Development of a Next Generation Risk Assessment framework for inhalation safety of consumer products

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Unilever- Safety & Environmental Assurance Centre (SEAC)

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We use scientific evidence-based risk and impact assessment methodologies to ensure that the risks / impacts of adverse human health and/or environmental effects from exposure to chemicals used in our products, processes & packaging are acceptably low.

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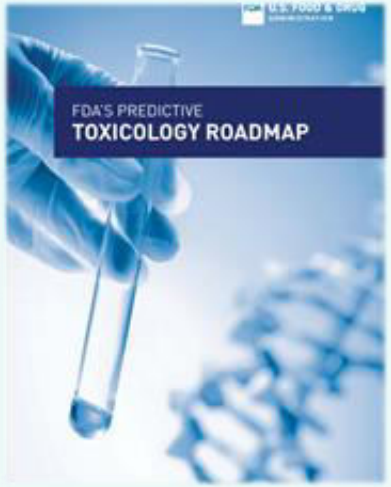
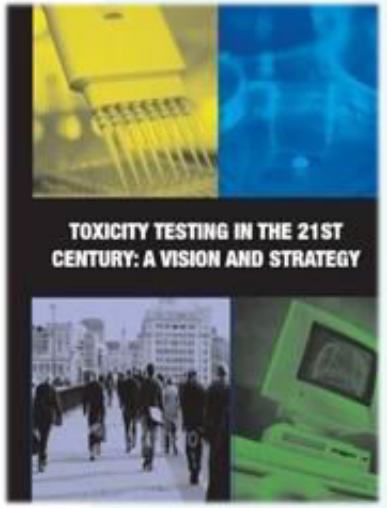
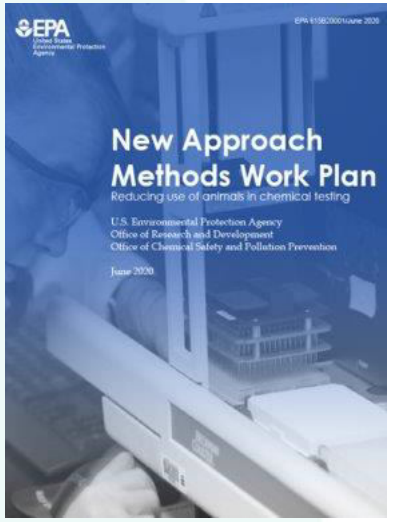
Next Generation Risk Assessment (NGRA)

Safety without animal testing







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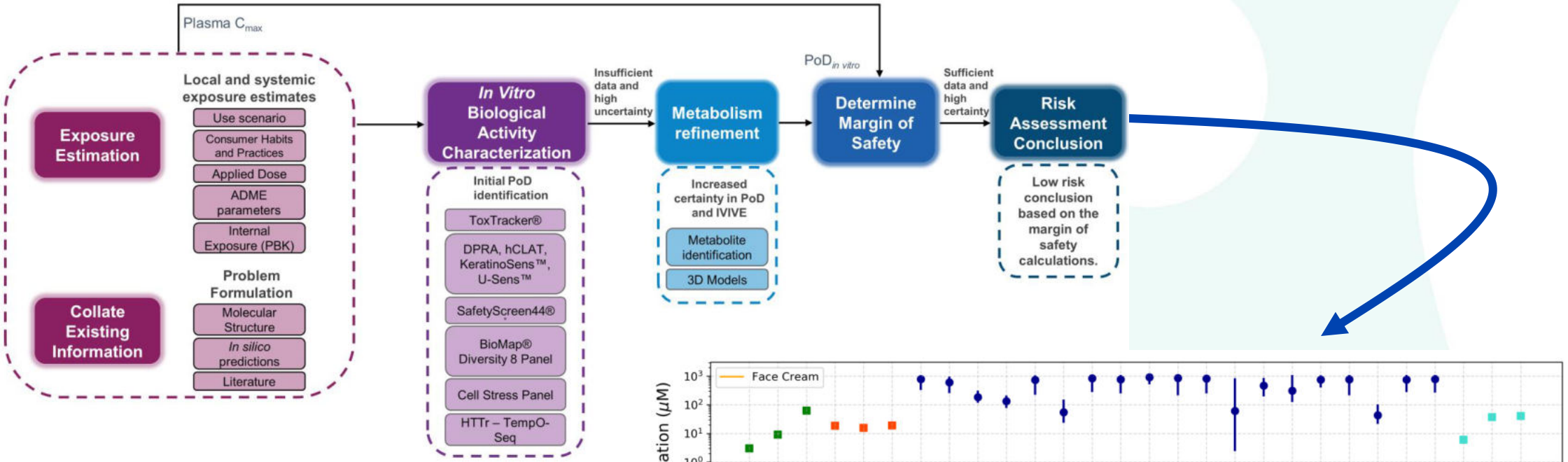
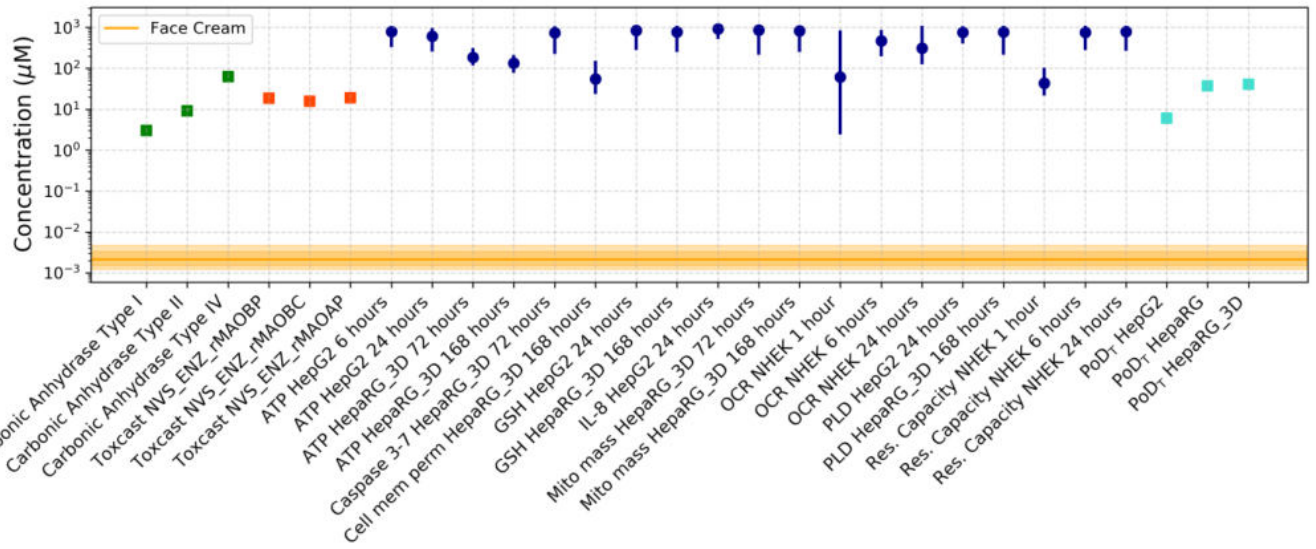
Next generation risk assessment (NGRA) – using tools and approaches to assure safety without animal testing





Moxon *et al.* (2020) et al., Toxicology in Vitro 63, 104746
Hatherell *et al.* (2020) et al., Toxicological Sciences, 176 (1), 11–33
Baltazar *et al.* (2020) et al., Toxicological Sciences, 176 (1) 236–252
Thomas R et al. (2019) et al., Toxicological Sciences 169 (2) 317–332,

Inhalation exposure depends on product type and habits & practices

Can we safely use x% of ingredient y in product z?

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Ongoing development of a Inhalation Framework

Collate Existing Information/ Problem Formulation

Exposure *

- Use scenario
- Consumer Habits and Practices
- Particle Size Distribution
- Tier 1 – screening assessment
- Tier 2 – in silico exposure modelling e.g. ConsExpo
- Tier 3 – Experimental data
- Regional Lung Deposition modelling

Hazard data

- Molecular Structure
- *In silico* predictions (PCA)
- Protein content
- Existing in vivo data
- Read Across

Data Generation

Acute and Chronic

- ALI Upper Airway (Irritation, remodelling, clearance mechanism dysfunction, inflammation)
- ALI Lower Airway (Lung Fibrosis, inflammation)
- Lower Airway (Macrophage clearance, biopersistency, surfactant disruption)
- Microphysiological Systems

Determine Point of Departure and Margin of Exposure / BER

- Exposure based waiving
- DNEL derivation
- Chemical Sensitiser benchmarking
- *In vitro* concentration-response modelling

Risk Assessment Conclusion

Risk decision based upon Weight of Evidence

*Consumer Exposure in Inhalation risk assessment

General strategy to developing an inhalation toolbox

Case study based approach

New polymers for use in antiperspirants & silanes for use in general purpose cleaners

- Chemistry
- Potential hazards
- Existing information

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Exposure- led

Exposure is calculated using consumer habits and practices.
A tiered modelling approach is applied to simulate realistic consumer exposure

- **Product type**: formulation & hardware
- **Particle size distribution**
- **Consumer habits and practices**:
  - E.g. antiperspirant: application 2x/day, 2s per axillae, exposure duration 10 min, room volume $10m^3$.
- **Tiered modelling approach**.
- **In vitro exposure doses** are informed by predictions from **MPPD** (Multiple Path Particle Dosimetry) model.

