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

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Unilever- Safety & Environmental Assurance Centre (SEAC)

About Unilever

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Unilever Safety & Environmental Assurance Centre (SEAC)

SEAC Purpose: **Protecting People & Planet**

Trusted Impactful Innovations designed for Safety & Sustainability

Safety and Environmental Science

We want consumers to be confident that our products are safe for them and their families, and better for the environment. The scientists at Unilever's Safety and Environmental Assurance Centre (SEAC) play a key role in ensuring that our products are safe and environmentally sustainable.

Learn more about our science and scientists

Safe and sustainable by design

How we build safety and sustainability into every product innovation.

SAFETY BY DESIGN »

Keeping people and the environment safe

The science-based approaches we use to keep our consumers, workers and the environment safe.

SAFETY RISK ASSESSMENTS »

Reducing our environmental impact

How we harness the latest science to minimise our environmental footprint.

OUR SCIENTIFIC APPROACH »

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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Assuring inhalation safety: Inhalation exposure depends on product type and habits & practices

Several Unilever products lead to an unintentional inhalation exposure :

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

Household cleaning products

Hairsprays (pump and aerosol)

Shampoos

Anti-perspirant/ deodorant aerosols

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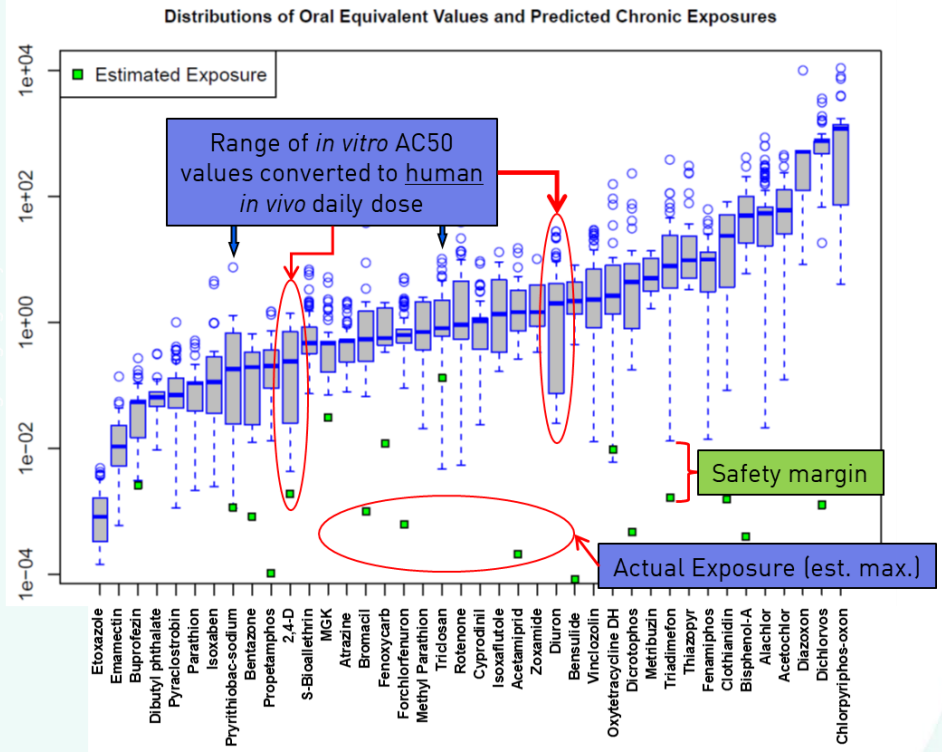
Safety without animal testing - Next Generation Risk Assessment (NGRA)

*NGRA is defined as **an exposure-led, hypothesis-driven** risk assessment approach that **integrates New Approach Methodologies (NAMs)** to assure **safety without the use of animal testing***



Slide from Dr Rusty Thomas, EPA, with thanks

Rotroff, *et al.* Tox.Sci 2010

The hypothesis underpinning this type of NGRA is that **if there is no bioactivity observed at consumer-relevant concentrations, there can be no adverse health effects.**

General strategy to developing an inhalation toolbox

Hypothetical Case study based approach

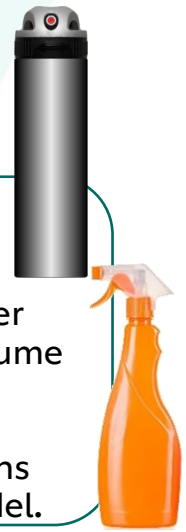
New polymers for use in antiperspirants & silanes for use in general purpose cleaners

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

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 practice**s:
  - 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.



Hypothesis-driven

Identification of key hazard concerns for the chemicals of interest

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

- NAMs identification and evaluation using benchmark compounds

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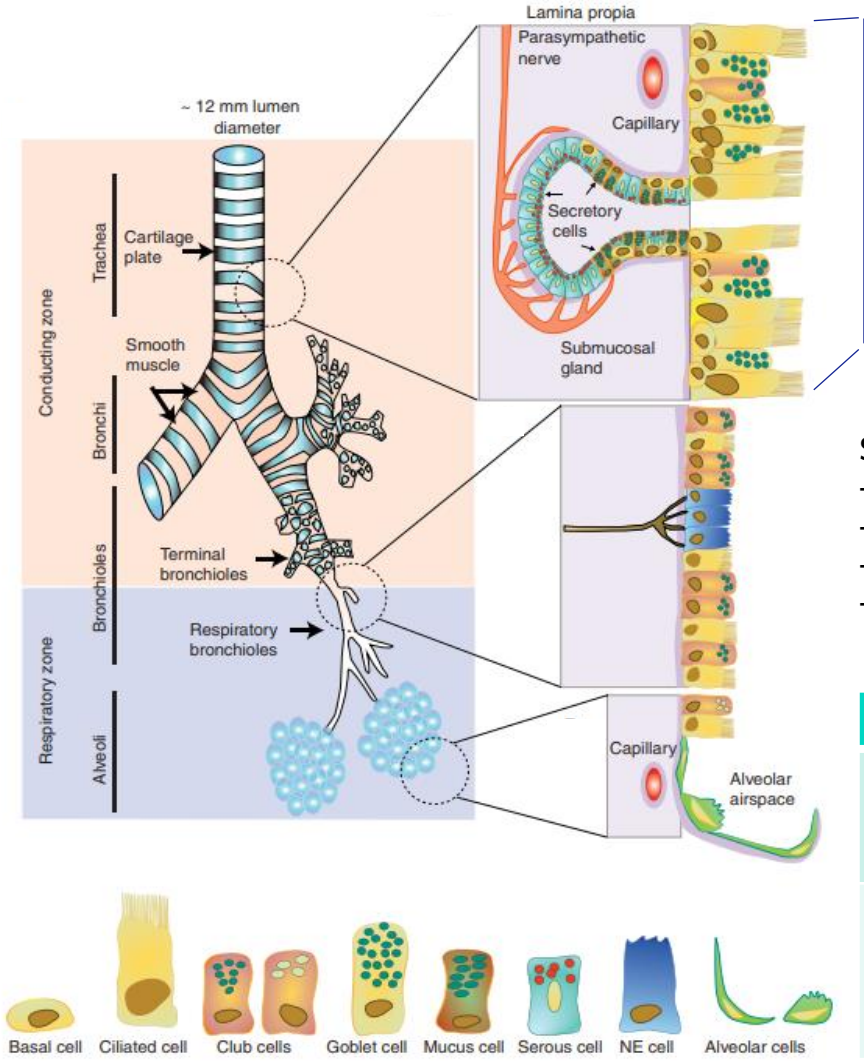
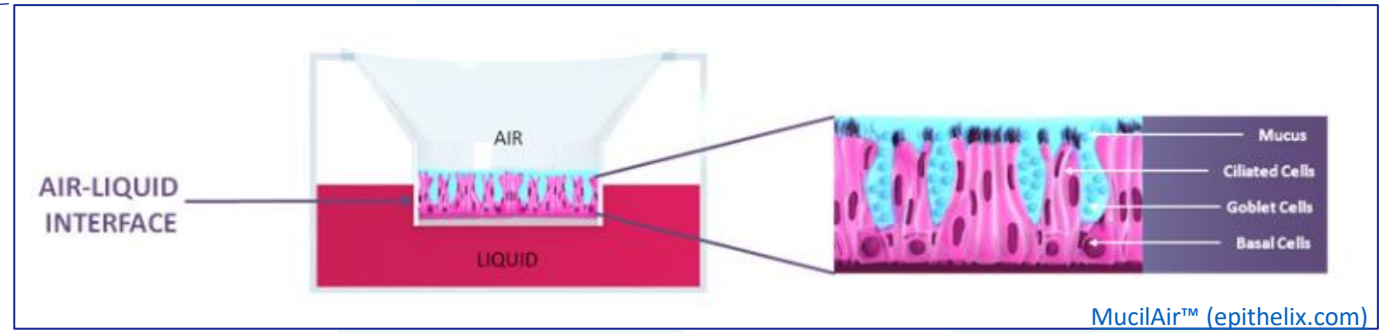
Upper Airway – The MucilAir™-HF cell system (Epithelix)



modified after Bustamante-Marin, et al. 2017



MucilAir™ (epithelix.com)

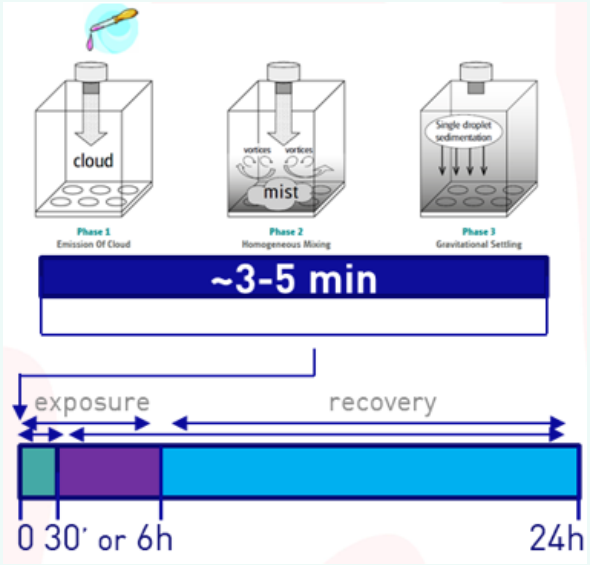
Reconstituted cells system using human primary bronchial cell cocultured with human airway fibroblast.

