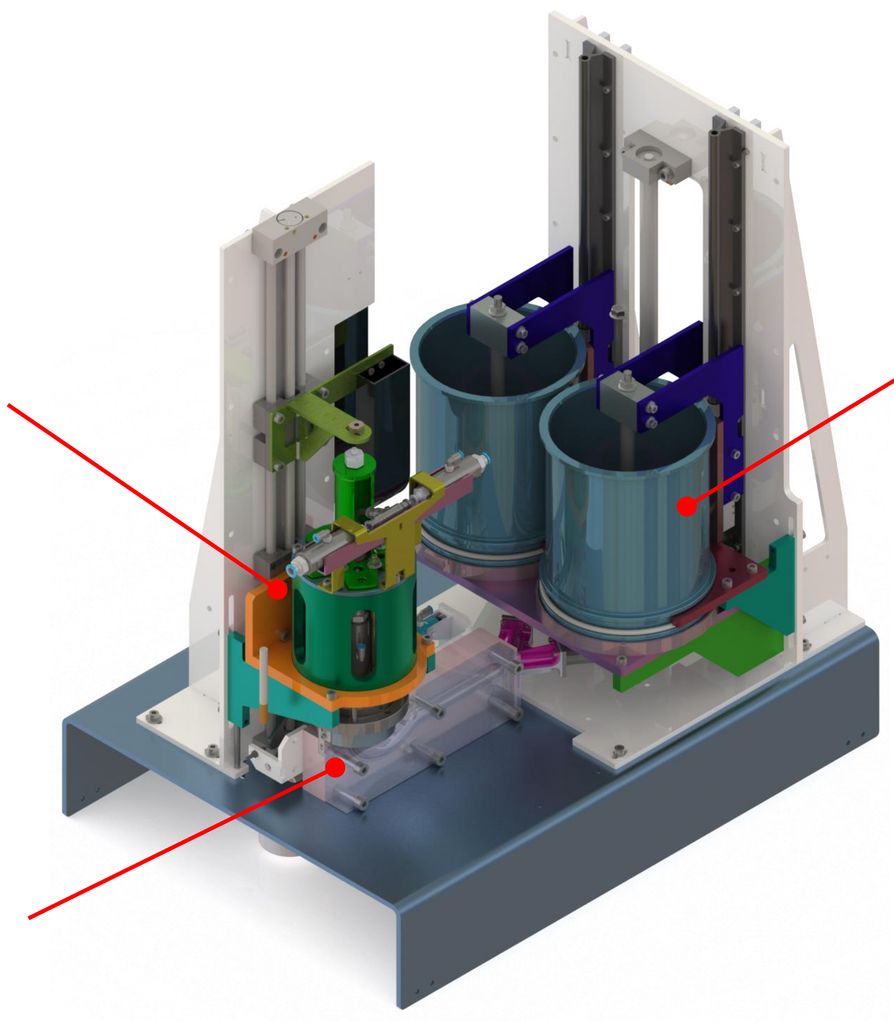

Figure 1: Total view of the InHALES system. The system is modular; individual parts (mouth pump, trachea, main bronchi, lung pumps) can be modified or exchanged without changing the overall system structure of function.

Lung pumps

- Capable of inhaling a total volume (both pumps together) of 0–6000 mL
  - Free choice of residual volume
  - Free choice of inhaled volume
  - Free choice of inhalation timing and profile
  - Entirely made of stainless steel, heat-sterilizable
- Five positions per pump where transwell inserts (24-well format) with cell cultures can be placed **(Figure 1.2)**

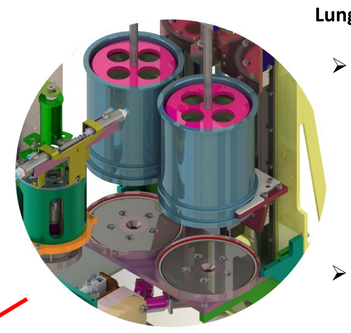

Figure 1.2: The lung pumps in opened position.

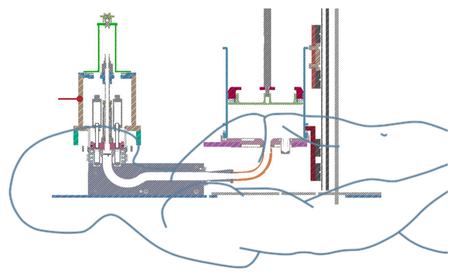

Figure 1.4: The individual modules (mouth pump, trachea, main bronchi, and lung lumen) are arranged in a way that mimics human physiology.