General strategy to developing an inhalation toolbox

Case study based approach

New polymers for use in antiperspirants & silanes for use in general purpose cleaners

- Chemistry; phys-chem properties
- Potential hazards
- Existing information

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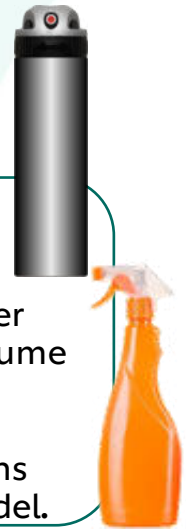

Hypothesis-driven

Identification of key hazard concerns for the chemicals of interest

- Lung fibrosis
- Impairment of mucociliary clearance
- Lung surfactant inhibition
- Biopersistency /Clearance

- **Cell models type:** upper airways (MucilAir) and Lower airways EpiAlveolar)
- **Biomarkers aligned to MIE and KE of critical AOPs (e.g. AOP 173, AOP 148, AOP 202)**

Clippinger et al. 2018. Toxicology in Vitro 52 (2018) 131–145
Halappanavar et al. 2020. Particle and Fibre Toxicology 17:16

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General strategy to developing an inhalation toolbox – benchmark chemicals

For each benchmark chemical:

- Exposure scenario was defined and classified as high or low risk
- *In vitro* and *in vivo* hazard data collated

Modulators of cilia beating frequency or/and mucus production	Inflammation/ fibrosis, cytotoxicity	Negative controls (history of safe use)/case studies
• Benzalkonium chloride • LPS • Carboxymethylcellulose • Acrolein • Isoproterenol • HC067047 • Chlorocresol • Nicotine • CFTRinh-172 • TNF-alpha	• Polyhexamethylene guanidine phosphate (PHMG) • Dimethyloctadecyl [3-(trimethoxysilyl) propyl] ammonium chloride • Akemi Antifleck Super • Acrolein • Amiodarone • Doxorubicin • Min-u-Sil5 (crystalline silica) • Aerosil 200 (amorphous silica)	• Coumarin • Sulforaphane • Acudyne™ DHR polymer • Gantrez™ ES-425

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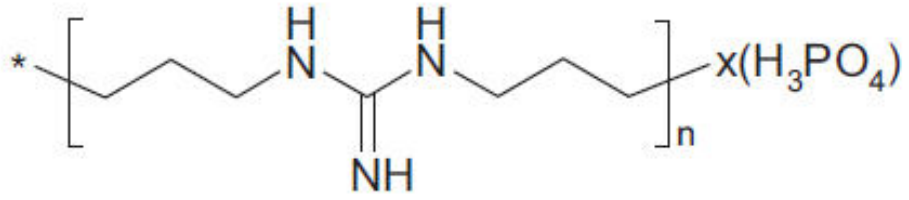
Introduction to benchmark case study chemical - Polyhexamethylene guanidine phosphate (PHMG)

- PHMG caused **acute interstitial pneumonia and pulmonary fibrosis in humans** after exposure during normal used of household humidifiers (Kim et al. 2016).
- PHMG induced ROS generation, production of inflammatory fibrotic cytokines in co-culture cells composed of lung epithelial cells, macrophages and mast cells (Calu-3, THP-1 and HMC-1 ALI co-culture model) (Kim et al. 2016).
- In A549 cells PHMG exposure resulted in changes in gene expression relevant to the progression of cell death included induction of genes related to apoptosis, autophagy, fibrosis, and cell cycle (Jung et al. 2014).

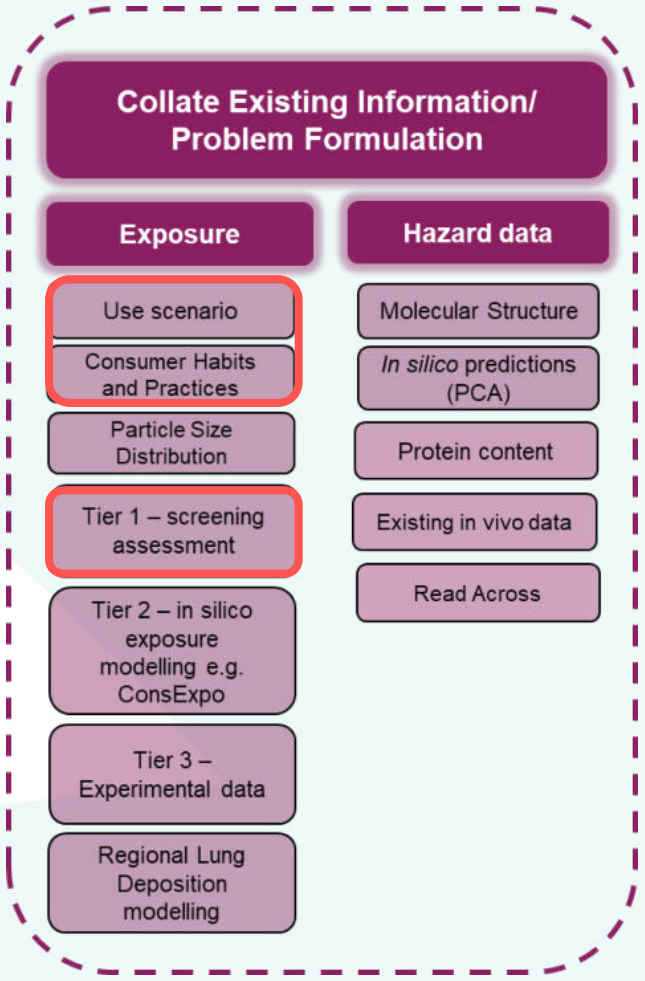


Polyhexamethyleneguanidine phosphate (n/x=1~2)
(PHMG phosphate)
CAS RN 89697-78-9

Kim et al (2016). J Toxicol Sci 41(6): 711-717

Kim et al (2016). Arch Toxicol 90(3): 617-632
Jung et al 2014). Toxicology in Vitro 28(4): 684-692.

Exposure assessment- Use scenario & Tier 1 assessment



Parameters used to calculate Tier 1 screening assessment – airborne concentration (mg/m³):

- Concentration of PHMG in the disinfectant (µg/ml): 1276
- Disinfectant volume (mL): 10
- Frequency (number of applications): 2
- Volume of the room (m³): 27
- Degree of ventilation: 1 (assumed no ventilation)

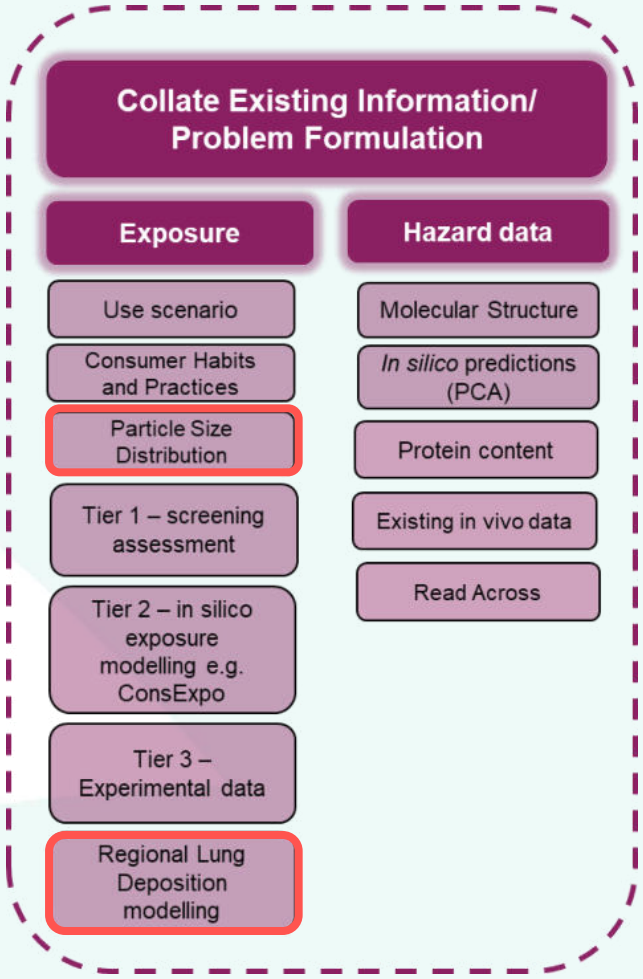
Airborne PHMG level estimated (mg/m3)

$$= \frac{10\ \text{ml/addition} \times 2\ \text{additions} \times 1276\ \text{ug/ml} \times 1}{27\ \text{m}^3}$$

= 0.95 mg/m³

Park et al (2015). Indoor Air **25**(6): 631-640.