Selection Criteria:
- Exposure at the ALI
- Stable cells system which allows repeated exposure
- Allows measurement of biomarkers of relevant AOP's
- Mechanistic approach; allowing measurement for mycolitic activity as well as for inflammation (AOP 148, 411, 424 &425)

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	airway remodelling, Asthma, COPD, lung fibrosis

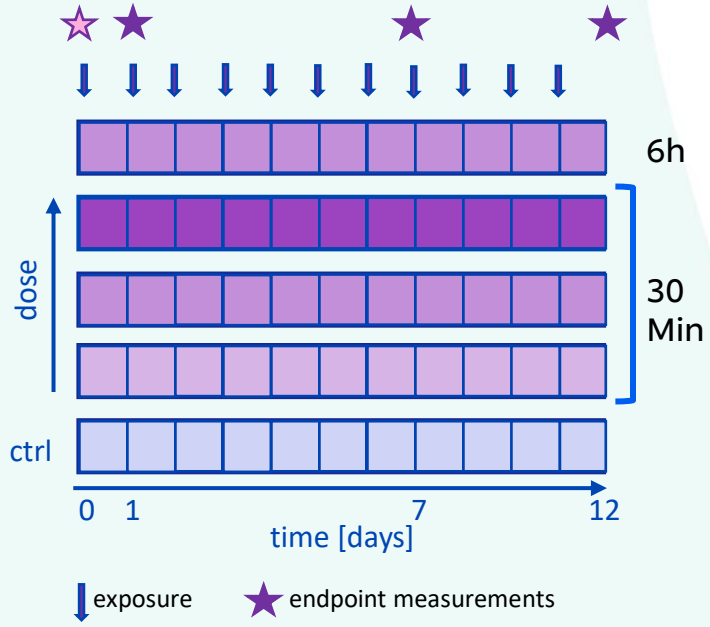
Huang et al., Drug Discovery and Development—Present and Future 2011 8
Sivars et al., Toxicol Sci. 2018 162(1):301-308

Upper Airway – Experimental design



modified after VITROCELL®

- Cells were exposed with nebulised compound if possible 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 and additional MCC measurement was taken 30min after exposure

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Upper Airway – results 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	Negative controls (history of safe use)
• Benzalkonium chloride ✗ • LPS ✗ • Carboxymethylcellulose ✗ • Acrolein ✗ • Isoproterenol ✓ • Chlorocresol ✗ • Nicotine ✗ • CFTRinh-172 ✗ • TNF-alpha ✗	• TNF-alpha ✓ • Benzalkonium chloride ✓ • Acrolein ✗ • LPS ✗ • Isoproterenol ✗	• Coumarin ✓ • Sulforaphane ✓ • Acudyne™ DHR polymer ✓ • Gantrez™ ES-425 ✓

✓ results as expected ✗ unexpected outcomes

→ Gaps identified: Interindividual variability, dosing, variability/sensitivity of the cell model.

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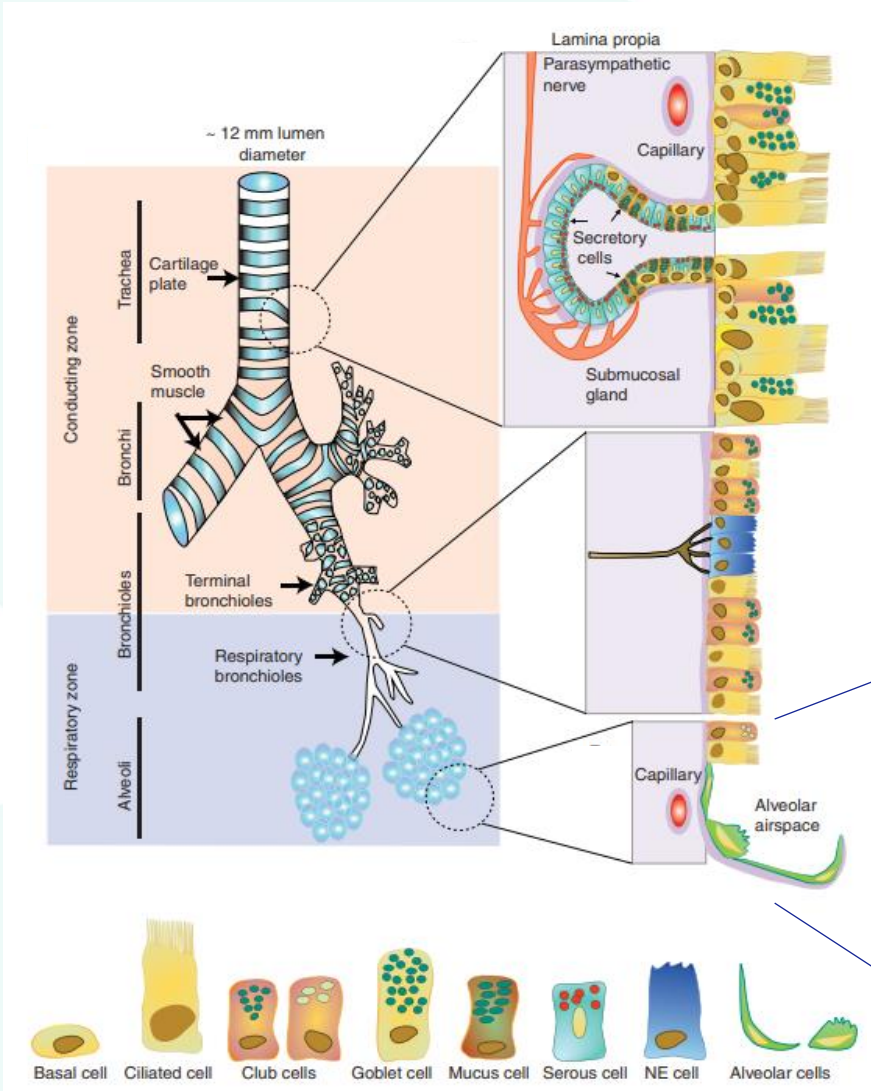
Lower Airway – The EpiAlveolar™ cell system (MatTek)



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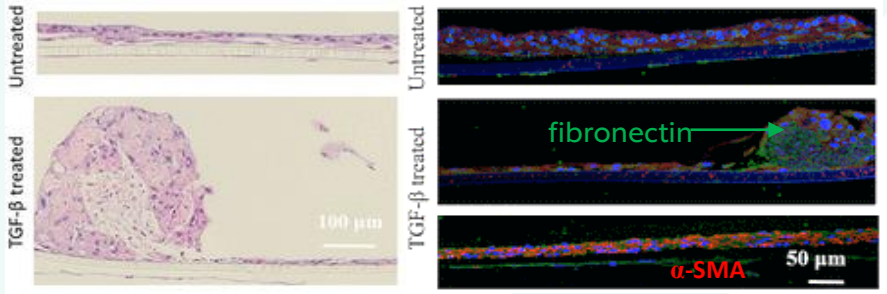
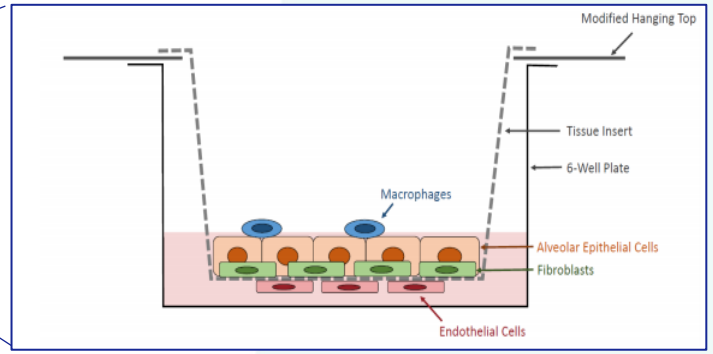


Barosova et al., *ACS Nano* 2020, 14, 4, 3941−3956

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



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
- Mechanistic approach; allowing measurement oxidative stress and inflammation (AOP173)
- Co-culture of cells including immune competent cells/macrophages and fibroblast

