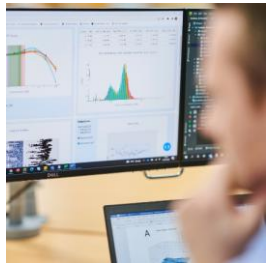
A non-animal toolbox for a next-generation risk assessment (NGRA) approach for inhalation safety: applicability of NAMs informed by adverse outcome pathways (AOPs) associated with pulmonary toxicity

Renato Ivan de Ávila, PhD

(Renato.de-Avila@unilever.com)

bts CONGRESS
driving excellence in toxicology
07 – 09 May 2025

SERS
Safety, Environmental & Regulatory Science



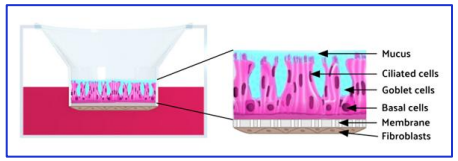
Unilever

Human-relevant strategy for selecting NAMs for lung toxicity NGRA

Broad coverage of bioactivity readouts relevant to inhalation hazards which can provide *in vitro* PoDs

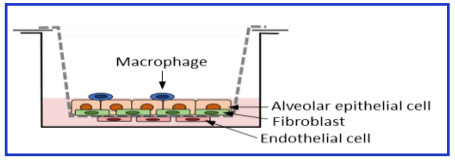
	Effects	Readouts	Acute toxicity	Chronic effect
Upper respiratory tract MucilAir™-HF model	Tissue functionality changes	Mucus secretion, cilia beating (CBF), mucociliary clearance (MCC)	Irritation, enhanced chance of airway infection	Goblet cell hyperplasia, asthma, chronic obstructive pulmonary disease (COPD)
	Cytotoxicity, barrier integrity, inflammatory modulation	Trans-epithelial electrical resistance (TEER), cytokine/ chemokine modulation	Local cytotoxicity, irritation, inflammation	Airway remodelling, Asthma, COPD, lung fibrosis
Lower respiratory tract EpiAlveolar™ model	Barrier integrity, inflammatory and transcriptomic modulation	TEER, cytokine/ chemokine modulation, transcriptomics analysis	Local cytotoxicity, inflammation, wound healing	Airway remodelling/ scarring, lung fibrosis

Upper respiratory tract: MucilAir™-HF
→ impairment of mucociliary clearance (MCC) (**AOP148**)



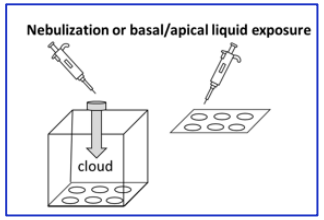
Reconstituted cells system using human primary bronchial cell cocultured with human airway fibroblast.

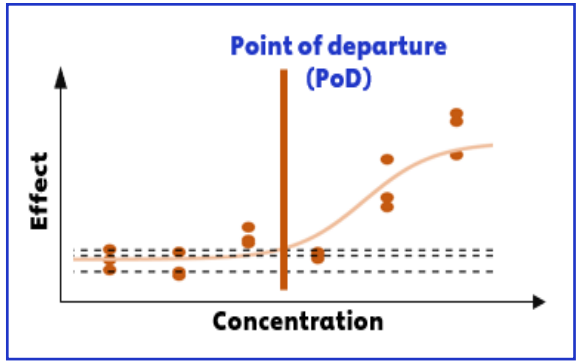
Lower respiratory tract: EpiAlveolar™
→ Lung inflammation and fibrosis **(AOP 173)**



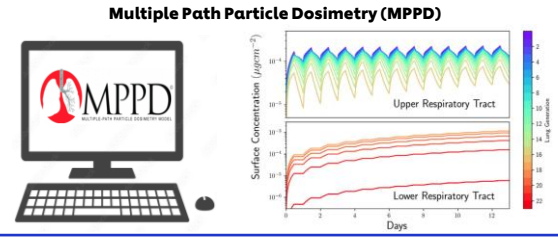
Primary human alveolar epithelial cells, pulmonary endothelial cells and monocyte-derived macrophages

