Assessing Respiratory Toxicity of Chemicals in Two Human *in vitro* Systems

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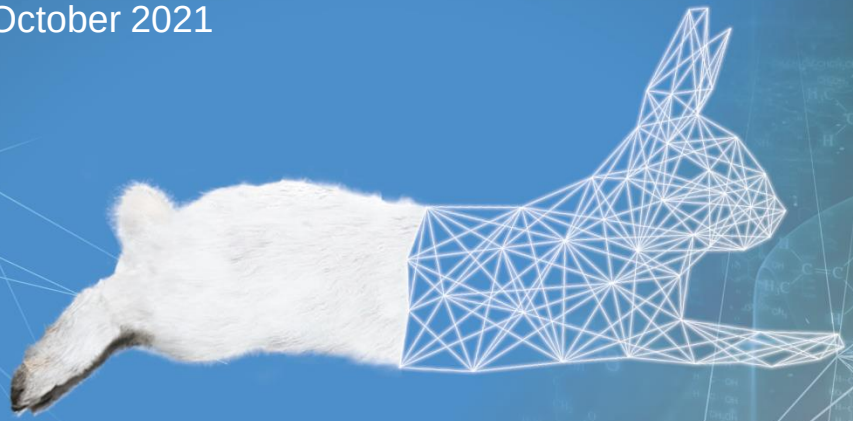
PETA Science Consortium International e.V.

CORESTA SSPT – NAM Symposium
19 October 2021



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FUNDING

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WORKSHOPS AND WEBINARS

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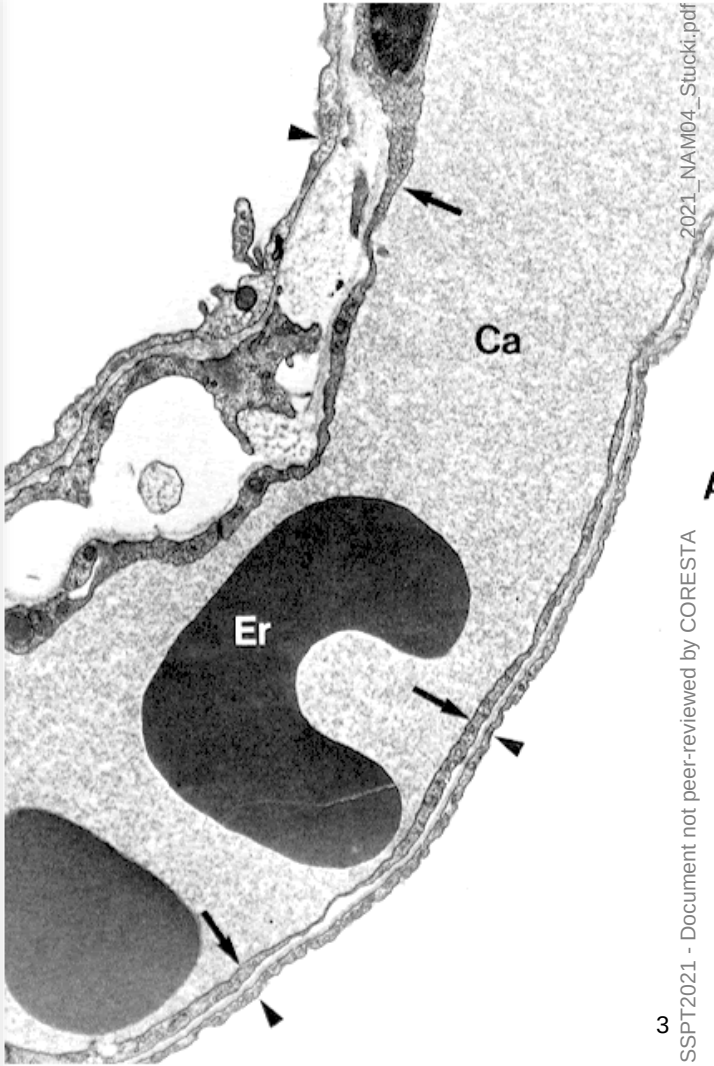
RETROSPECTIVE REVIEWS

The human lungs

- 100-150 m^2 surface area
- <1 µm air-blood barrier thickness
- 7-10,000 km of blood vessels
- 17,000 breaths per day
- 7,000 L of air per day
- 40+ different cell types