Exposure assessment- Lung deposition calculations – Multiple path particle dosimetry model (MPPD)



- Calculation of **Dose Rate (µg/cm²/min)** using **MPPD** default settings (breathing scenario, lung volume, etc) for different lung sizes **using the stochastic human model** (1st percentile (smallest); 40th (nearly the median); 99th (largest)
  - MMAD: 80 nm
  - GSD: 1 (assumed to be monodispersed)
  - With and without clearance
- Calculation of **total and regional dose (µg/cm²)** was adjusted by the exposure duration of 11hrs per day

MPPD
MULTIPLE-PATH PARTICLE DOSIMETRY MODEL
VERSION 2.0

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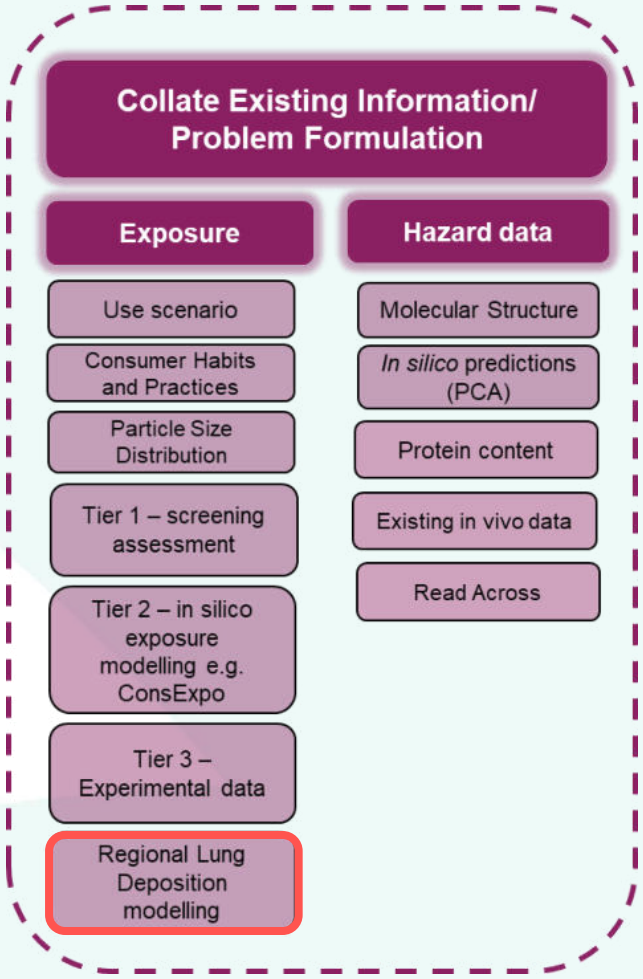
Kim et al (2016). J Toxicol Sci 41(6): 711-717.

Exposure assessment- Lung deposition calculations – Multiple path particle dosimetry model (MPPD)



Predicted **total and regional dose (µg/cm²) based on consumer exposure scenario using the 40th percentile**

Lung deposition (µg/cm²)	Day 1 exposure	Day 7 exposure*	Day 12 exposure*
Tracheobronchial deposition	0.09	0.6	1.0
Pulmonary deposition	0.0007	0.005	0.008
Total	0.001	0.008	0.01

*Assuming worst case scenario of no clearance between exposures.

MPPD
MULTIPLE-PATH PARTICLE DOSIMETRY MODEL
VERSION 2.0

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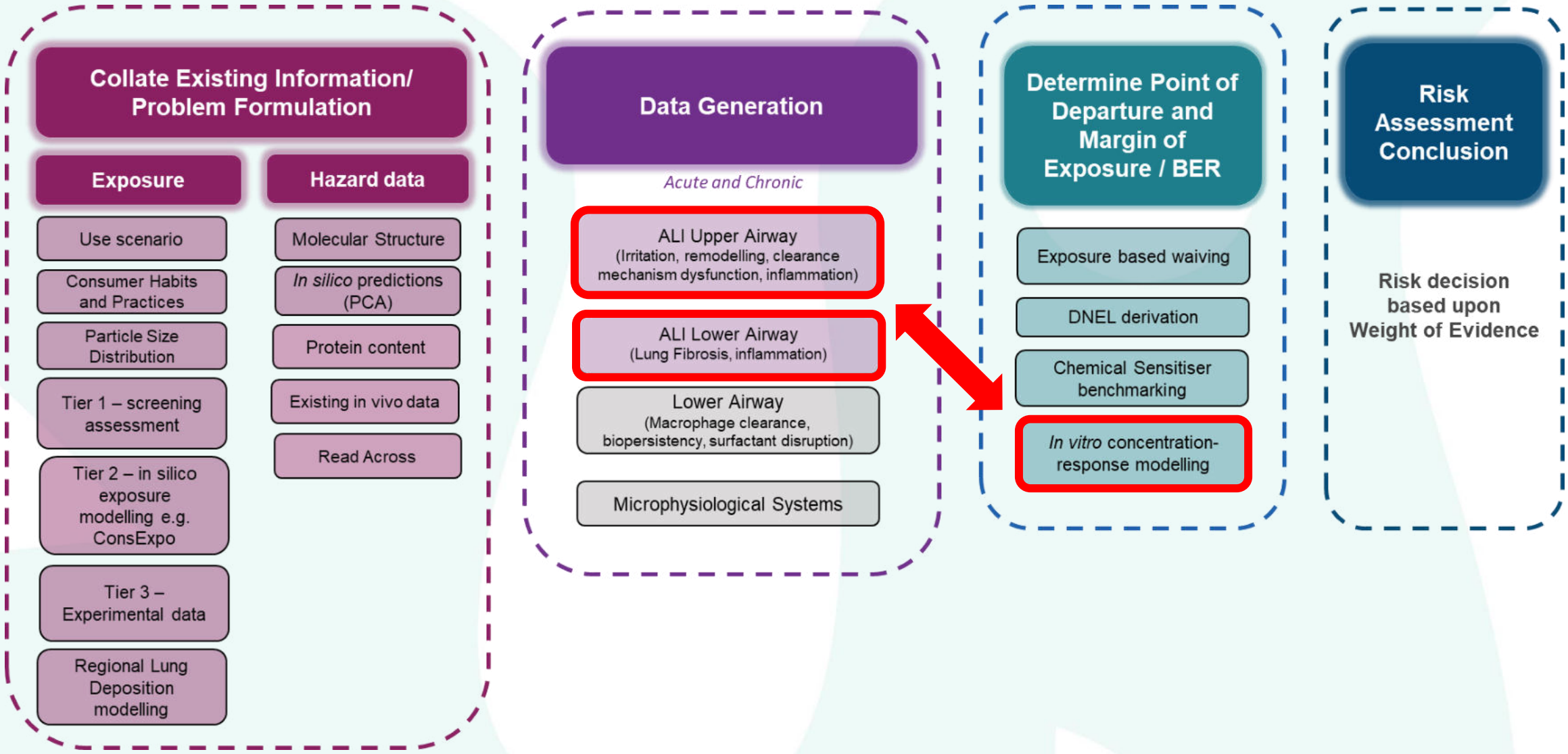
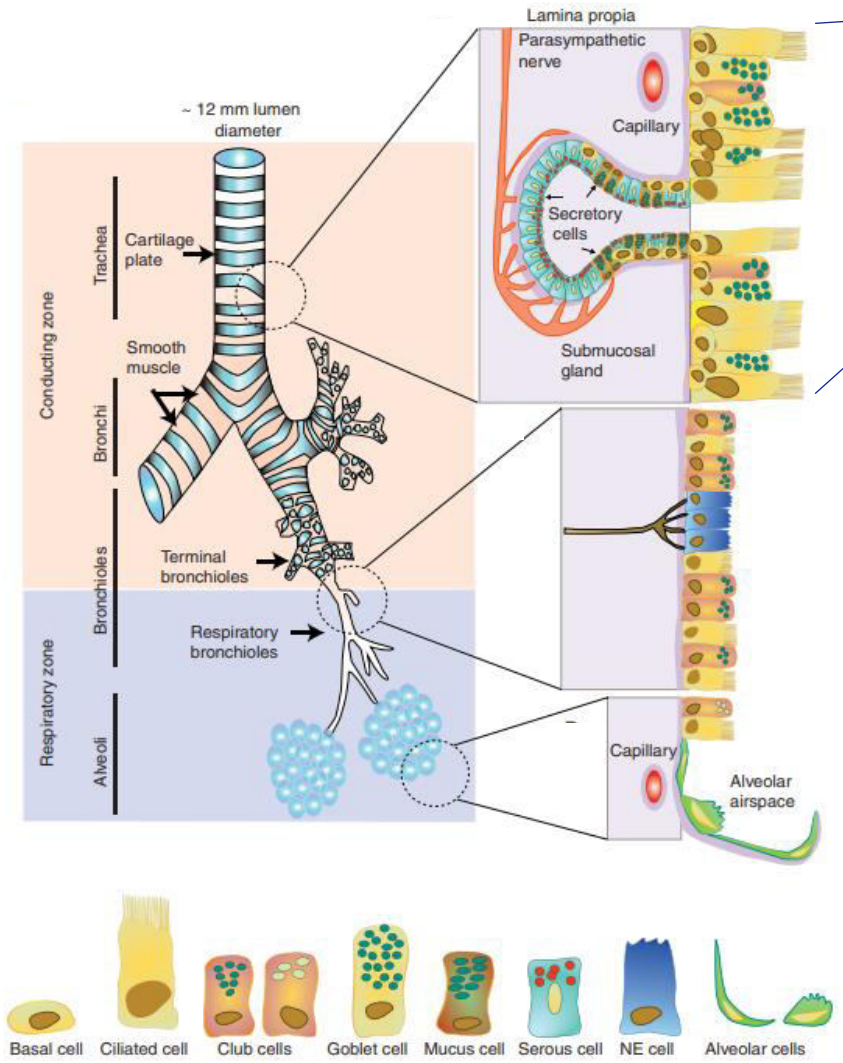
Kim et al (2016). J Toxicol Sci 41(6): 711-717.

