ALTERNATIVE APPROACHES TO INHALATION TOXICITY TESTING

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INTRODUCTION

Inhalation is a major route of exposure to substances such as air pollution, drugs, nanomaterials, agrochemicals, solvent vapors, or inhaled smoke. Inhaled substances may cause portal-of-entry effects in the respiratory tract or enter the systemic circulation; therefore, it is important to characterize the benefits or risks that they may present. Regulatory testing for inhalation toxicity is often conducted following test guidelines from the Organisation for Economic Co-operation and Development (OECD).

Scientific and ethical drivers have led to interest in developing human-relevant, mechanistically-based approaches that don't use animals. To advance these approaches, international multi-stakeholder collaborations have formed between industry, government, non-profit organizations, method developers, and academia. This poster highlights progress and ongoing collaborative work focused on determining federal agency needs[1,2], curating existing data, identifying gaps in *in silico* or *in vitro* methods available to assess toxicity following inhalation exposure, and adverse outcome pathway development.

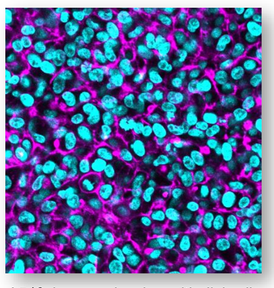
While the ideal testing approach will vary depending on the test substance and purpose of the study, proof-of-concept testing is being conducted to show the utility of non-animal approaches to predict the toxicity of inhaled substances. A collaborative approach will enable researchers to build upon the experiences of others and most efficiently optimize and standardize testing approaches that will be fit for regulatory decision-making.

NON-TESTING APPROACHES

Models to describe ADME (absorption, distribution, metabolism, and elimination): • Multiple-path particle dosimetry (MPPD) model • Computational fluid dynamics (CFD) modelling • Physiologically based pharmacokinetic (PBPK) modeling	Predict the aerodynamic behaviors determining initial inhaled deposition of substances within the airway and subsequent distribution via clearance mechanisms. Hybrid CFD/PBPK models can aid in prediction of regional dose plus systemic absorption.
Quantitative structure-activity relationships models (e.g., MultiCASE or UL REACH*Across*)	*In silico* software that use quantitative parameter(s), such as physicochemical properties, to predict acute inhalation toxicity
Grouping and read-across	Applying data from one substance(s) to predict the same property or effect for a structurally 'similar' substance

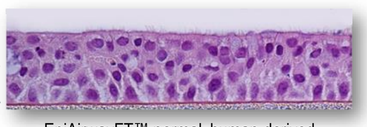
IN VITRO SYSTEMS

Cell lines

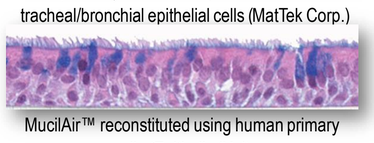


A549, human alveolar epithelial cells

3-D tissue models

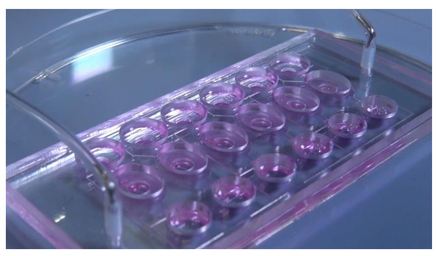


EpiAirwayFT™ normal, human-derived tracheal/bronchial epithelial cells (MatTek Corp.)



MucilAir™ reconstituted using human primary cells (Epithelix Sàrl)

Lung-on-a-chip



Breathing lung-on-a-chip (AlveoliX AG)

Air-liquid interface systems

In vitro exposure devices can be used to expose cells or tissues cultured at the air-liquid interface

The PETA International Science Consortium awarded the Institute for In Vitro Sciences, ScitoVation, and VITO NV equipment from VITROCELL® so that they can offer *in vitro* inhalation testing services.