Anatomical and physiological differences

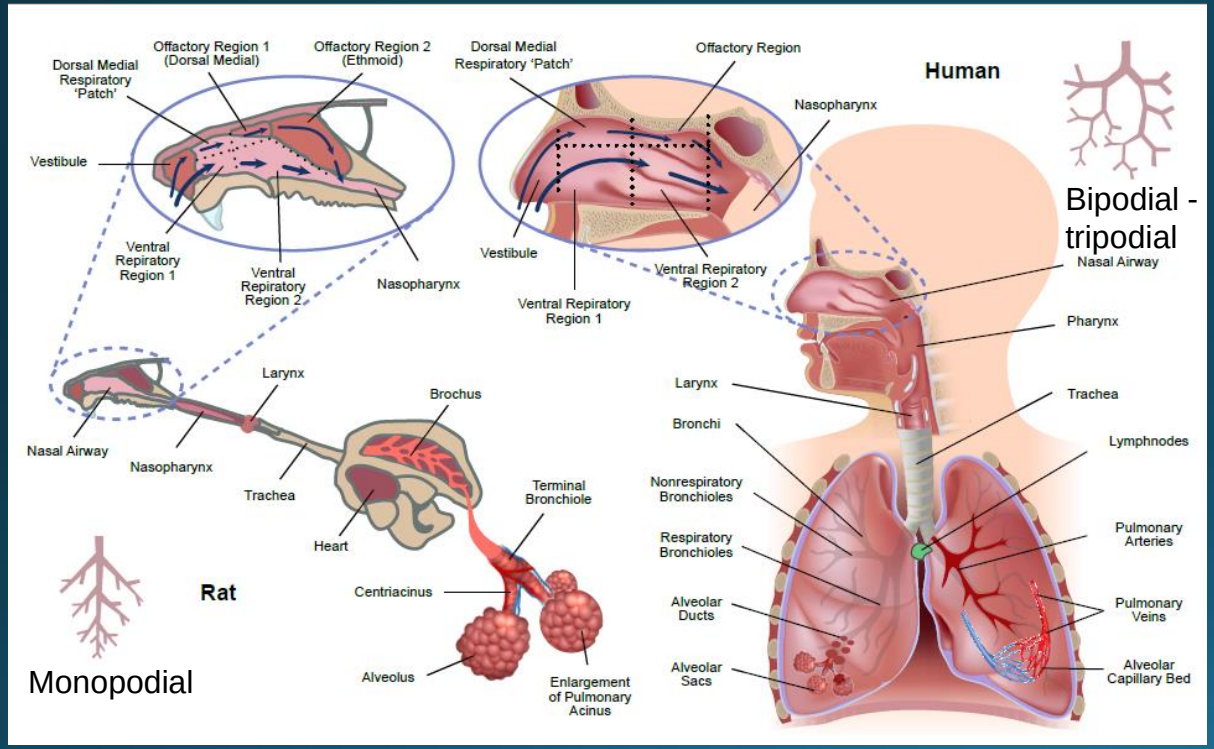


Illustration modified from Dr. Jack R. Harkema,
Professor of Comparative Pathology, Michigan State University

Ventilation rates and breathing mode

Airway architecture and branching pattern

Cell type distribution and mucous composition

Metabolic activity

The INSPiRE Initiative:
IN vitro System to Predict REspiratory toxicity

Toxicology in Vitro 52 (2018) 131–145

Contents lists available at ScienceDirect

Toxicology in Vitro

journal homepage: www.elsevier.com/locate/toxinvit

Review

Pathway-based predictive approaches for non-animal assessment of acute inhalation toxicity

Amy J. Clippinger[a,*], David Allen[b], Holger Behrsing[c], Kelly A. BéruBé[d], Michael B. Bolger[e], Warren Casey[f], Michael DeLorme[g], Marianna Gaça[h], Sean C. Gehen[i], Kyle Glover[j], Patrick Hayden[k], Paul Hinderliter[l], Jon A. Hotchkiss[m], Anita Iskandar[n], Brian Keyser[o], Karsta Luettich[n], Lawrence Milcha[...], Hans Raabe[c], En[...], Peter S. Thorne[x]

Contents lists available at ScienceDirect

Toxicology in Vitro

journal homepage: www.elsevier.com/locate/toxinvit

Alternative approaches for acute inhalation toxicity testing to address global regulatory and non-regulatory data requirements: An international workshop report

Amy J. Clippinger[a,*], David Allen[b], Annie M. Jarabek[c], Marco Corvaro[d], Marianna Gaça[e], Sean Gehen[f], Jon A. Hotchkiss[g], Grace Patlewicz[h], Jodie Melbourne[a], Paul Hinderliter[i], Miyoung Yoon[j], Dongeun Huh[k], Anna Lowit[l], Barbara Buckley[c], Michael Bartels[m], Kelly BéruBé[n], Daniel M. Wilson[g], Ian Indans[o], Mathieu Vinken[p]

vito

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Goals of the INSPiRE initiative

- Present a case study on how *in vitro* approaches may be used for acute toxicity testing
- Compare a 2D cell line with 3D human reconstructed lung tissues
- Derive *in vitro* point of departure (POD)
- Strengthen scientific confidence in *in vitro* models *en lieu* of animal testing

Initial considerations for human *in vitro* respiratory toxicity testing

CHEMICAL OR SUBSTANCE TO TEST?

WHAT EXPOSURE SYSTEM?

WHICH IN VITRO/EX VIVO SYSTEM?

WHAT KIND OF CELLS?

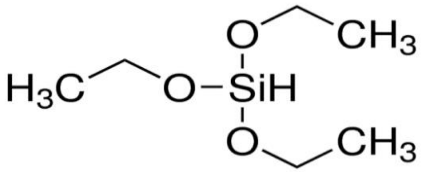
WHAT ENDPOINTS/ READOUTS?

Picking a chemical or substance to test?

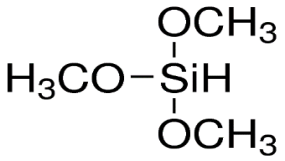
- Know your substance:
  - Physicochemical properties of the substance?
  - Locally metabolized?
  - *in vitro* or *in vivo* data available?
  - Known Adverse Outcome Pathways?