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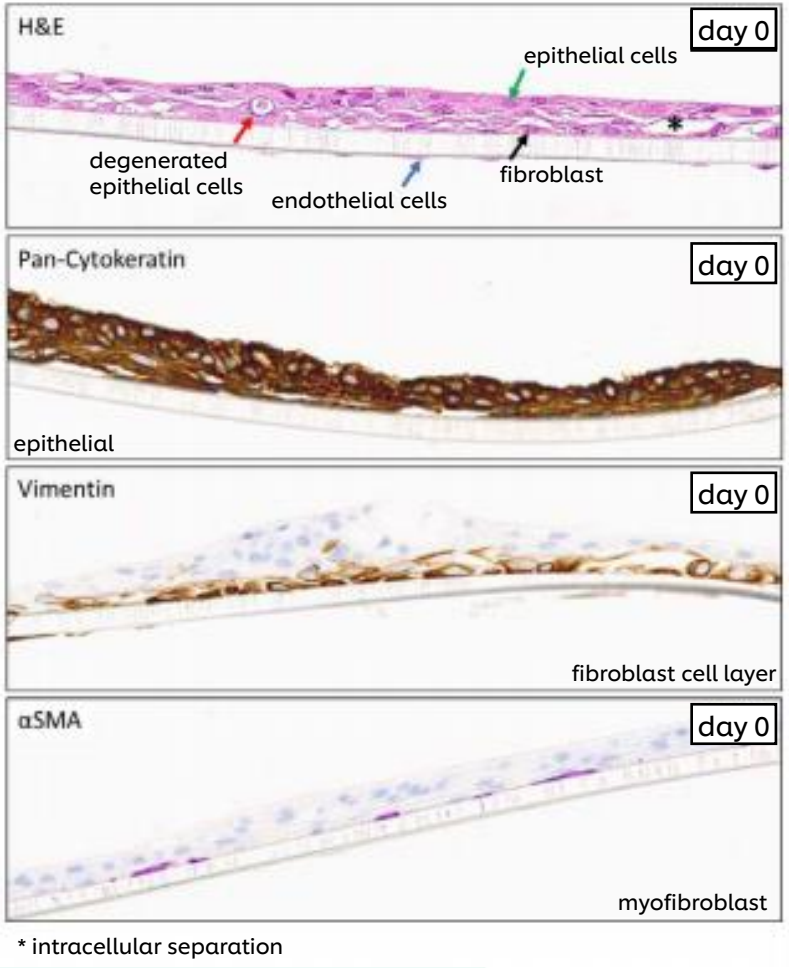
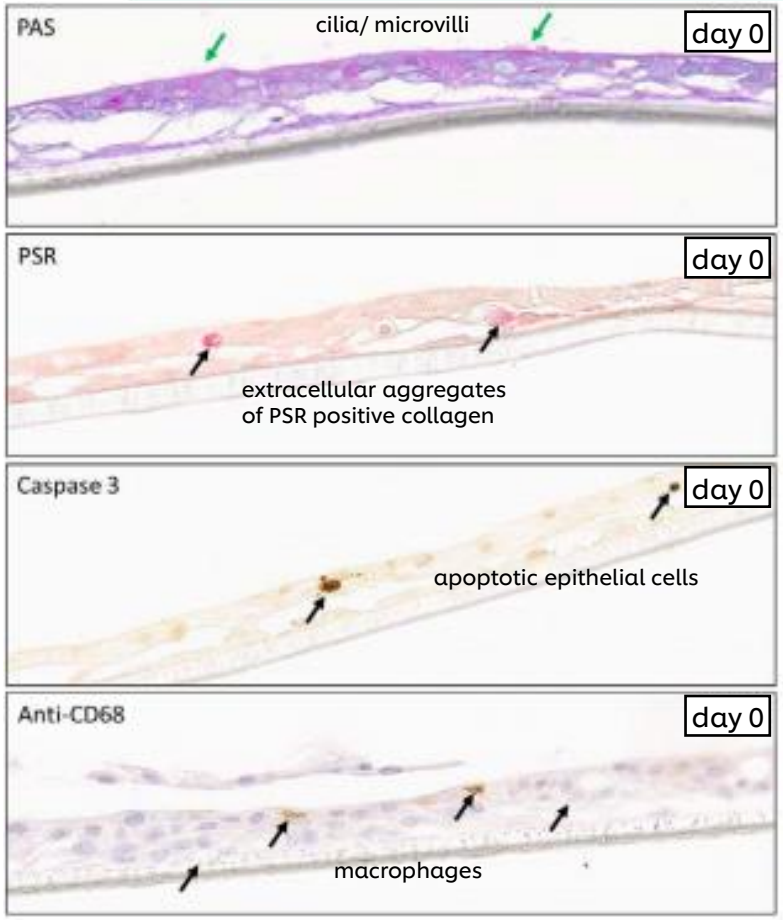
Morphology of EpiAlveolar™ cell model

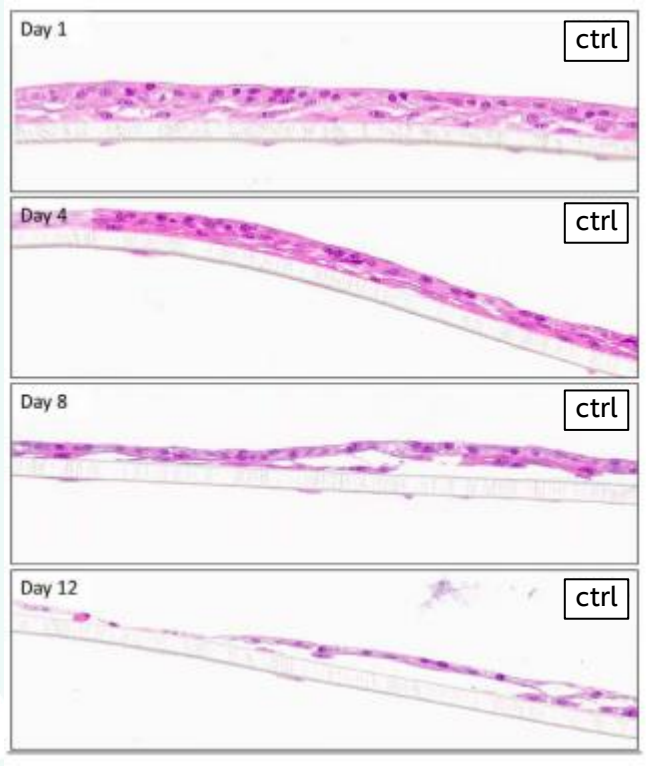


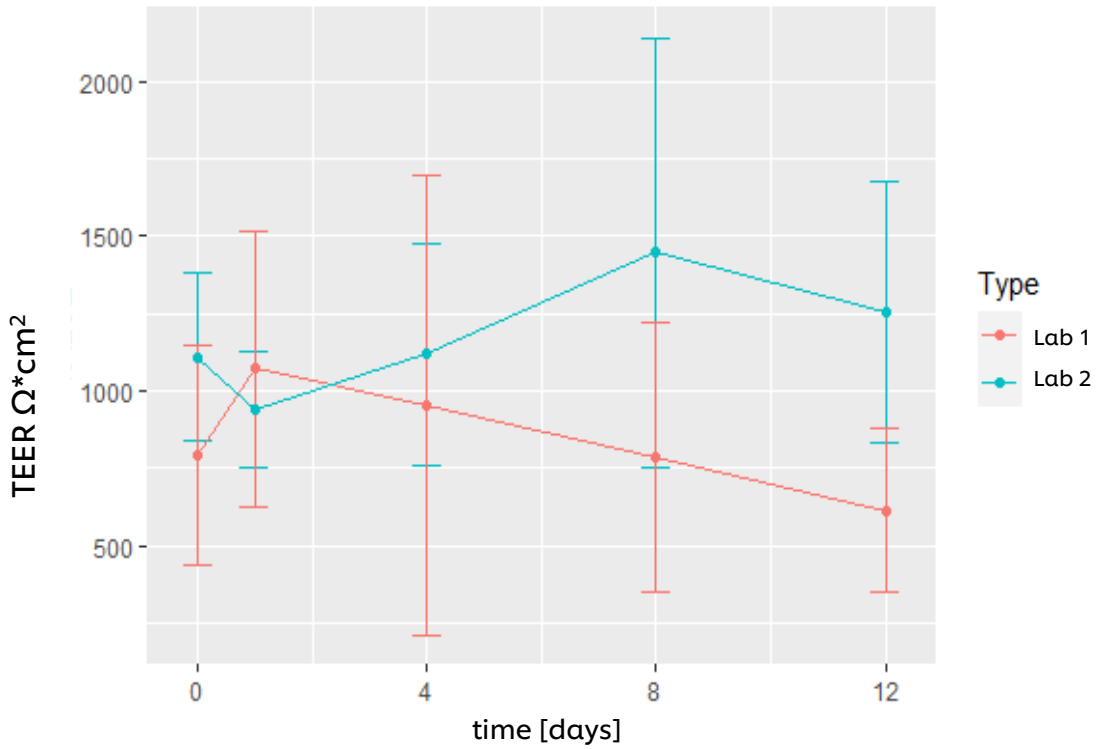
* intracellular separation



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

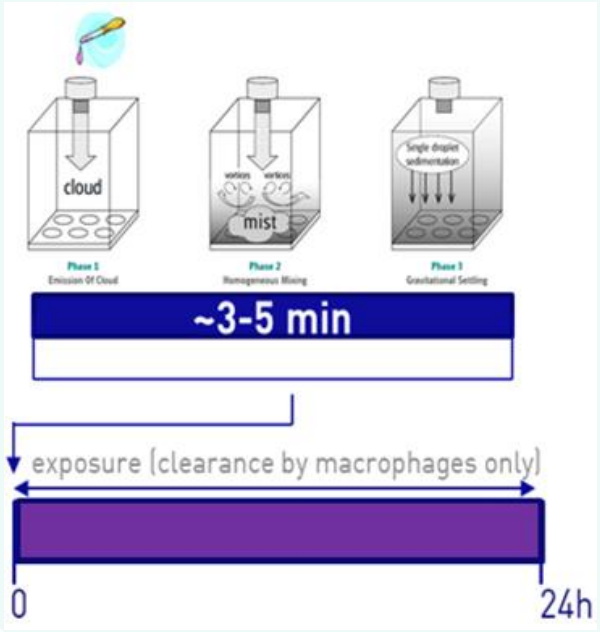
Morphological changes of the EpiAlveolar™ cell model over time