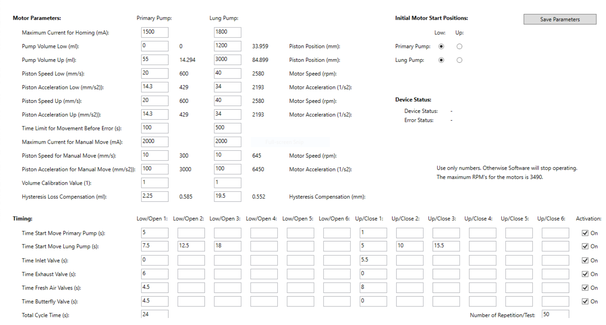

Figure 1.5: Pump displacement volumes; the timing, acceleration, and maximum speed of piston movement; the opening and closing of valves; and cycle number and duration can be chosen freely in the system's software.

Initial system testing, results, and discussion

The system "puffed" and "inhaled" a fluorescent test aerosol (propylene glycol (PG), glycerol (G), and water, labelled with disodium fluorescein (DSF)) or smoked 3R4F reference cigarettes (University of Kentucky).

The applied puffing and inhalation cycle is shown in **Figure 2**. 50 puffs were taken, either from a constant flow of the fluorescent test aerosol or from five 3R4F cigarettes.

In the mouth and the lung pumps, cell culture inserts containing phosphate-buffered saline (PBS) were exposed.

In the tracheal model (not providing slots for cell culture inserts in the prototype), patches of adhesive tape were placed at the positions indicated in **Figure 3**.

Upon test exposures, the deposited mass of DSF or nicotine and eight representative carbonyl compounds was quantified in the exposed PBS samples. DSF and nicotine were quantified in individual samples (five samples per pump per repetition). For the quantification of carbonyl compounds, the five samples obtained per pump were pooled.

Quantification of aerosol deposition on the patches of adhesive tape was only performed for the fluorescent test aerosols; the deposited material was eluted in PBS and fluorometrically quantified.

Particle size distributions in the aerosols were measured at different locations in the system using a TSI 3321 Aerodynamic Particle Sizer®.

The results are shown in **Figures 4 and 5**. All experiments were conducted in five independent repetitions.

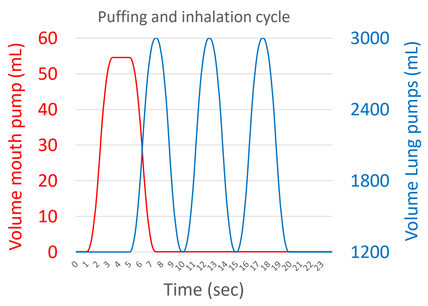

Figure 2: The puffing and inhalation cycle applied during the test exposures. The cycle was repeated 50 times per exposure; software settings are shown in Figure 1.5.

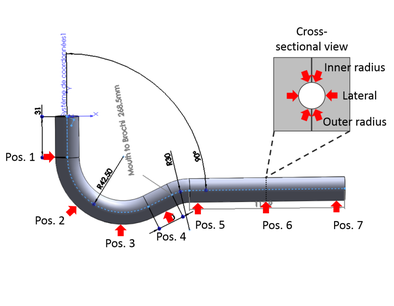

Figure 3: Positions in the tracheal model where patches of adhesive tape were placed for exposures.

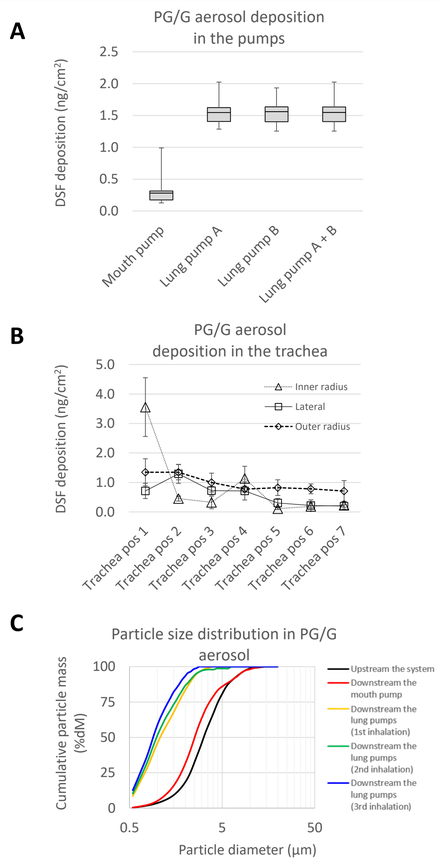

Figure 4: A) deposition of disodium fluorescein in the mouth pump and the two lung pumps (called A and B). 25 values per pump; error bars show lowest and highest values. **B)** deposition of DSF in the trachea (at position listed in **Figure 3**). Error bars show standard deviations (10 values per position). **C)** The cumulative, mass-based particle size distribution of the PG/G aerosol at different locations in the system.