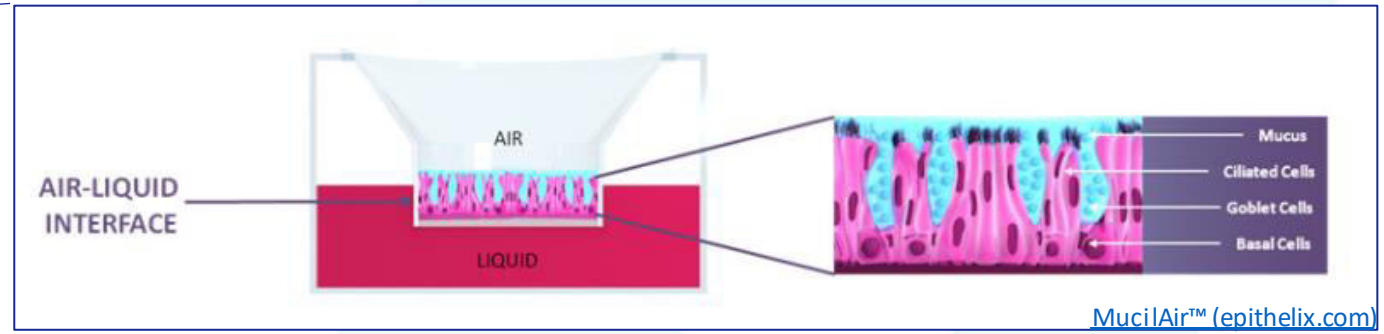
Ongoing development of a Inhalation Framework



Upper Airway – The MucilAir™-HF cell system (Epithelix)



modified after Bustamante-Marin, et al. 2017



MucilAir™ (epithelix.com)

Reconstituted cells system using human primary bronchial cell cocultured with human airway fibroblast.

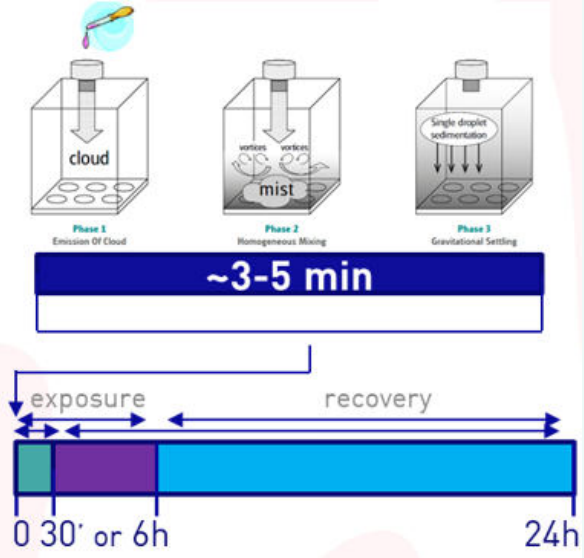
selection criteria:
- Exposure at the ALI
- Stable cells systems which allows repeated exposure
- Allows measurement of biomarkers of relevant AOP's
- Measurement for mycolitic activity as well as for inflammation and wound healing

functionality	biomarker	acute	chronic
mycolitic activity	mucus secretion, cilia beating (CBF), mucociliary clearance (MCC)	irritation, enhanced chance of airway infection	goblet cell hyperplasia, asthma, COPD
barrier function	tissue integrity (TEER, LDH), cytokine/chemokine release, extracellular matrix accumulation	local cytotoxicity, inflammation, wound healing	airway remodelling, Asthma, COPD, lung fibrosis

toxic endpoint of concern for PHMG (but more concise for lower airways)

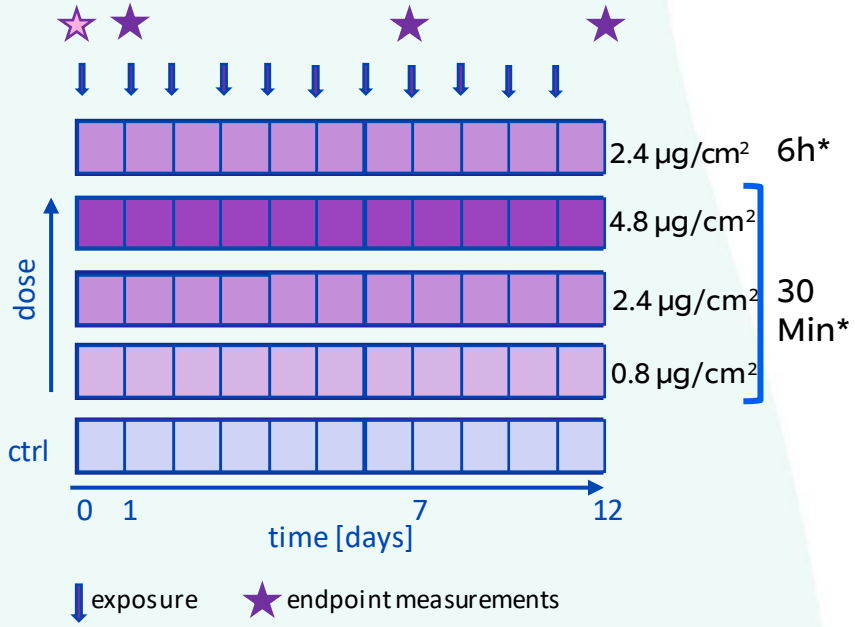
Huang et al., Drug Discovery and Development—Present and Future 2011 8
Sivars et al., Toxicol Sci. 2018 162(1):301-308