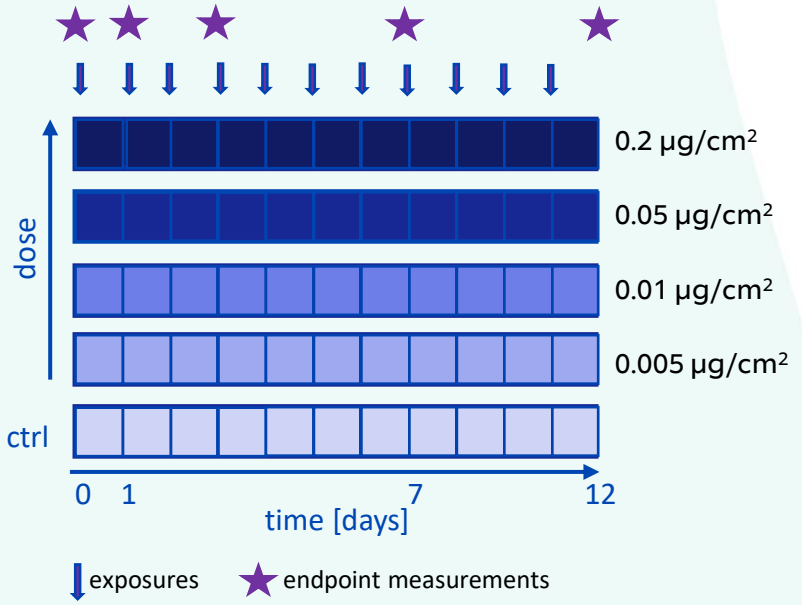
- Thinning of the EpiAlveolar tissue from a 2-4 cell layer down to a single cell layer
- Barrier functions remains stable over time, with some variability between laboratories

Lower Airway – Experimental design



modified after VITROCELL®

- Cells were exposed with nebulised compound 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

Lower Airway – results 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

Inflammation/ fibrosis, cytotoxicity	Negative controls (history of safe use)/case studies
• Amiodarone ✅ • Doxorubicin ✅ • Min-u-Sil5 (crystalline silica) ❌ • Aerosil 200 (amorphous silica) ❌ • LPS ❌ • PHMG ✅	• Sulforaphane ✅

✅ results as expected ❌ unexpected outcomes

➡ Gaps identified: dosing, variability/sensitivity of the cell model.

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Case Study

Hypothetical inclusion of a novel preservative in Hairsprays

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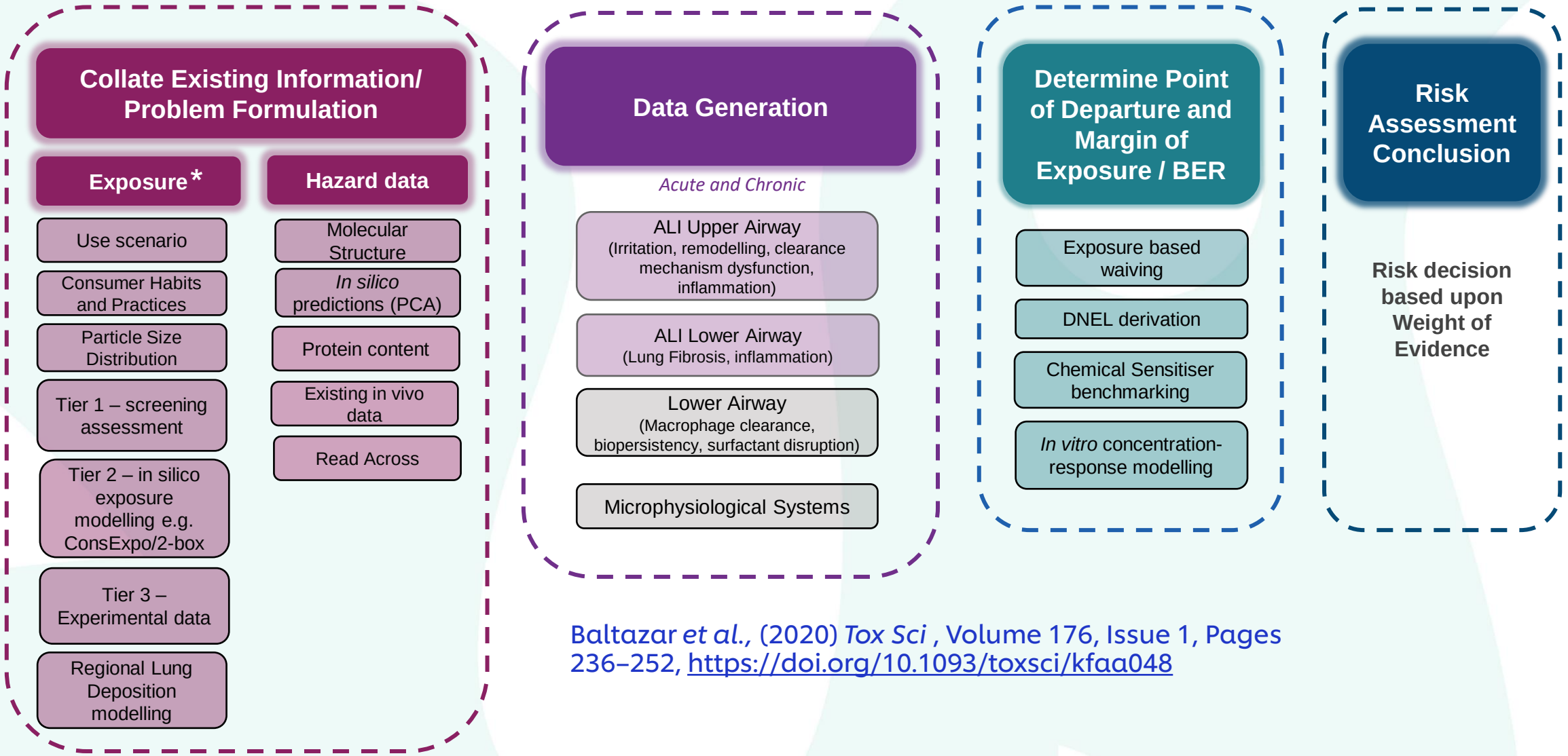
Ongoing development of an Inhalation Framework



Baltazar *et al.*, (2020) *Tox Sci* , Volume 176, Issue 1, Pages 236–252, https://doi.org/10.1093/toxsci/kfaa048

*Consumer Exposure in Inhalation risk assessment

Hypothetical Case study – 0.25% of a novel preservative in a hairspray aerosol

We have applied this framework to the chemical polyhexamethyleneguanidine phosphate (PHMG) to look at exposures:

(a) for an hypothetical case study imagining it was a new ingredient for a hairspray.

(b) that are known to be adverse in humans after during normal used of household humidifiers (Park et al 2015. Indoor Air 25(6): 631-640).