Silanes

– Highly reactive
– Triethoxysilane more stable



Triethoxysilane
(GHS 2, CAS # 998-30-1)



Trimethoxysilane
(GHS 1, CAS# 2487-90-3)

What exposure system to use?

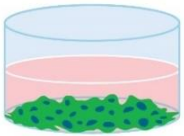
Pipetting		ALI exposure
Easier to calculate exposure dose	+	Physiologically relevant
No special equipment needed	+	Final formulation can be used
May disturb surface lining fluid	−	Special equipment needed
Limited to (particles in) liquids	−	Monitoring exposure dose more challenging

Petersen EJ, Sharma M, Clippinger AJ, et al. Use of Cause-and-Effect Analysis to Optimize the Reliability of *In Vitro* Inhalation Toxicity Measurements Using an Air-Liquid Interface. doi:10.1021/acs.chemrestox.1c00080

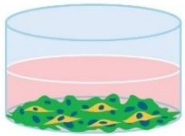
Which *in vivo* / *ex vivo* system to use?

Do not allow for exposures at ALI

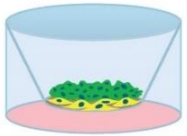
Allow for exposures at ALI



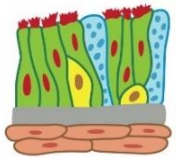
Submerged mono-cultures



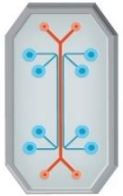
Submerged co-cultures



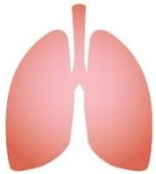
Co-cultures grown at the ALI



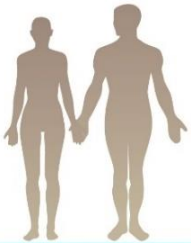
3D reconstructed human tissues grown at the ALI



Microfluidic human lung-on-a-chip



Human PCLS



Human *in vivo*

PHYSIOLOGICAL RELEVANCE

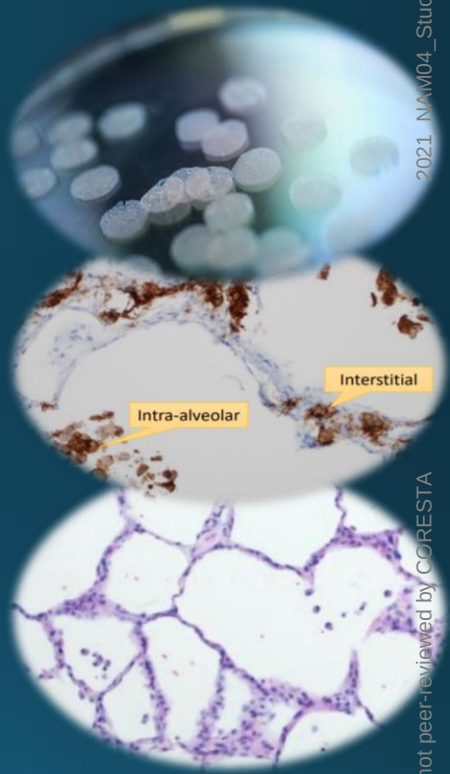
COMPLEXITY

Clippinger et al 2018

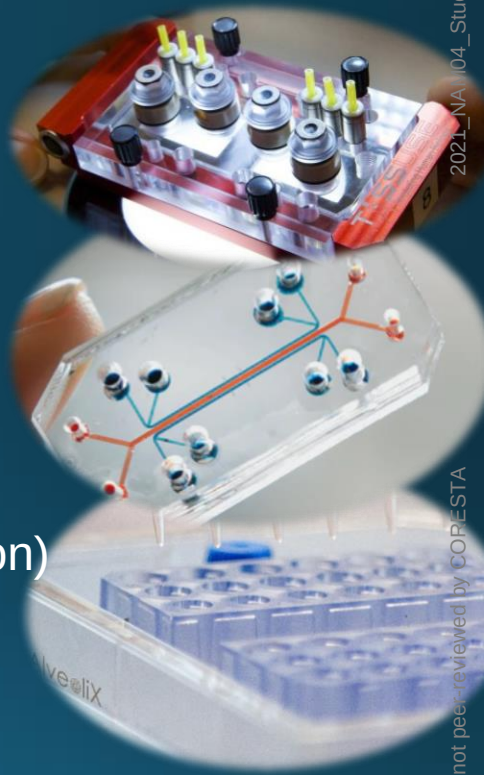
Human precision-cut lung slices

+
- PCLS from healthy and diseased donors
- All relevant cells and structures present
- Culture for 28+ days & cryopreservation possible

−
- Cross-section
- Multiple cell types may make readout more challenging
- Obtainment of (suitable) donor tissues



Microphysiological Systems / Organs-on-chip

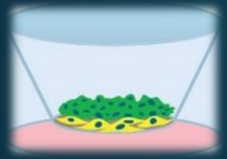


+
- Influencing microenvironment of tissues/cells
- Mechanical stretch or „blood“ flow possible
- Allow combination of different tissues (e.g., Lung-Liver)

