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

Iris Muller & Maria Baltazar

Unilever- Safety & Environmental Assurance Centre (SEAC)
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

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.
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)
- Anti-perspirant/deodorant aerosols
- Shampoos
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.

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

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

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

- 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 10m³.
- 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
Upper Airway – The MucilAir™-HF cell system (Epithelix)

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)

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

modified after Bustamante-Marín, et al. 2017

Huang et al., Drug Discovery and Development—Present and Future 2011 8
Sivars et al., Toxicol Sci. 2018 162(1):301-308
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 day 12.

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.
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

<table>
<thead>
<tr>
<th>Modulators of cilia beating frequency or/and mucus production</th>
<th>Inflammation</th>
<th>Negative controls (history of safe use)</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Benzalkonium chloride ●</td>
<td>• TNF-alpha ✔</td>
<td>• Coumarin ✔</td>
</tr>
<tr>
<td>• LPS ⚠</td>
<td>• Benzalkonium chloride ✔</td>
<td>• Sulforaphane ✔</td>
</tr>
<tr>
<td>• Carboxymethylcellulose ●</td>
<td>• Acrolein ●</td>
<td>• Acudyne™ DHR polymer ✔</td>
</tr>
<tr>
<td>• Acrolein ●</td>
<td>• LPS ⚠</td>
<td>• Gantrez™ ES-425 ✔</td>
</tr>
<tr>
<td>• Isoproterenol ✔</td>
<td>• Isoproterenol ⚠</td>
<td></td>
</tr>
<tr>
<td>• Chlororesol ⚠</td>
<td>• TNF-alpha ⚠</td>
<td></td>
</tr>
<tr>
<td>• Nicotine ⚠</td>
<td>•</td>
<td></td>
</tr>
<tr>
<td>• CFTRinh-172 ⚠</td>
<td>•</td>
<td></td>
</tr>
<tr>
<td>• TNF-alpha ⚠</td>
<td>•</td>
<td></td>
</tr>
</tbody>
</table>

Gaps identified: Interindividual variability, dosing, variability/sensitivity of the cell model.
Lower Airway – The EpiAlveolar™ cell system (MatTek)

modified after Bustamante-Marin, et al. 2017

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<th>acute</th>
<th>chronic</th>
</tr>
</thead>
<tbody>
<tr>
<td>barrier function</td>
<td>tissue integrity (TEER, LDH), mitotoxicity,</td>
<td>local cytotoxicity, inflammation, wound</td>
<td>airway remodelling/scarring, lung fibrosis</td>
</tr>
<tr>
<td></td>
<td>cytokine/chemokine release, extracellular</td>
<td>healing</td>
<td></td>
</tr>
<tr>
<td></td>
<td>matrix accumulation</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

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

primary human alveolar epithelial cells, pulmonary endothelial cells and monocyte-derived macrophages
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
Lower Airway – Experimental design

- 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 day 12.

modified after VITROCELL®

![Diagram of experimental design](image-url)
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

<table>
<thead>
<tr>
<th>Inflammation/ fibrosis, cytotoxicity</th>
<th>Negative controls (history of safe use)/case studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amiodarone ✔️</td>
<td>Sulforaphane ✔️</td>
</tr>
<tr>
<td>Doxorubicin ✔️</td>
<td></td>
</tr>
<tr>
<td>Min-u-Sil5 (crystalline silica) ✗</td>
<td></td>
</tr>
<tr>
<td>Aerosil 200 (amorphous silica) ✗</td>
<td></td>
</tr>
<tr>
<td>LPS ✗</td>
<td></td>
</tr>
<tr>
<td>PHMG ✔️</td>
<td></td>
</tr>
</tbody>
</table>

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

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

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*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

\[ \text{Oligomer, MW=500-700 g/mol} \]

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

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 m³
Hypothetical Case study – Tier 1 exposure assessment

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

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

1 \text{ 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.

