Collaborative efforts to advance *in vitro* inhalation toxicity testing towards regulatory acceptance

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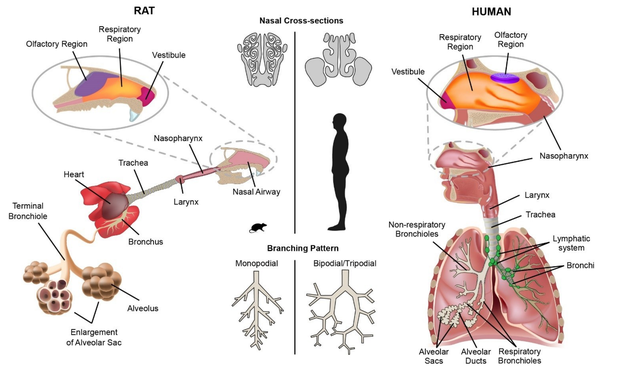
Abstract/Poster
#3596/P750

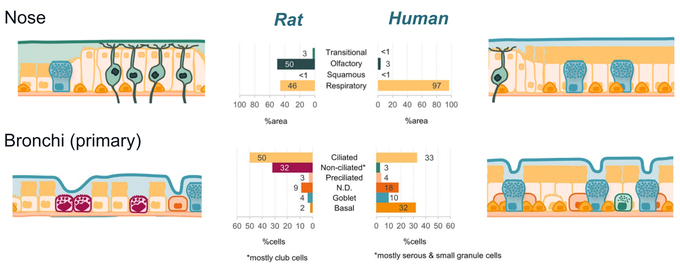
PETA SCIENCE CONSORTIUM INTERNATIONAL e.V.

Assessing the state-of-the-science



Anatomy of the rat and human respiratory tracts with representative nasal cross section and typical branching pattern of bronchi.

Regulatory agencies worldwide have requirements to assess the potential effects of inhaled chemicals on humans. While inhalation toxicity testing has traditionally been conducted in rats, differences in the respiratory tracts of humans and rats limit the precision with which rats can reliably predict human effects. Therefore, *in vitro* models are increasingly being used to assess the toxicity of inhaled substances. Gaining regulatory acceptance for these approaches involves coordinated efforts from stakeholders across various geographies and sectors. Here, we present a collaborative effort to establish scientific confidence in an *in vitro* approach for assessing the portal-of-entry effects of inhaled substances on the respiratory tract. Effectively advancing *in vitro* testing approaches within the inhalation toxicity space involves coordinated efforts from method development to optimization, standardization, confidence-building, and regulatory acceptance.

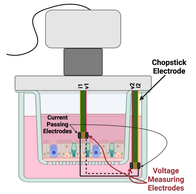


Cell types in the nasal (upper image) and bronchial regions (lower image) differ substantially in rats and humans

Illustrations from **Stucki, Sauer, Allen, et al. *Regul Toxicol Pharmacol*. 2024.150:105648**

Acknowledgements: We extend our sincere gratitude to each of the collaborating organizations that contributed to this work.

Method standardization: reporting recommendations



Standardizing *in vitro* practices by developing minimum reporting recommendations helps facilitate repeatability and reproducibility, thus enabling cross laboratory comparisons of data and regulatory acceptance. A paper on the Minimum Information for Reporting on the TEER Assay (MIRTA) is the result of the work of the RespTox Collaborative, an international, cross-sector consortium of experts conducting *in vitro* inhalation toxicity testing (**Sharma, Huber, Arnesdotter, et al. *Arch Tox*. 2024**).

TEER: Trans-Epithelial/Endothelial Electrical Resistance

Model characterization: metabolism

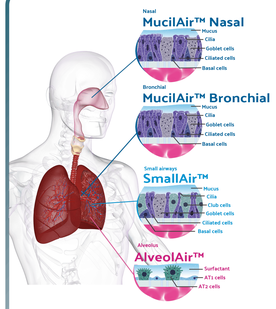


Image courtesy of Epithelix

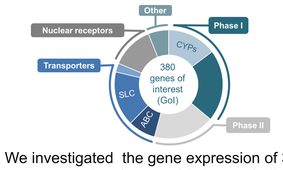
Reconstructed tissue models

Understanding local metabolic processes enhances the scientific confidence in *in vitro* reconstructed human respiratory epithelial (RHRE) models and their ability to evaluate substances undergoing local biotransformation. In partnership with Epithelix, BASF, and Helmholtz Munich, we performed RNA sequencing to assess the metabolic capabilities of Epithelix's RHRE models from various regions of the respiratory tract (nasal, bronchial, small airway, and alveolar) and from up to five donors.

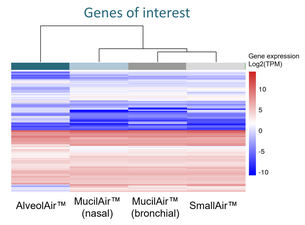
Join us on Tuesday, March 18, to learn more about this work! (Abstract #1176)

Tuesday, March 18, 11:00 AM to 12:30 PM, Room W304E, Convention Center
Symposium Session: Assessing the Metabolic Competence of *In Vitro* Models and Their Applicability in Regulatory Toxicology

Explore our curated database for commercially available human **lung tissue models** and other organs!