−
- Various materials used → may influence dose (e.g. absorption)
- Choice of cell culture medium for multiple “organs”
- Standardisation and comparability difficult

www.thepsci.eu/chips

2D monocultures vs reconstructed tissues

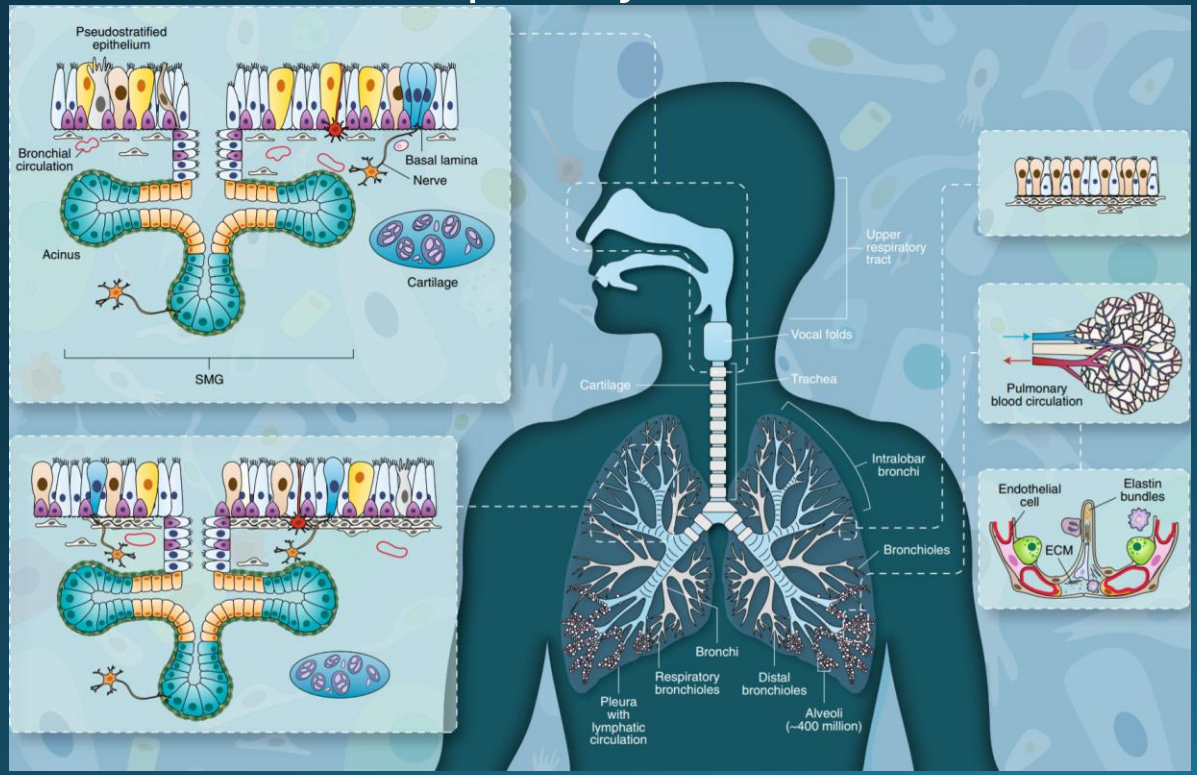


2D monocultures		3D reconstructed tissues
Simpler & less expensive	+	Primary cells differentiated to *in situ*-like epithelium
Higher throughput	+	ALI cultures for months
Cell lines and their limitations	−	Generally more expensive
Often short-term ALI cultures	−	

Petersen EJ, Sharma M, Clippinger AJ, et al. Use of Cause-and-Effect Analysis to Optimize the Reliability of *In Vitro* Inhalation Toxicity Measurements Using an Air-Liquid Interface. doi:10.1021/acs.chemrestox.1c00080

What kind of cells to use?

Respiratory tract



https://www.nature.com/articles/s41556-019-0357-7.pdf

Cell types

Airways

- **Basal cells:** TP63, KRT5, NGFR
  Function as multipotent stem cells
- **Club cells:** SCGB1A1, SCGB3A2 (data from mice)
  Immunomodulatory functions
- **Goblet cells:** MUC5AC, FOXA3, SPDEF
  Secrete mucins
- **Ciliated cells:** FOXJ1, β-tubulin IV
  Remove mucus from the lung
- **NE cells:** ASCL1, CALCA
  Act as sensory cells; communicate with neurons
- **Rare cells**
  Ionocytes: FOXI1, CFTR high
  Tuft (brush) cells: TRPM5, GNG13