12-day exposure scheme for both tissue models:

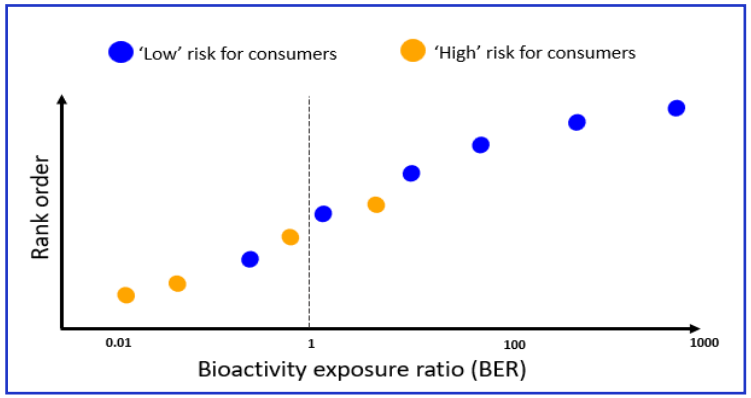




Exposure data: inhalation particle deposition



Bioactivity exposure ratio (BER):
the ratio between the *in vitro* PoD and predicted human exposure



Unilever

Evaluation of the NAM toolbox: selection of test substances

Benchmark chemicals: exposure scenarios are associated either with no effects in humans or have been reported to cause adverse respiratory effects

11 benchmark chemicals investigated in **14 human low- or high risk exposure scenarios**

No.	Reference Material	Risk classification	Risk classification reasoning	Product
1	**BE PVM/MA**	Low	Safe use in cosmetic products	Hair spray
2	**Coumarin**	Low	Safe use in cosmetic products	Anti-perspirant
3	**Acrylate copolymer**	Low	Safe use in cosmetic products	Hair spray
4	**Amorphous silica**	Low	Safe use in cosmetic products	Anti-perspirant
		Low	Safe under recommended exposure limit	Occupational scenario
5	**Carboxymethylcellulose sodium salt (CMC)**	Low	Safe use in nasal sprays	Nasal spray
6	**Benzalkonium chloride (BAC)**	Low	Safe use in nasal sprays/ophthalmic products	Nasal spray
		Low	Safe use in homecare products	Cleaning spray
7	**Crystalline silica**	Low	Safe under permissible exposure limit	Occupational scenario
		High	Silicosis after cumulative exposure	Occupational scenario
8	**Polyhexamethyleneguanidine phosphate (PHMG)**	High	Serious adverse lung effects in humans	Humidifier
9	**Akemi**	High	Acute lung toxicity	Tile coating product
10	**Doxorubicin**	High	Interstitial lung disease in cancer patients	Therapeutic dose
11	**Amiodarone**	High	Alveolar/interstitial pneumonitis with a subacute onset	Therapeutic dose