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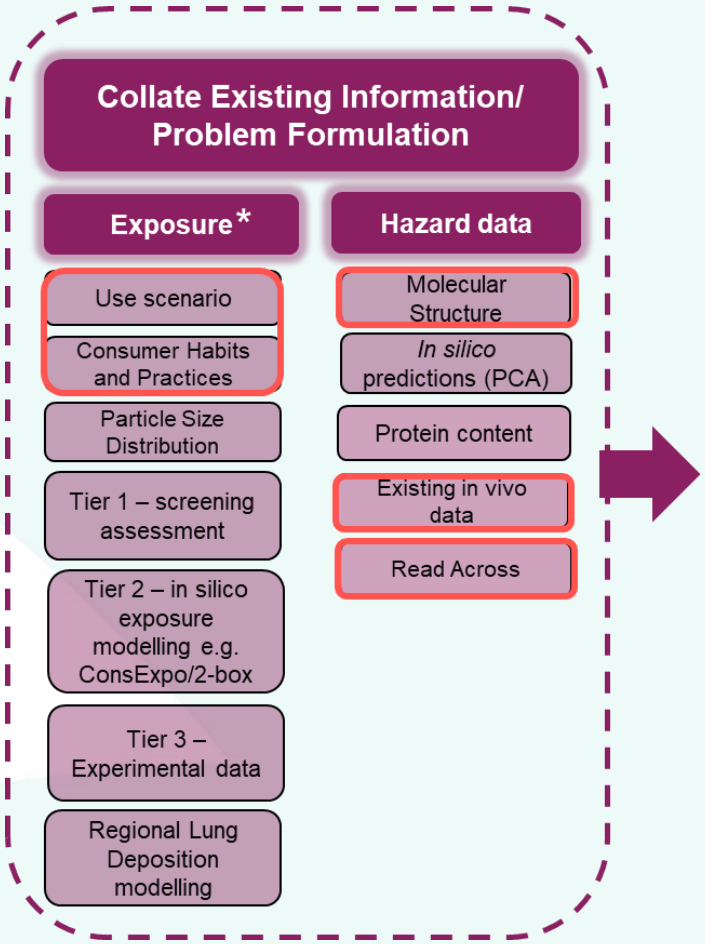
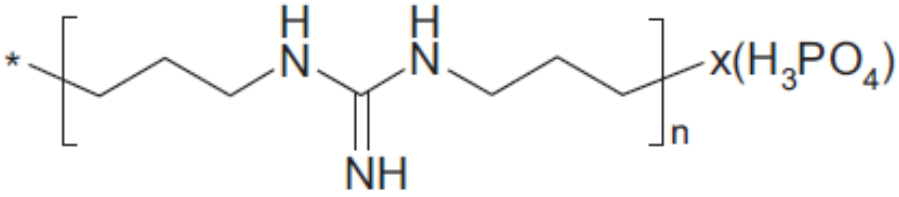
Hypothetical Case study – 0.25% of a novel preservative in a hairspray aerosol



Chemical identify



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

Oligomer, MW= 500-700 g/mol

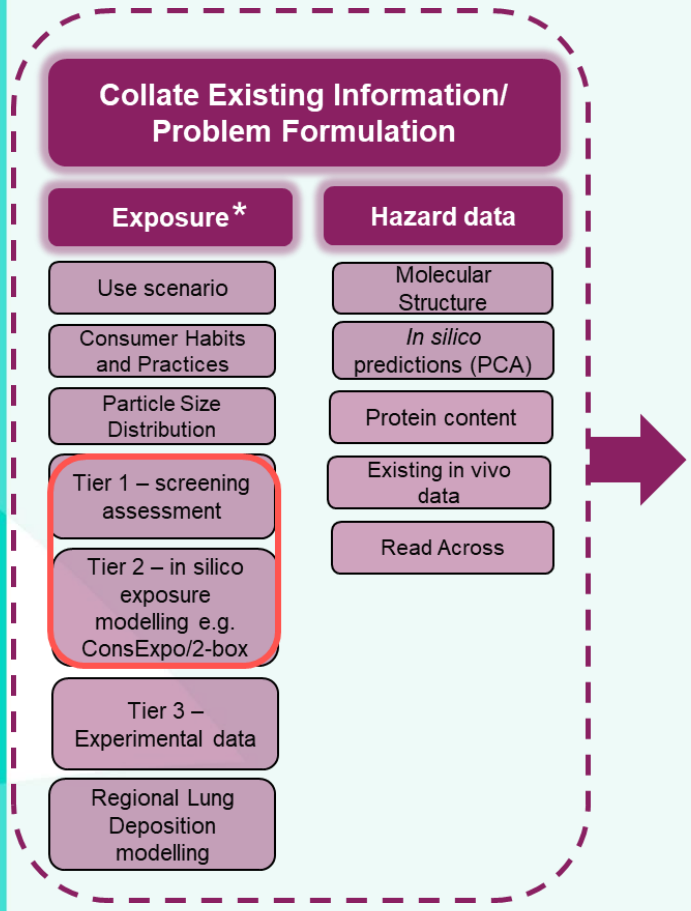
Assumptions:

- No existent animal or human
- No read-across available

Use scenario & Consumer habits and practices:

- **Spray rate:** 0.6 g/s
- **Spray duration:** 10s
- **Number application per day:** 1
- **Breathing zone:** 1 m^3

Hypothetical Case study – Tier 1 exposure assessment



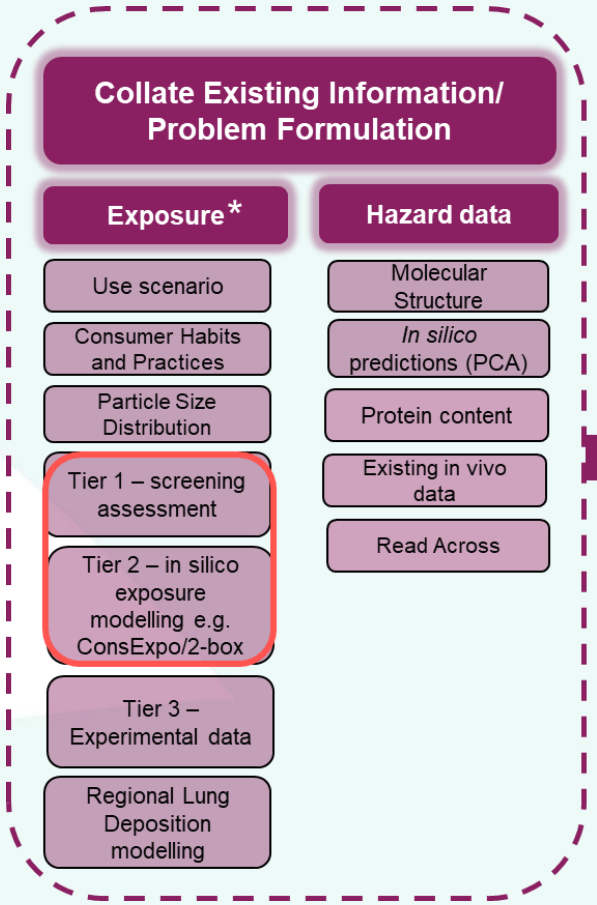
$$\text{Tier 1 Exposure} = \frac{\text{Weight of Ingredient in the Spray Formulation}}{\text{Room Volume}} \left[\frac{mg}{m^3}\right]$$

$$= \frac{0.6\ g/s \times 10s \times 1 \times (0.25/100)}{1\ m^3} = 15\ mg/m^3$$

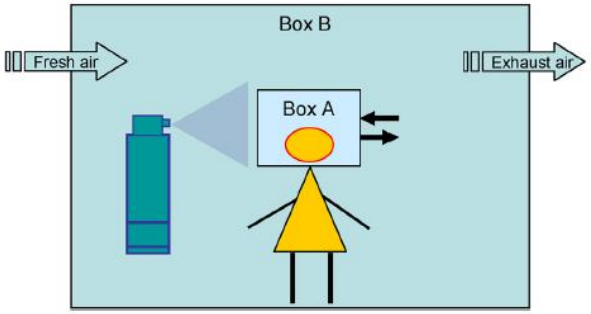
This is a conservative approach that assumes that 100% of the substance in the consumer product or article will be released at once and homogenously into the room and there is no ventilation. The duration of exposure is 24 hours and all released material is 100% inhalable

1. Guidance on Information Requirements and Chemical Safety Assessment Chapter R.15: Consumer exposure assessment Version 3.0 - July 2016
2. Steiling et al., 2014. : Principle considerations for the risk assessment of sprayed consumer products. Toxicology Letters 227 (2014) 41–49

Hypothetical Case study – Tier 2 - 2-Box Indoor Air Dispersion model developed by RIFM



Input	Spray rate (mg/min)	36000
	Inclusion level (%)	0.25
	Emission duration (min)	0.1667
	Number of applications	1
	Zone 1 volume (m3)	1
	Zone 2 volume (m3)	19.1
	Air flow (1 -> outside) (m3/min)	0
	Air flow (2 -> outside) (m3/min)	1.89
	Air flow (1 -> 2) (m3/min)	7.24
	Time in zone 1 (min)	1
	Time in zone 2 (min)	9
	Body weight (kg)	60
	Inhalation rate (L/min)	20
	Initial zone 1 concentration (mg/m3)	0
	Initial zone 2 concentration (mg/m3)	0
	Time step (min)	0.02
	Exposure duration (min)	10
Output	Mean zone 1 for 1st minute (mg/m3)	2.690339
	Mean zone 2 for next 9 minutes (mg/m3)	0.505035
	Time-weighted average (mg/m3)	0.7



Images from: Steiling et al., 2014. Principle considerations for the risk assessment of sprayed consumer products. Toxicology Letters 227 (2014) 41–49

http://www.rifm.org/uploads/Inhalation%20Modeling%202-Box%20Webinar%201.17.2012.pdf