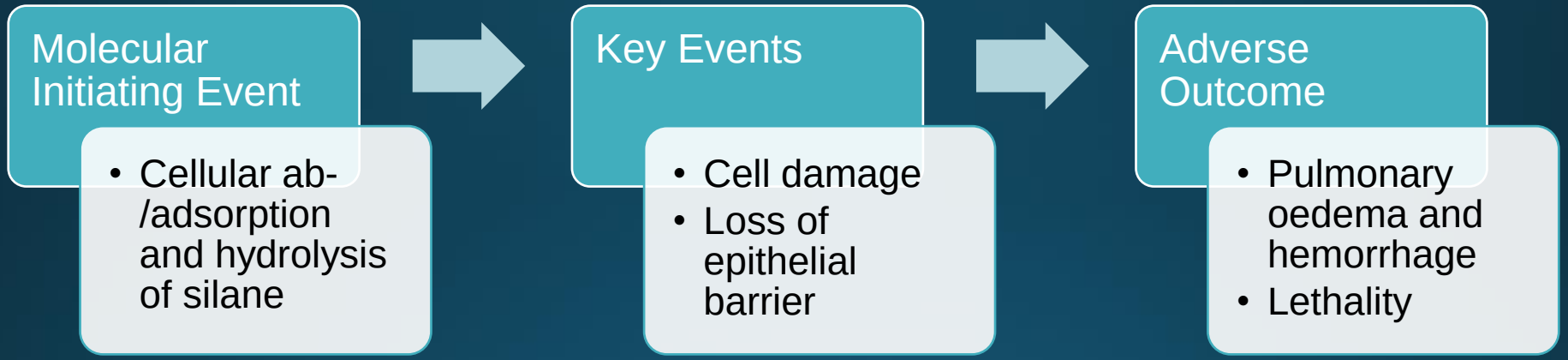
Alveoli

- **AEC2s:** SFTPC, DC-LAMP
  Stem cells; produce surfactant
- **AEC1s:** PDPN, AGER
  Large surface area; facilitate gas exchange

Immune cells

- **Dendritic cells**
- **Alveolar resident macrophages, interstitial macrophages**
- **Basophils, eosinophils**
- **Lymphoid cells (T and B cells)**

What endpoints/readouts to measure?

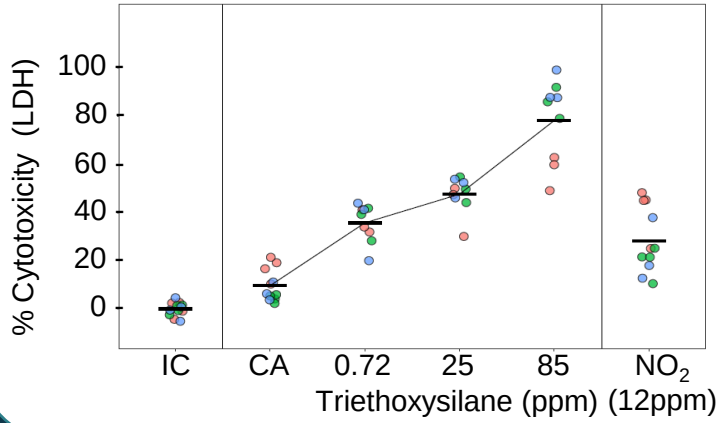


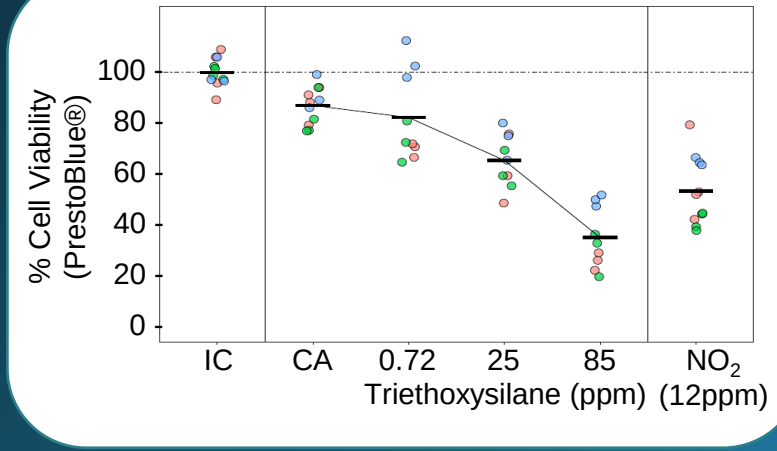
- Si content in cells and basal medium

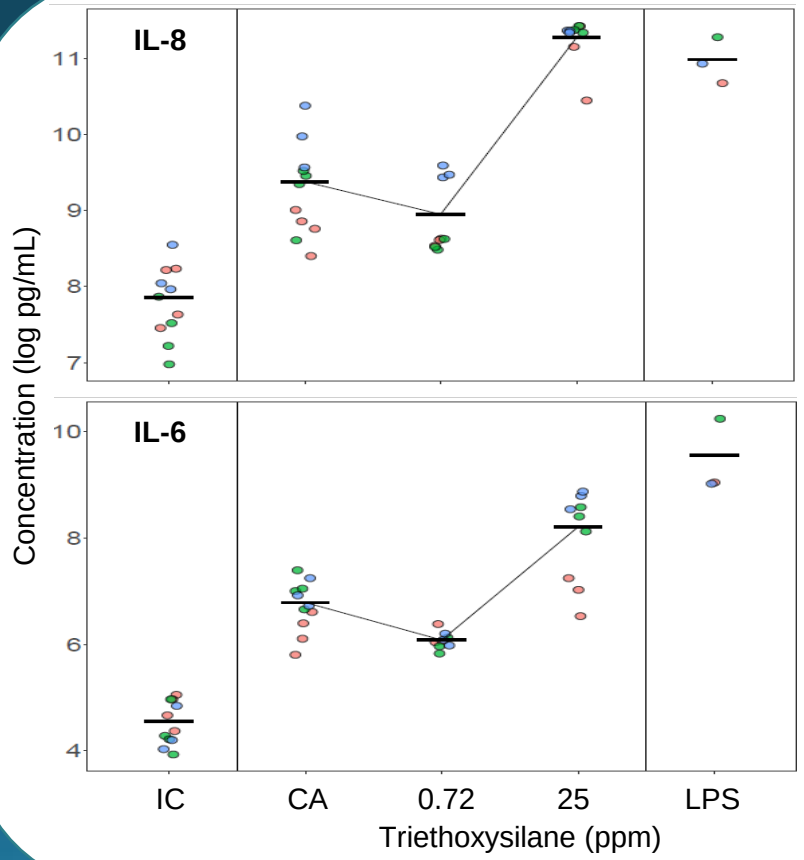
- Evident toxicity (cytotoxicity, cell viability, histology)
- Sub-toxic effects (inflammatory cytokines, cilia beating frequency)
- Trans-epithelial electrical resistance (TEER)