Upper Airway – Experimental design



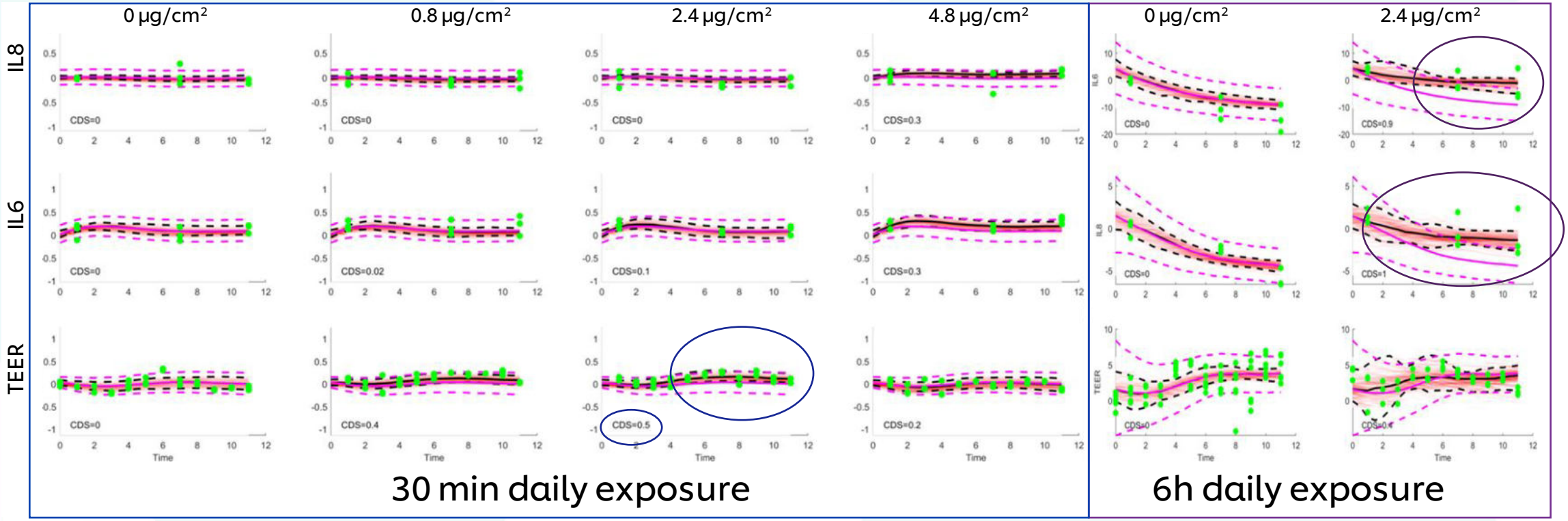
- Cells were exposed with nebulised PHMG using the VITROCELL®cloud chamber
- Daily exposure duration was aligned to adjust for mucociliary clearance of the upper airway (Paul et al. , Pulmonary Medicine 2013; Gizurarson, Biol. Pharm.Bull. 2015, 38(4); Herve et al., Chest 1993 103(1)).

- Repeated exposure was conducted on a daily basis for up to 12 days and the different biomarkers were measured at least for day 0, day 1, day 4, day 7 and day12
- All Endpoints were measured after a recovery period 24h after exposure, with the exception of day 0 were and additional MCC measurement was taken 30min after exposure



* exposure is aligned with short use of a homecare/personal care product and was not aligned to a long term exposure of PHMG in humidifier!

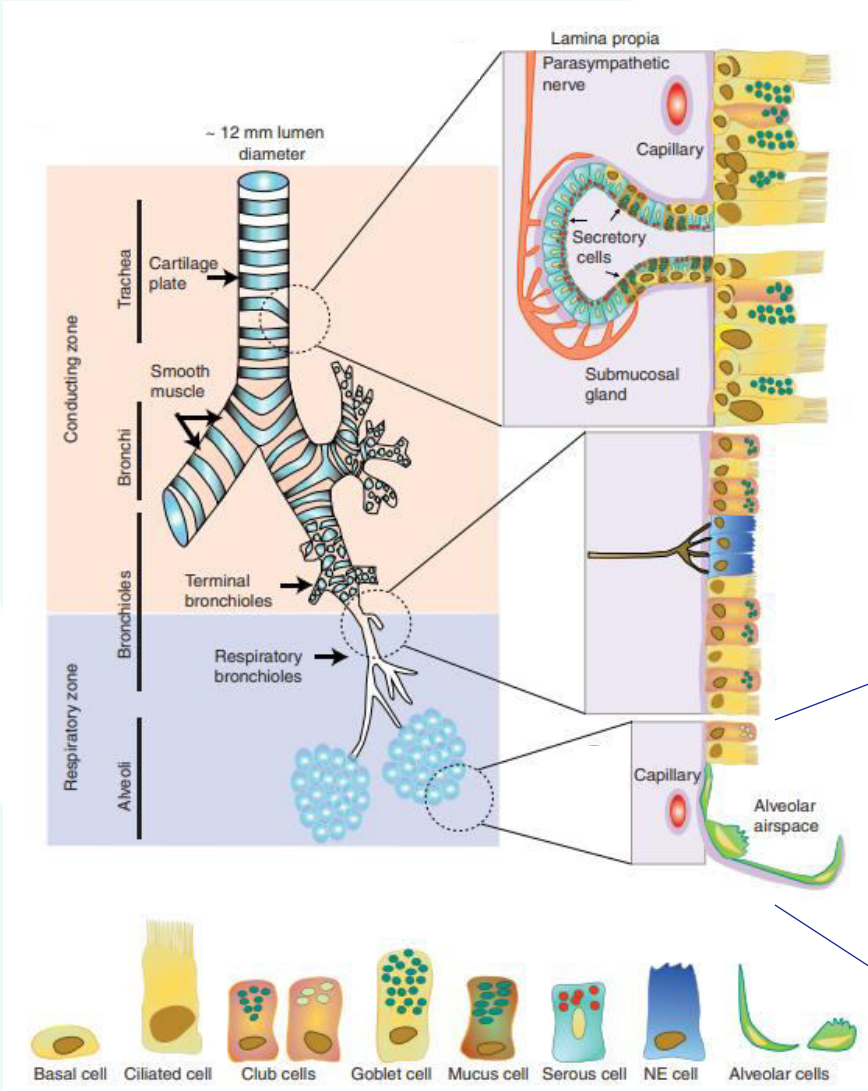
PHMG causes a slight inflammatory response in MucilAir™ cell model



Pink dashed line – 95% cred range of control.
Black dashed line – 95% cred range of mean response
Green dots – data points

- PHMG causes slight inflammation in the 6 h treated tissues only
- TEER measurements shows statistically significant increase at a daily exposure at 2.4µg/cm²
- The results indicate that exposure duration has an effect on experimental outcome
- The mathematical model is still under construction => **preliminary results**

Lower Airway – The EpiAlveolar™ cell system (MatTek)



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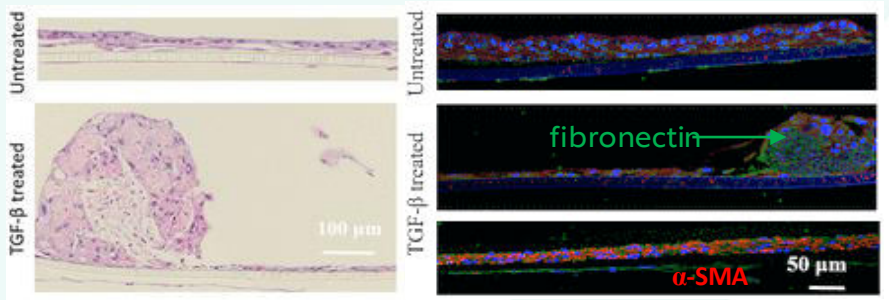
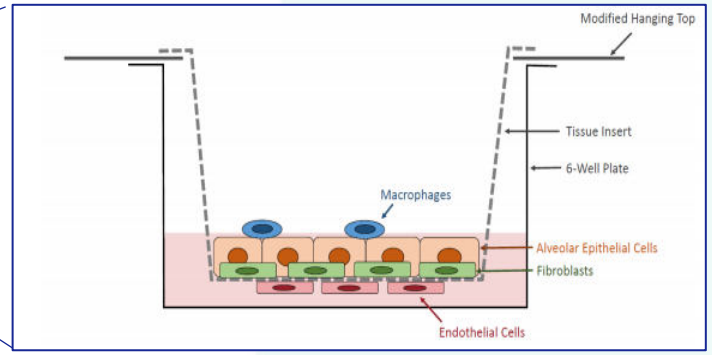


Barosova et al., *ACS Nano* 2020, 14, 4, 3941−3956

functionality	biomarker	acute	chronic
barrier function	tissue integrity (TEER, LDH), mitotoxicity, cytokine/ chemokine release, extracellular matrix accumulation	local cytotoxicity, inflammation, wound healing	airway remodelling/scarring, lung fibrosis

