A holistic approach to human-relevant *in vitro* inhalation toxicology testing

Amy J. Clippinger[1]*, Monita Sharma[1], Nuria Roldan[1], Adam Bettmann[1], Andreas O. Stucki[1]

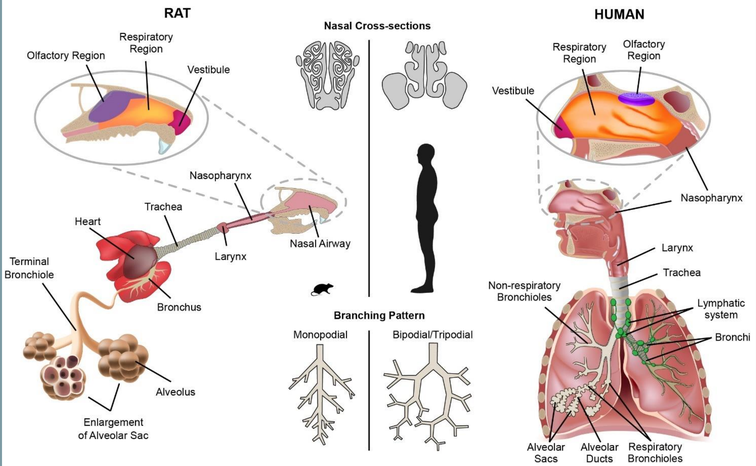
PETA Science Consortium International e.V., Stuttgart, Germany *AmyJC@thepsci.eu

PETA SCIENCE CONSORTIUM INTERNATIONAL e.V.

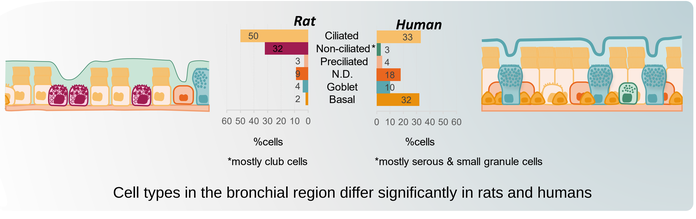
Learn more about the Science Consortium's inhalation work at:

Assessing the State-of-the-Science

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.



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



Cell types in the bronchial region differ significantly 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.

Model Characterization: Metabolism

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' RHRE models from various regions of the respiratory tract (nasal, bronchial, small airway, and alveolar) and up to five donors.

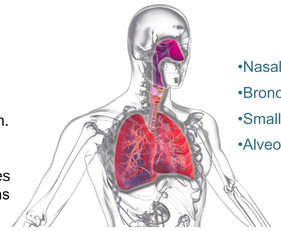
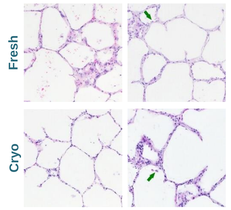


Image from the Epithelix website

hPCLS



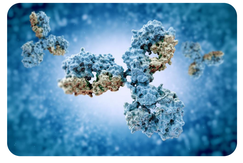
In collaboration with the Institute for In Vitro Sciences (IIVS), we helped fund the development of a protocol for the cryopreservation and long-term maintenance of human precision-cut lung slices (hPCLS). Cryopreserved tissues retained viability, protein content, and cell-specific markers (**Patel,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

Currently, fresh and cryopreserved human lung slices are being assessed for cell sub-populations and cell-specific metabolic capabilities by single-cell RNA sequencing. The results will be compared to native lung tissues.

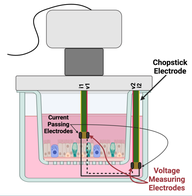
Method Optimization: Antibodies

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.



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

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

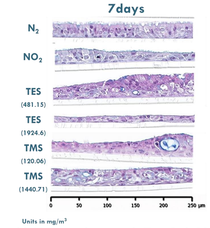
Human bronchial epithelial cell line

MucilAir™

Human bronchial epithelial tissue model

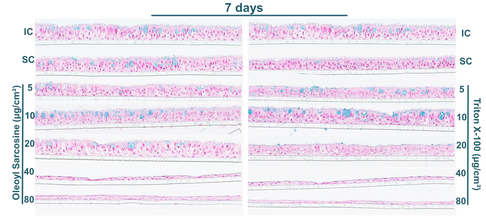
Cellular effects	BEAS-2B	MucilAir™
Cell viability (PrestoBlue®)	✓	✓
Cytotoxicity (LDH)	✓	✓
Inflammatory markers (IL-6, CXCL-8)	✓	✓
Cilia beating frequency (CBF) and Average Active Area (AAA)		✓
Barrier integrity (TEER)		✓
Histology (H&E staining)		✓

Silanes
Triethoxysilane (TES), Trimethoxysilane (TMS)



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

Surfactants
Oleoyl Sarcosine, Triton X-100

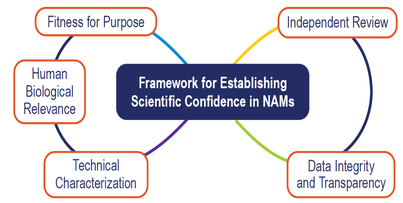


Stucki et al. Manuscript in preparation

Towards International Guidance

Multi-lab testing is being organized to evaluate the use of a reconstructed human respiratory epithelial model to assess portal-of-entry effects of liquid chemicals. 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

Funding and Training Opportunities

The Science Consortium provides equipment to help expand access to *in vitro* testing (www.thepsci.eu/funding). For example, we've donated:
- Materials such as reconstructed human tissue models and recombinant antibodies
- Equipment such as voltohmmeters for TEER Assay, *in vitro* exposure devices (VITROCELL; MedTec Biolab), flow cytometers and automatic dispensers

We also provide free training opportunities, such as:
- Travel awards for conferences and hands-on training (e.g., the Institute for In Vitro Sciences Practical Methods Training Course)
- Webinars including the ongoing EPIC webinar series on the use of NAMs in risk assessment