# The Importance of Understanding Drivers of Irritation *In Vivo* for Selection of Chemicals Used in the Development and Evaluation of *In Vitro* Eye Irritation Assays: Cosmetics Europe Analysis

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## Introduction

Cosmetics Europe's Task Force Eye Irritation (TFEI) is actively involved in the development of alternative methods to assess eye irritation potential of cosmetic ingredients using *in vitro* methods, based on optimizing current *in vitro* test methods, applied research projects and collaborative activities with external partners. Selecting chemicals for use in the development and evaluation of *in vitro* eye irritation assays based on a thorough understanding of what drives irritation in classification of ocular effects of chemicals in the *in vivo* rabbit Draize test is therefore a critical and essential element that enables identification and evaluation of predictive capacity and applicability domain at an early stage of development. To facilitate understanding of the importance of such drivers of irritation, Cosmetics Europe TFEI has undertaken an in depth analysis of the publicly available external databases containing *in vivo* eye irritation data for 258 chemicals and solutions of chemicals tested in the Draize eye irritation test. This analysis is based on having good quality *in vivo* data that has allowed a clear understanding of the different ocular tissues effects that drive classification. These include corneal opacity (CO), iritis (IR), conjunctival redness (CR), conjunctival chemosis (CC), days to clear and/or persistence of effects. In addition, all 258 chemicals were screened for their commercial availability, assurance that they cover the whole range of irritation potential and represent relevant classes and physical states. Until today such an analysis is unprecedented, and it will have important implications for *in vitro* methods development, evaluation and validation activities.

## Strategy for Building a Master Chemicals List

1. Collection of chemicals with good quality *in vivo* data from existing databases (e.g. ECETOC, ZEBET and Laboratoire National de la Santé (Gautheron)) and generation of UN GHS classifications for eye irritation
2. Identification of the *in vivo* drivers of UN GHS classification, as depicted in Table 1
3. Differentiation of chemicals according to the drivers of classification as depicted in Table 2 and Figure 1, using the following prioritization scheme:
   - **a) Category 1:**
     - Chemicals classified based on severity (mean scores of Days 1-3)
     - Chemicals classified based on persistence at Day 21, but not severity
     - Chemicals classified based on CO = 4, but not severity or persistence
     - Choice of endpoint driving classification dependent on the number of animals
   - **b) Category 2:**
     - Choice of endpoint driving classification (2B: severity; 2A: severity and persistence at Day 7) dependent on the number of animals
   - **c) No Category:**
     - CO = 0 → chemicals showing CO scores equal to zero in all animals and all observed timepoints
     - CO > 0 → chemicals showing at least one CO score higher than 0 at any timepoint in any animal

**Table 1: List of the *in vivo* drivers of UN GHS classification**

| Category 1 | | | | | Category 2 | | | NC | |
|---|---|---|---|---|---|---|---|---|---|
| Severity (Mean scores of Days 1-3) | | Persistence at Day 21 | | | Severity (Mean scores of Days 1-3) | | | CO = 0 | CO > 0 |
| CO mean ≥ 3 in 67% of the animals | IR mean > 1.5 in 67% of the animals | CO | IR | CR and/or CC | CO mean ≥ 1 in 67% of the animals | IR mean ≥ 1 in 67% of the animals | CR mean and/or CC mean ≥ 2 in 67% of the animals | Chemicals with at least 1 animal with a mean score of days 1-3 for at least one endpoint above the classification cut-off marked with ** | |
| CO = 4 in at least 1 animal | | | | | Persistence at Day 7 | | | | |
| | | | | | CO | IR | CR and/or CC | | |

**Figure 1: Distribution of chemicals according to drivers of classification for 258 chemicals (64 Cat1, 30 Cat 2A, 14 Cat 2B and 150 NC) from the ECETOC, ZEBET and Gautheron databases**

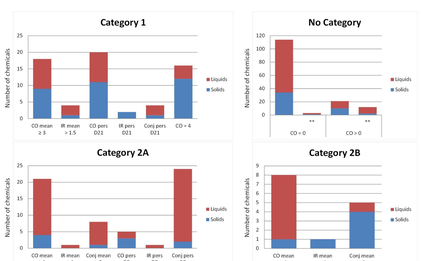


**Table 2: Drivers of classification for several representative chemicals selected from 3 publically available databases: ECETOC, ZEBET and Gautheron**