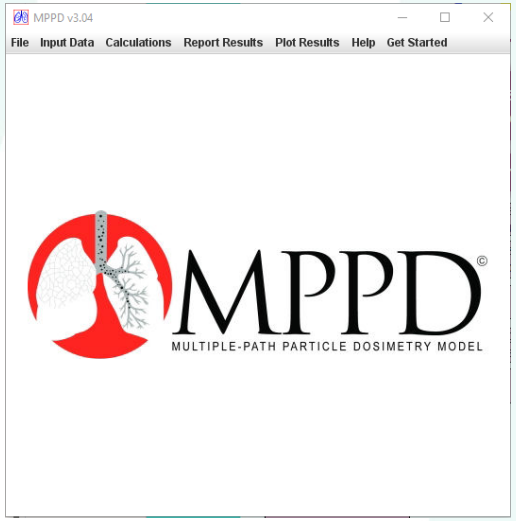
Hypothetical Case study – Regional Lung Deposition Modelling

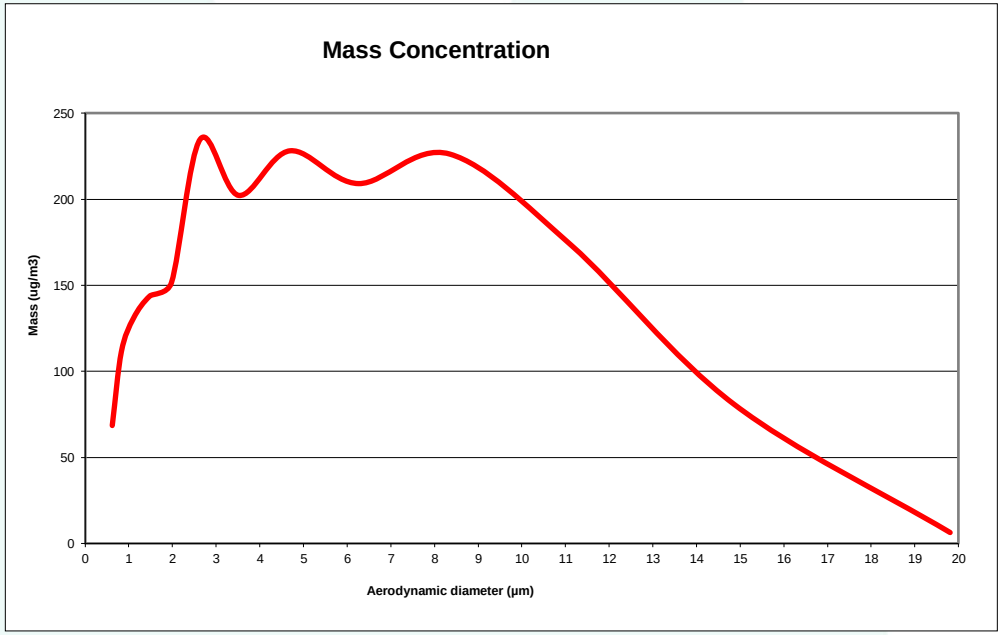
Collate Existing Information/ Problem Formulation

Exposure*	Hazard data
Use scenario	Molecular Structure
Consumer Habits and Practices	*In silico* predictions (PCA)
Particle Size Distribution	Protein content
Tier 1 – screening assessment	Existing in vivo data
Tier 2 – in silico exposure modelling e.g. ConsExpo/2-box	Read Across
Tier 3 – Experimental data	
Regional Lung Deposition modelling	

Measured Particle Size Distribution

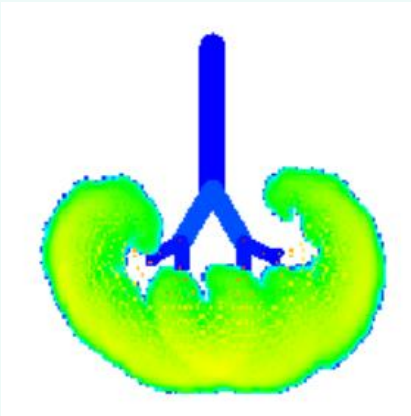
Mean Mass Aerodynamic Diameter : 3.64±2.62µm

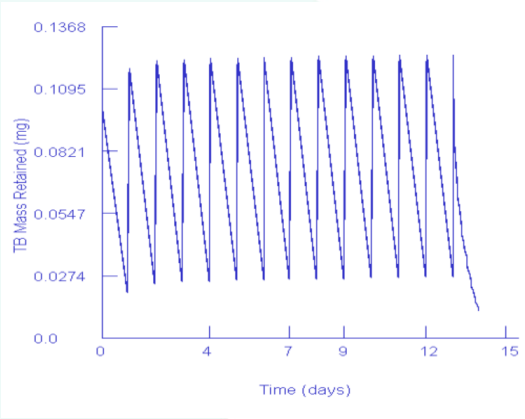


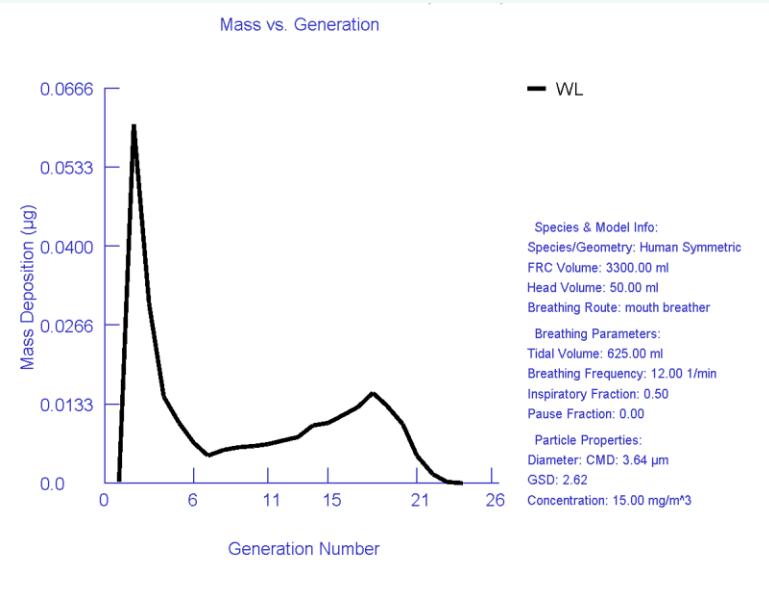


Hypothetical Case study – Regional Lung Deposition for repeated exposures

Lung Geometry : Yeh-Schum Symmetric with default clearance







	Airborne Concentration	Day 1 $\mu g/cm^2$ Upper	Day 1 $\mu g/cm^2$ Lower	Day 12 $\mu g/cm^2$ Upper	Day 12 $\mu g/cm^2$ Lower
Tier 1	15 mg/m³	0.086	0.0011	0.129	0.0136
Tier 2	0.7 mg/m³	0.004	5.48E-05	0.006	6.35E-04

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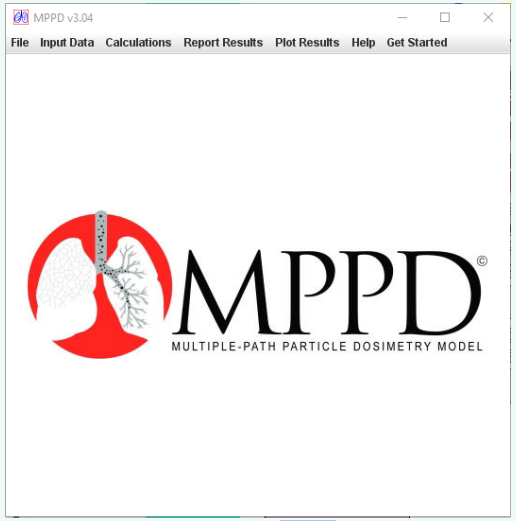
PHMG Humidifier exposures associated with adverse effects in humans

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 (m^3): 27
- Degree of ventilation: 1 (assumed no ventilation)



MMAD: 80 nm

GSD: 1

Airborne PHMG level estimated (mg/m3)

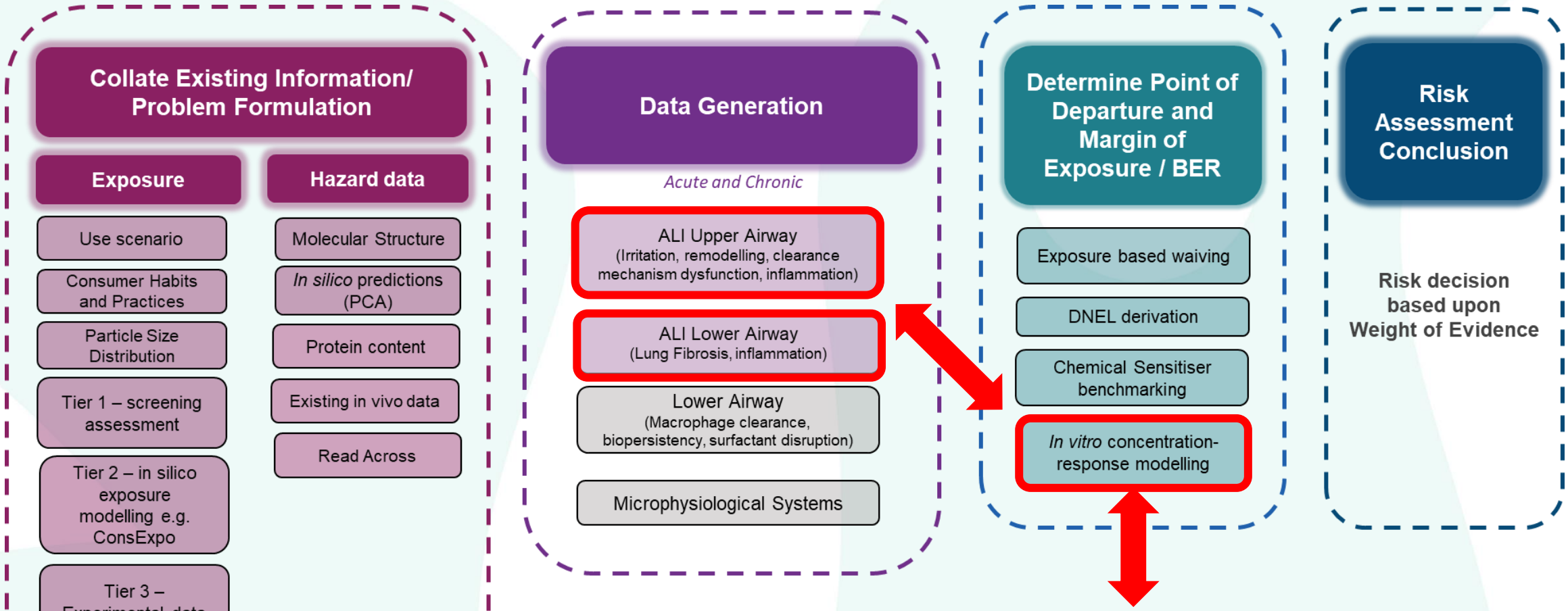
$$= \frac{10 \text{ ml/addition} \times 2 \text{ additions} \times 1276 \text{ ug/ml} \times 1}{27 \text{ m}^3}$$