WORKSHOPS

In addition to free webinars, two inhalation workshops were co-organized by the PETA International Science Consortium and the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

2015: Design of an *In Vitro* System to Assess the Inhalation Toxicity of Nanomaterials[3]

2016: Alternative Approaches for Acute Inhalation Toxicity Testing to Address Global Regulatory and Non-Regulatory Data Requirements[4]

1. Establish a database of existing acute inhalation toxicity data

NICEATM Integrated Chemical Environment

https://ice.ntp.niehs.nih.gov/

2. Optimize (Q)SAR models

3. Prepare a review on mechanisms of acute inhalation toxicity, dosimetry considerations, & available non-animal methods[5]

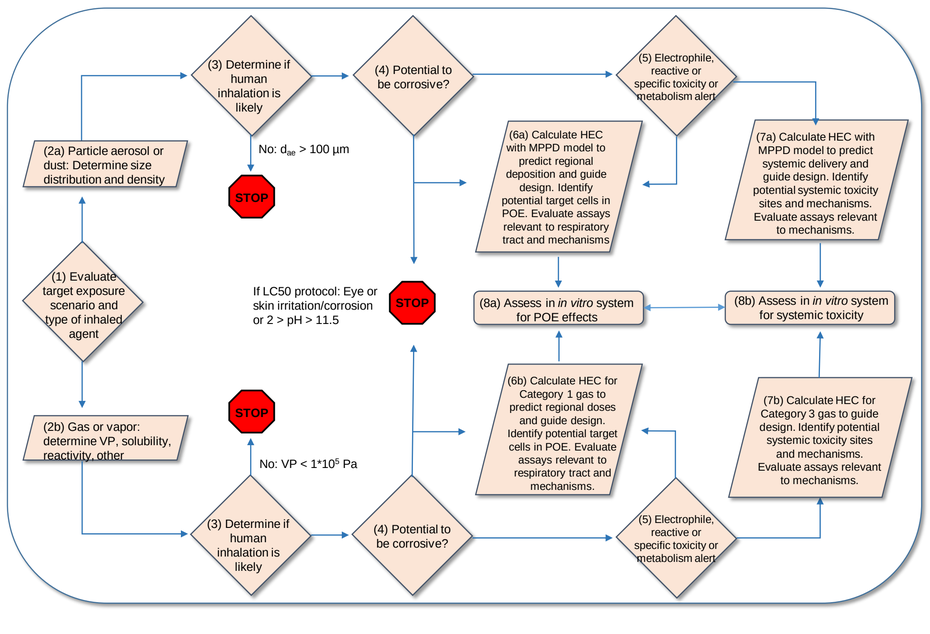
4. Design a non-animal testing approach and conduct a proof-of-concept study

Potential Endpoints:
Cytotoxicity/cell viability
Tissue integrity (TEER)
Reactive oxygen species
Oxidative stress
Pro-inflammatory signaling
Cilia beat frequency

Potential In Vitro Systems:
Cell lines (e.g., A549, BEAS-2B, Calu3 cells)
3D reconstructed human tissue models

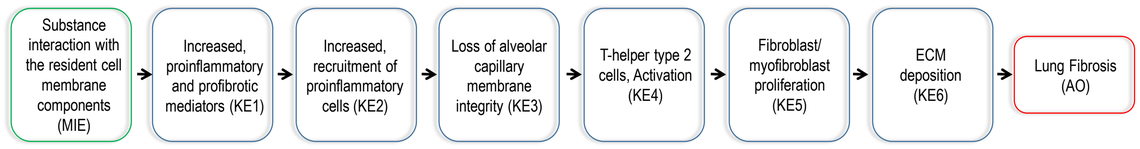
Integrated approaches to testing and assessment

To foster education in this area, a free webinar series on alternative approaches for inhalation toxicity testing, featuring presentations from experts in the field, can be accessed online: www.piscltd.org.uk/inhalation-webinars



ADVERSE OUTCOME PATHWAYS

An adverse outcome pathway (AOP) is a conceptual framework describing a sequential chain of causally linked events at different levels of biological organization that lead to an adverse health or ecotoxicological effect. AOPs can be used to organize existing data and design non-animal testing strategies. The **AOP Wiki** is an interactive and virtual platform for AOP development created by the European Commission's Joint Research Centre, the EPA, and the OECD: https://aopwiki.org/



AOP 173: Increased substance interaction with the resident cell membrane components leading to lung fibrosis
Sabina Halappanavar, Monita Sharma, Hakan Wallin, Ulla Vogel, Kristie Sullivan, Amy J. Clippinger (*manuscript in preparation*)

