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

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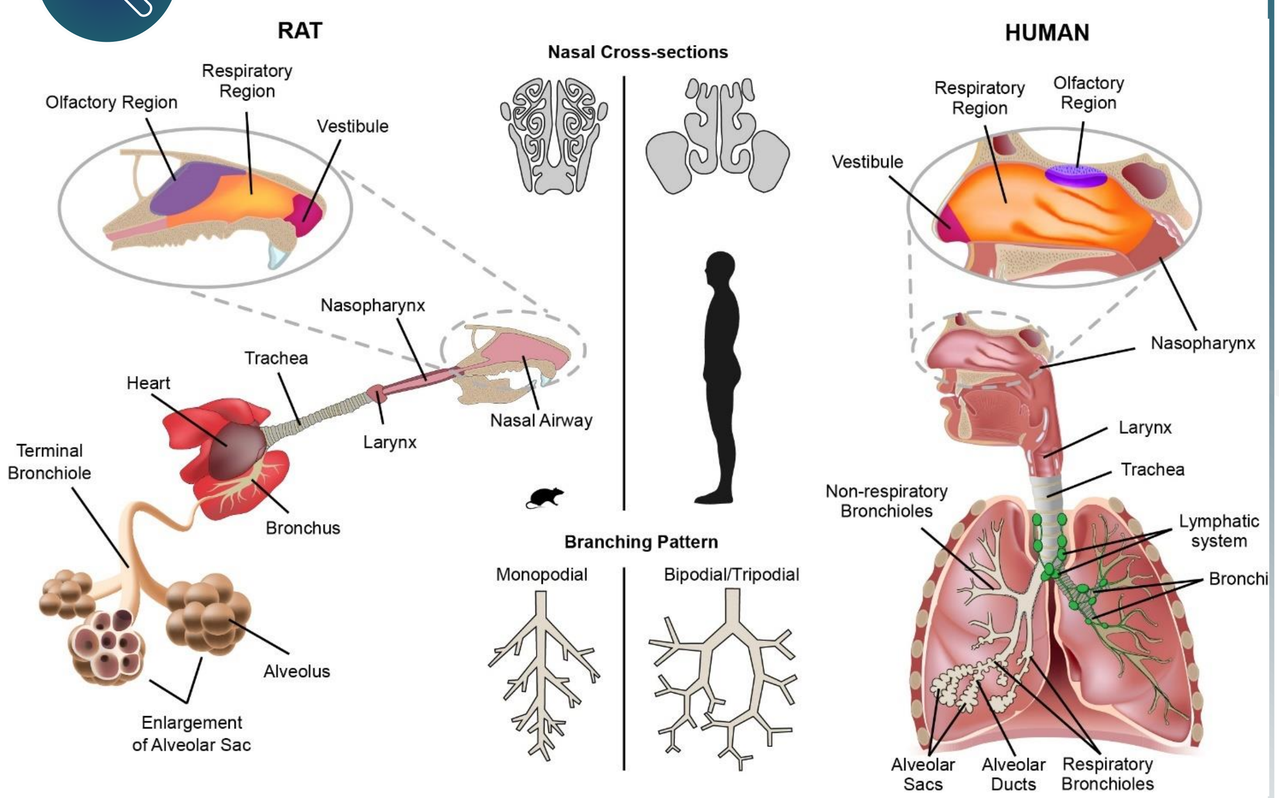
Learn more about the Science Consortium's inhalation work at:

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. Illustration from Stucki et al. (2024).

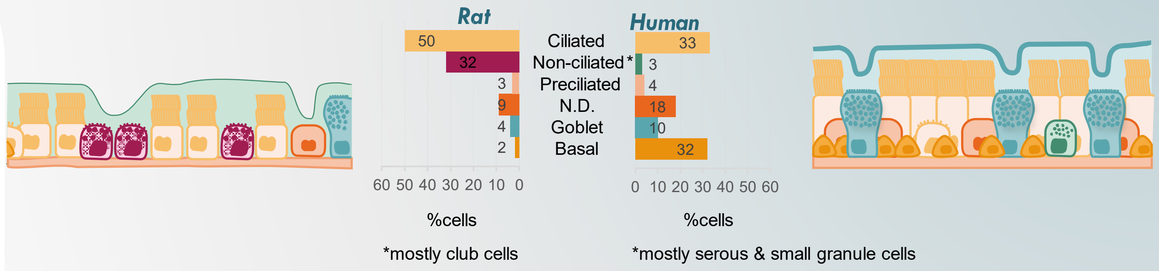
Adverse Outcome Pathways (AOPs)

In collaboration with industry, regulatory agencies, and non-profit organisations, we mapped two AOPs covering adverse lung effects. **AOP 411** together with AOPs 424 and 425 form a network for the common AO of decreased lung function**.** **AOP 173** has pulmonary fibrosis as the AO and has been endorsed by the Organisation of Economic Co-operation and Development (OECD).

