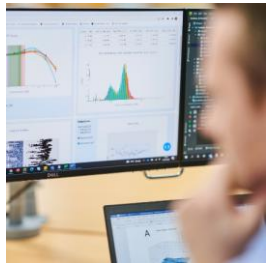
A non-animal toolbox in NGRA for inhalation safety: applicability of NAMs informed by AOPs associated with pulmonary toxicity

NGRA 中的吸入安全性的非动物工具箱：基于AOP的肺毒性 NAM 的可用性

Jin Li, PhD

李津

SERS
Safety, Environmental & Regulatory Science



Unilever

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)

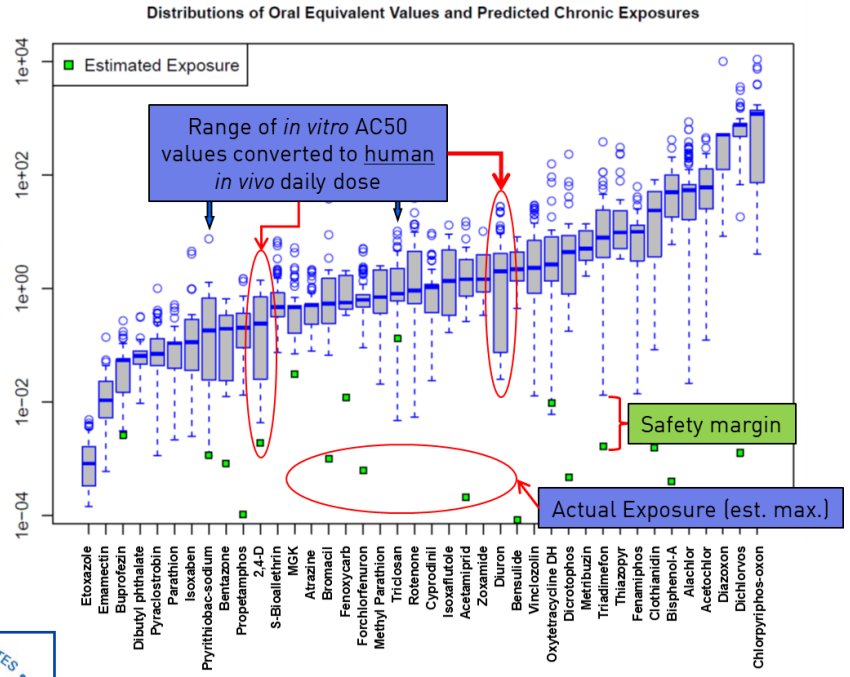
Shampoos

Anti-perspirant/ deodorant aerosols

Unilever

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

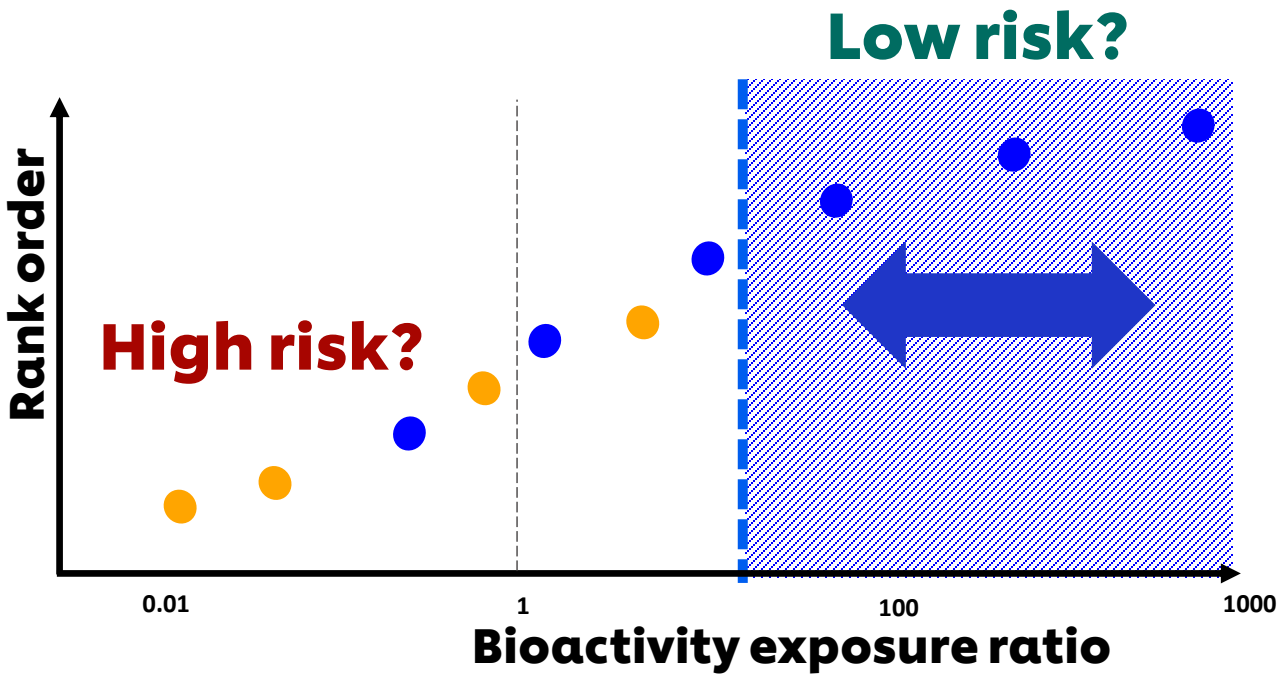
Rotroff, *et al.* Tox.Sci 2010
DOI:10.1093/toxsci/kfq220

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.**

Unilever: Testing the Performance of NAMs in an NGRA
联合利华：在 NGRA 中测试 NAM 的标准

Benchmarking using chemical-exposure scenarios