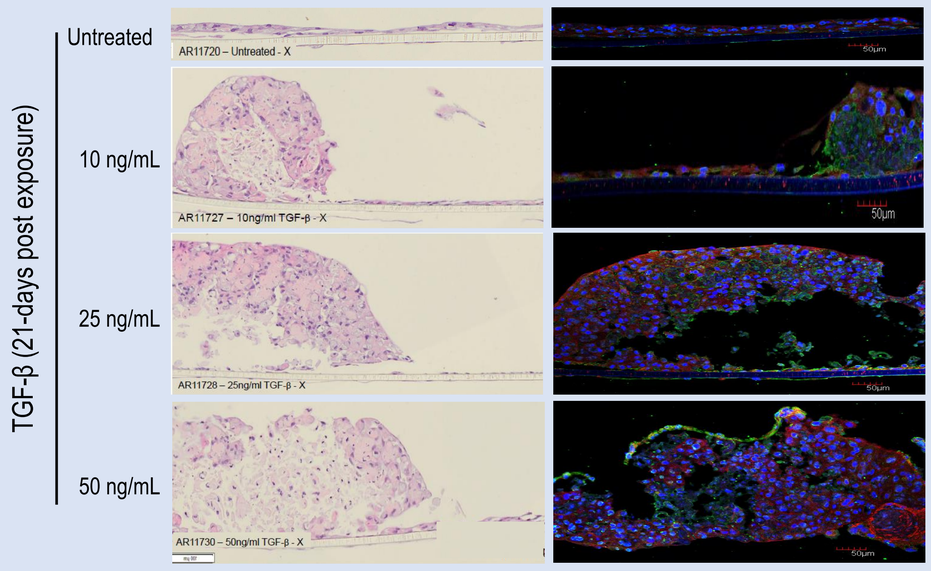
IN VITRO SYSTEM TO PREDICT THE DEVELOPMENT OF PULMONARY FIBROSIS

The PETA International Science Consortium funded MatTek Corporation to develop a model of the lower respiratory tract. Cell types:

- Alveolar epithelial cells
- Pulmonary endothelial cells
- Human fibroblasts
- Monocyte-derived macrophages (optional)

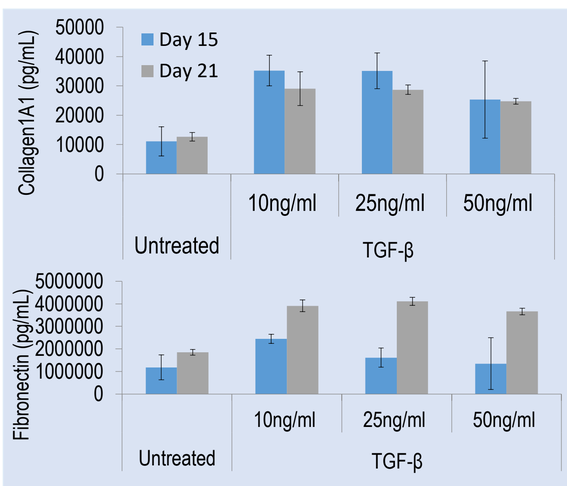
Aim: Develop a human-relevant *in vitro* test system to predict pulmonary fibrosis and enable effective risk assessment.

Method: A three-dimensional reconstructed human tissue model of the lower respiratory tract (MatTek EpiAlveolar™) was treated with a known pro-fibrotic stimuli (TGF-β) to assess if the *in vitro* system can predict the human outcome.



The EpiAlveolar™ model was treated with TGF-β at different concentrations (10, 25, or 50 ng/mL) as a positive control. Hematoxylin and eosin staining and immunostaining (for fibronectin (green), nuclei (blue), and alpha-smooth muscle actin (red)) was performed 21-days post exposure.

Observation: Tissue contraction and an increase in cell number was observed 21-days post-exposure for all tested concentrations of TGF-β.



EpiAlveolar™ model was treated with TGF-β at different concentrations (10, 25, or 50 ng/mL). Supernatant was collected after day 15 and 21 and assessed using the Bio-Plex® Multiplex Immunoassay System. Levels of pro-fibrotic biomarkers are expressed as an absolute concentration in the media (pg/mL). The graphs represent n=1 experiment with 3 tissue replicates per treatment and each tissue sample run in duplicate.

Observation: A trend towards an increase in collagen 1A1 and fibronectin was observed after day 15 and 21 of treatment with TGF-β.

Next step: Test the *in vitro* system using other test substances, including multi-walled carbon nanotubes.

CONCLUSION

The development, implementation, and global regulatory acceptance of non-animal approaches for inhalation toxicity testing is an ambitious but attainable goal, with success necessitating collaboration among diverse stakeholders. Non-animal approaches have the potential to better protect human health by using 21st century science rooted in contemporary understanding of human mechanisms of toxicity.

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