Development of a Next-Generation Risk Assessment Framework Informed by Adverse Outcome Pathways (AOPs)

Matt Dent

Unilever- Safety & Environmental Assurance Centre (SEAC)

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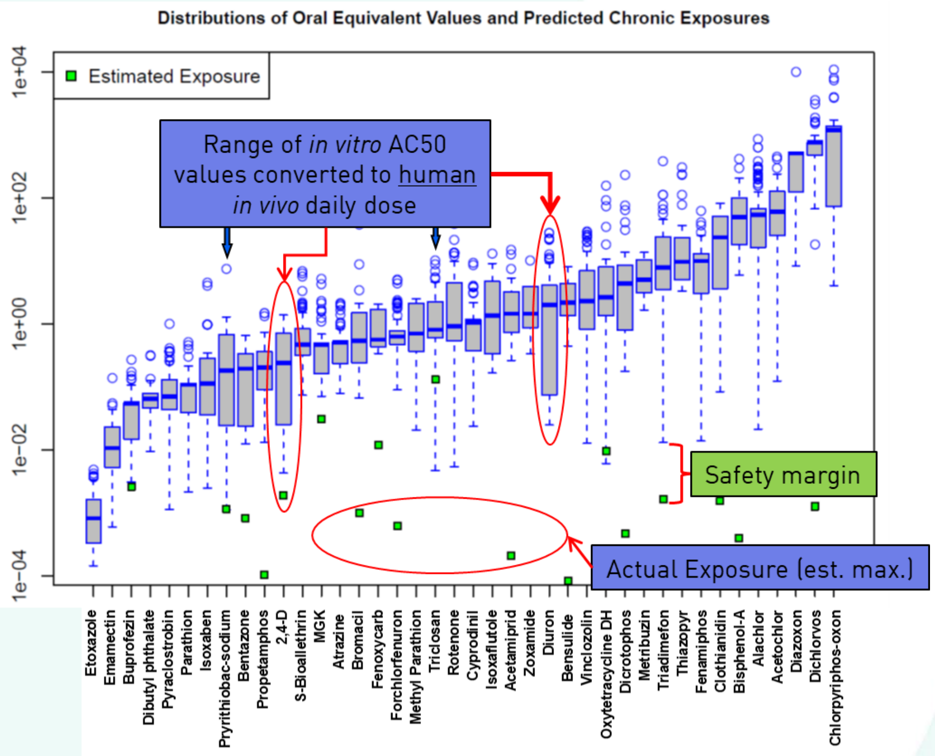
Safety without animal testing - Next Generation Risk Assessment (NGRA)

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.**

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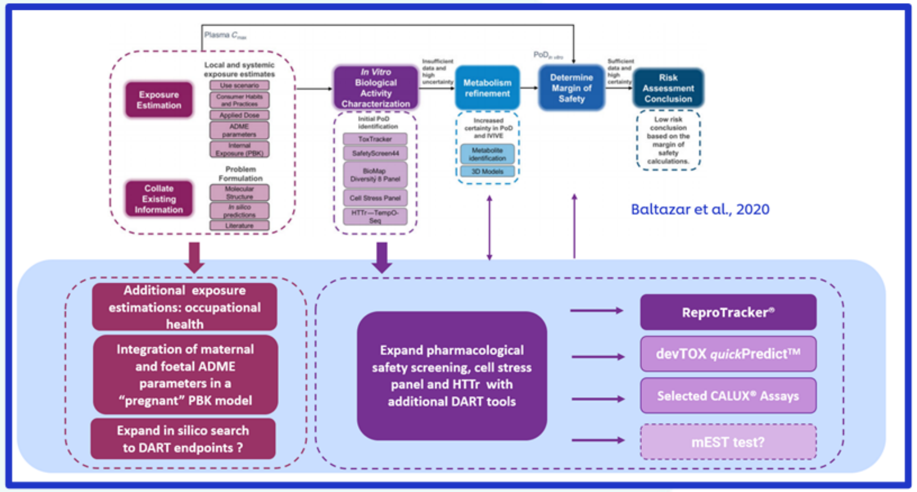
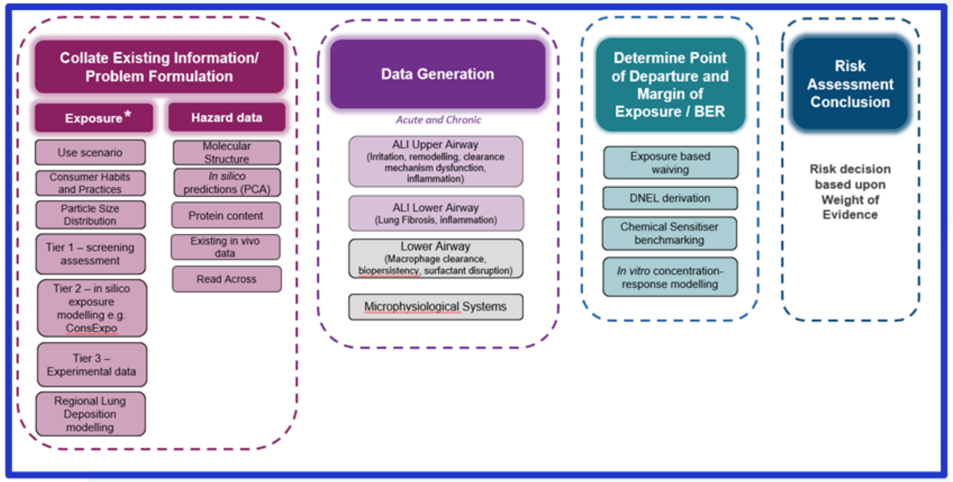
Our Exposure-led NGRA approaches