Find more information about AOPs under aopwiki.org

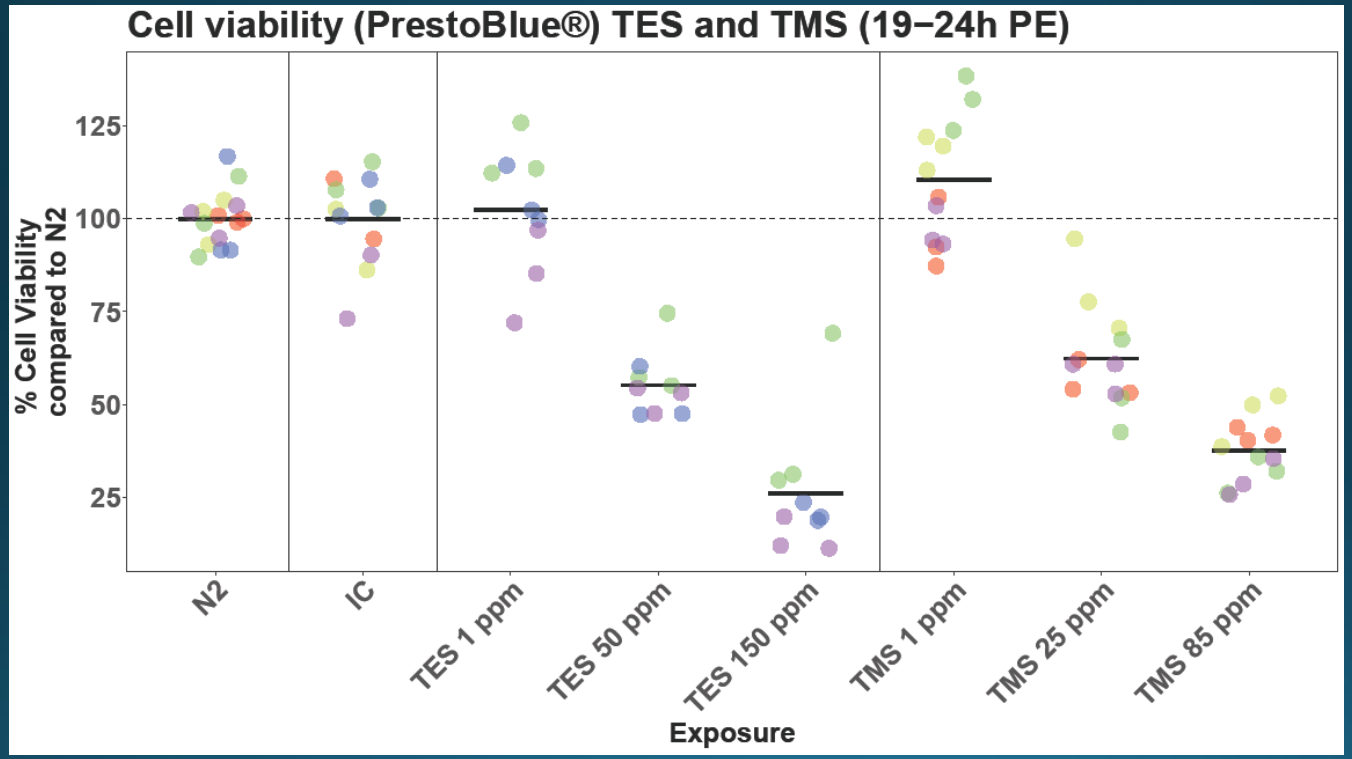
Cell response to triethoxysilane exposure (60 min)