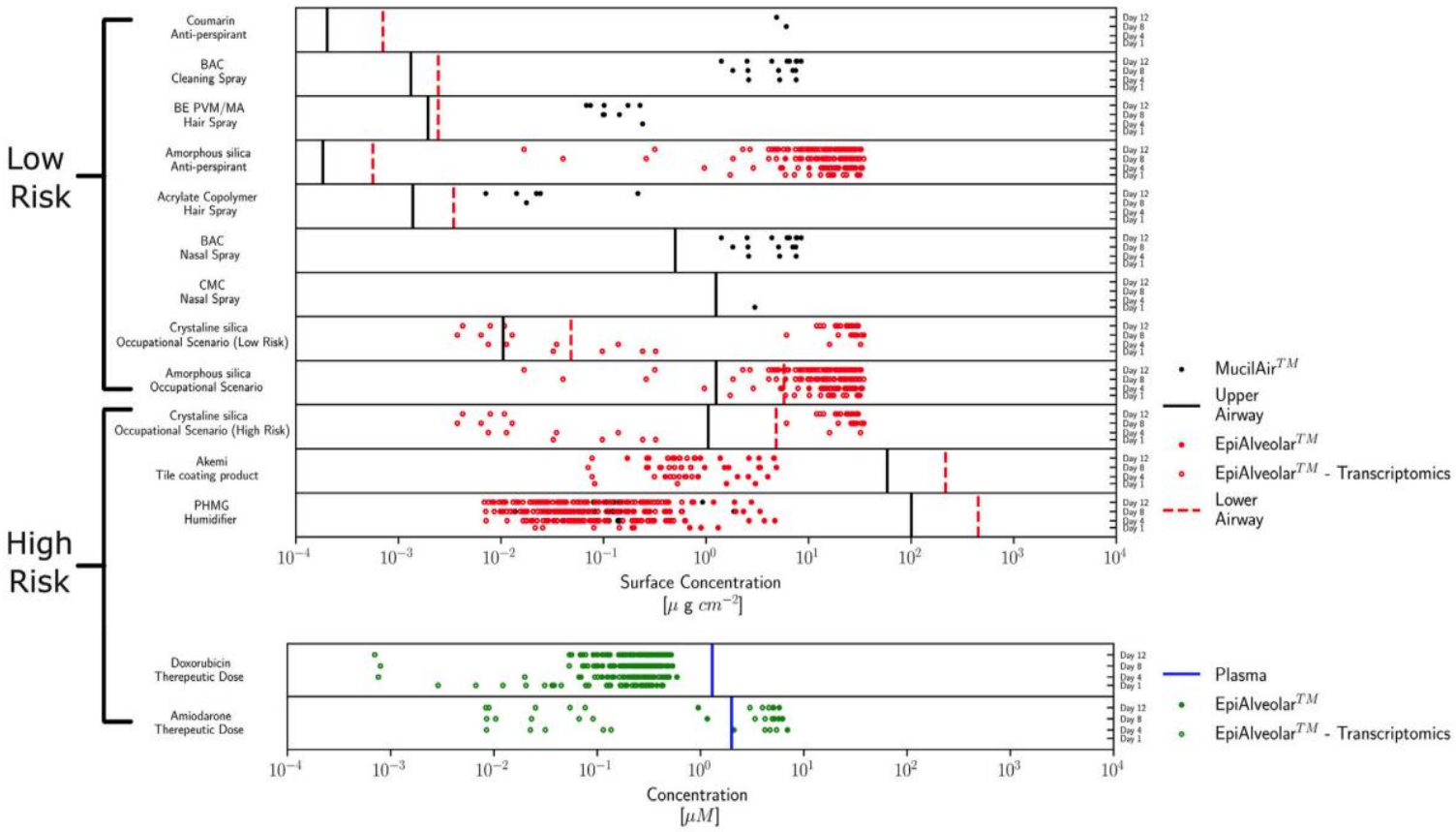
Tested in MucilAir™-HF only – Tested in EpiAlveolar™ only - Tested in both tissue models

Unilever

In general, for high-risk exposure-chemical scenarios *in vitro* PoDs were lower than the predicted exposure

11 benchmark chemicals investigated in
14 human low- or high risk exposure scenarios

- The obtained PoDs were combined with exposure estimates to calculate BER values
- BER is able to separate the low- and high-risk benchmark exposure scenarios for 12 out of the 14 scenarios
  - ✓ **Low-risk:** PoDs occurred at higher concentrations than the corresponding human exposure values. **Except:** crystalline and amorphous silica occupational scenarios
  - ✓ **High-risk:** clear overlap between the PoDs and human exposure (lung deposited mass or Cmax)