DART

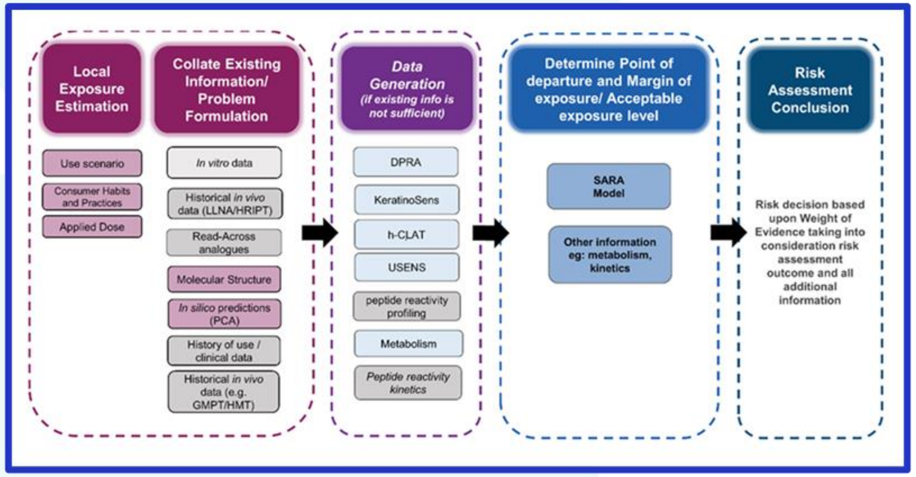


Rajagopal et al (2022). Front. Toxicol., 07 March 2022

Inhalation

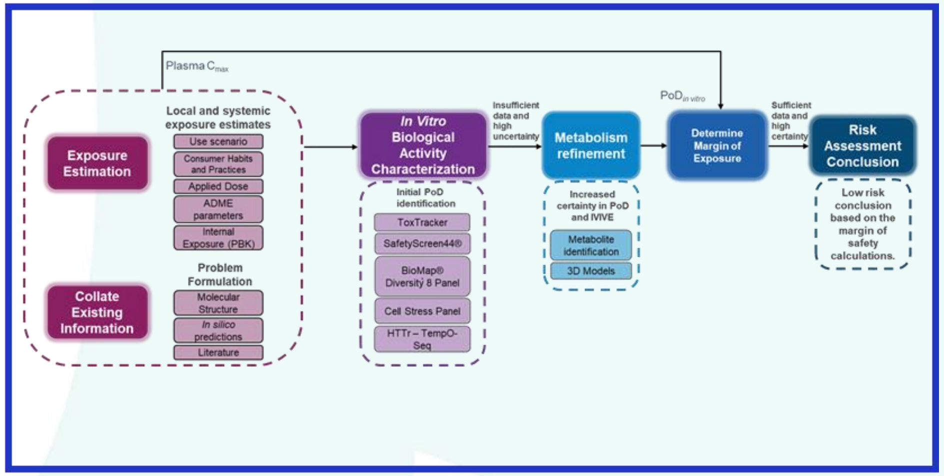


Skin Sensitisation



Reynolds et al (2021) Reg Tox Pharmacol, **127**, 105075

Systemic safety



Baltazar *et al.*, (2020) *Tox Sci* , Volume 176, Issue 1, Pages 236–252

Next generation approaches for inhalation –identification of key areas of lung toxicity

APPLIED IN VITRO TOXICOLOGY
Volume 4, Number 2, 2018
Mary Ann Liebert, Inc.
DOI: 10.1089/aivt.2017.0034

MEETING REPORT

Air–Liquid Interface *In Vitro* Models for Respiratory Toxicology Research: Consensus Workshop and Recommendations

Ghislaine Lacroix,[1] Wolfgang Koch,[2] Detlef Ritter,[2] Arno C. Gutleb,[3] Søren Thor Larsen,[4] Thomas Loret,[1] Filippo Zanetti,[5] Samuel Constant,[6] Savvina Chortarea,[7,8] Barbara Rothen-Rutishauser,[7] Pieter S. Hiemstra,[9] Emeric Frejafon,[1] Philippe Hubert,[1] Laura Gribaldo,[10] Peter Kearns,[11] Jean-Marc Aublant,[12] Silvia Diabaté,[13] Carsten Weiss,[13] Antoinette de Groot,[14] and Ingeborg Kooter[15]

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Toxicol In Vitro. Author manuscript; available in PMC 2019 September 25.

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Pathway-Based Predictive Approaches for Non-Animal Assessment of Acute Inhalation Hazard Determination

Amy J. Clippinger[a], David Allen[b], Holger Behrsing[c], Kelly A. BéruBé[d], Michael B. Bolger[e], Michael DeLorme[f], Marianna Gaça[g], Sean C. Gehen[h], Kyle Glover[i], Patrick Hayden[j], Paul Hinderliter[k], Jon A. Hotchkiss[l], Anita Iskandar[m], Brain Keyser[n], Karsta Luettich[m], Lan Ma-Hock[o], Anna Maione[j], Patrudu Makena[n], Jodie Melbourne[a], Lawrence Milchak[f], Sheung P. Ng[p], Alicia Paini[q], Kathryn Page[r], Grace Patlewicz[s], Pilar Prieto[q], Hans Raabe[c], Emily Reinke[t], Clive Roper[u], Jane Rose[v], Monita Sharma[a], Wayne Spoo[n], Peter S. Thorne[w], Daniel M. Wilson[l], Annie M. Jarabek[x]

Target Site Exposure
- Solubility
- Vapor pressure
- Particle size, density, distribution
- Chemical reactivity

Molecular Initiating Events
- Oxidation of cellular molecules
- Acetylcholinesterase inhibition
- Cytochrome C oxidase inhibition
- DNA/protein alkylation
- Modulation of ion channels
- Receptor binding e.g.,
  - *Activation of EGFR (via phosphorylation)*
  - *Activation of TRPA1 receptor*
  - *Activation of glucocorticoid receptor*
  - *Activation/inhibition of G protein coupled receptors*
  - *Inhibition of muscarinic acetylcholine receptors*
  - *Inhibition of NMDA receptors*
  - *Binding to hormone receptor*

Cellular Key Events
- ROS formation
- Antioxidant (e.g., glutathione) depletion
- Inhibition of energy (ATP) production
- Cytotoxicity
- Collagen deposition
- Increased mucous production
- Cytoskeleton disruption
- Cytokine/chemokine production
- Surfactant depletion
- Modulation of signal transduction pathways
- Inhibition of nucleotide synthesis
- Protein modification
- Modulation of protein synthesis
- Effects on the blood
- Vitamin interference

Tissue / Organ Key Events
- Cell proliferation
- Inflammatory response
- Cell transformation
- Squamous cell metaplasia
- Loss of epithelial barrier function
- Reduced ciliary beat frequency
- Goblet (mucous) cell hyperplasia, metaplasia, and proliferation
- Respiratory failure
- Tracheitis
- Bronchiolitis
- Alveolitis
- Pulmonary edema
- Bronchoconstriction
- Alveolar distention
- Smooth muscle remodeling
- Change in lung mechanics (resistance, compliance, pressure-volume curves, FEV1)

Organism / Population Responses
- Systemic toxicity
- Acute lethality
- Target organ effects (e.g., hepatotoxicity)
- Airway hyperreactivity
- Chemical narcosis

General strategy to developing an inhalation toolbox

Hypothetical Case study based approach

New ingredients for use in consumer products

Problem formulation: chemistry; physico-chemical 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.
Multiple Path Particle Dosimetry (MPPD) model predicts local lung exposure (dose/unit area)

Hypothesis- driven

Proposed NAM toolbox with sufficient biological coverage for the assessment of local lung toxicity for inhaled chemicals

- Lung inflammation and fibrosis
  EpiAlveolar™ cell model
  (Link to AOP 173)
- Impairment of mucociliary clearance
  The MucilAir™-HF cell model
  (Link to AOP 148)
- Lung surfactant inhibition
  (Link to AOP 302)
- Biopersistency/Clearance (Link to AOPs 173 & 303)