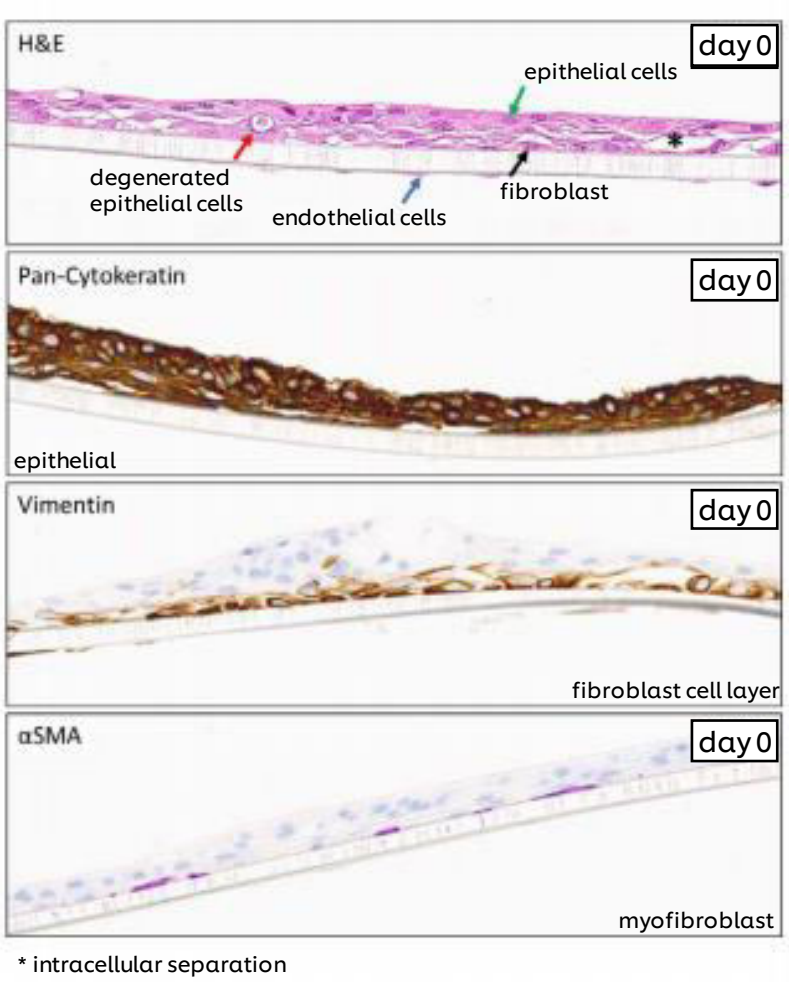
toxic endpoint of concern for PHMG

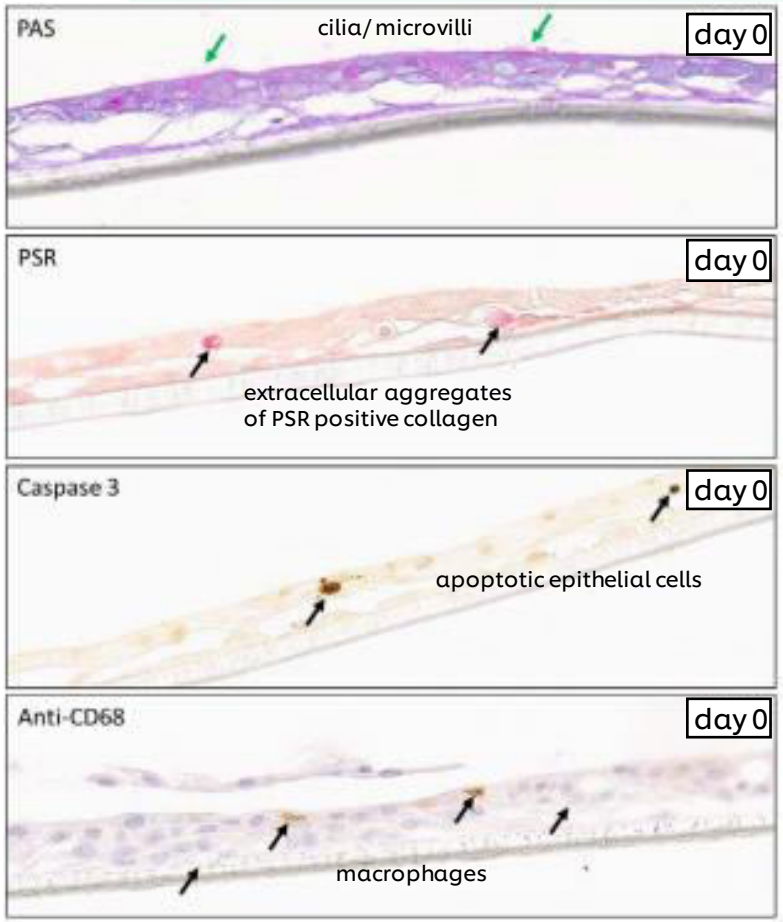


primary human alveolar epithelial cells, pulmonary endothelial cells and monocyte-derived macrophages

selection criteria:
- Exposure at the ALI
- Stable cells systems which allows repeated exposure
- Allows measurement of biomarkers of relevant AOP's
- Co-culture of cells including immune competent cells/macrophages and fibroblast

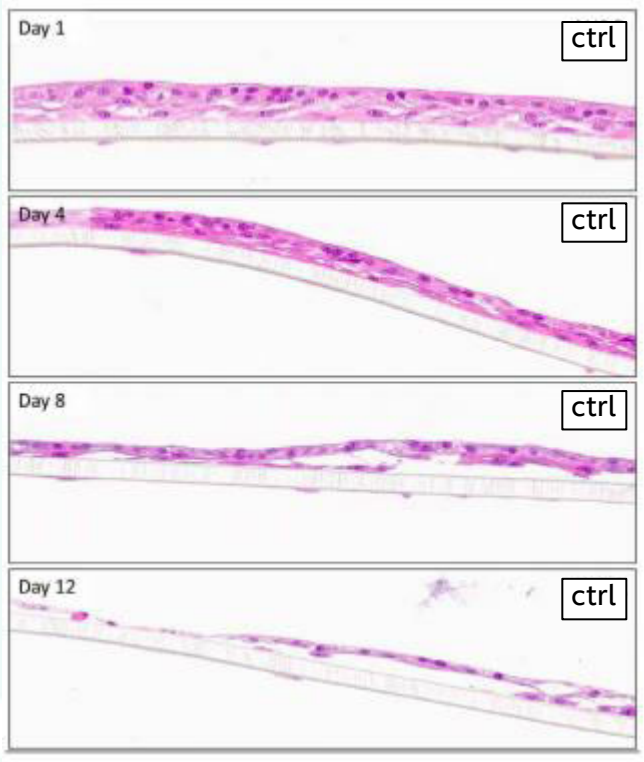
Morphology of EpiAlveolar™ cell model

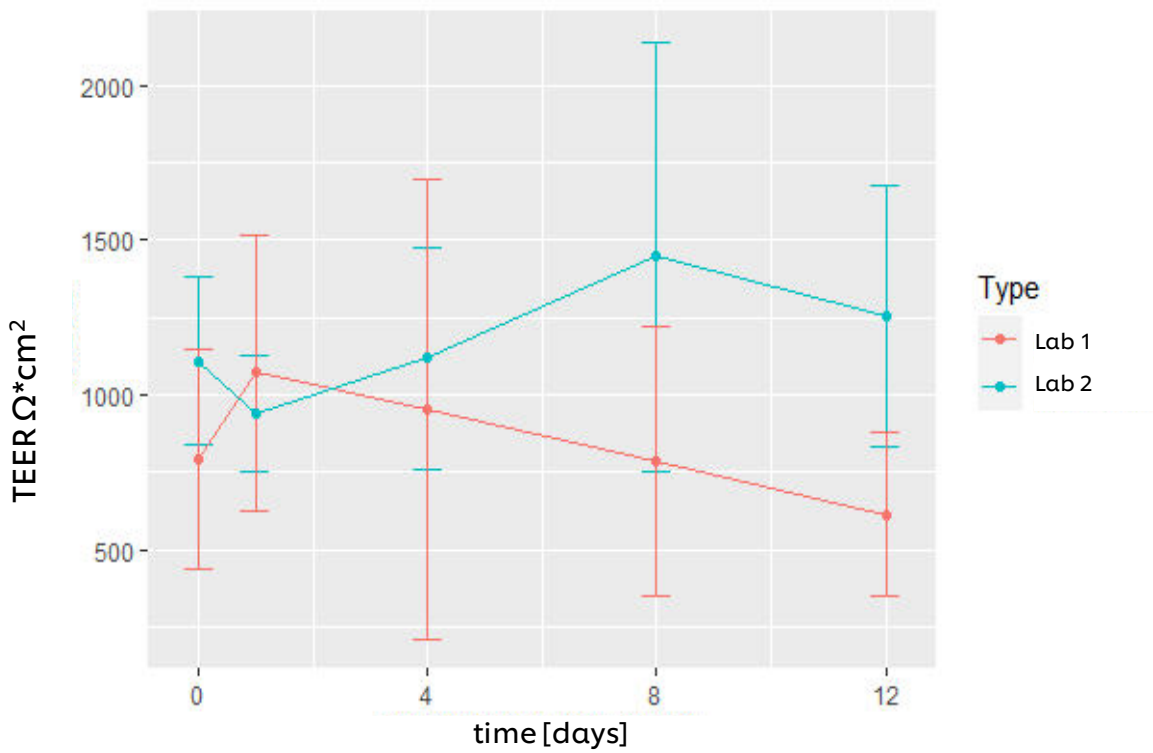




→ No staining with prosurfactant C (marker for AT2 cells) could be detected. However inclusion of AT2 cells were shown in Borosva et al., 2020

