Cosmetics Europe Eye Programme: Relevance to Integrated Approaches on Testing and Assessment for Serious Eye Damage/Eye Irritation.

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Introduction

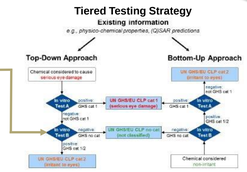
Overall aim of Cosmetics Europe (CE) eye program is to advocate towards better recognition by regulators/external scientific organizations of safety assessment approaches using testing strategies based on alternative methods. The CE program comprises three core focus areas: 1) methods evaluation through optimization/refinement of existing *in vitro* test methods; 2) mechanistic understanding and guidance for industry on selection of chemicals for use in development/evaluation of alternative methods/testing strategies through provision of a comprehensive database of existing *in vivo* data analyzed by drivers of classification and 3) data integration/evaluation of testing strategies/approaches. The outcome of each project provides a means to inform different elements of the modules within the OECD guidance on integrated approaches on testing and assessment (IATA). This poster describes how each project of the eye program contributes to the different modules across the three parts of the IATA.

Focus Area 1: Methods Evaluation

EURL ECVAM / CE Reconstructed human Cornea-Like Epithelium (RhCE) Eye Irritation Validation Study (EIVS)

Study Design

- Discrimination of UN GHS No Cat. from Classified (Cat. 1/Cat. 2) for chemicals in the framework of a Bottom-Up/Top-Down test strategy (Scott *et al*. 2010).
- Chemical selection balanced for classification (Cat. 1/Cat. 2/No Cat) and liquid/solid.
- 105 chemicals tested in EpiOcular™ EIT and SkinEthic™ HCE in replicate tissues in 3 independent laboratories in 3 independent qualified runs.
- 60 solid chemicals tested in EpiOcular™ EIT with an optimised solids protocol.

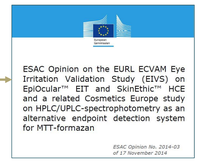


Outcome: EpiOcular™ EIT (60% Cell Viability Cut-Off PM)

Performance	Solids Protocol (Opt)	Liquids Protocol (Orig)	Liquids (Orig) + Solids (Opt)	Study Acceptance Criteria	
				Acceptable	Unacceptable
Sensitivity	93.5%	98.3%	**95.7%**	≥ 90%	< 80%
Specificity	60.7%	66.7%	**63.0%**	≥ 60%	< 50%
Accuracy	78.0%	81.9%	**79.7%**	≥ 75%	< 65%

Reproducibility	% Concordance of Classifications (Original Liquids & Solids Protocols)	Study Acceptance Criteria Acceptable
Within Laboratory (WLR)	**95.2%**	≥ 85%
Between Laboratory (BLR)	**93.3%**	≥ 80%

Issue of ESAC opinion


ESAC Opinion on the EURL ECVAM Eye Irritation Validation Study (EIVS) on EpiOcular™ EIT and SkinEthic™ HCE and a related Cosmetics Europe study on HPLC/UPLC-spectrophotometry as an alternative endpoint detection system for MTT-formazan

ESAC Opinion No. 2014-03 of 17 November 2014

Adoption by OECD

OECD/OCDE 492 Adopted: 28 July 2015

OECD GUIDELINE FOR THE TESTING OF CHEMICALS

Reconstructed human Cornea-like Epithelium (RhCE) test method for identifying chemicals not requiring classification and labelling for eye irritation or serious eye damage

Focus Area 2: Mechanistic Understanding and Importance of Drivers of Classification

Chemical Selection Guidance for Evaluation of *In Vitro* Test Methods

CE compiled a database of Draize data from external lists that were created to support past validation activities. This database contains 681 independent *in vivo* studies on 634 individual chemicals representing a wide range of chemical classes. A description of all the ocular effects observed *in vivo*, i.e. degree of severity and persistence of corneal opacity (CO), iritis (IR), and/or conjunctiva effects (Conj), was added for each individual study in the database, and the studies were categorised according to their UN GHS/EU CLP classification and the main effect driving the classification.