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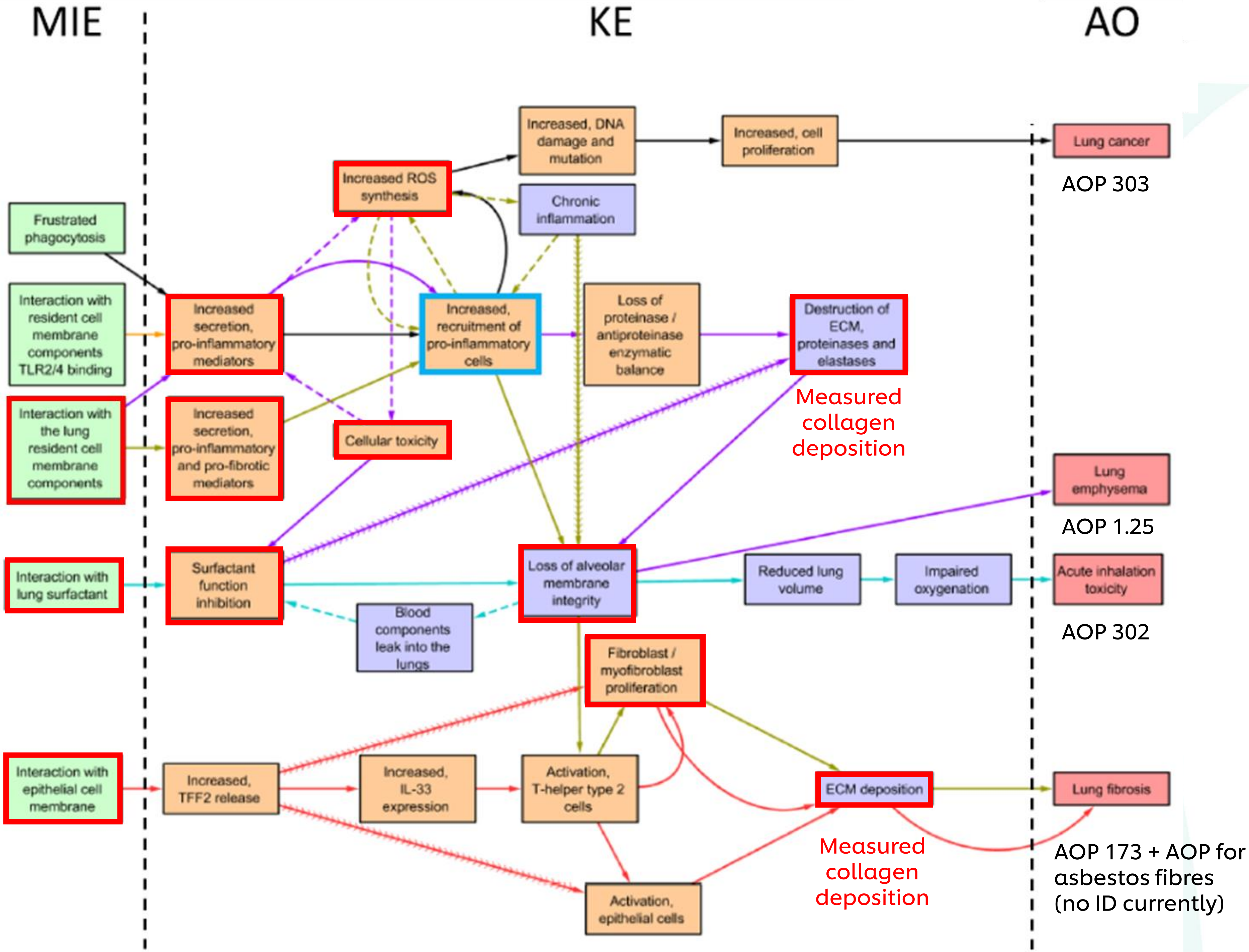
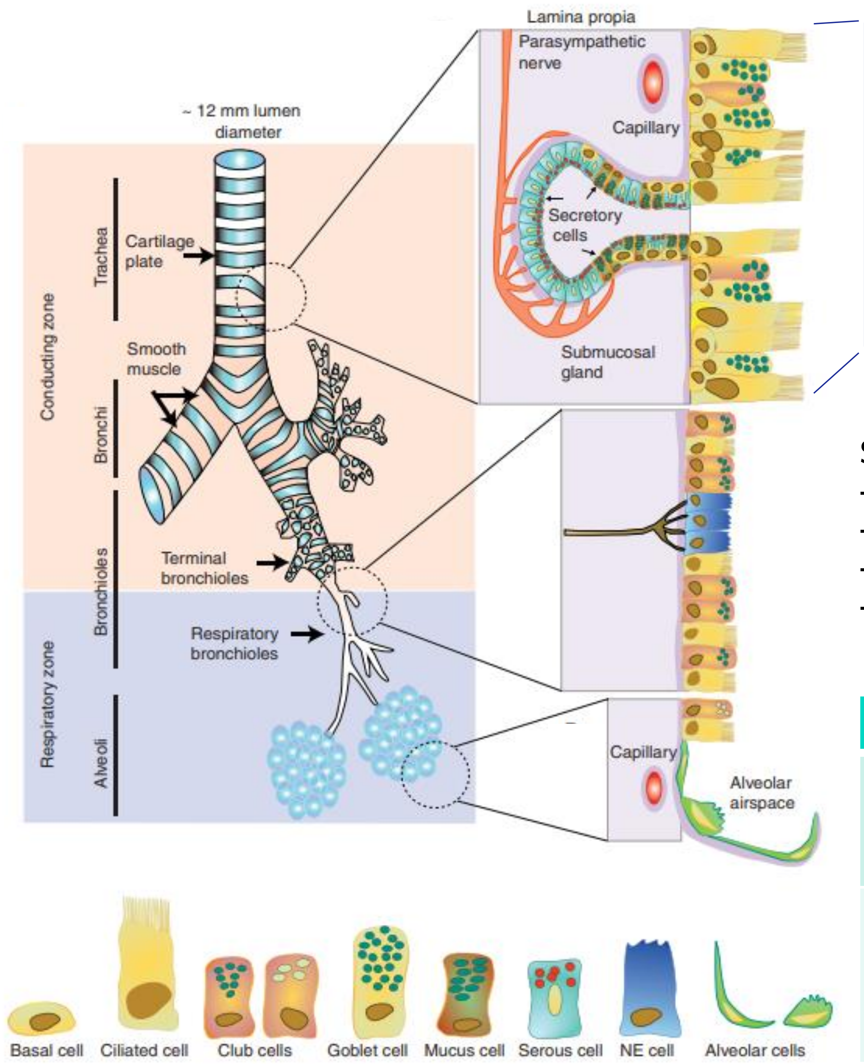
From mechanistic point of view our strategy covers multiple MIE, KEs



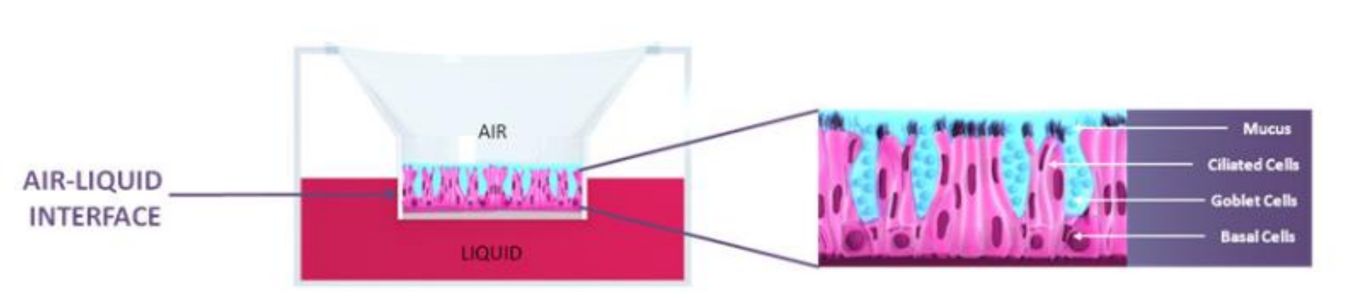
MIEs/KEs covered by current toolbox

Halappavar 2020. Particle and Fibre Toxicology volume 17, 16

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



modified after Bustamante-Marin, et al. 2017



MucilAir™ (epithelix.com)