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

#### Input

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spray rate (mg/min)</td>
<td>36000</td>
</tr>
<tr>
<td>Inclusion level (%)</td>
<td>0.25</td>
</tr>
<tr>
<td>Emission duration (min)</td>
<td>0.1667</td>
</tr>
<tr>
<td>Number of applications</td>
<td>1</td>
</tr>
<tr>
<td>Zone 1 volume (m³)</td>
<td>1</td>
</tr>
<tr>
<td>Zone 2 volume (m³)</td>
<td>19.1</td>
</tr>
<tr>
<td>Air flow (1 -&gt; outside) (m³/min)</td>
<td>0</td>
</tr>
<tr>
<td>Air flow (2 -&gt; outside) (m³/min)</td>
<td>1.89</td>
</tr>
<tr>
<td>Air flow (1 -&gt; 2) (m³/min)</td>
<td>7.24</td>
</tr>
<tr>
<td>Time in zone 1 (min)</td>
<td>1</td>
</tr>
<tr>
<td>Time in zone 2 (min)</td>
<td>9</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>60</td>
</tr>
<tr>
<td>Inhalation rate (L/min)</td>
<td>20</td>
</tr>
<tr>
<td>Initial zone 1 concentration (mg/m³)</td>
<td>0</td>
</tr>
<tr>
<td>Initial zone 2 concentration (mg/m³)</td>
<td>0</td>
</tr>
<tr>
<td>Time step (min)</td>
<td>0.02</td>
</tr>
<tr>
<td>Exposure duration (min)</td>
<td>10</td>
</tr>
</tbody>
</table>

#### Output

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean zone 1 for 1st minute (mg/m³)</td>
<td>2.690339</td>
</tr>
<tr>
<td>Mean zone 2 for next 9 minutes (mg/m³)</td>
<td>0.505035</td>
</tr>
<tr>
<td>Time-weighted average (mg/m³)</td>
<td>0.7</td>
</tr>
</tbody>
</table>
Hypothetical Case study – Regional Lung Deposition Modelling

Collate Existing Information/Problem Formulation

Exposure *
- Use scenario
- Consumer Habits and Practices
- Particle Size Distribution
- Tier 1 – screening assessment
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- Tier 3 – Experimental data

Hazard data
- Molecular Structure
- In silico predictions (PCA)
- Protein content
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- Read Across

Measured Particle Size Distribution

Mean Mass Aerodynamic Diameter : 3.64±2.62µm

![Particle Size Distribution Graph](image)
Hypothetical Case study – Regional Lung Deposition for repeated exposures

Lung Geometry: Yeh-Schum Symmetric with default clearance

<table>
<thead>
<tr>
<th>Tier</th>
<th>Airborne Concentration</th>
<th>Day 1 $\mu g/cm^2$</th>
<th>Upper</th>
<th>Lower</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tier 1</td>
<td>15 mg/m$^3$</td>
<td>0.086</td>
<td>0.0011</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tier 2</td>
<td>0.7 mg/m$^3$</td>
<td>0.004</td>
<td>5.48E-05</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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)

\[ \text{Airborne PHMG level estimated (mg/m³)} = 10 \text{ mL/addition} \times 2 \text{ additions} \times 1276 \text{ µg/ml} \times 1 \text{ } 27 \text{ m³} \]

\[ = 0.95 \text{ mg/m³} \]

<table>
<thead>
<tr>
<th>Mass</th>
<th>Upper µg/cm²</th>
<th>Lower µg/cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Day</td>
<td>0.07268</td>
<td>0.00136</td>
</tr>
<tr>
<td>12 Day</td>
<td>0.109848</td>
<td>0.015757</td>
</tr>
</tbody>
</table>

MMAD: 80 nm
GSD: 1

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)

**Collate Existing Information/Problem Formulation**
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- Acute and Chronic
  - ALI Upper Airway
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  - ALI Lower Airway
    - (Lung Fibrosis, inflammation)
  - Lower Airway
    - (Macrophage clearance, biopersistence, 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
Case study: PHMG causes a mild inflammatory response in MucilAir™ cell model

- 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

Day 12

Log conc. [µg/cm²]

PoD_{UA} - upper airways
PoD_{LA} - lower airways
Hairspray exposure Tier 2
0.7 mg/m³ 10 min/day
Exposure_{UA} - Upper airway
Exposure_{LA} - Lower airway

Bioactivity-exposure ratio (BER)

<table>
<thead>
<tr>
<th>BER</th>
<th>Hairspray exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>BER_{UA}</td>
<td>366</td>
</tr>
<tr>
<td>BER_{LA}</td>
<td>110</td>
</tr>
</tbody>
</table>
Benchmarking against existent known human exposures to PHMG associated with adverse effects in humans

Day 12

Log conc. [µg/cm²]

Exposure

UA - Upper airway

LA - Lower airway

Humidifier exposure
0.95 mg/m³ 11h/day

PoDUA - upper airways
PoDLA - lower airways

Bioactivity-exposure ratio (BER)

<table>
<thead>
<tr>
<th></th>
<th>Hairspray exposure</th>
<th>Humidifier exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>BERUA</td>
<td>366</td>
<td>20</td>
</tr>
<tr>
<td>BERLA</td>
<td>110</td>
<td>4.4</td>
</tr>
</tbody>
</table>

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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