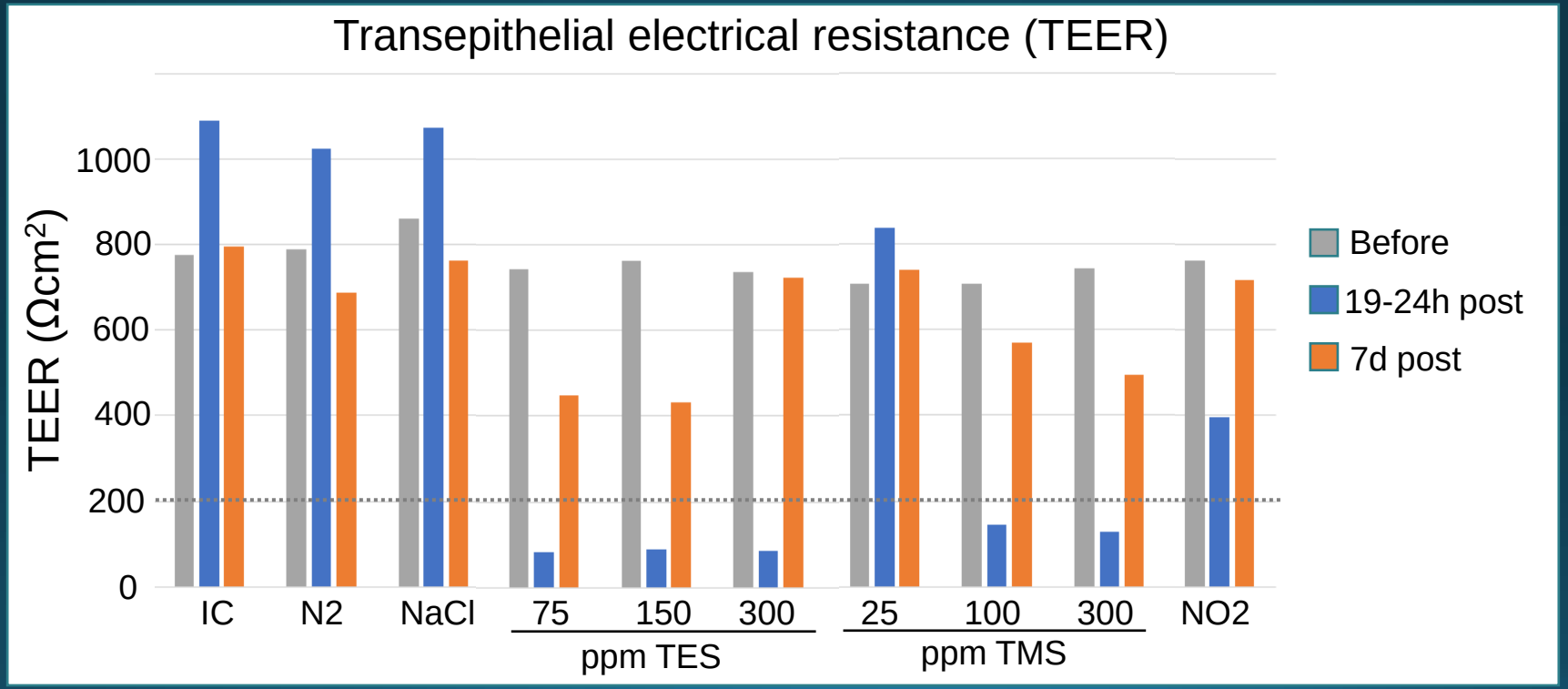




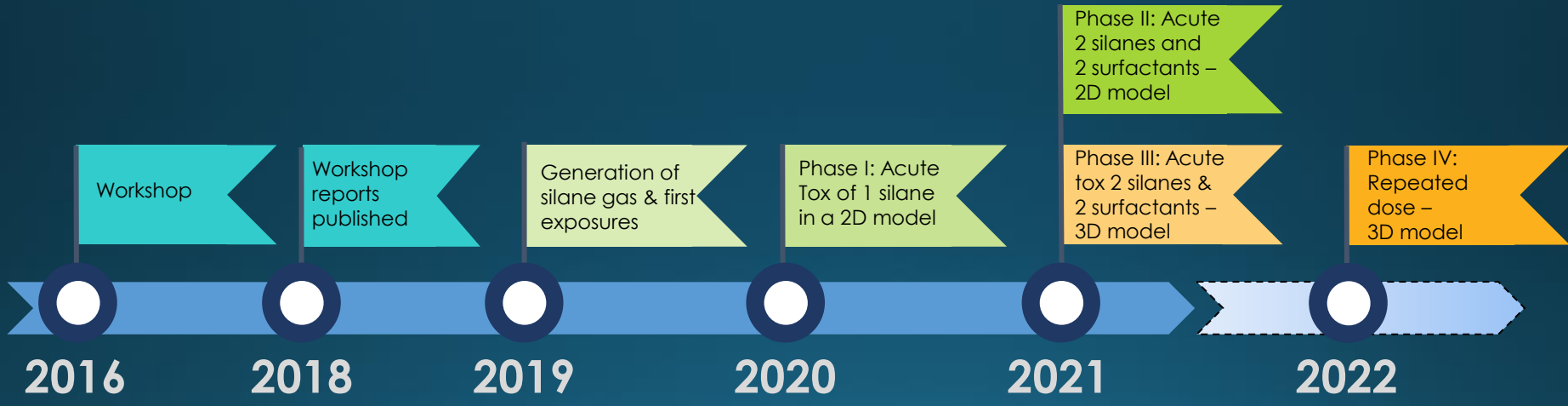
2D cell model response to silane exposure (30 min)



Silane toxicity to the epithelial barrier in 3D model



INSPiRE Initiative – Next steps



Take home

- Developments in recent years allow for human-relevant exposures of lung cells or tissues
- No size fits all: depending on the substance to test – *in vitro* methods may need adaptation (e.g., addition of endpoints)
- Rather than only having one *in vitro* assay, a battery of assays may be needed to answer a specific question (e.g., OECD TG 497 – Defined approaches on skin sensitisation)
- Especially in combination with *in silico* models, *in vitro* models have the potential to replace inhalation testing in animals
  - Chlorothalonil human health draft risk assessment - 90-day subchronic rat inhalation study waived by EPA OPP based on *in silico* and *in vitro* methods using MucilAir® tissue model *https://www.regulations.gov/document/EPA-HQ-OPP-2011-0840-0080*
- Talk to regulators **early** in the development process to discuss your NAM strategy and to find out whether animal testing is even necessary