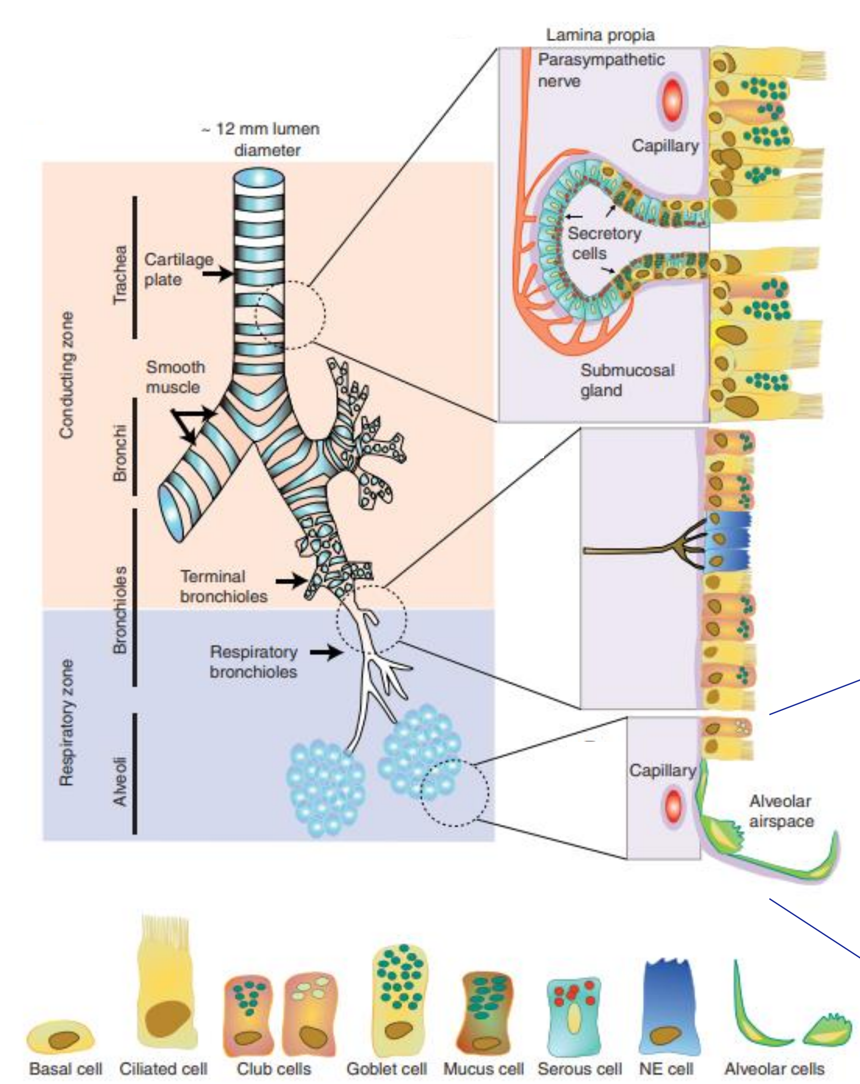
Reconstituted cells system using human primary bronchial cell cocultured with human airway fibroblast.

Selection Criteria:
- Exposure at the ALI
- Stable cells system which allows repeated exposure
- Allows measurement of biomarkers of relevant AOPs
- 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

Lower Airway – The EpiAlveolar™ cell system (MatTek)



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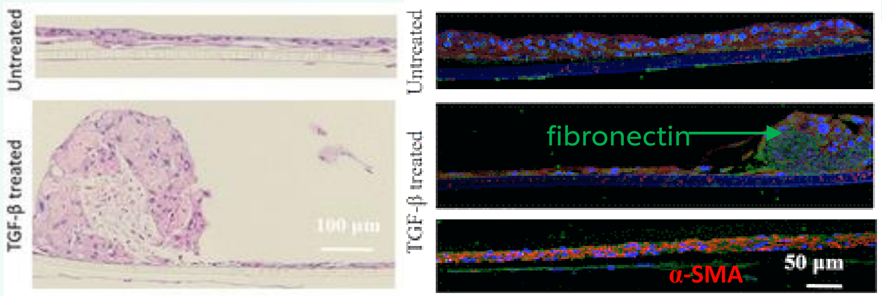
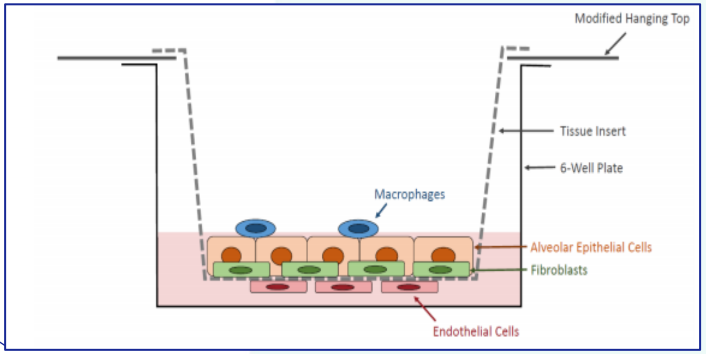


Barosova et al., *ACS Nano* 2020, 14, 4, 3941−3956

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

Case Study

Hypothetical inclusion of a novel preservative in Hairsprays

Ongoing development of an 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/2-box
- 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

Existing data

- Exposure based waiving
- DNEL derivation
- Chemical Sensitiser benchmarking

Determine Point of Departure and Margin of Exposure / BER

- *In vitro* concentration-response modelling
- Calculation of Bioactivity:Exposure ratio

Risk Assessment Conclusion

Risk decision based upon Weight of Evidence

* Consumer Exposure in Inhalation risk assessment

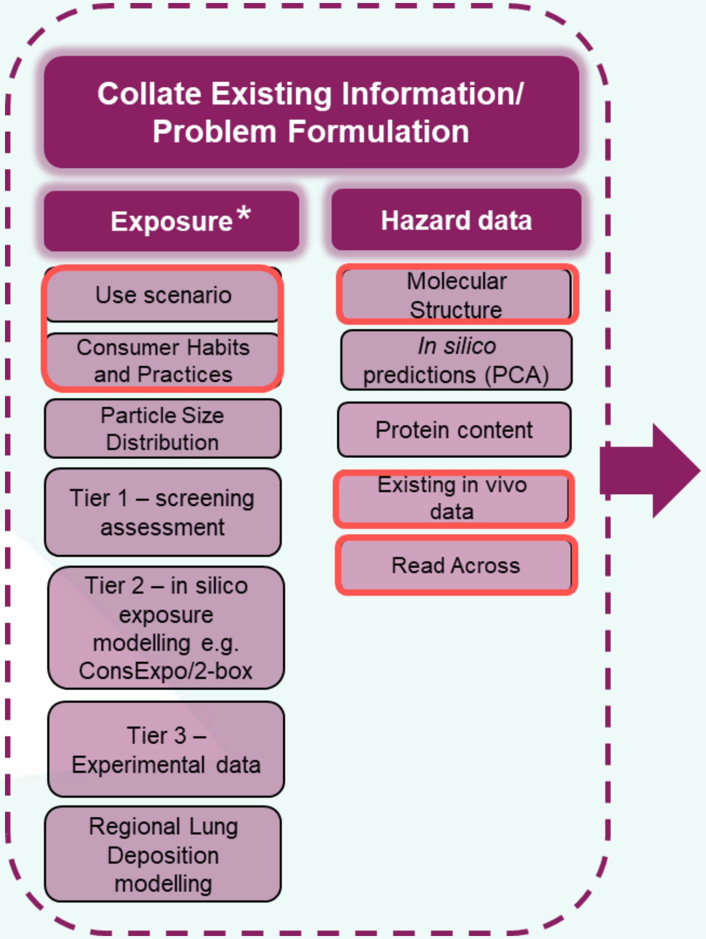
Hypothetical Case study – 0.25% of a novel preservative in a hairspray aerosol

