Lung surfactant inhibition – an alternative acute inhalation toxicity test

Jorid Birkelund Sørli and Emilie Da Silva

jbs@nfa.dk
eds@nfa.dk
Endpoint replacement

• Acute inhalation toxicity

  • Accepted tests OECD TG 403/436/433
  • Endpoints: death (403 and 436) or evident toxicity (433)

  • No accepted alternatives
<table>
<thead>
<tr>
<th>Impregnation product</th>
<th>In vitro test</th>
<th>In vivo test</th>
<th>Correlation</th>
<th>Toxic for humans</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;Wood impregnation&quot;</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>&quot;Stain repellent super&quot;</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>&quot;Liquid stain protection&quot;</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>&quot;Faceal oleo MG&quot;</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>&quot;HG textile&quot;</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>&quot;HG leather &quot;</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Sørli et al, 2015
The airways

Adapted from Ungaro et al., 2012

http://www.sharinginhealth.ca/respiratory/respiratory.html
Alternative integrated testing strategy gap

- Most **cell-based** lung toxicity assays do not incorporate lung surfactant

BUT

- Lung surfactant has a vital function in the lungs

http://usmle.biochemistryformedics.com/respiratory-distress-syndrome-case-discussion
Lung surfactant inhibits surface tension

Low surface tension
Normal alveoli

High surface tension
Collapsed alveoli
Perez-Gil & Weaver, Physiology, 2010
**Constrained drop surfactometer**

- Measures surface tension
- Temperature control
- Flow through for realistic exposure
- Compatible with different aerosol generators
- Fast, easy, and low cost test
Drop shape

- The contact angle and the shape can be used to calculate the surface tension (ADSA, Zuo et al. 2004)

<table>
<thead>
<tr>
<th>Water</th>
<th>Lung surfactant</th>
</tr>
</thead>
<tbody>
<tr>
<td>70 mN/m</td>
<td>93º</td>
</tr>
</tbody>
</table>
Alternative integrated testing strategy

- Lung surfactant function is a point-of-entry measure

- Lung surfactant should be added to air-liquid interface cell-based assays for more realistic exposure scenarios

- Lung surfactant function can only be assessed in a dynamic assay, thus this should be done separately

- Disruption of lung surfactant function can be included in an IATA to determine the potential for causing acute inhalation toxicity
Applicability domain

• Impregnation products
  • Frequently used both by professionals and by consumers
  • Make surfaces water and dirt repellant -> easy to clean
  • Cause acute inhalation toxicity regularly

• Inhaled pharmaceuticals (marketed formulations Sørli et al 2015)
  (excipients Sørli et al 2018, in review)
  • Treatment for lung disease are inhaled and can potentially interact with lung surfactant

• Nano particles can reach deep into the lungs and interact with the lung surfactant
Future work and next steps

• Enhancers in biopharmaceuticals (Sørli et al, paper in review)

• Testing of:
  • Nanoparticles in EU project SmartNanoTox
  • Per-fluorinated compounds in collaboration with The Norwegian Institute of Public Health
  • Surveillance of problematic impregnation or spray products by the Danish poison center and the Danish EPA
  • Cleaning products in spray form
  • Chemicals with a known reported LD50 after inhalation

• Plan to submit the Pre-submission form to EURL-ECVAM
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References

- The need for an alternative test
  - Da Silva and Sørli, Applied In Vitro Toxicology (2018)

- Description of the method

- Use of the CDS to measure LS function
  - Impregnation product and LS interaction

- Inhaled pharmaceuticals and LS interaction
  - Sørli et al, in review (2018)

- Nanoparticle LS interaction
  - Valle et al, ACS nano (2015)
  - Valle et al, Acs Sustainable Chemistry & Engineering (2014)
  - Hu et al, Nanoscale (2013)

- Mouse inhalation bioassay and impregnation products
  - Larsen et al, Toxicology letters (2014)
  - Sørli et al, Altex (2015)