A.I.S.E. *In vitro* Programme: Alternative Test Methods to Classify Detergents for Eye Effects

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On behalf of the A.I.S.E. *In Vitro* Technical Team
**In Vitro Test Methods for Serious Eye Damage / Irritation**

- **Corrosive**
  - BCOP (Bovine Corneal Opacity & Permeability Test)
  - ICE (Isolated Chicken Eye Test)
  - FL (Fluorescein Leakage)
  - STE (Short Time Exposure Test)
  - CM (Cytosensor Microphysiometer)
  - HET-CAM (Hen’s egg test)
  - IRE (Isolated Rabbit Eye Test)

- **Irritant**
  - **In vitro methods to affirm Corrosive/ Cat. 1**

- **Non-irritant**
  - **In vitro methods to affirm Not Classified**

- **In vivo**
  - Draize Test
  - Low Volume Eye Test

**In vitro** methods to classify Moderate/ Cat. 2
A.I.S.E. *In Vitro* Programme

- Reversibility of ocular tissue lesions are not evaluated *per se* in the current OECD TG methods accepted to identify serious eye damage / Cat. 1

- Persistence of tissue effects (at Day 21) count for a significant proportion of serious eye damage / Cat. 1 classification
  - Cosmetics Europe / EURL-ECVAM joint activity: 55.5% (106/191) of Cat. 1 chemicals from public and new databases are classified based on persistence of effects only (Adriaens *et al.* 2014, Arch Tox 88, 701-723)

- A.I.S.E. *in vitro* programme
  - Investigated the use of *in vitro* test methods to classify detergents and cleaning products
  - Considered both non-extreme-pH and extreme-pH products
  - Aimed to generate *in vitro* data which correctly reflect the hazard potential to man, including identification of potential serious eye damage effects (EU CLP/ UN GHS Cat. 1)
  - Reviewed literature and existing data from A.I.S.E. members
  - Conducted *in vitro* testing of representative product formulations
  - Focus on histopathology as an additional endpoint to the Isolated Chicken Eye (ICE) test to evaluate:
    - Predictive capacity for identification of eye Cat. 1 detergents
    - Reproducibility between independent laboratories for histopathology

www.aise.eu
Background Isolated Chicken Eye Test

Liquids 30 µL
Solids 30 mg

10 seconds application
20 mL rinse

Evaluation parameters
- Corneal thickness
  (Swelling)
- Corneal Opacity
- Fluorescein Retention

Superfusion apparatus
Histopathology in ICE
## Semi-Quantitative Scoring System for ICE Cornea

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Observation</th>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epithelium: erosion</td>
<td>Very slight</td>
<td>½</td>
<td>Few single cells up to the entire single superficial layer</td>
</tr>
<tr>
<td></td>
<td>Slight</td>
<td>1</td>
<td>Up to 3 layers are gone</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>2</td>
<td>Up to 50% of the epithelial layer is gone</td>
</tr>
<tr>
<td></td>
<td>Severe</td>
<td>3</td>
<td>Epithelial layer is gone up to the basement membrane</td>
</tr>
<tr>
<td>Epithelium: vacuolation</td>
<td>Very slight</td>
<td>½</td>
<td>Few scattered cells</td>
</tr>
<tr>
<td></td>
<td>Slight</td>
<td>1</td>
<td>Groups of vacuolated cells or single string of cells with small vacuoles</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>2</td>
<td>Up to 50% of the epithelium consists of vacuolated cells</td>
</tr>
<tr>
<td></td>
<td>Severe</td>
<td>3</td>
<td>50 – 100% of the epithelium consists of vacuolated cells</td>
</tr>
<tr>
<td>Epithelium: necrosis</td>
<td>Normal</td>
<td>-</td>
<td>&lt; 10 necrotic cells</td>
</tr>
<tr>
<td></td>
<td>Very slight</td>
<td>½</td>
<td>10 – 20 necrotic cells</td>
</tr>
<tr>
<td></td>
<td>Slight</td>
<td>1</td>
<td>20 – 40 necrotic cells</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>2</td>
<td>Many necrotic cells but &lt; 50% of the epithelial layer</td>
</tr>
<tr>
<td></td>
<td>Severe</td>
<td>3</td>
<td>50 – 100% of the epithelial layer is necrotic.</td>
</tr>
<tr>
<td>Stroma: pyknotic nuclei</td>
<td>Normal</td>
<td>-</td>
<td>&lt; 5 pyknotic nuclei</td>
</tr>
<tr>
<td></td>
<td>Slight</td>
<td>1</td>
<td>5 – 10 pyknotic nuclei</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>2</td>
<td>&gt; 10 pyknotic nuclei</td>
</tr>
<tr>
<td>Stromal disorder of fibres</td>
<td>Present</td>
<td>P</td>
<td>Irregular appearance of the fibres.</td>
</tr>
<tr>
<td>Endothelium: necrosis</td>
<td>Present</td>
<td>P</td>
<td>The endothelium consists of only one layer, so a grade is not relevant</td>
</tr>
</tbody>
</table>