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

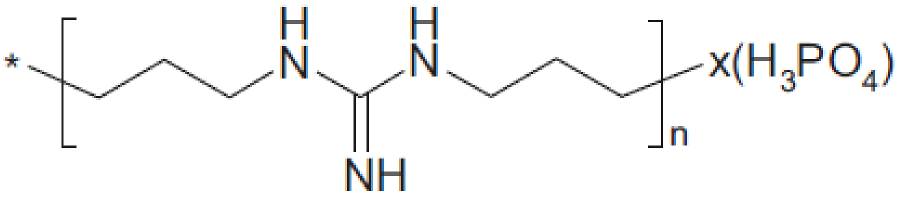
(a) for an hypothetical case study imagining it was a new ingredient for a hairspray.

(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).

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

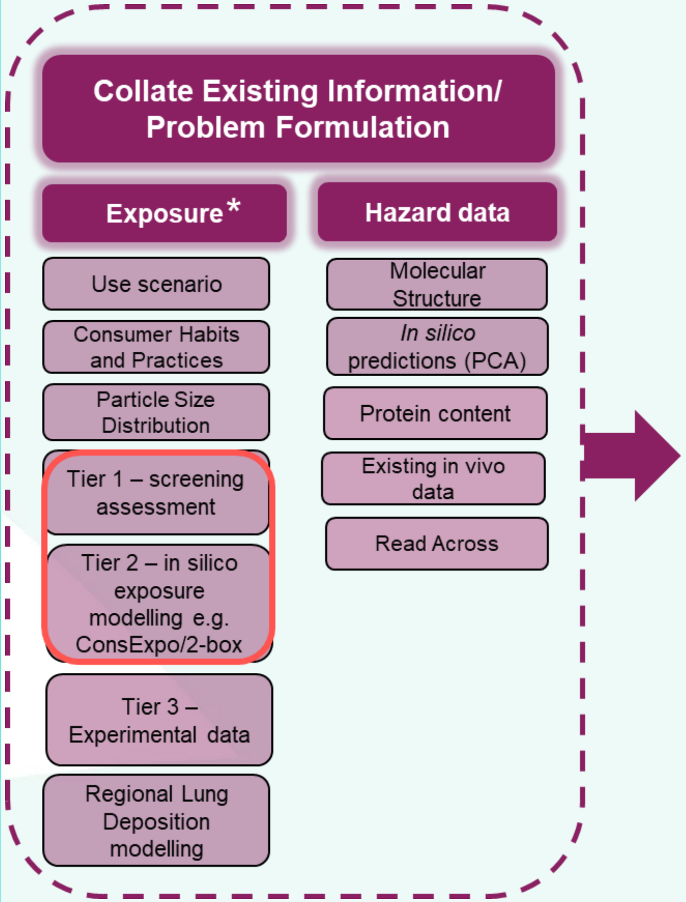
Assumptions:

- 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



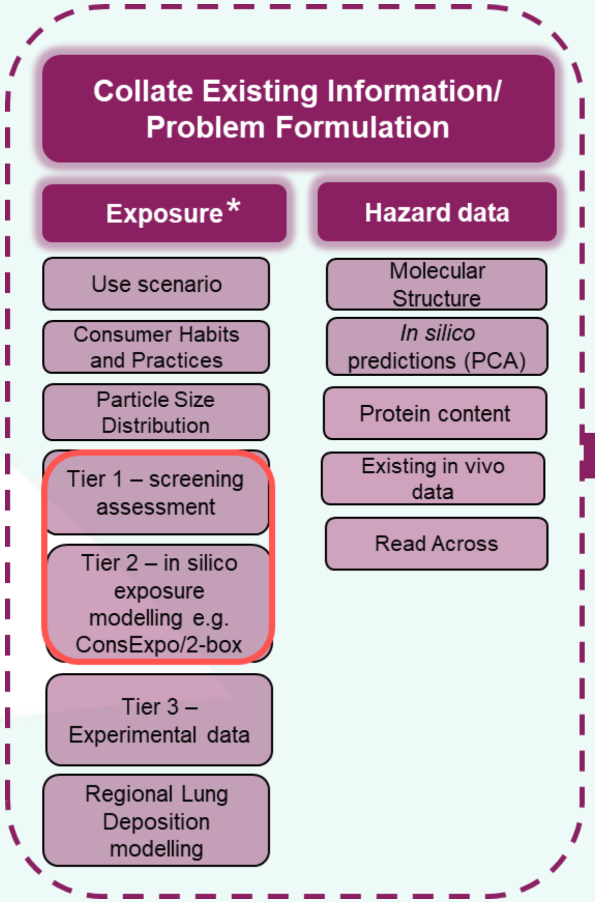
$$\text{Tier 1 Exposure} = \frac{\text{Weight of Ingredient in the Spray Formulation}}{\text{Room Volume}} \left[\frac{mg}{m^3}\right]$$

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

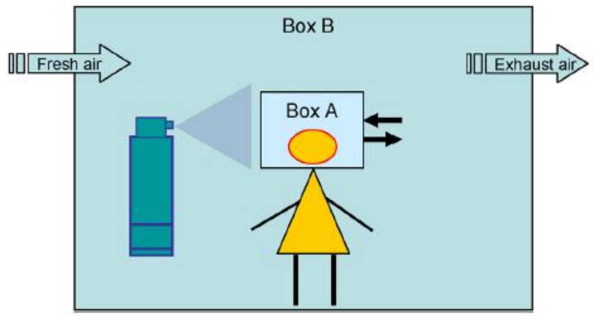
This is a conservative approach that assumes that 100% of the substance in the consumer product or article will be released at once and homogenously into the room and there is no ventilation. The duration of exposure is 24 hours and all released material is 100% inhalable