Other inhalation related PSCI projects

Generating an Alternative System to Predict Pulmonary Fibrosis (GASPP)

- MatTek EpiAlveolar commercially available
- Used in EU-Horizon 2020 project PATROLS

Development of adverse outcome pathways (AOP)

- AOP 173: Substance interaction with the lung resident cell membrane components leading to lung fibrosis
- AOP 411: Oxidative stress leading to decreased lung function

FBS and Animal-Component free Testing (FACT)

- A549 cells sucessfully transitioned to animal-free medium

Precision-cut lung slices (PCLS)

- Study on cryopreservation ongoing

Awards

- 3D Tissues with MatTek and Epithelix
- CellTox Sampler with MedTec Bio
- VITROCELL inhalation exposure systems
- Travel Grants

Webinars and Workshops

- Several inhalation related webinars in 2016, 2018, 2020 and 2021
- https://www.thepsci.eu/inhalation-webinars/

Please visit www.thepsci.eu/our-work/inhalation/

Selected resources for respiratory *in vitro* methods

- https://www.thepsci.eu/inhalation-publications/
- Petersen EJ, Sharma M, Clippinger AJ, Gordon J, Katz A, Laux P, Leibrock LB, Luch A, Matheson J, Stucki AO, Tentschert J, Bierkandt FS. Use of Cause-and-Effect Analysis to Optimize the Reliability of In Vitro Inhalation Toxicity Measurements Using an Air-Liquid Interface. *Chem Res Toxicol*. 2021;34:1370−1385
- Welch J, Wallace J, Lansley AB, Roper C. Evaluation of the toxicity of sodium dodecyl sulphate (SDS) in the MucilAir™ human airway model in vitro. *Regul Toxicol Pharmacol*. 2021. Epub ahead of print.
- Hargrove MM, Dobrzansk BP, Li L, Constant S, Wallace J, Hinderliter P, Wolf DC, Charlton A. Use of the MucilAir Airway Assay, a New Approach Methodology, for Evaluating the Safety and Inhalation Risk of Agrochemicals. *Appl In Vitro Toxicol.* 2021;7(2):50-60.
- Marescotti D, Serchi T, Luettich K, Xiang Y, Moschini E, Talikka M, Martin F, Baumer K, Dulize R, Peric D, Bornand D, Guedj E, Sewer A, Cambier S, Contal S, Chary A, Gutleb AC, Frentzel S, Ivanov NV, Peitsch MC, Hoeng J. How complex should an in vitro model be? Evaluation of complex 3D alveolar model with transcriptomic data and computational biological network models. *ALTEX*. 2019; 36(3):388-402.
- Behrsing H, Hill E, Raabe H, Tice R, Fitzpatrick S, Devlin R, Pinkerton K, Oberdörster G, Wright C, Wieczorek R, Aufderheide M, Steiner S, Krebs T, Asgharian B, Corley R, Oldham M, Adamson J, Li X, Rahman I, Grego S, Chu PH, McCullough S, Curren R. *In vitro* exposure systems and dosimetry assessment tools for inhaled tobacco products: Workshop proceedings, conclusions and paths forward for in vitro model use. *Altern Lab Anim*. 2017;45(3):117-158.
- Behrsing H, Raabe H, Manuppello J, Bombick B, Curren R, Sullivan K, Sethi S, Phipps R, Tesfaigzi Y, Yan S, D'Ruiz C, Tarran R, Constant S, Phillips G, Gaça M, Hayden P, Cao X, Mathis C, Hoeng J, Braun A, Hill E. Assessment of *in vitro* COPD models for tobacco regulatory science: Workshop proceedings, conclusions and paths forward for *in vitro* model use. *Altern Lab Anim.* 2016;44(2):129-166.
- Clippinger AJ, Allen D, Behrsing H, BéruBé KA, Bolger MB, Casey W, DeLorme M, Gaça M, Gehen SC, Glover K, Hayden P, Hinderliter P, Hotchkiss JA, Iskandar A, Keyser B, Luettich K, Ma-Hock L, Maione A, Makena P, Melbourne J, Milchak L, Ng S, Paini A, Page K, Patlewicz G, Prieto P, Raabe H, Reinke E, Roper C, Rose J, Sharma M, Spoo W, Thorne PA, Wilson DM, Jarabek AM. Pathway-based predictive approaches for non-animal assessment of acute inhalation toxicity. *Toxicol In Vitro*. 2018;52:131-145.

Selected resources for *in vitro* methods in general

- PETA Science International Consortium https://www.thepsci.eu/
- NICEATM https://ntp.niehs.nih.gov/whatwestudy/niceatm/
- EPA‘s List of NAMs: https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/alternative-test-methods-and-strategies-reduce
- EURL ECVAM https://ec.europa.eu/jrc/en/eurl/ecvam
- Tracking System for Alternative methods towards Regulatory acceptance (TSAR) https://tsar.jrc.ec.europa.eu/
- Frontiers in *in vitro* Toxicology Research Topic on Chemical Testing Using NAMs https://www.frontiersin.org/research-topics/19075/
- Non-Animal Technologies (NAT) Database: https://nat-database.org/
- AOP wiki: https://aopwiki.org

Thank you!

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