| Chemicals | GHS Classification | Drivers of Classification: Severity – Cut-off values | Severity – Number of animals | Specific observations – CO = 4 or other observations | Specific observations – Number of animals | Persistence – Cut-off time | Persistence – Number of animals | Comments | CAS # | Physical Form | Data Source |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Promethazine HCL | 1 | CO mean ≥ 3 | 2/3 | | | | | Delayed effects | 58-33-3 | S | Gautheron |
| Quinacrine | 1 | IR mean > 1.5 | 2/3 | | | | | | 69-05-6 | S | ECETOC |
| 3,6-Dimethyloctanol | 1 | CO mean ≥ 1 | 3/3 | | | CO pers D21 | 1/3 | Delayed effects | 151-19-9 | L | ZEBET |
| 2,5-Dimethylhexanediol | 1 | CO mean ≥ 1 | 3/3 | | | IR pers D21 | 1/3 | | 110-03-2 | S | ZEBET |
| Butyl cellosolve | 1 | CO mean ≥ 1 | 3/3 | | | Conj pers D21 | 3/3 | | 111-76-2 | L | ECETOC |
| Methyl thioglycolate | 1 | CO mean ≥ 1 | 3/3 | CO = 4 | 1/3 | | | CO = 4 at D1 in 1/3 fully reversed by D10 | 2365-48-2 | L | ECETOC |
| Tetra aminopyrimidine sulphate | 2A | CO mean ≥ 1 | 2/3 | | | CO pers D7 | 1/3 | | 5392-28-9 | S | Gautheron |
| iso-Butanal | 2B | CO mean ≥ 1 | 2/3 | | | | | | 78-84-2 | L | ZEBET |
| Methyl cyanoacetate | 2A | IR mean ≥ 1 | 3/3 | | | IR pers D7 | 2/3 | | 105-34-0 | L | ECETOC |
| m-Dinitrobenzene | 2B | IR mean ≥ 1 | 2/3 | | | | | | 99-65-0 | S | ZEBET |
| N-Lauroyl sarcosine Na salt (10%) | 2A | Conj mean ≥ 2; CO mean ≥ 1 | 3/3; 2/3 | | | Conj pers D7 | 1/3 | | 7631-98-3 | S (L as tested) | Gautheron |
| Sodium monochloroacetate | 2B | Conj mean ≥ 2 | 3/3 | | | | | | 3926-62-3 | S | ZEBET |
| N,N-Dimethyl guanidine sulphate | NC | CO = 0 ** | ** Conj 1/3 | | | | | | 598-65-2 | S | ECETOC |
| 4-Chloro-4-nitrodiphenylether | NC | CO = 0 | | | | | | | 1836-74-4 | L | ZEBET |
| Tetra aminopyrimidine sulphate | NC | 0 < CO < 1 | | | | | | | 5392-28-9 | S | ECETOC |
| Methanol | NC | 0 < CO < 1 ** | ** CO 1/3 | | | | | | 67-56-1 | L | Gautheron |

## Conclusion

Our analysis clearly demonstrates the importance of understanding the *in vivo* tissue effects which drive eye irritation classification according to the UN GHS system. This builds on recent more general publications in this area (Adriaens *et al.*, Archives of Toxicology 2013). The availability of such a comprehensive list of the drivers of irritation for identified chemicals will be a critical and essential tool when selecting chemicals for development, evaluation and validation of new *in vitro* eye irritation tests. Valuable insights can be taken from our analysis of the DroI: 1) high involvement of corneal effects driving Cat 1, Cat 2A and Cat 2B classifications; 2) when persistence of effects at day 21 drives Cat 1 classification, this is primarily based on corneal effects; 3) the importance of conjunctival effects in classification of Cat 2A versus 2B; 4) low prevalence of iris effects driving classification. Also the physical form of the chemical can have an impact e.g. many more solids than liquids are classified Cat 1 when based on the criterion of CO=4.

Importantly, some inconsistencies within the *in vivo* data could be identified, e.g. : 1) tetra aminopyrimidine sulphate (CAS-No. 5392-28-9) which is classified differently (Cat 2A and NC) in two independent *in vivo* studies; 2) methyl thioglycolate (CAS No. 2365-48-2), which is identified as Cat 1 based on CO=4, even though corneal opacity fully reversed by day 10 of the study.

As such, provided here is a strategy for selecting reference chemicals based on understanding ocular effects that drive irritation in the *in vivo* rabbit Draize test in classification of chemicals. It is proposed that use of this approach would facilitate early and accurate assessment of the performance of a new method, within tiered testing strategies for example, that suggested in the EURL-ECVAM 2005 expert meeting (Scott *et al.*, Toxicology In Vitro 2010).

Presented in this poster is an excerpt of the overall analysis that has been completed for >500 chemicals which will be published in a peer-reviewed journal.