Category 1 28.1% (n=165)					Category 2 * 13.5% (n=79)			No Category 58.4% (n=343)			
Severity * in ≥ 60% of the animals 27.3% (n=45)		Persistence on Day 21 in at least one animal 46.7% (n=77)		Severe CO in at least one animal 20.6% (n=34)	Severity * in ≥ 60% of the animals			in at least one observation time in at least one animal		in all observation times in all animals	
CO mean ≥ 3	IR mean > 1.5	CO	Conj	IR	CO=4	CO mean ≥ 1	Conj mean ≥ 2	IR mean ≥ 1	CO > 0 **	CO > 0	CO = 0 **	CO = 0
73.3% (n=33)	26.7% (n=12)	80.5% (n=62)	19.5% (n=15)	0% (n=0)	100% (n=34)	60.8% (n=48)	38% (n=30)	1.3% (n=1)	8.7% (n=30)	13.1% (n=45)	1.7% (n=6)	76.4% (n=262)

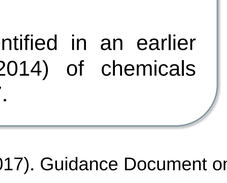
* Mean scores calculated from gradings at 24, 48, and 72 hours after instillation of the test chemical; ** at least one animal with a mean score of days 1-3 above the classification cut-off for at least one endpoint


Cosmetics Europe compilation of historical serious eye damage/eye irritation in vivo data analysed by drivers of classification to support the selection of chemicals for development and evaluation of alternative methods/strategies: the Draize eye test Reference Database (DRD)

João Barroso[1,2] · Uwe Pfannenbecker[3] · Els Adriaens[4] · Nathalie Alépée[5] · Magalie Cluzel[6] · Ann De Smedt[7] · Jalila Hibatallah[8] · Martina Klaric[1] · Karsten R. Mewes[9] · Marion Millet[10] · Marie Templier[10] · Pauline McNamee[11]

Arch Toxicol (2017) 91:521–547 DOI 10.1007/s00204-016-1679-x

REVIEW ARTICLE


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 in vitro test methods

Els Adriaens · João Barroso · Chantra Eskes · Sebastian Hoffmann · Pauline McNamee · Nathalie Alépée · Sandrine Besson-Touya · Ann De Smedt · Bart De Wever · Uwe Pfannenbecker · Magalie Taillhardat · Valérie Zuang

Arch Toxicol (2014) 88:701–723 DOI 10.1007/s00204-013-1156-8

IN VITRO SYSTEMS

Key Outcomes:

- CO most important tissue effect driving (78.2%) Cat. 1
- Cat. 2 mostly driven by CO mean ≥ 1 and Conj mean ≥ 2

This confirms the trends identified in an earlier analysis (Adriaens et al., 2014) of chemicals registered between 1981-2007.

OECD IATA for Serious Eye Damage and Eye Irritation*

Part 1; Module 3
In vitro data from OECD adopted test methods on serious eye damage and eye irritation

Part 1; Module 5
Other data from non-OECD adopted test methods on serious eye damage and eye irritation

Part 1; Module 7
Physicochemical properties (existing, measured or estimated

Part 2; Module 9
Phases and elements of WoE approaches

Part 3; Modules
New testing *in vitro*

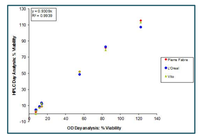
Focus Area 1: Methods Evaluation

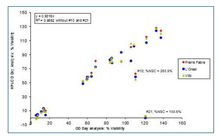
Use of HPLC/UPLC-Spectrophotometry (HPLC/UPLC) in Reconstructed human Tissue (RhT) Test Methods

Approach

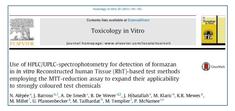
- Addresses a known limitation of the MTT-reduction assay in RhT test methods regarding possible interference of strongly coloured chemicals with measurement of formazan by absorbance (OD).
- 3 independent laboratories qualified their HPLC/UPLC systems to measure formazan from tissue extracts.
- Up to 26 chemicals (coloured and non-coloured) were then tested in RhT test methods for eye/skin irritation and skin corrosion. Dataset subsequently extended to include ingredients with cosmetics functionality.