- Chemicals with well-defined human exposures
- Traditional safety assessment available
- High certainty in the risk classification for each chemical-exposure scenario from a consumer goods perspective
- Risk class is relative to consumer health (N.B. drugs = high-risk)



‘Low’ risk for consumers from systemic perspective

‘High’ risk for consumers from systemic perspective

Protectiveness

How many of the high-risk exposure scenarios are identified as uncertain/high risk?
(i.e. BER < threshold)
有多少高风险暴露情景被确定为不确定/高风险?
(即 BER <阈值)

Utility

How many of the low-risk scenarios are identified as low-risk at this early tier stage in a risk assessment framework?
(i.e. BER > threshold)
在风险评估框架的早期阶段，有多少低风险情景被确定为低风险？ (即 BER >阈值)

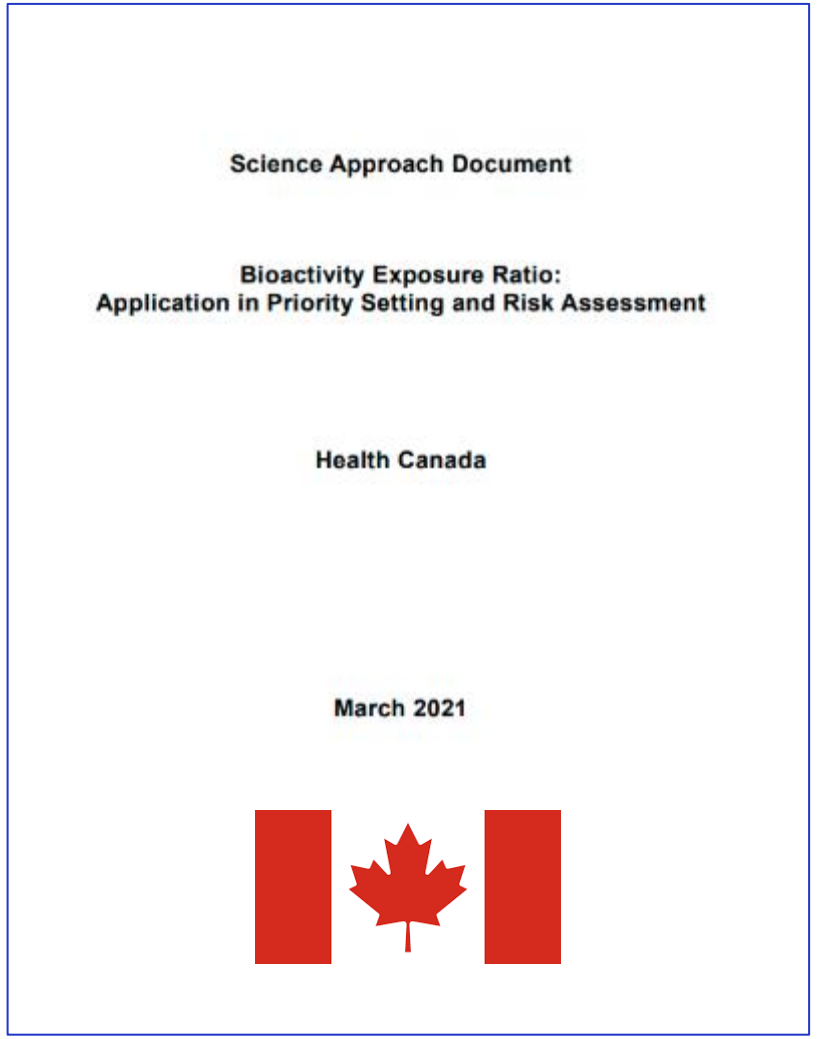
Bioactivity: Exposure Ratio (BER)
生物活性：暴露 比率(BER)