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



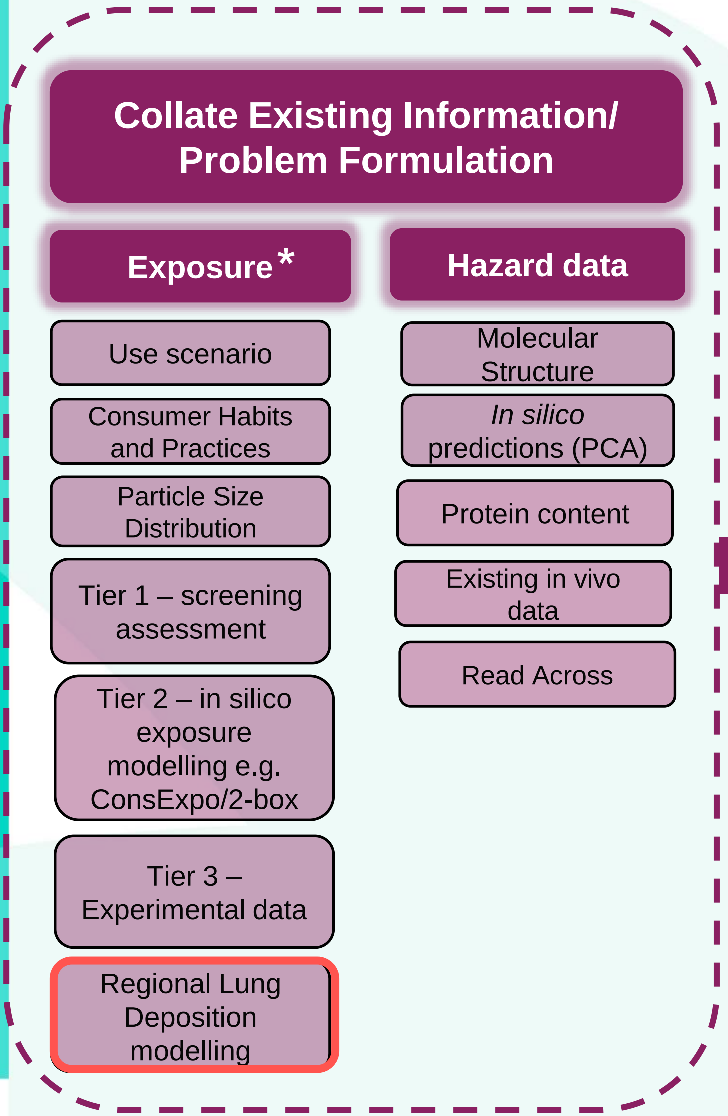
Input	Spray rate (mg/min)	36000
	Inclusion level (%)	0.25
	Emission duration (min)	0.1667
	Number of applications	1
	Zone 1 (Box A) volume (m3)	1
	Zone 2 (Box B) volume (m3)	19.1
	Air flow (1 -> outside) (m3/min)	0
	Air flow (2 -> outside) (m3/min)	1.89
	Air flow (1 -> 2) (m3/min)	7.24
	Time in zone 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

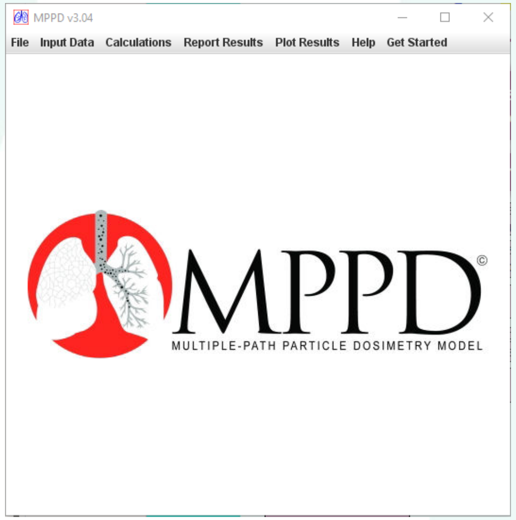
http://www.rifm.org/uploads/Inhalation%20Modeling%202-Box%20Webinar%201.17.2012.pdf

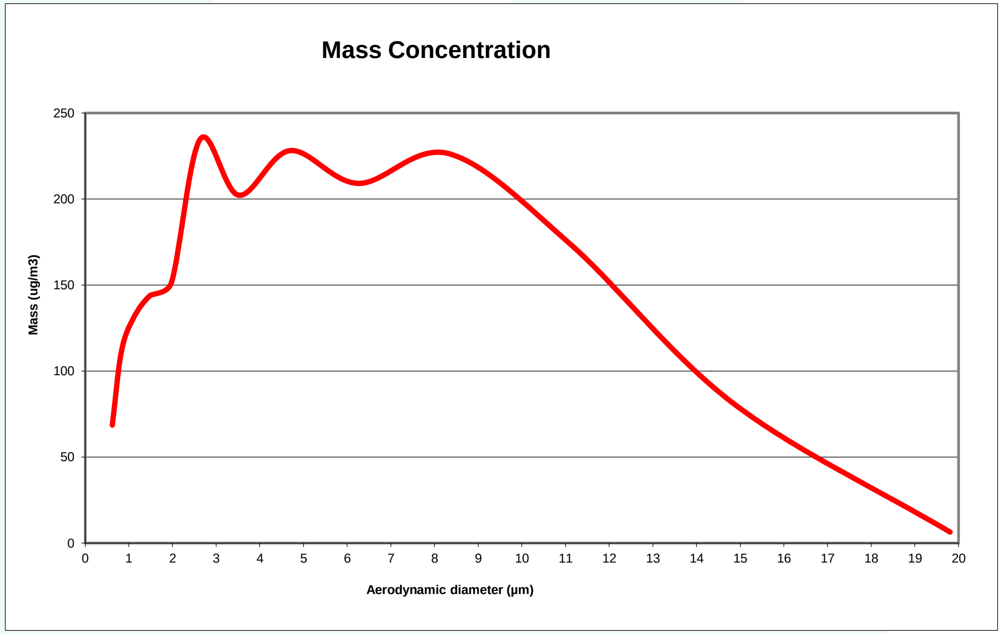
Hypothetical Case study – Regional Lung Deposition Modelling



Measured Particle Size Distribution

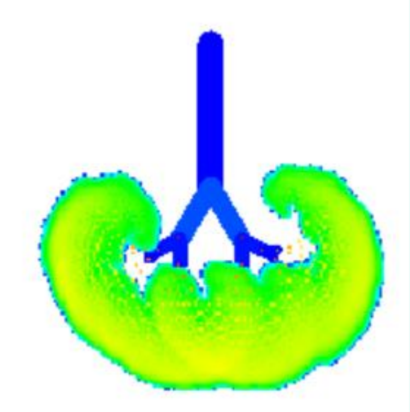
Mean Mass Aerodynamic Diameter : 3.64±2.62µm

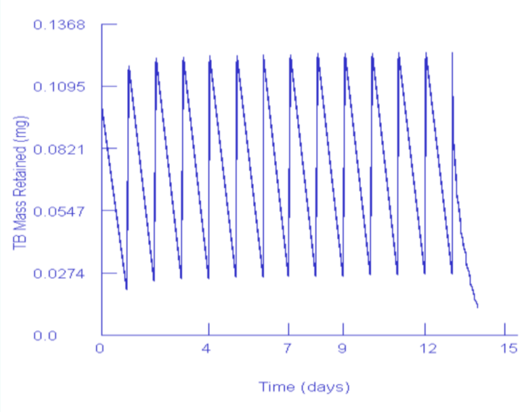


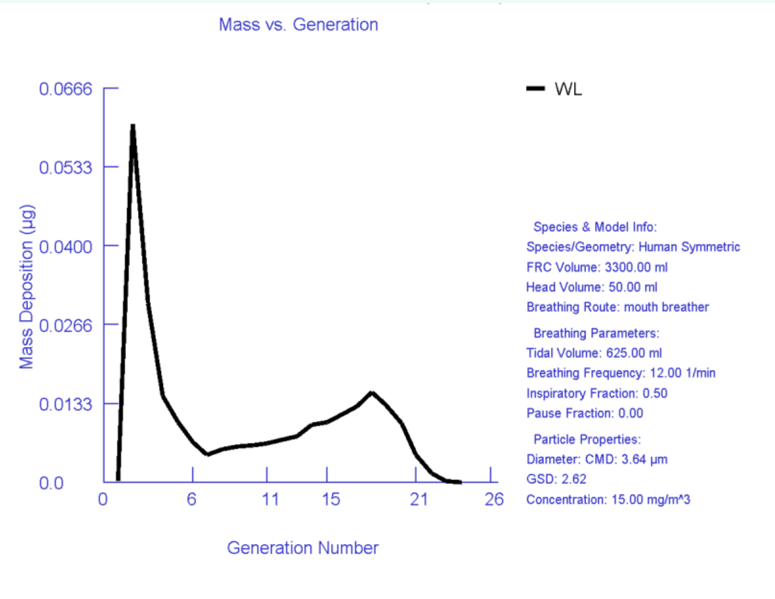


Hypothetical Case study – Regional Lung Deposition for repeated exposures

Lung Geometry : Yeh-Schum Symmetric with default clearance







		Day 1 $\mu g/cm^2$		Day 12 $\mu g/cm^2$	
	Airborne Concentration	Upper	Lower	Upper	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