Results (Example: EpiOcular™ EIT)





- Formazan measurement by HPLC/UPLC is highly reproducible.
- HPLC/UPLC and OD gave almost identical tissue viabilities for chemicals not exhibiting colour interference.
- Independent of the test system used, HPLC/UPLC can measure formazan for strongly coloured chemicals when this is not possible by absorbance only.


Toxicology in Vitro

Use of HPLC/UPLC-spectrophotometry for detection of formazan in in vitro Reconstructed human Tissue (RhT)-based test methods employing the MTT-reduction assay to expand their applicability to strongly coloured test chemicals

N. Alépée*, J. Barroso [2], A. De Smedt [3], B. De Wever [4], J. Hibatallah [5], M. Klaric [6], K.R. Mewes [7], M. Millet [7], U. Pfannenbecker [8], M. Taillhardat [9], M. Templier [9], P. McNamee [10]


Toxicology in Vitro

Assessment of cosmetic ingredients in the in vitro reconstructed human epidermis test method EpiSkin™ using HPLC/UPLC-spectrophotometry in the MTT-reduction assay

N. Alépée [1], J. Hibatallah [2], M. Klaric [3], K.R. Mewes [4], U. Pfannenbecker [5], P. McNamee [6]

Adopted in 2015 into:

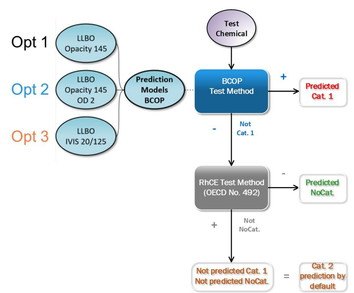
- OECD TG 492 - RhCE eye irritation
- OECD TG 431 - RHE skin corrosion
- OECD TG 439 - RHE skin irritation

Focus Area 3: Data Integration

Development / Refinement of Testing Strategies (TS)

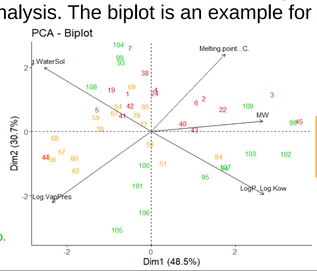
An initial database of chemicals was curated for which *in vivo* and partial *in vitro* data exist. After integration of all *in vitro* data on an industry platform level, remaining data gaps were identified and further *in vitro* testing conducted resulting in a comprehensive *in vivo/in vitro* database of more than 110 chemicals to date. Building on proposed CON4EI testing strategies, the comprehensive database was analysed to determine the robustness of such testing strategies, an example of which is below (also see poster abstract #307) and to identify opportunities for refinement using e.g. physico-chemical properties (also see poster abstract #416).

Example of a testing strategy



Relationship between physico-chemical properties and UN GHS

The relationship between LogP, melting point, vapor pressure, water solubility, surface tension, MW, number of H bond donors/acceptors and UN GHS classification of chemicals was explored by using principal components analysis. The biplot is an example for neat liquids.



GHS (n = 48)
Cat 1 – Chemical No.
Cat 2 – Chemical No.
No Cat – Chemical No.

*Reference: OECD (2017). Guidance Document on an Integrated Approach on Testing and Assessment for Serious Eye Damage and Eye Irritation. Series on Testing and Assessment No. 263. ENV Publications, Organisation for Economic Cooperation and Development, Paris.