POD from *in vitro* Bioactivity Assays
体外生物活性测定中的 POD

Systemic exposure in humans (from PBK)
人体全身暴露（来自 PBK模型）

'Bioactivity exposure ratios (BERs). BERs are analogous to the traditional margin of exposure used in risk assessment in that chemicals with a lower BER possess a higher potential for risk'
生物活性：暴露 比率 BERs 类似于风险评估中使用的传统暴露边际，BER 较低的化学品具有较高的潜在风险

Kuo *et al* (2022)

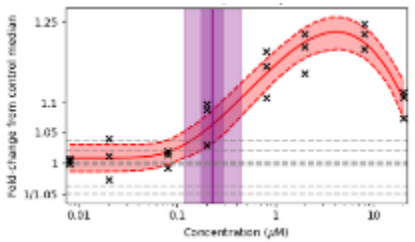


Science approach document - Bioactivity exposure ratio: Application in priority setting and risk assessment - Canada.ca

科学方法文献 - 生物活性:暴露比：在优先级设定和风险评估中的应用 - Canada.ca

Our approach NGRA in action for systemic toxicity

Point of departure (POD) derived from concentration-response data



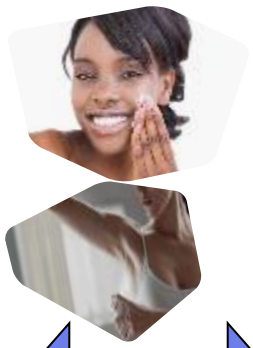
Systemic toolbox of assays (NAMs) which cover a broad biological space – measurements of bioactivity

- **Transcriptomics**
- **Cellular stress assays**
- **Receptor binding/enzymatic assays**
- **Others**

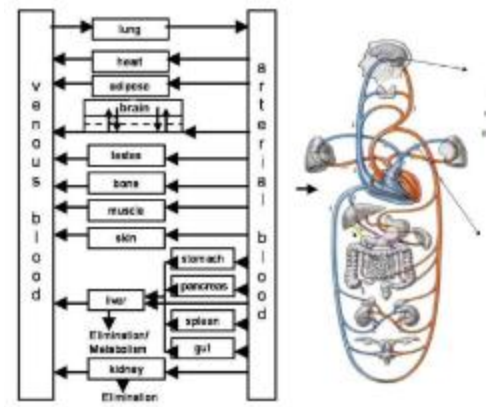
Calculation of Bioactivity exposure ratio (BER)