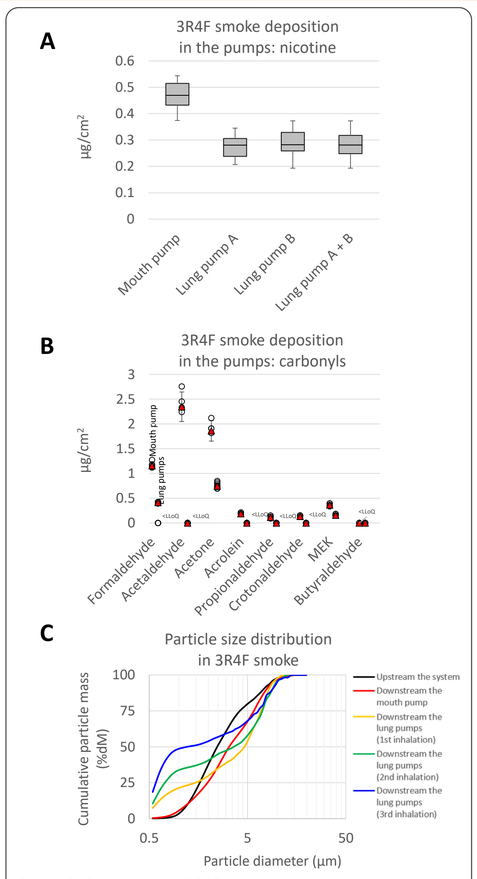

Figure 5: Deposition of 3R4F smoke constituents in the mouth pump and the two lung pumps (called A and B). **A)** Nicotine and **B)** eight representative carbonyl compounds. Individual experimental repetitions (black circles) and averages (red triangles) are shown. **C)** The cumulative, mass-based particle size distribution of the smoke at different locations in the system.

- A prototype of the system was built and tested in cell-free experiments.
- Basic proof-of-concept:
  - System functionality
  - Repeatability of exposures
  - Uniformity of aerosol delivery to replica positions
- Complex aerosol dynamics in the system. This is considered the result of particle sizes, the partitioning of aerosol constituents between the particulate and the gas phase, and the complexity of the system and is in line with the complexity of aerosol deposition described in the human respiratory tract.
  - Different aerosol types and aerosol constituents show different patterns of deposition.
  - Changes in particle size distributions during an aerosol's passage through the system are specific to the aerosol type.
  - A 1:1 comparison to *in vivo* data was not performed, as relevant parameters (temperature, humidity, bronchioles, and alveolar surface) are not yet covered in the system.
- Next steps of system characterization will include cell culture exposures and the comparison of the observed *in vitro* responses to *in vivo* outcomes of aerosol inhalation.

References:

1. Carvalho TC, Peters JI, Williams RO. *International Journal of Pharmaceutics* 2011, **406**(1–2):1-10.
2. Heyder J. *Proceedings of the American Thoracic Society* 2004, **1**(4):315-320.
3. Pankow JF. *Chem Res Toxicol* 2001, **14**(11):1465-1481.
4. Thorne D, Adamson J. *Exp Toxicol Pathol* 2013, **65**(7):1183-1193.
5. Paur HR, Mulhopt S, Weiss C, Diabate S. *J Verbrauch Lebensm* 2008, **3**(3):319-329.
6. Hervé, P. In vitro Experiments: Development of a New Aerosol Exposure System. Master Thesis at the école d'ingénieurs CESI, Saint-Nazaire, 2017
7. Ochs M, Weibel ER (eds.). McGraw-Hill; 2008.
8. Nordlund M, Belka M, Kuczaj AK, Lizal F, Jedelsky J, Elcner J, Jicha M, Sauser Y, Le Bouhellec S, Cosandey S. *Inhalation toxicology* 2017, **29**(3):113-125.

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