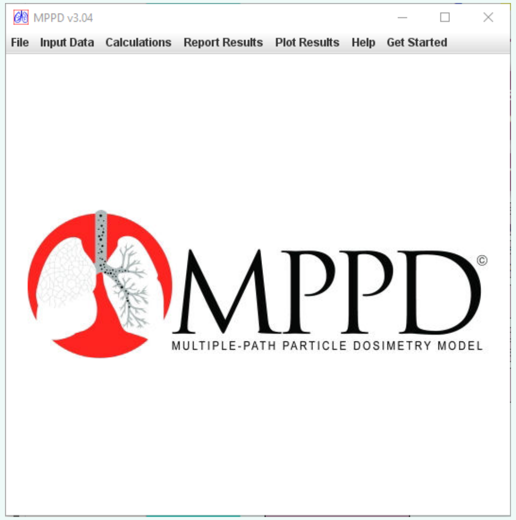
Parameters used to calculate Tier 1 screening assessment – airborne concentration (mg/m³):

- Concentration of PHMG in the disinfectant (µg/ml): 1276
- Disinfectant volume (mL): 10
- Frequency (number of applications): 2
- Volume of the room (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



MMAD: 80 nm

GSD: 1

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

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

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Determine Point of Departure and Margin of Exposure / BER

- *In vitro* concentration-response modelling
- Calculation of Bioactivity:Exposure ratio

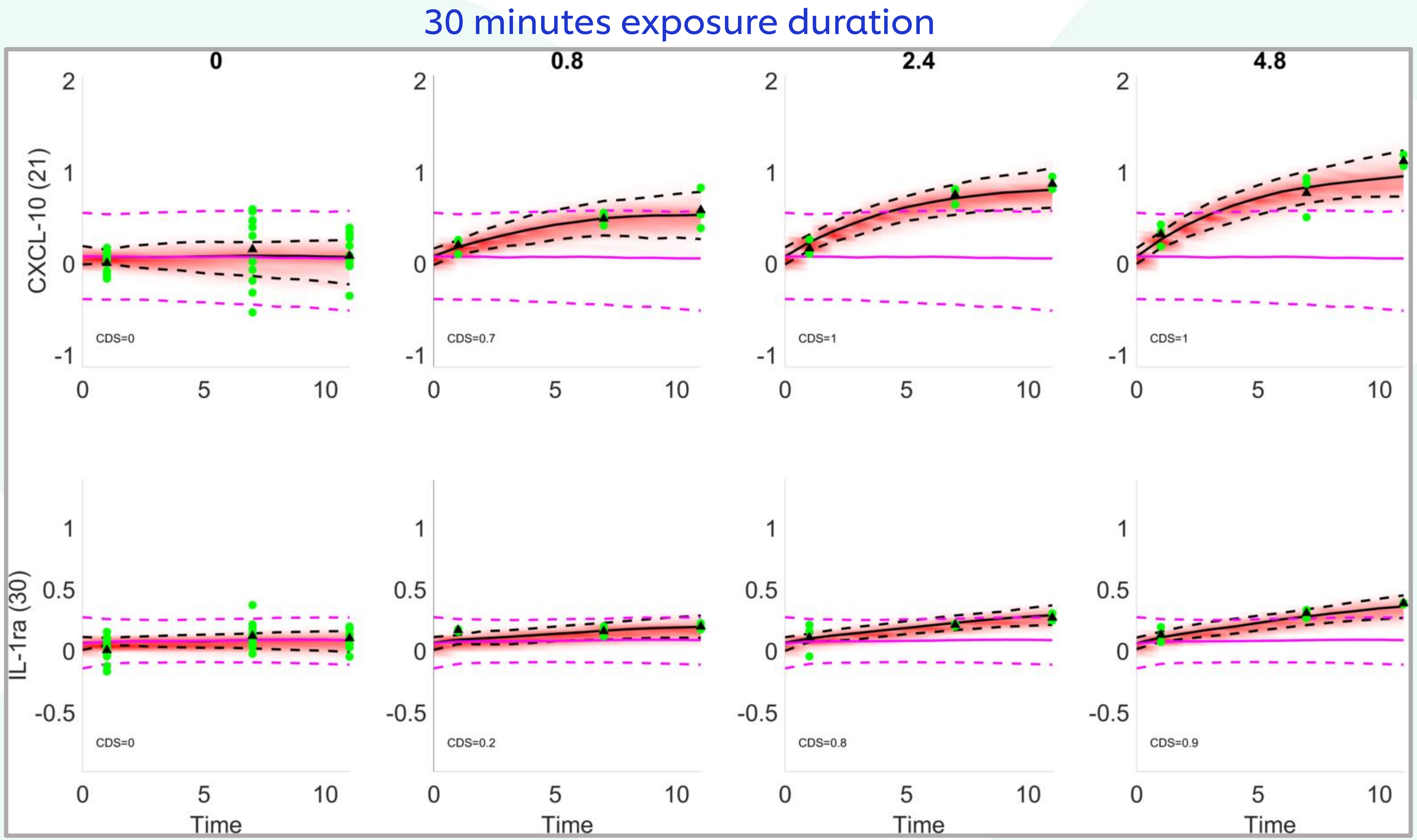
Method for calculating a *Point of Departure (PoD) using a probabilistic model of concentration and time dependent biological responses (state space model)*