The BER is defined as the ratio between the POD and the relevant exposure metric

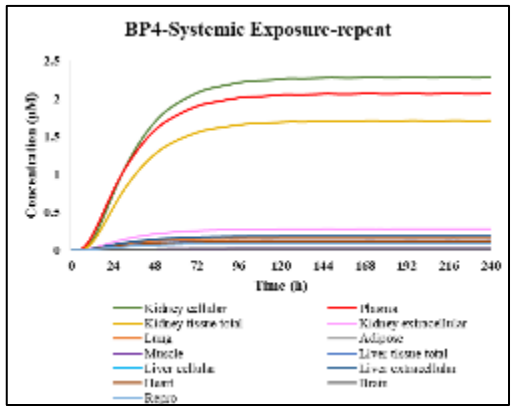
Exposure models (PBK, free/total concentration)



Skin pen

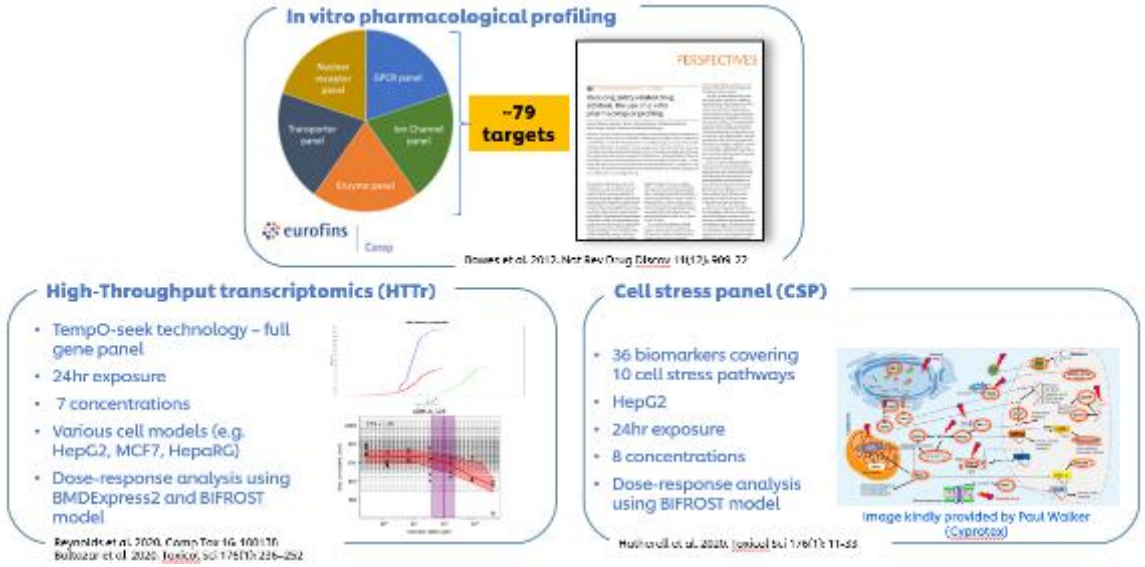


Exposure estimation: Plasma C_{max}, organ distribution, AUC

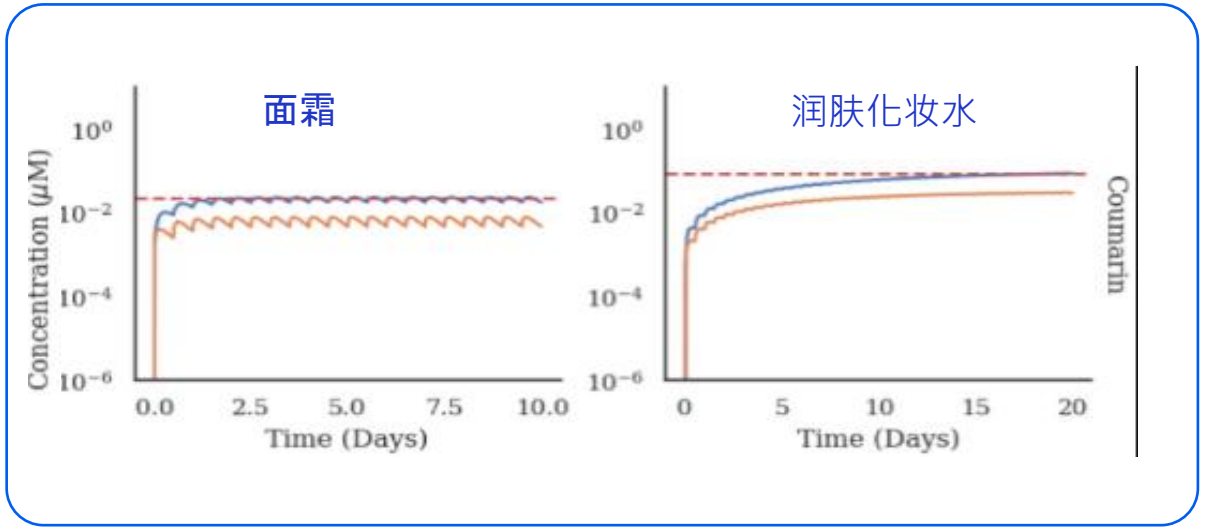


活性：暴露 比率(BER) / 评估结果

生物 活性



暴露



确定最低（最敏感）出发点，以 **μM 表示**

确定以 **μM 表示的**现实最坏情况血浆暴露量 （C_{max}）

$$\text{生物活性暴露率} = \frac{\text{生物 活性}}{\text{暴露}}$$

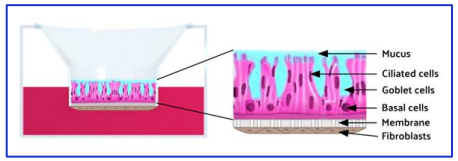
BER 越大，暴露于消费者身体发生毒性的风险就越低

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

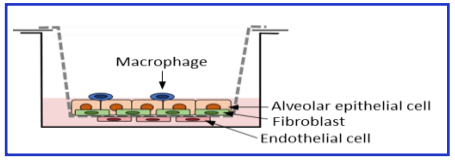
	Effects	Readouts	Acute toxicity	Chronic effect