	Molecular Initiating Event (MIE)	Key Events (KEs)	Adverse Outcome (AO)
AOP 411	Oxidative stress	1 → 2	Decreased lung function
AOP 173	Substance interaction with the resident pulmonary cell membrane components	1 → 2 → 3 → 4 → 5 → 6	Pulmonary Fibrosis

In silico and *in vitro* models are increasingly being used in integrated approaches to assess the toxicity of inhaled substances. Gaining regulatory acceptance for these newer approaches involves concerted efforts from stakeholders across various geographies and sectors. Here, we present a collaborative effort to establish scientific confidence in non-animal methods for assessing the toxicity of inhaled substances on the respiratory tract. This work involves analyzing the state of the science to identify and address gaps in research as well as developing and standardizing methods to facilitate their regulatory implementation.

Cell types in the bronchial region differ significantly in rats and humans



Hence, we explore the applicability of available human *in vitro* models and participate in method development to address current gaps

Filling research gaps

INSPIRE

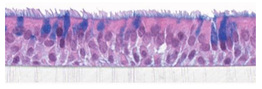
Conducted in collaboration with the Flemish Institute for Technological Research (VITO), the INSPIRE Initiative *(IN vitro* Systems to PredIct REspiratory toxicity), aims to:

- build scientific confidence in *in vitro* testing approaches to predict respiratory toxicity,
- identify relevant cellular effects, generation and exposure methods, and model systems that may be most appropriate for use, depending on the purpose of testing.

BEAS-2B

Human bronchial epithelial cell line

MucilAir™

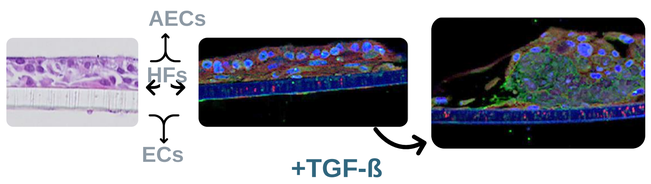


Human bronchial epithelial tissue model

Endpoints	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)		✓
Morphology (H&E staining)		✓

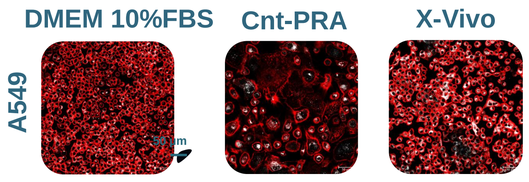
Method development and standardization

Model of the Distal Lung



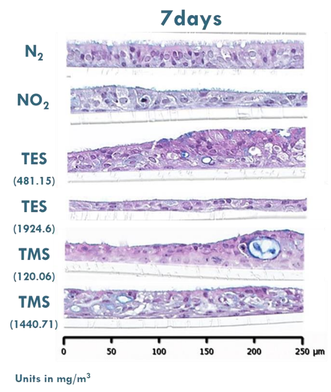
We helped fund the development of a reconstructed alveolar tissue model that includes primary alveolar epithelial cells (AECs), human fibroblasts (HFs), and endothelial cells (ECs) (EpiAlveolar™, MatTek).

FBS-free media

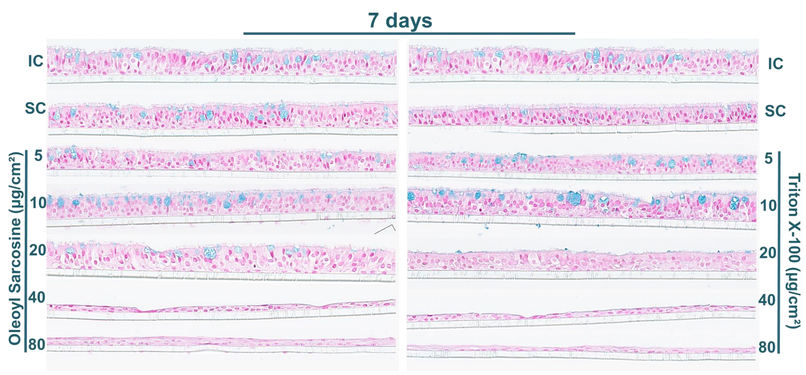


In collaboration with the Luxembourg Institute of Science and Technology (LIST), the A549 cell line was transitioned to chemically-defined media and characterized. This work is being extended to other respiratory epithelial cell lines and fibroblasts.