Cazelle *et al.*, Toxicology in Vitro 28: 657-666, 2014
Histopathology Decision Criteria

- Histopathology decision criteria recommended to be used in addition to the standard validated ICE test method (OECD TG 438) for the identification of EU CLP / UN GHS eye Cat. 1 non-extreme pH detergents (2<pH<11.5)

<table>
<thead>
<tr>
<th>Tissue layer</th>
<th>Effects triggering eye serious damage (EU CLP / UN GHS Cat. 1) identification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epithelium</td>
<td>- erosion ≥ moderate (score 2) in at least 2 out of 3 eyes</td>
</tr>
<tr>
<td></td>
<td>- and/or, any vacuolation (≥ very slight, score ½) observed in the mid and/or lower parts in at least 2 out of 3 eyes</td>
</tr>
<tr>
<td></td>
<td>- or, if erosion ≥ moderate (score 2) in 1 out of 3 eyes + vacuolation ≥ very slight in mid and/or low part (score ½) is observed in at least another eye out of the 3 eyes</td>
</tr>
<tr>
<td></td>
<td>- and/or, necrosis ≥ moderate (score 2) observed in at least 2 out of 3 eyes</td>
</tr>
</tbody>
</table>

Cazelle et al., Toxicology in Vitro 28: 657-666, 2014
**Histopathology as an Additional Endpoint to ICE**

- Main tissue effects correlated to *in vivo* EU CLP / UN GHS Cat. 1 classification:
  - Control
  - Epithelial erosion (at least moderate level)
  - Epithelial vacuolation (mid and / or lower layers)

- A.I.S.E. decision criteria for identification of EU CLP / UN GHS Cat. 1 (Draize + LVET Cat. 1 only) for surfactants and non-extreme pH detergents:
  - Increase in accuracy: from 39% to 70% (n=23)
  - Decrease in false negative rate: from 78% to 33% (n=18)
  - Acceptable false positive rate: from 0% to 20% (n=5)

<table>
<thead>
<tr>
<th>Correct predictions</th>
<th>Severity</th>
<th>Persistence</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICE</td>
<td>50% (4/8)</td>
<td>0% (0/10)</td>
<td>22% (4/18)</td>
</tr>
<tr>
<td>ICE + histopathology</td>
<td>88% (7/8)</td>
<td>50%-70*% (5-7*/10)</td>
<td>67-83*% (12-15*/18)</td>
</tr>
</tbody>
</table>

* Improved decision criteria based on epithelial necrosis effects observed for pure surfactants
Histopathology: useful additional endpoint to the validated ICE test method prediction model
- Non-extreme pH detergent and cleaning products, surfactants and surfactant dilutions

Allows a better prediction of EU CLP / UN GHS Cat. 1
- Identifies Cat. 1 classified in vivo based on persistence of effects and avoids misclassification of Cat. 1 classified in vivo based on severity of effects

Proposed use: to change from “no prediction can be made“ into EU CLP / UN GHS Cat. 1 (“up-classification“ only)

Does not appear useful for pH-extreme products as classification is based mainly on severity of effects

Between laboratory reproducibility study is ongoing
Update on OECD developments

- Two Standard Project Submission Forms (SPSFs) were submitted to OECD in 2014 for revision of TG 438 and GD160 to include histopathology as an additional endpoint to the ICE for identification of non-pH-extreme UN GHS Cat. 1 surfactants and surfactant-containing mixtures.

- In April 2015, the OECD Working Group of the National Coordinators for the Test Guidelines (WNT) agreed to consider revising the OECD TG 438 and GD 160.

- First draft of revised OECD TG 438 from September 23, 2015 was discussed at the last OECD eye irritation / corrosion expert group meeting November 9-10, 2015.

- Decision at OECD Expert Group Meeting in November 2015 to show reproducibility of results: Transfer of histopathology to other test institutes.
Status of Ongoing Activities

- Development of a comprehensive histopathology atlas.
- First draft of revised OECD GD 160 from September 14, 2016 to be discussed at the next OECD eye irritation / corrosion expert group meeting November 3-4, 2016.
- Nov/Dec 2016: Results of histopathology reproducibility study.
- April 2017: Discussion of revised OECD TG 438 and GD 160 at OECD WNT Meeting.