Defining a safe threshold: animal testing *versus* non-animal NAMs

- Traditional Margin of Safety ($MoS_{animal\ data}$ for local lung effects) > 25* → **low risk**

  *Uncertainty safety factor of 25 to account for uncertainties related to interspecies (animal-to-human: 2.5-safety factor) and inter-individual (human-to human: 10-safety factor) variabilities[1]

- *In vitro* Bioactivity Exposure Ratio ($BER_{NAM\ data}$) > 3 → **low risk (?)**

  *Uncertainty safety factor of 3 applied in the chlorothalonil acute inhalation risk assessment to cover potential variation in sensitivity among human population (intraspecies)[2]

- **$BER_{NAM\ data}$ > 3 would be protective for all benchmark chemicals**, particularly driven by the transcriptomics PoDs for the high-risk exposure scenarios, e.g., Amiodarone and Crystalline silica

Amiodarone - high risk therapeutic dose

Day	Min PoD	Biomarker	BER	Risk
4	6.95	Cytokine: MMP-1 (Lab 2)	3.47	Low
	0.0084	Transcriptomics: LV30	0.0042	High
8	1.31	Cytokine: ICAM-1 (Lab 1)	0.65	High
	5.20	Cytokine: ICAM-1 (Lab 2)	2.60	High
	0.0084	Transcriptomics: LV30	0.0042	High
12	0.97	Cytokine: ICAM-1 (Lab 1)	0.48	High
	5.03	Cytokine: ICAM-1 (Lab 2)	2.51	High
	0.0083	Transcriptomics: LV30	0.0041	High

- Note some differences in EpiAveolar PoDs among Laboratories 1 and 2

Crystalline silica – high risk occupational scenario

Day	Min PoD	Biomarker	BER	Risk
1	0.032	Transcriptomics: LV131	0.071	High
4	0.0075	Transcriptomics: LV110	0.0041	High
8	34.53	Cytokine: MMP-7 (Lab 2)	11.14	Low
	0.0037	Cytokine: LV110 (Lab 2)	0.0012	High
12	30.51	Cytokine: MMP-7 (Lab 2)	6.32	Low
	0.0042	Transcriptomics: 110	0.00087	High

[1]ECHA (2012). Guidance on information requirements and chemical safety assessment: chapter R.8: characterisation of dose [Concentration]-Response for human health.
[2]EPA (2021). Document ID: EPA-HQ-OPP-2011-0840-0080. Available at https://www.regulations.gov/document/EPA-HQ-OPP-2011-0840-0080

Concluding remarks

- Strategy of selecting **non-animal NAMs informed by AOPs** associated with pulmonary toxicity can provide relevant biological coverage
- **Further evaluation of the performance of NAM toolbox can build confidence in the protectiveness of the approach**: testing a wider substance dataset with varied mechanisms of action, uses, and balanced low and high-risk benchmarks
- There is a need to **establish scientific confidence** by improving the reproducibility, standardization of protocols, and *in vitro* culture methodologies
- **Benchmarking decision outcomes provides an alternative to the traditional validation of NAMs:**

  apical effects in rodent studies *vs.* NAMs in the context of making protective safety decisions

Acknowledgements

Epithelix Sarl:

- **Samuel Constant**
- **Bernadett Boda**

Charles River Laboratories:

- **Joanne Wallace**

Institute for *In Vitro* Sciences (IIVS):

- **Holger Peter Behrsing**
- **Vivek Patel**

Unilever:

- **Iris Müller**
- **Hugh Barlow**
- **Alistair Mark Middleton**
- **Mathura Theiventhran**
- **Danilo Basili** (now Société des Produits Nestlé S.A.)
- **Anthony M. Bowden**
- **Ouarda Saib**
- **Patrik Engi**
- **Tymoteusz Pietrenko**
- **Matthew Dent**
- **Carl Westmoreland**
- **Zoë Deag**
- **Claire Peart**
- **Mark Liddell**
- **Beate Nicol**

seac.unilever.com

Unilever