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

Nuria Roldan[1*], Monita Sharma[1], Adam Bettmann[1], Andreas O. Stucki[1] and Amy J. Clippinger[1]
[1] PETA Science Consortium International e.V., Stuttgart, Germany *nuriar@thepsci.eu
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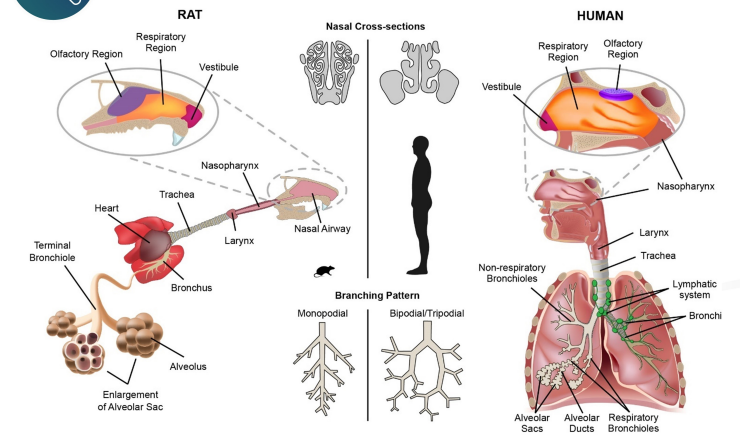
Assessing the state-of-the-science



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).

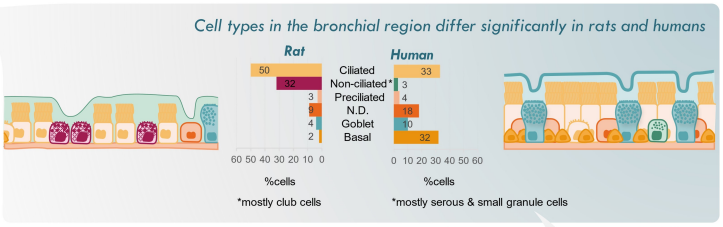
Adverse Outcome Pathways (AOPs)

In collaboration with industry, regulatory agencies, and non-profit organisations, we mapped two AOPs associated with adverse lung effects induced by a wide range of potential chemical stressors.
AOP 411 together with AOPs 424 and 425 form a network for the common adverse outcome of decreased lung function. **AOP 173** has pulmonary fibrosis as an adverse outcome, and it 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

Advancing non-animal methods within the inhalation toxicity space requires multiple, coordinated efforts. These efforts enable a better understanding of the advantages and limitations of *in vitro* methods, which is key to designing integrated testing approaches to effectively protect human health.

Here, we present on our efforts to increase scientific confidence in *in vitro* inhalation toxicity test methods by analysing the **state-of-the-science**, **addressing research gaps**, and optimising and standardising methods to facilitate their **regulatory implementation**.



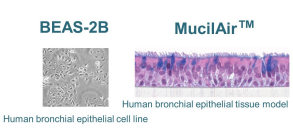
Hence, we explore the applicability of available human *in vitro* models and participate in method development to address current gaps.

Filling research gaps

INSPIRE

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.

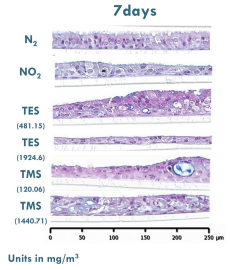
This is done in collaboration with the Flemish Institute for Technological Research (VITO).



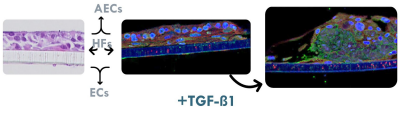
Surfactants
Triton X-100, Oleoyl sarcosine

Silanes (TES, TMS)
Triethoxysilane, trimethoxysilane

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)		✓

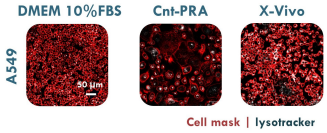


Model of the Distal Lung



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



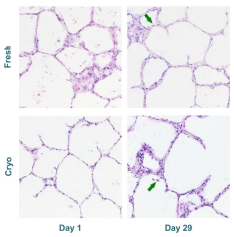
In collaboration with the Luxembourg Institute of Science and Technology (LIST), the A549 cell line was transitioned to chemically defined media and characterized. This work is being extended to other respiratory epithelial cell lines and fibroblasts.

Metabolism

Reconstructed tissue models

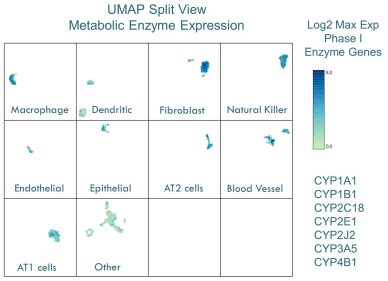
Understanding local metabolic processes is important for enhancing the usability of *in vitro* inhalation models and assessing their ability to evaluate substances undergoing local biotransformation. To address this, human reconstructed airway models (Epithelix) from various regions of the respiratory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

hPCLS

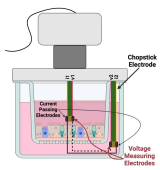


In collaboration with researchers at the Institute for In Vitro Sciences (IIVS), we funded the development of a protocol for the cryopreservation and long-term maintenance of human precision cut lung slices (hPCLS). This model was successfully assessed for viability, protein content, and cell specific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

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



Regulatory implementation



We work to standardise in vitro practices by developing minimum reporting standards that aim at facilitation 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.

TEER: Trans-Epithelial/Endothelial Electrical Resistance

Funding opportunities

In vitro reagents & test systems	Equipment	Travel awards
▪ 3D human reconstructed tissue models	▪ Electrode and voltohmeter for TEER Assay	▪ Conferences
▪ Recombinant antibodies	▪ Exposure devices (aerosols, vapors, particles, complex mixtures)	▪ Hands-on training
	▪ Other (flow cytometer, automatic dispensers)	
~14 Awardees	~12 Awardees	~42 Awardees

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