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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Abstract

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 publically available external databases containing *in vivo* eye irritation data for 258 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 Gautheron) and generation of UN GHS classifications
2. Creation of a list of *in vivo* drivers of UN GHS classification, as depicted in Table 1
3. Distribution 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			CO = 4 in at least 1 animal	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 **	
						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

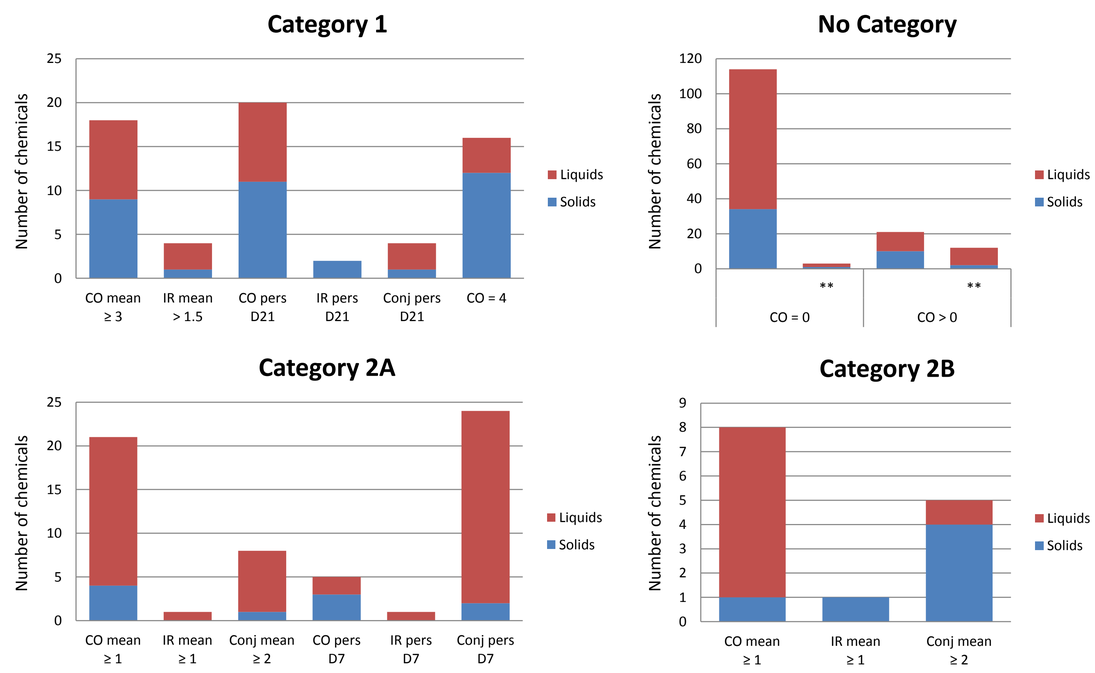

Table 2: Drivers of classification for several representative chemicals selected from 3 publically available databases: ECETOC, ZEBET and Gautheron

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

The importance of understanding drivers of irritation *in vivo* when selecting chemicals for development, evaluation and validation of *in vitro* eye irritation test methods is clearly demonstrated in the analysis presented here. Using such an approach, it is possible to identify key considerations such as: 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. It is also interesting to note that physical form can have an impact e.g. many more solids than liquids are classified Cat 1 when based on the criterion of CO=4. Furthermore, important challenges are identified from this analysis regarding: 1) consistency of data an example of which is tetra aminopyrimidine sulphate which is classified differently (Cat 2A and NC) in two independent *in vivo* studies and 2) interpretation of data an example of which is methyl thioglycolate which is identified as Cat 1 based on CO=4 even though the 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, and of its possible contribution to a tiered testing strategy according to the one, for example, published from the ECVAM 2005 expert meeting (Scott *et al*., 2010).