Risk Assessment Conclusion

Risk decision based upon Weight of Evidence

* Consumer Exposure in Inhalation risk assessment

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



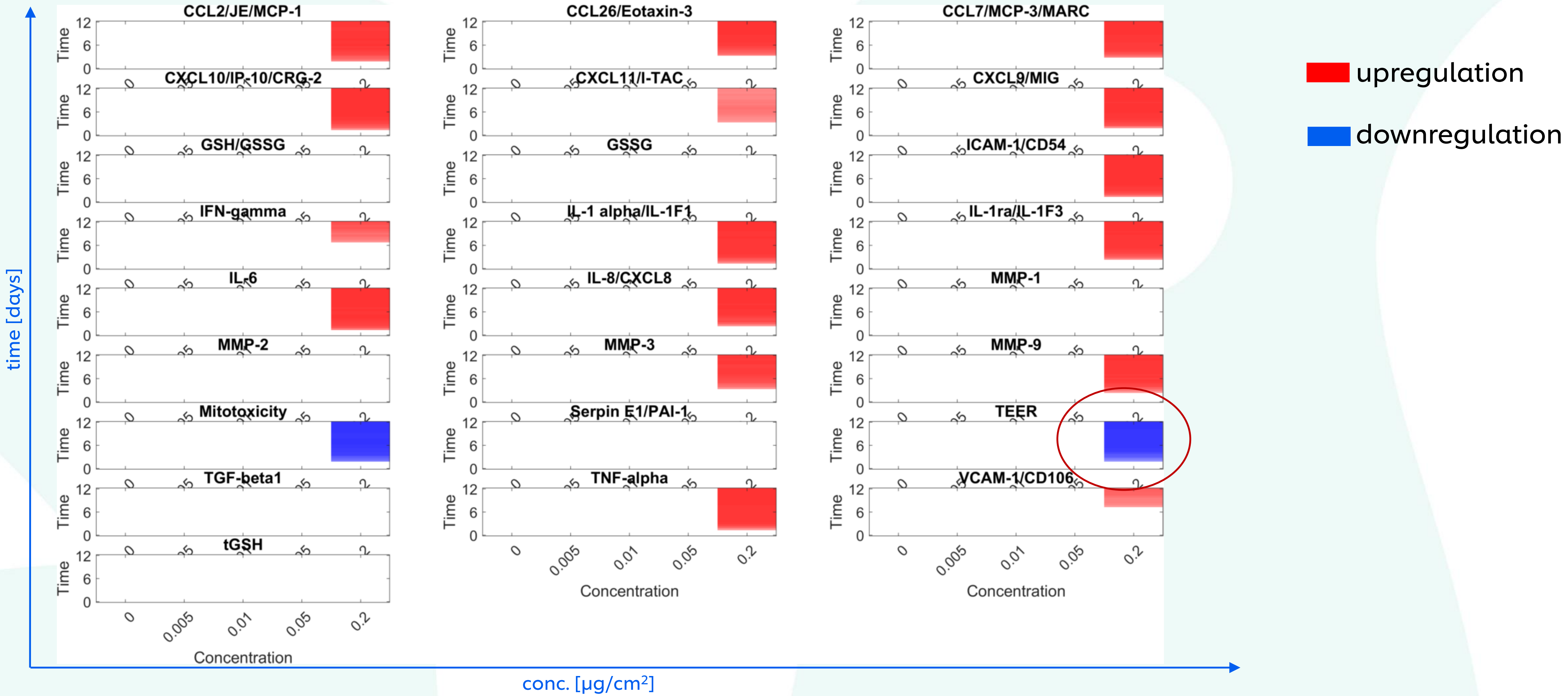
Pink dashed line: 95% cred range of control.

Black dashed line: 95% cred range of mean response

Green dots: data points

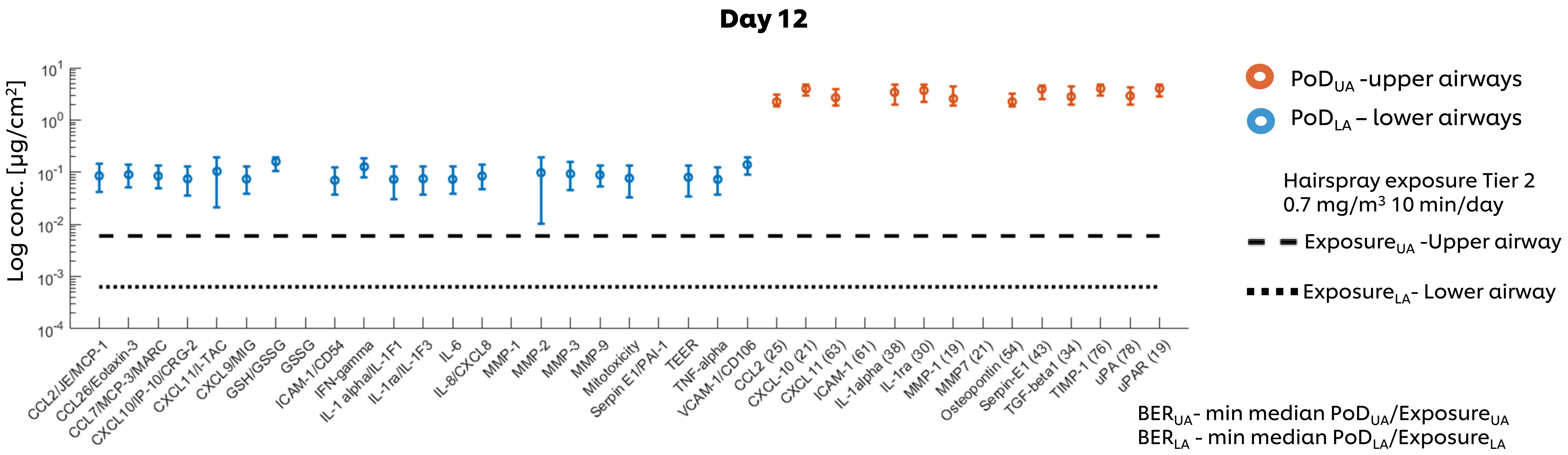
- Out of 26 biomarkers, only 2 showed significant changes, across dose and time
- Other biomarkers that had borderline dose-response were not considered for the BER plots
- PHMG was not cytotoxic in this model up to the dose tested

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

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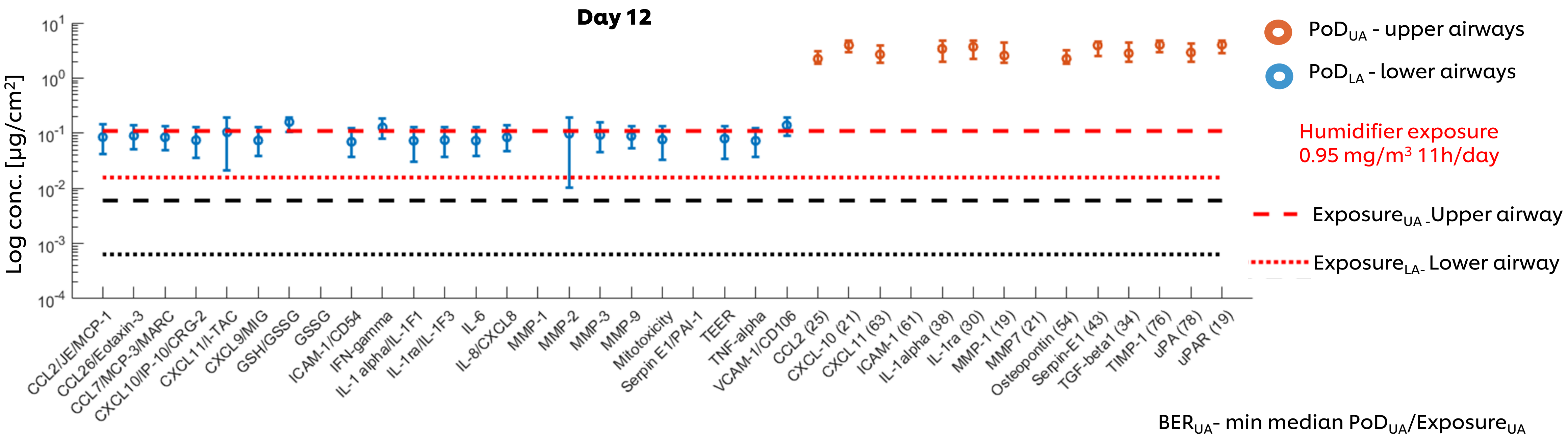
Benchmarking against existent known human exposures to PHMG associated with adverse effects in humans



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.

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

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- Ouarda Saib
- Hugh Barlow
- Ellen Edwards

Epithelix:

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- Bernadett Boda

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IIVS:

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- Vivek Patel
- Adam Wahab
- Pooja Naik

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Thank You for your attention!

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