Upper respiratory tract MucilAir™-HF model	Tissue functionality changes	Mucus secretion, cilia beating (CBF), mucociliary clearance (MCC)	Irritation, enhanced chance of airway infection	Goblet cell hyperplasia, asthma, chronic obstructive pulmonary disease (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**)



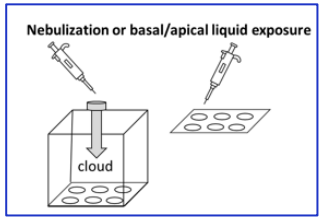
Reconstituted cells system using human primary bronchial cell cocultured with human airway fibroblast.

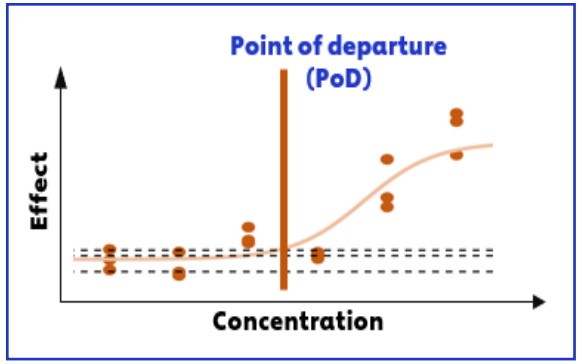
Lower respiratory tract: EpiAlveolar™
→ Lung inflammation and fibrosis **(AOP 173)**



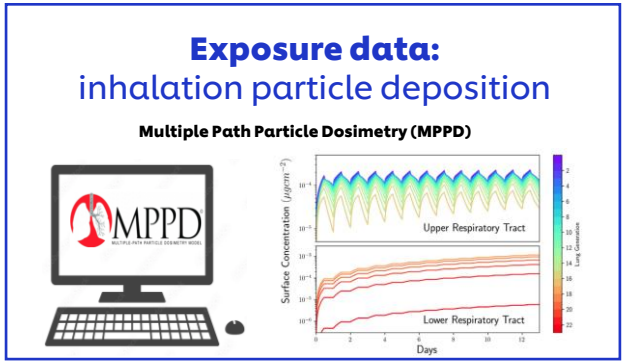
Primary human alveolar epithelial cells, pulmonary endothelial cells and monocyte-derived macrophages

12-day exposure scheme for both tissue models:

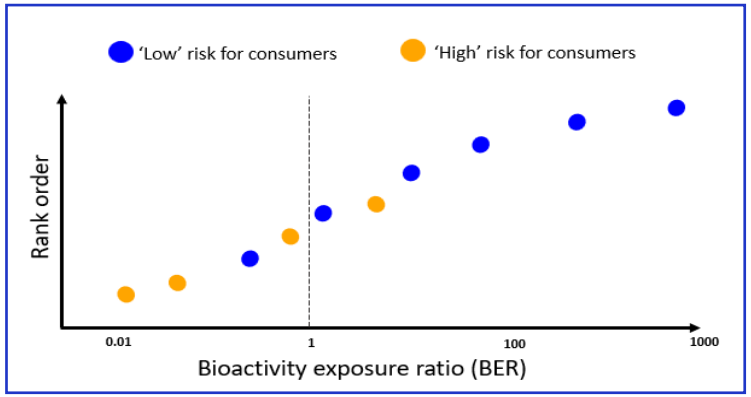




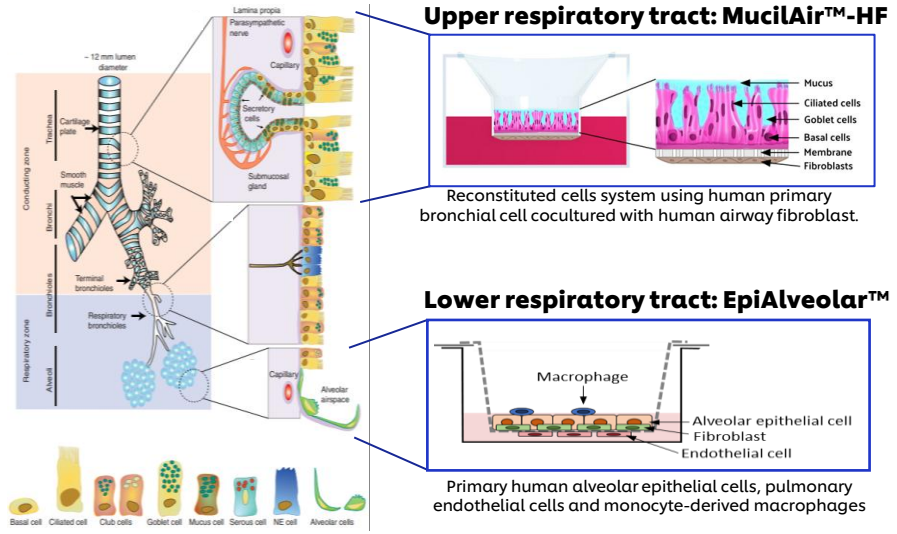
Exposure data:
inhalation particle deposition



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



Human-relevant strategy for selecting NAMs for lung toxicity NGRA



Upper respiratory tract: MucilAir™-HF

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

Lower respiratory tract: EpiAlveolar™

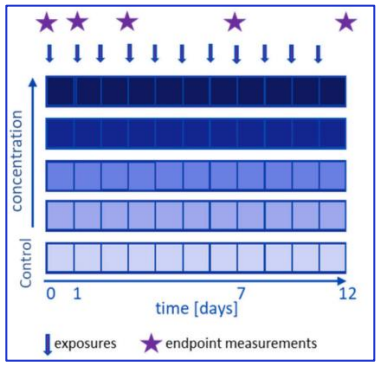
Primary human alveolar epithelial cells, pulmonary endothelial cells and monocyte-derived macrophages

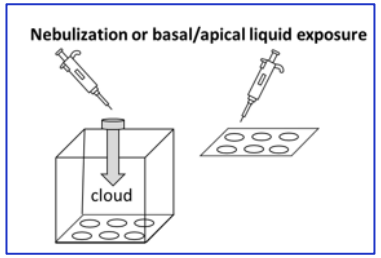
modified after Bustamante-Marin et al. (2017)
DOI: 10.1101/cshperspect.a028241

Selection Criteria:

- *体内样肺毒物暴露：气液界面 （ALI） 暴露*
- *允许重复曝光*
- *稳定的组织系统，从生理学上概括了人类呼吸道上皮的许多方面*
- - *允许测量相关 AOP 的生物标志物：*
- MucilAir-HF™ *系列*
- *粘液溶解活性和炎症的测量 （AOP 148、411、424 和 425）*
- EpiAlveolar ™
- *细胞（包括免疫感受态细胞/巨噬细胞和成纤维细胞）的氧化应激、纤维化和炎症共培养的测量 （AOP 173,1.25， 303,302）*

12-day exposure scheme:





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	**Butyl ester of poly (methyl vinyl ether-alt-maleic acid monoethyl ester) copolymer (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

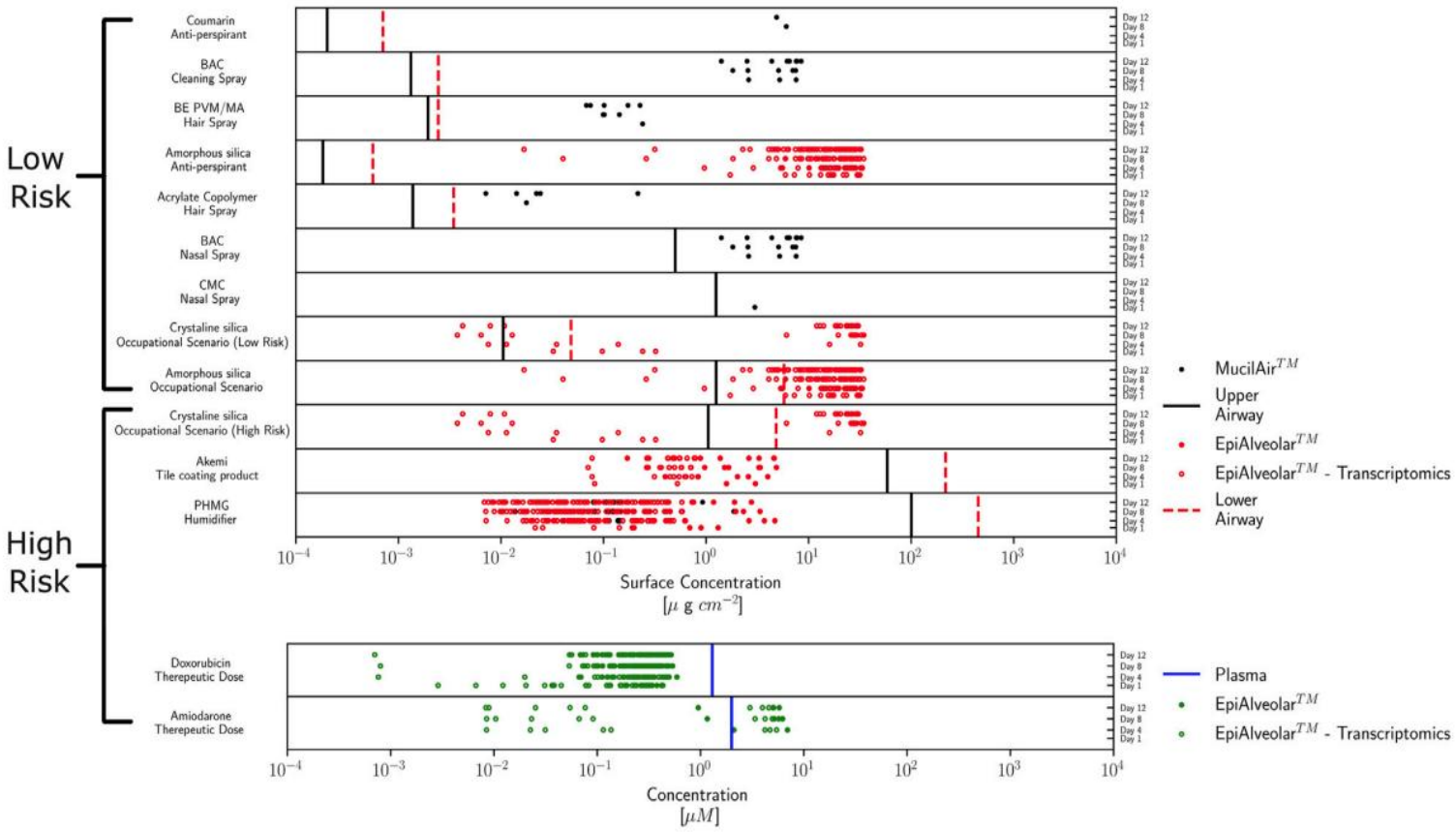
Tested in MucilAir™-HF only – **Tested in EpiAlveolar™ only** - **Tested in both tissue models**