Morphological changes of the EpiAlveolar™ cell model over time





- Thinning of the EpiAlveolar tissue from a 2-4 cell layer down to a single cell layer
- Barrier functions remains stable over time, with some variability between laboratories

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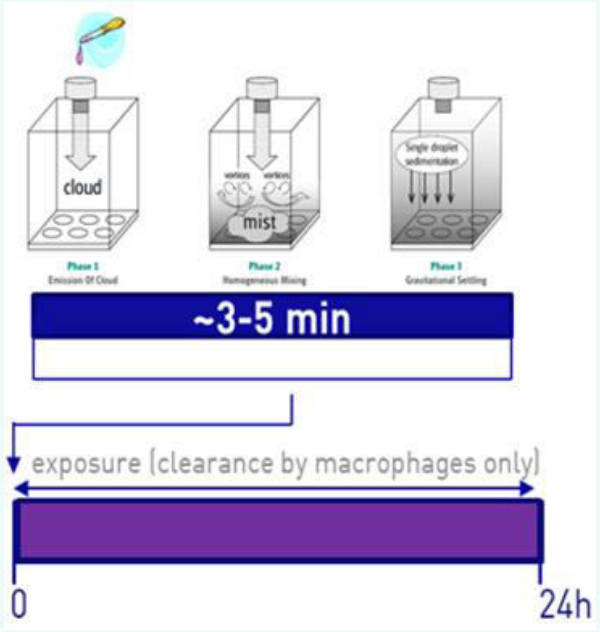
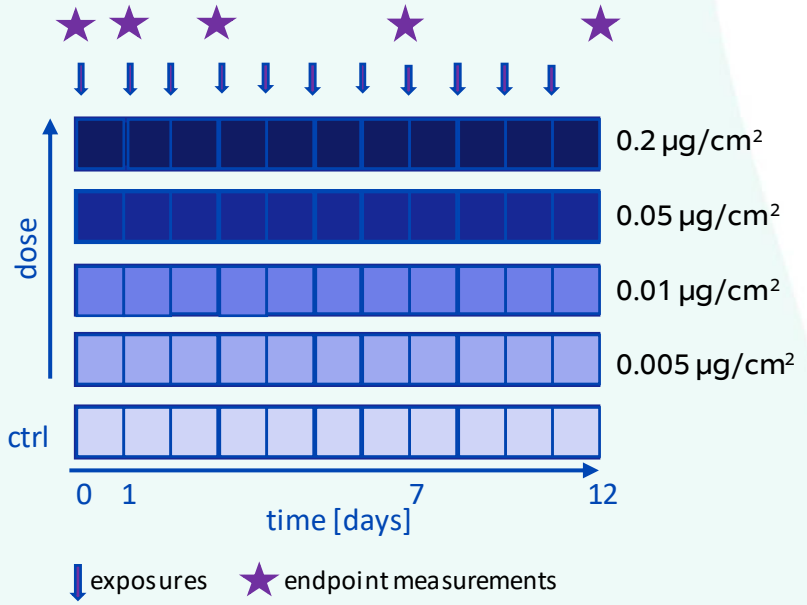
Lower Airway – Experimental design



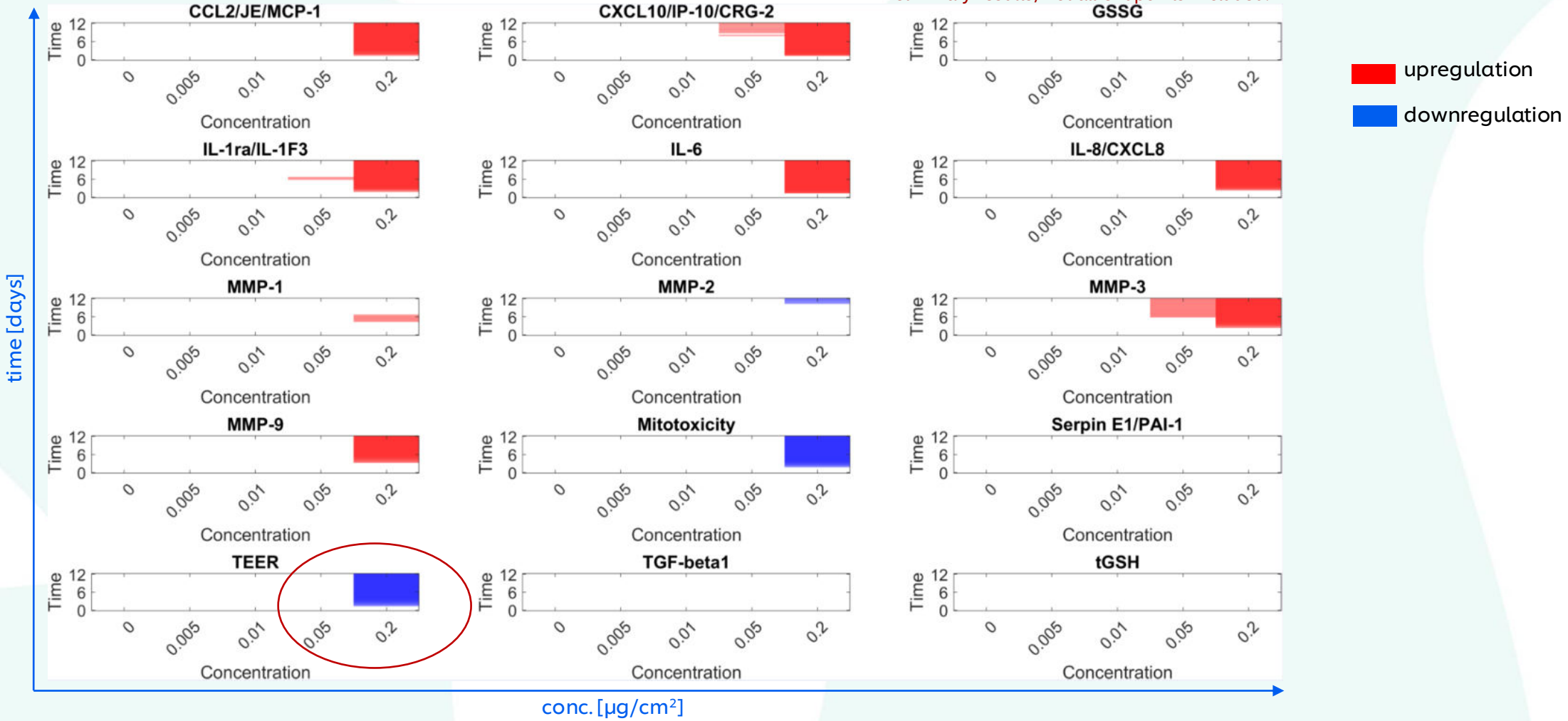
- Cells were exposed with nebulised PHMG using the VITROCELL®cloud chamber
- Cells were exposed for 24h without recovery

- Repeated exposure was conducted on a daily basis for up to 12 days and the different biomarkers were measured at least for day 0, day 1, day 4, day 7 and day12



PHMG causes cytotoxicity in EpiAlveoloar™ cell model

Preliminary results, not all endpoints included!



- Daily exposure of 0.2 µg/cm² leads to loss of tissue integrity (TEER) accompanied by increased release of pro-inflammatory cytokine markers and ECM accumulation.
- These results might reflect the *in vivo* situation in humans where PHMG leads to acute interstitial pneumonia which is characterised by diffuse alveolar damage.