Silanes (TES, TMS)
Triethoxysilane, trimethoxysilane



Surfactants
Triton X-100, Oleoyl sarcosine

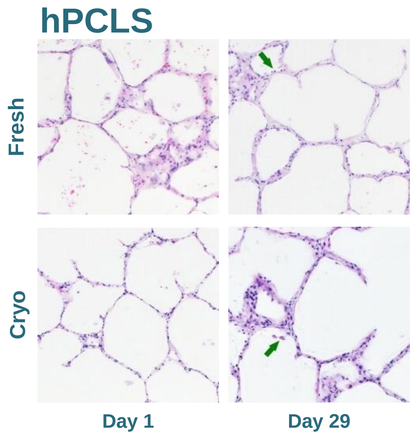


Metabolism

Reconstructed tissue models

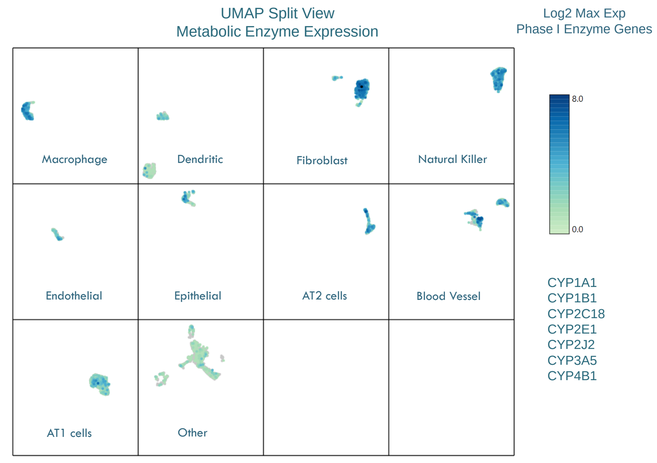
Understanding local metabolic processes is important for enhancing the usability of *in vitro* inhalation models and assessing their ability to evaluate substances undergoing local biotransformation. To address this, human reconstructed airway models (Epithelix) from various regions of the respiratory tract (nasal, bronchial, small airway, and alveolar) and up to five donors are being analyzed using RNA sequencing and compared to primary human tissues.

hPCLS

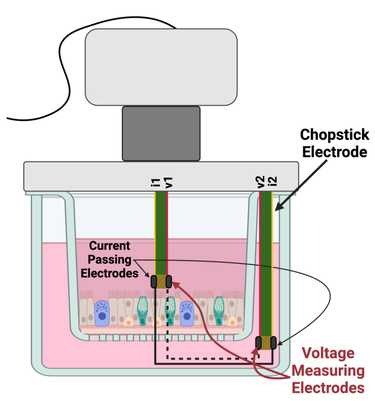


In collaboration with the Institute for In Vitro Sciences (IIVS), we funded the development of a protocol for the cryopreservation and long-term maintenance of human precision cut lung slices (hPCLS). This model was successfully assessed for viability, protein content, and cell specific markers.

Currently, this model is being assessed for cell sub-populations and compared to native lung tissues with a focus on cell-specific metabolic capabilities by single-cell RNA sequencing.



Reporting recommendations for assays



We work to standardize *in vitro* practices by developing minimum reporting recommendations that aim to facilitate repeatability and reproducibility, thus better enabling data interpretation and comparison across laboratories.

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 et al. *Under Review*).

TEER: Trans-Epithelial/Endothelial Electrical Resistance

Funding and training opportunities

In vitro reagents & test systems	Equipment	Travel awards	Training and webinars
•3D human reconstructed tissue models	•Electrode and voltohmeter for TEER Assay	•Conferences	•EPIC Webinar Series on the Use of NAMs in Risk Assessment (ongoing)
•Recombinant antibodies	•Exposure devices (VITROCELL; MedTech Biolab)	•Hands-on training	•Inhalation specific webinar series (2018, 2020, 2021)
	•Other (flow cytometer, automatic dispensers)		