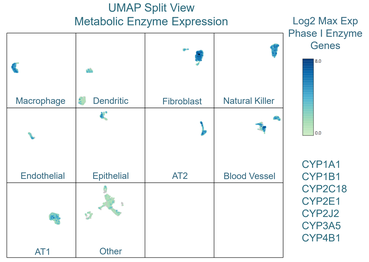


We investigated the gene expression of 380 genes associated to xenobiotic metabolism



RHRE types clustering based on their similarities in expression of the genes of interest

hPCLS



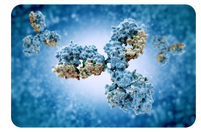
In collaboration with the Institute for In Vitro Sciences (IIVS), we helped fund the development of a protocol for the cryopreservation and long-term maintenance of human precision-cut lung slices (hPCLS). Cryopreserved tissues retained viability, protein content, and cell-specific markers (**Patel, Amin, Wahab, et al. *Toxicol Sci*. 2023. 191(2):253-265**), which expands the accessibility of this model system.

Currently, fresh and cryopreserved hPCLS are being assessed for cell sub-populations and cell-specific metabolic capabilities by single-cell RNA sequencing.

Method optimization: antibodies

In collaboration with Unilever and Abcalis, we are developing animal-free recombinant antibodies against Interleukin 6 (IL-6) and Interleukin 8 (IL-8/CXCL-8). Animal-free recombinant antibodies offer advantages over animal-derived ones, including being highly specific for their intended targets and consistent and reproducible across batches.

Once developed, these antibodies will be made available to the scientific community.



Proof of concept testing: The INSPIRE Initiative

Our INSPIRE (*IN vitro* Systems to PredIct REspiratory toxicity) Initiative aims to (1) build scientific confidence in *in vitro* testing approaches to predict respiratory toxicity and (2) identify relevant cellular effects, exposure methods, and model systems that may be most appropriate for use, depending on the purpose of testing.

Single exposure testing is complete and repeat exposure experiments are starting. Testing is being conducted at the Flemish Institute for Technological Research (VITO).

BEAS-2B

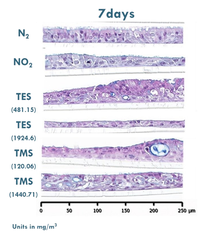
Human bronchial epithelial cell line

MucilAir™

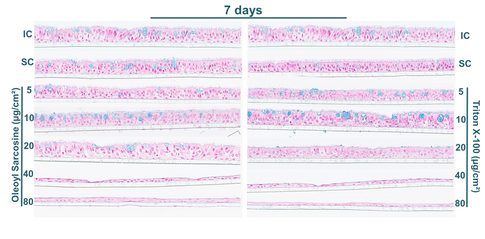
Human bronchial epithelial tissue model

Cellular effects	BEAS-2B	MucilAir™
Cell viability (PrestoBlue®)	✓	✓
Cytotoxicity (LDH)	✓	✓
Inflammatory markers (IL-6, CXCL-8)	✓	✓
Cilia beating frequency (CBF) and Average Active Area (AAA)		✓
Barrier integrity (TEER)		✓
Histology (H&E staining)		✓

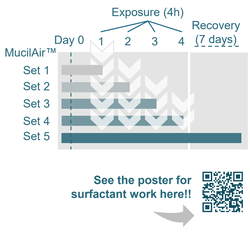
Silanes (single dose)
Triethoxysilane (TES)
Trimethoxysilane (TMS)



Surfactants (single dose)
Oleoyl Sarcosine, Triton X-100



Surfactants (repeat dose)
Oleoyl Sarcosine, Triton X-100



See the poster for surfactant work here!!

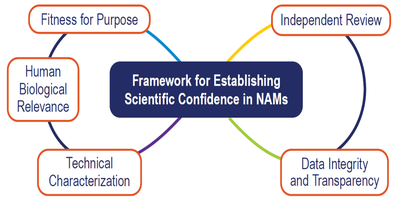
Manuscript in preparation

Sharma, Stucki, Verstraelen, et al. *Tox Sci*. 2023. 195(2):213-230

Towards international guidance

Multi-laboratory testing is being organized to evaluate the use of a reconstructed human respiratory epithelial model to assess portal-of-entry effects of chemicals delivered as liquids. These data will support the submission of a proposal to the Organisation for Economic Co-operation and Development (OECD) for an *in vitro* test guideline. The intent is that this will be one of multiple methods that can be used to cover the varied information needs for inhalation toxicity.

The data and method will be evaluated using an established scientific confidence framework to ensure fitness for purpose, human biological relevance, technical characterization, data integrity and transparency, and independent review.



van der Zalm, Barroso, Brown, et al. *Arch Toxicol*. 2022. 96:2865-2879