= 0.95 mg/m³

Mass	Upper $\mu g/cm^2$	Lower $\mu g/cm^2$
1 Day	0.07268	0.00136
12 Day	0.109848	0.015757

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

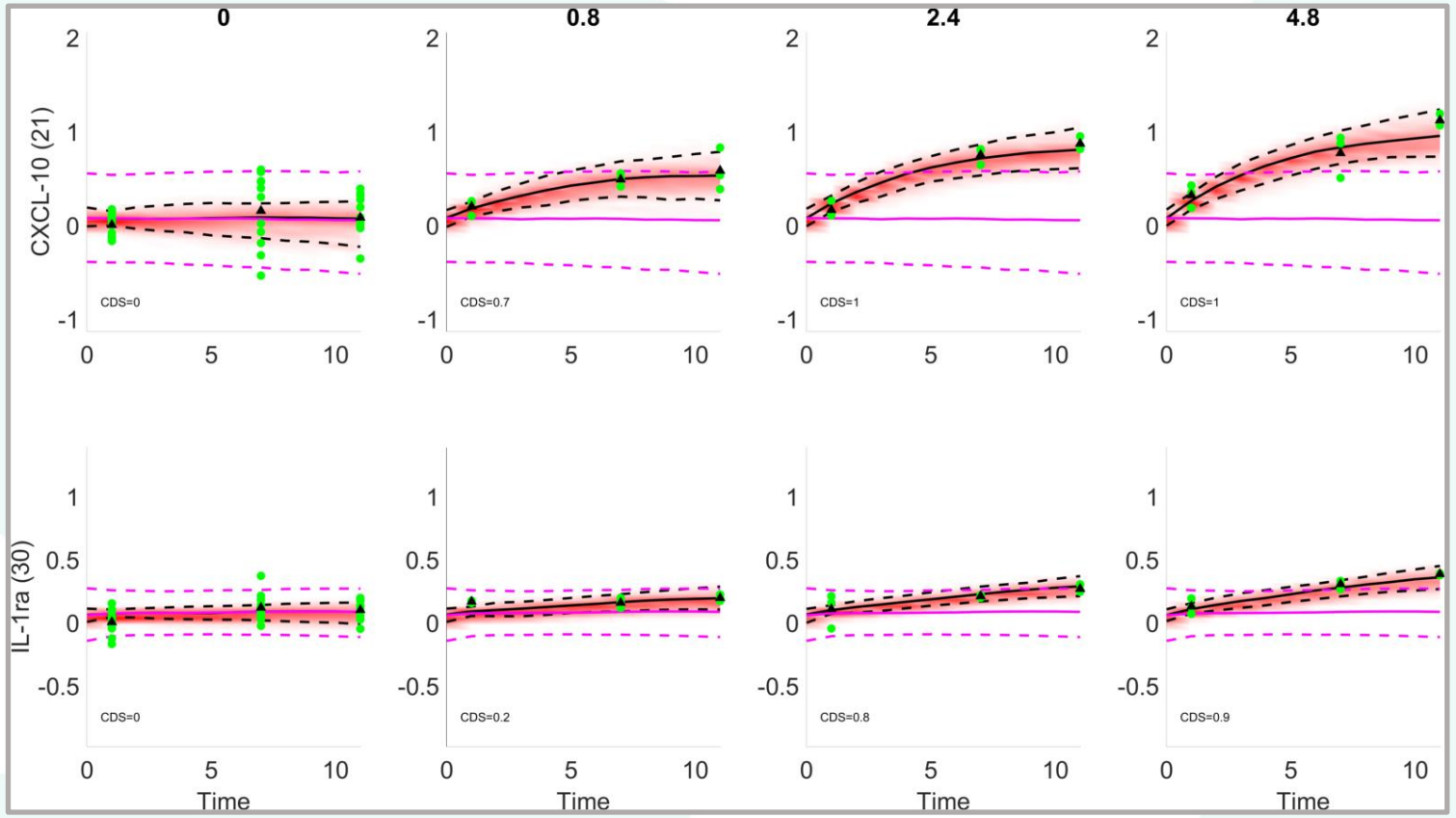
Ongoing development of an Inhalation Framework



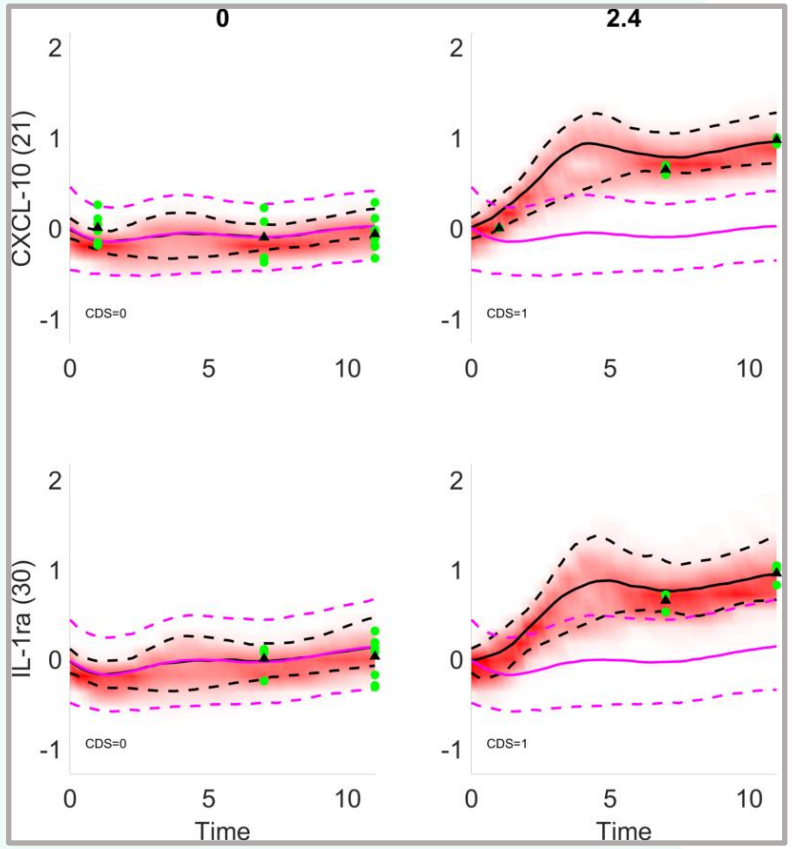
- Method for calculating a *Point of Departure (PoD) using a probabilistic model of concentration and time dependent biological responses (state space model)*

Case study: PHMG causes a mild inflammatory response in MucilAir™ cell model

30 minutes exposure duration



6h exposure duration



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

- Out of 26 biomarkers, only 2 showed significant changes, across dose and time
- Other biomarkers that had borderline dose-response were not considered for the BER plots
- PHMG was not cytotoxic in this model up to the dose tested

