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
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
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

Exposure-led

• 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

• NAMs identification and evaluation using benchmark compounds

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

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Lung fibrosis
Impairment of mucociliary clearance
Lung surfactant inhibition
Biopersistency/Clearance
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-Marin, 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
Upper Airway – Experimental design

- 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
- \textit{In vitro} and \textit{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>Chlorocresol</td>
<td></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)

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

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

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

modified after Bustamante-Marin, et al. 2017
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
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.
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></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)

### 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 polyhexamethylenebiguanidine 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

\[
\begin{align*}
\text{Oligomer, MW=} & \quad 500-700 \text{ g/mol} \\
\text{Polyhexamethyleneguanidine phosphate (n/x=1-2)} & \\
\text{(PHMG phosphate)} & \\
\text{CAS RN 89697-78-9} & \\
\end{align*}
\]

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

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

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

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.

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

<table>
<thead>
<tr>
<th>Input</th>
<th></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>

<table>
<thead>
<tr>
<th>Output</th>
<th></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>

For more details, please refer to the following publication:


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
- Existing in vivo data
- Read Across

Measured Particle Size Distribution
Mean Mass Aerodynamic Diameter: 3.64±2.62µm

Read Across*

Measured Particle Size Distribution

Mean Mass Aerodynamic Diameter: 3.64±2.62µm

Mass Concentration
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>Day 12 $\mu g/cm^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Upper</td>
<td>Lower</td>
</tr>
<tr>
<td>Tier 1</td>
<td>15 mg/m$^3$</td>
<td>0.086</td>
<td>0.0011</td>
</tr>
<tr>
<td>Tier 2</td>
<td>0.7 mg/m$^3$</td>
<td>0.004</td>
<td>5.48E-05</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)

\[
= 10 \text{ ml/addition} \times 2 \text{ additions} \times 1276 \text{ µg/ml} \times 27 \text{ m}^3
\]

\[
= 0.95 \text{ mg/m}^3
\]

<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>

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

- 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

Bioactivity-exposure ratio (BER)

<table>
<thead>
<tr>
<th></th>
<th>Hairspray exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>BER&lt;sub&gt;UA&lt;/sub&gt;</td>
<td>366</td>
</tr>
<tr>
<td>BER&lt;sub&gt;LA&lt;/sub&gt;</td>
<td>110</td>
</tr>
</tbody>
</table>
Benchmarking against existent known human exposures to PHMG associated with adverse effects in humans

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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Thank You for your attention!

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