Unilever

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

- 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)

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



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

Unilever

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**

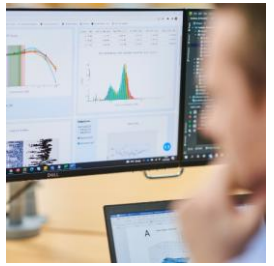
seac.unilever.com

Unilever

NAMs/NGRA Collaborations in China

在中国的合作

SERS
Safety, Environmental
& Regulatory Science



Unilever

Safety Science Journey with China 安全科学中国之旅

Unilever

中国消费者产品安全合作中心

Unilever China CPSC Centre
联合利华中国消费者产品安全合作中心

中国消费者产品安全合作中心

5. Milestones on NAMs Journey: moving Steadily

2015 PBPK training by CMU

2011 TT21 Symposium

2014, Oct/Nov
2 TATT set-up

NAMs/NGRA on Human Health (July,2024 Shenyang)

NAMs/NGRA on Environmental Safety (Sept 2024 @Tianjin)

Oct 2024 化学物质预测模型工具学组

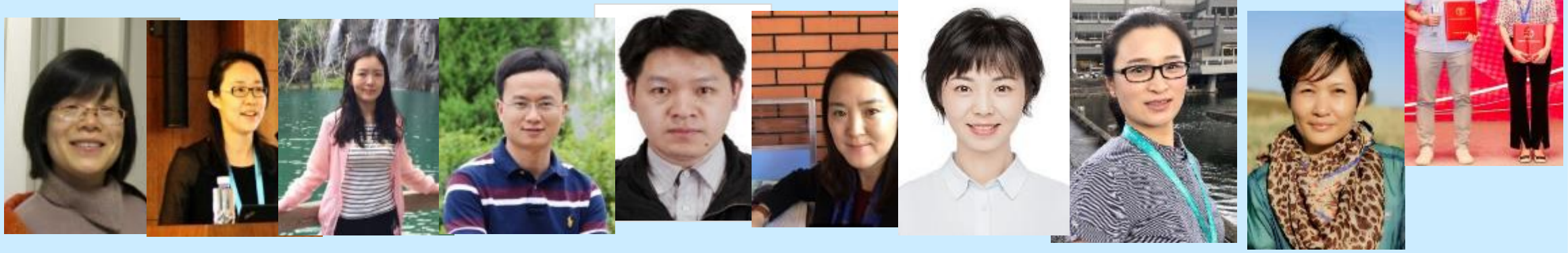
中国环境科学学会化学品环境风险防控专业委员会“化学物质预测模型工具学组”

中国毒理学替代奖 (2016-2023)

2025-2028

Previous Award Winners

9 Innovation award winners



2016 2017 2018 2019 2021 2023

2025 中国毒理学会 替代法发展奖

姓名	工作单位	专业贡献
史 薇	南京大学	**有机**污染物的迁移、转化和毒性影响，**化学品的高通量**筛选和风险评估
秦建华	中国科学院大连化学物理研究所	**微流控器官芯片、疾病模型、干**细胞、药物评价和分析检测
韦艳宏	中山大学	环境化学物质的心血管毒性作用，环**境心**脏毒理学中的分子机制、生物标**志物和体外模型**
徐 平	军事医学研究院	**蛋白**质组学、分子生物学和生物化学，**重点是泛素信号**传导、翻译后修饰和**参与肝**纤维化研究
庄树林	浙江大学	计算毒理学和污染物毒代动力学，环**境健康**

Thank You for your attention!

seac.unilever.com

Unilever