The importance of understanding drivers of classification in vivo for selection of chemicals used in the development and evaluation of in vitro serious eye damage/eye irritation assays: Cosmetics Europe analysis.

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Introduction

A thorough understanding of which of the factors assessed in the in vivo Draize eye test are responsible for driving UN GHS classification is critical for an adequate selection of chemicals to be used in the development and evaluation of alternative methods and for properly assessing their predictive capacity and limitations. For this purpose, Cosmetics Europe undertook to compile an extensive database of chemicals tested in Draize eye tests. The data are available in the Draize rabbit eye test, the hazard potential of a test chemical is determined based on its effect on corneal opacity (CO), irritation (IR), conjunctival redness (CR), and conjunctival chemosis (CC) in combination with full reversibility or persistence of any effect on day 21 after instillation. In order to achieve full description of the in vivo Draize eye test, it is clear that alternative methods, alone or in combination, need to address the main ocular tissue effects that drive classification. An evaluation of the various in vivo drivers of classification compiled in the DRD was performed to establish which of these are most important and from a regulatory point of view. This approach will facilitate an early and thorough assessment of the performance of a new alternative method and will help better identifying its limitations and applicability within testing strategies such as those suggested by Scott et al. (2010). Taken together, the key goals for conducting the DRD were: i) enable a comprehensive analysis and understanding regarding in vivo drivers of classification based on the Draize eye test; ii) further evaluate the variability of the Draize eye test based on data obtained from repeat studies; iii) enable a critical review of the UN GHS/EU CLP classification criteria for eye damage/irritation; iv) make available an extensive database of chemicals with OECD Test Guideline 405 in vivo data, beyond those generally used historically, for further method development and validation and v) to provide guidance for selecting reference chemicals based on understanding ocular tissue effects that drive classification in the in vivo rabbit Draize eye test.

Strategy for Developing the DRD

The DRD was primarily compiled using different sources of historical in vivo Draize eye test data i.e. ECETOC, ZEBET, Laboratoire National de la Santé (Gautheron), NICEATM, EUR L ECVAM, which were created to support past validation activities. These data were produced according to OECD Test Guideline 405 using proprietary and commercially available chemicals. The studies were classified according to the serious eye damage/eye irritation classification criteria defined by UN GHS and EU CLP which implemented UN GHS in the EU. According to these classification systems, there are several criteria derived from the four ocular tissue effects that drive the Draize eye test: CO (CR and CC), irritation (IR) and redness (RD) and the last three effects or (CR, IR and CC) categorized in two categories: Category 2A (irritant to eyes) when any of the eye effects in any animal is not fully reversible within 7 days of observation (Fig. 1A) or Category 2B (irritant to eyes) when any of the eye effects are not fully reversible within 14 days of observation (Fig.1B). The mean scores calculated from gradings of 0 to 4, and 72 hours period of administration of the test chemical (at least one animal with a mean score of 1.5 above the classification cut-off or at least one endpoint.

Table 1: List of the in vivo drivers of UN GHS classification for the chemicals requiring classification and subsequent use of chemicals not requiring classification. This table also contains the proportion (%) and number (n) of studies according to main driver of classification or according to the subgroups (No Cat).

<table>
<thead>
<tr>
<th>Category</th>
<th>1a (%)</th>
<th>2a (%)</th>
<th>2b (%)</th>
<th>2c (%)</th>
<th>2d (%)</th>
<th>No Cat (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO</td>
<td>28.1</td>
<td>13.5</td>
<td>58.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IR</td>
<td>21.3</td>
<td>15.6</td>
<td>56.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RD</td>
<td>13.5</td>
<td>12.1</td>
<td>44.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CR</td>
<td>12.1</td>
<td>10.2</td>
<td>34.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>12.1</td>
<td>10.2</td>
<td>34.5</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

The results of the analysis of the DRD clearly demonstrates the importance of understanding the in vivo tissue effects which drive eye damage/irritation classification according to the UN GHS/EU CLP systems. Builds on recent more general publications in this area (i.e. Barroso et al. 2013;); Adriaens et al. 2014*). Key conclusions drawn from the current analysis are:

**Key Conclusions**

- The most important drivers for Cat 1 Classification are CO mean ≥ 3 and IR mean ≥ 1.5 in the majority of animals (32 studies with 116 animals), (Fig. 2A) and Cat 2A studies showing CO persistence in the minority of the animals (Fig. 2B) have a similar distribution as those of the Cat 2A chemicals showing CO persistence on day 7 (Fig. 2C).

- In general, the CO scores of the Cat 1 chemicals classified based on CO persistence in the minority of the animals (Fig. 2B) have a similar distribution as those of the Cat 2A chemicals showing CO persistence on day 7 (Fig. 2C). In fact, based on the CO scores observed over the first three days, it is not possible to distinguish the Cat 1 studies with CO persistence in the minority of the animals (Fig. 2B) from the Cat 2A studies (Fig. 2C). The same is true for CR and CC (data not shown). Persistent effects of eye irritation in a minority of the animals should therefore not be used to drive a Cat 1 classification, nor should isolated extreme effects (CO ≥ 4) appearing late in the study, as these are most probably not related to the test chemical itself.

Figures 1A & B: Top left: Category 1 studies and bottom left: Category 2A studies.

References

- Adriaens et al. Retrospective analysis of the Draize test for serious eye damage/eye irritation. Importance of understanding the in vivo endpoints under UN GHS/EU CLP for the development and evaluation of alternative test methods. Archives of Toxicology 88, 101–124.