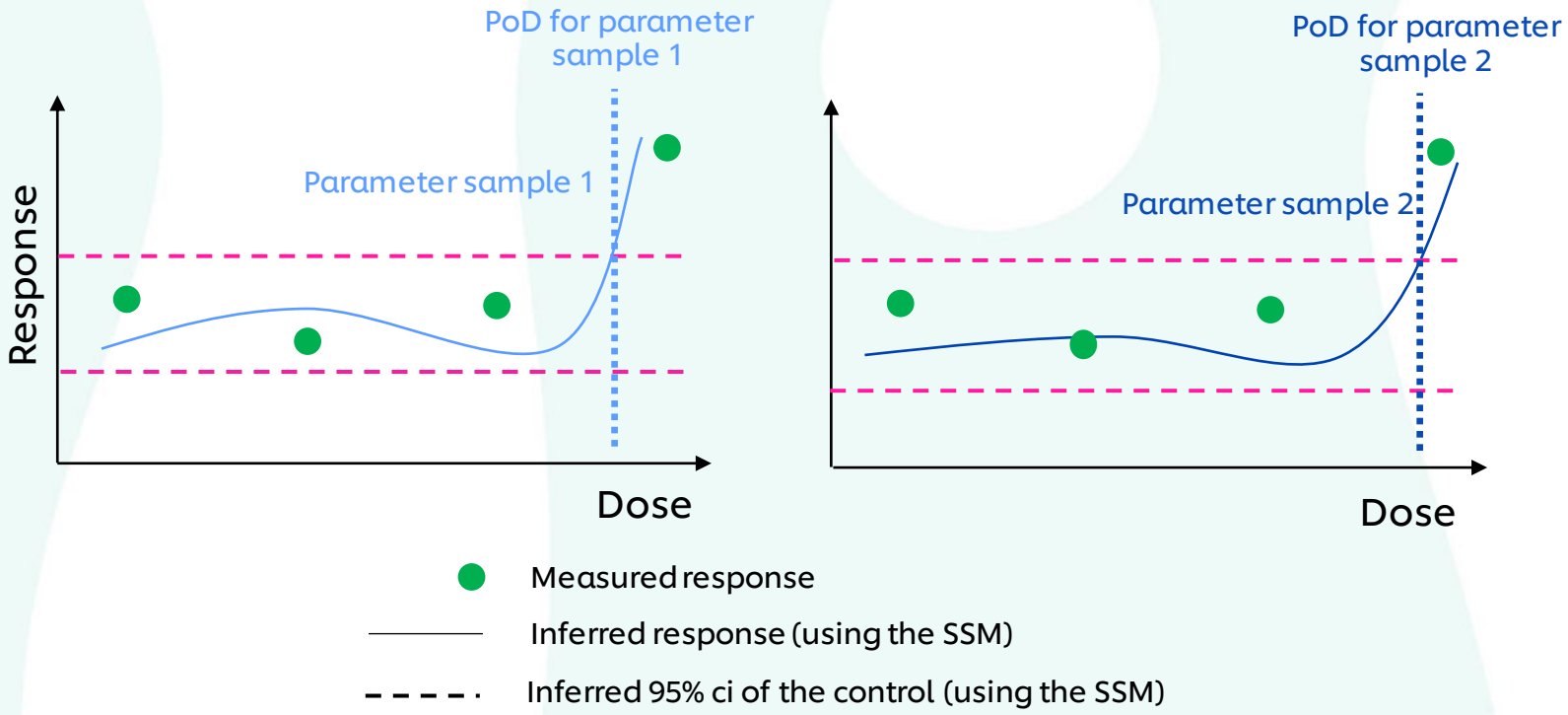
Method for calculating a Point of Departure (PoD) using the state space model (SSM)

Have multiple parameters samples obtained when training the model against the data

Use samples to:

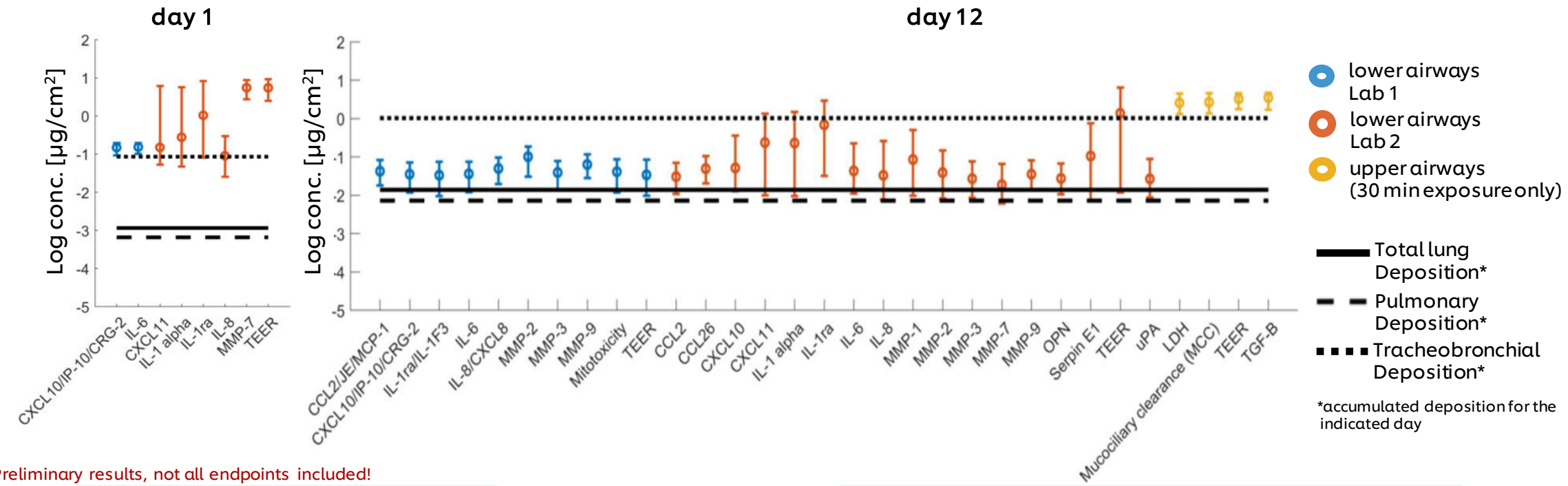
1. Infer the dose response.
2. Estimate the PoD (if CDS>0.5)

Repeat each timepoint/ endpoint!



Results in (approximate) PoD distribution, from which useful statistics can be estimated (median, 95%CI etc).

Comparing PoD to estimated lung deposition calculations



Preliminary results, not all endpoints included!

- the slightly lower PoD and higher deposition rate of substance at day 12 compared to day 1 indicates a lower margin of safety/exposure which will affect the risk assessment outcome
- Choice of cell model has a big impact on PoD calculation
- Donor/ lab variability is present but (at least for PHMG analysis) no significant effects on PoD

Gaps & Next steps

Exposure:

- Uncertainty in the estimates of regional deposition doses; limited human data to validate models and only available for total deposited fraction
- For chronic repeated dosing lack of ready- to- use models to predict steady state concentrations the lung (i.e. incorporation of multiple clearance mechanisms such as absorption, metabolism, mucociliary clearance and macrophage clearance)
- Uncertainty in the delivered dose in vitro, especially over repeated dosing scenario
- Compare MPPD outputs with Computational fluid dynamics (CFD) modelling results.

Data generation and concentration response modelling:

- Finalise the mathematical model and explore additional features (e.g. using the model to predict PoD at timepoints which were not measured)
- Uncertainty around exposure duration in vitro (30min-6h for upper airway, 24h for lower airway?)
- Evaluate the full data set including all benchmark substances and endpoints in the context of decision making, and where appropriate, extend the data set.
- Define most relevant endpoints to simplify experimental design
- Finalise and complete transcriptomic analysis
- Fill gaps to cover all endpoints of concern (e.g. biopersistence and surfactant inhibition)

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Acknowledgements

Unilever:

- Sophie Cable
- Anthony Bowden
- Alistair Middleton
- Joe Reynolds
- George Fitton
- Mathura Theiventhran
- Danilo Basili
- Mark Liddell
- Jade Houghton
- Tym Pietrenko
- Patrik Engi
- Ouarda Saib
- Hugh Barlow
- Ellen Edwards

Epithelix:

- Samuel Constant
- Bernadett Boda

Charles River Laboratories:

- Joanne Wallace
- Hazel Paulo
- Clive Roper (now Roper Toxicology consulting limited)

IIVS:

- Holger Behrsing
- Vivek Patel
- Adam Wahab
- Pooja Naik