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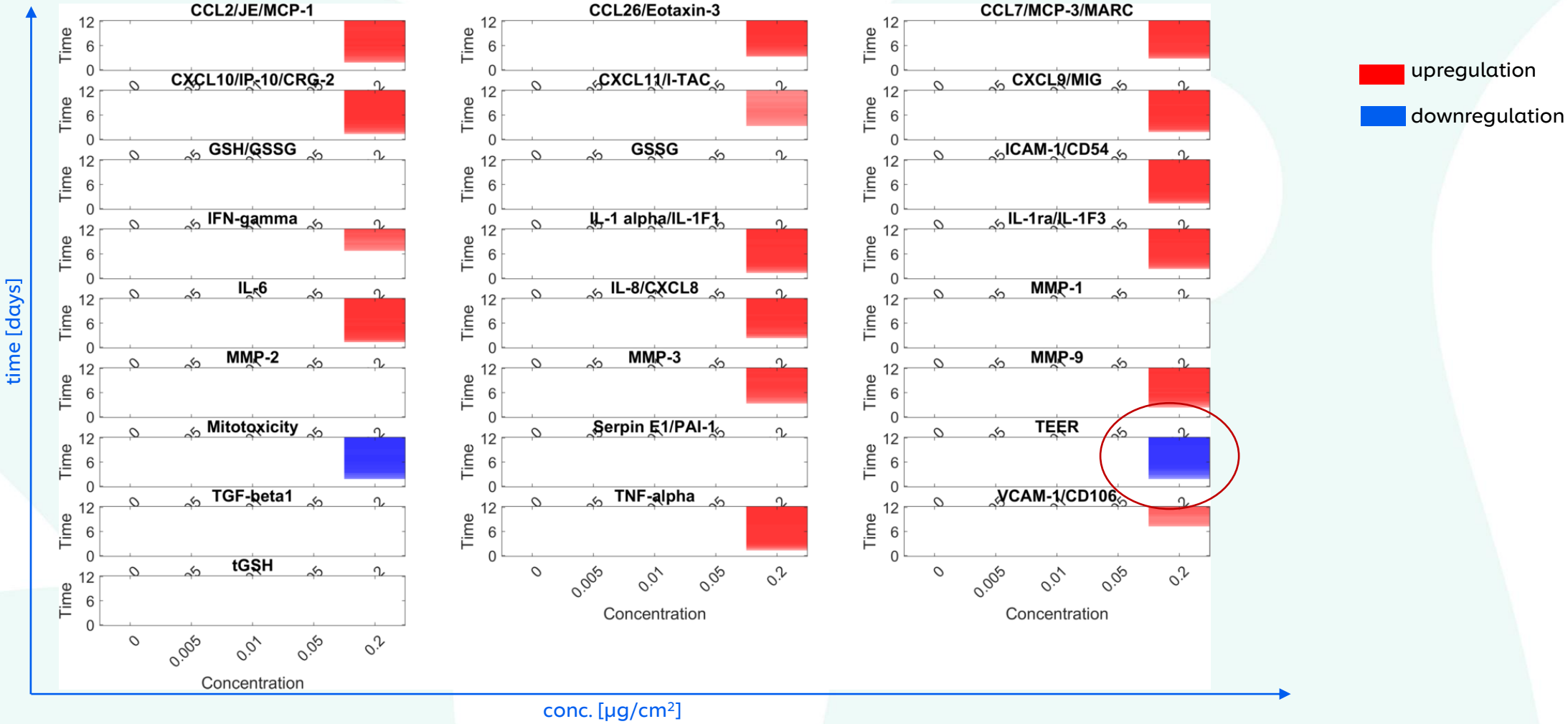
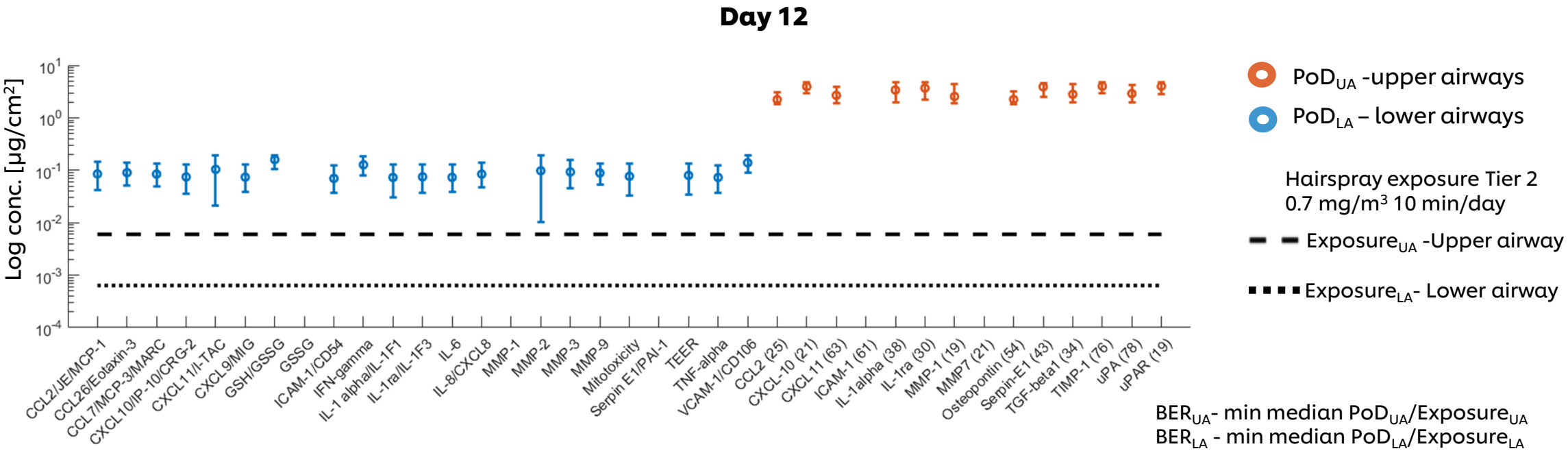
PHMG causes cytotoxicity in EpiAlveoloar™ cell model



- 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 (Kim et al (2016). Arch Toxicol 90(3): 617-632).

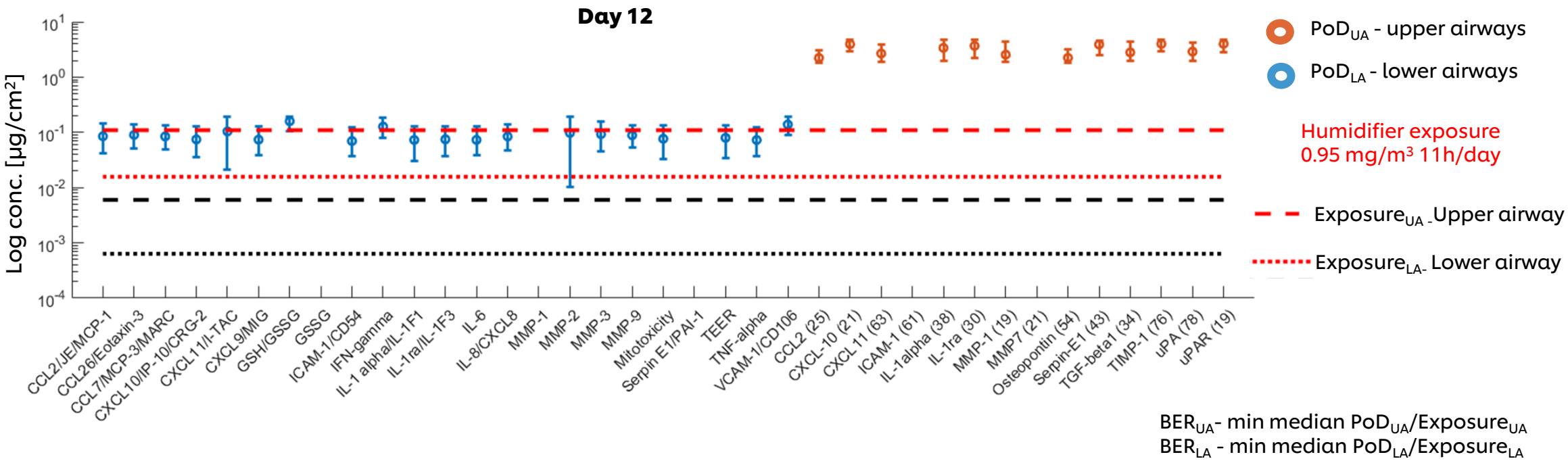
Hypothetical Case study: Calculation Bioactivity-exposure ratio (BER) for the hairspray exposure



BER$_{UA}$- min median PoD$_{UA}$/Exposure$_{UA}$
BER$_{LA}$ - min median PoD$_{LA}$/Exposure$_{LA}$

Bioactivity-exposure ratio (BER)	Hairspray exposure
BER$_{UA}$	366
BER$_{LA}$	110

Benchmarking against existent known human exposures to PHMG associated with adverse effects in humans



BER$_{UA}$- min median PoD$_{UA}$/Exposure$_{UA}$
BER$_{LA}$ - min median PoD$_{LA}$/Exposure$_{LA}$

Bioactivity-exposure ratio (BER)	Hairspray exposure	Humidifier exposure
BER$_{UA}$	366	20
BER$_{LA}$	110	4.4

Kim et al (2016). Arch Toxicol 90(3): 617-632
Jung et al 2014). Toxicology in Vitro 28(4): 684-692.
Park et al (2015). Indoor Air **25**(6): 631-640.

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Concluding remarks

- Evaluation of NGRA needs to be in the context of how to combine estimates of exposure and bioactivity to give reproducible decisions on safety with transparent measurement of uncertainty

- Large scale evaluation exercises & case studies can increase confidence in NAMs – for inhalation identification of benchmark chemical-exposures is urgently needed to allow us to assess the robustness of NAMs and define a *protective* BER.

- Through the process of this evaluation we can identify gaps in our approaches and design new testing strategies to address them

- Currently investigating other relevant endpoints such as surfactant inhibition and incorporating better clearance models

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