CON4EI: EpiOcular Eye Irritation Test (EIT)

1 MatTek In Vitro Life Sciences Laboratories, Bratislava, Slovakia;
2 Flemish Institute for Technological Research (VITO NV), Applied Bio & Molecular Systems, Mol, Belgium;
3 Adriaens Consulting bvba, Aalter, Belgium;
4 L'Oréal Research & Innovation, Aulnay-sous-Bois, France;
5 Institute of Industrial Organic Chemistry Branch Pszczyna, Department of Toxicological Studies, Pszczyna, Poland;
6 Envigo, Derbyshire, United Kingdom;
7 Cyprotex US, LLC, Kalamazoo, MI 49008, USA

Helena Kandarova[1], An Van Rompay[2], Els Adriaens[3], Nathalie Alépée[4], Agnieszka Drzewiecka[5], Przemyslaw Fochtman[5], Katarzyna Gruszka[5], Robert Guest[6], Gareth Maglennon[6], Jane Schofield[6], Jamin A. Willoughby[7], Sandra Verstraelen[2]

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Abstract

Assessment of the acute eye irritation potential is part of the international regulatory requirements for testing of chemicals. The objective of the CON4EI (CONsortium for *in vitro* Eye Irritation testing strategy) project is to develop tiered testing strategies for eye irritation assessment for all drivers of classification. For this, a set of 80 reference chemicals (38 liquids and 42 solids) was tested with eight different alternative methods. Here, the results obtained with reconstructed human cornea-like epithelium (RHCE) EpiOcular and the EpiOcular Eye Irritation Test (EIT) -adopted as OECD TG 492 - are shown.
The primary aim of this study was an evaluation of the performance of the test method to discriminate chemicals not requiring classification for serious eye damage/eye irritancy (No Category) from chemicals requiring classification and labelling (Category 1 and 2). In addition, the predictive capacity in terms of *in vivo* driver of classification was investigated. In a second step, it was investigated whether the EpiOcular EIT can be used as part of a tiered-testing strategy for eye irritation assessment. The chemicals were tested in two independent runs by MatTek IVLSL.
For the EpiOcular EIT, a sensitivity of 96.9% and specificity of 86.7% with an accuracy of 95% was obtained overall and for both runs separately (100% concordance). The results of this study seem promising with regard to the evaluation of inclusion of this test method in an integrated testing strategy for eye irritation assessment.
This research is funded by CEFIC-LRI. We acknowledge Cosmetics Europe for their contribution in chemical selection.

Keywords: CON4EI, EpiOcular EIT, eye irritation/corrosion, ocular irritation assay, *in vitro* novel irritation testing; testing strategy

Methods

CHEMICALS

Eighty chemicals were tested under blind conditions. The set of test substances was composed of 15 chemicals not requiring classification (No Cat) and 65 chemicals requiring classification (27 Cat 2 and 38 Cat 1).
The 80 chemicals were tested twice by MatTek IVLSL, Bratislava, Slovakia. Within a single experiment, Negative Control (NC) and Positive Control (PC) were concurrently tested on 2 tissues replicates.

TEST SYSTEM

The EpiOcular tissues are cultured at the air-liquid interface (**Figure 1**) which allows attainment of in vivo-like differentiation (**Figure 2**) and allows topical application of test articles. Normal human cells derived from tissue explants are used to produce the tissues. The EpiOcular tissues are produced under Good Manufacturing Practices (GMP).

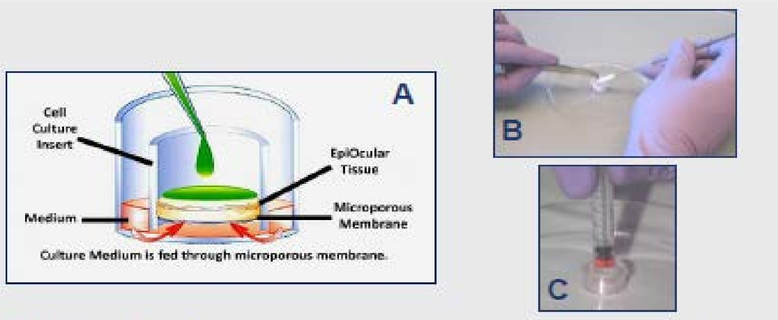


Figure 1. A. A schematic representation of topical EpiOcular™ tissue dosing. Application of solid materials **B.** with the Ted Pella spatula and **C.** with the stuffed syringe (with its head cut off)

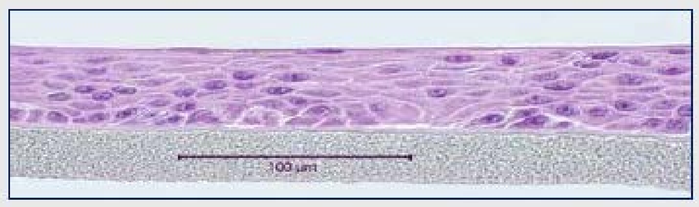
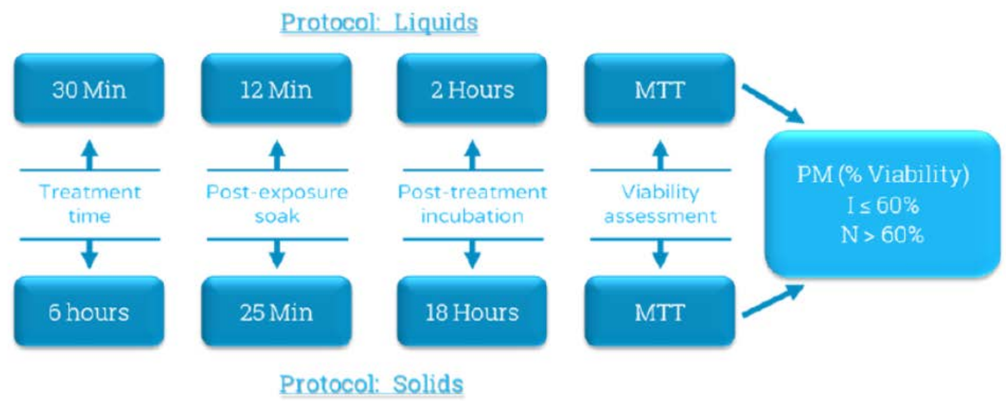


Figure 2. EpiOcular™ (OCL-200) model - representative formalin-fixed, paraffin embedded, H&E stained tissue cross-section. Tissue structure closely parallels human corneal epithelium.

ACCEPTANCE CRITERIA

The results of an experiment are accepted if:

- The negative control Optical Density (OD) should be > 1.0 and < 2.6.
- The mean relative viability of the positive control is: (a) 30 minute exposure: below 60% of control viability and (b) 6 hour exposure: below 60% of control viability
- The difference of viability between the two relating tissues of a single chemical is < 20% in the same run (for positive and negative control tissues and tissues of single chemicals).
- This applies also to the killed controls (single chemicals and negative killed control) and the colorant controls which are calculated as percent values related to the viability of the relating negative control.

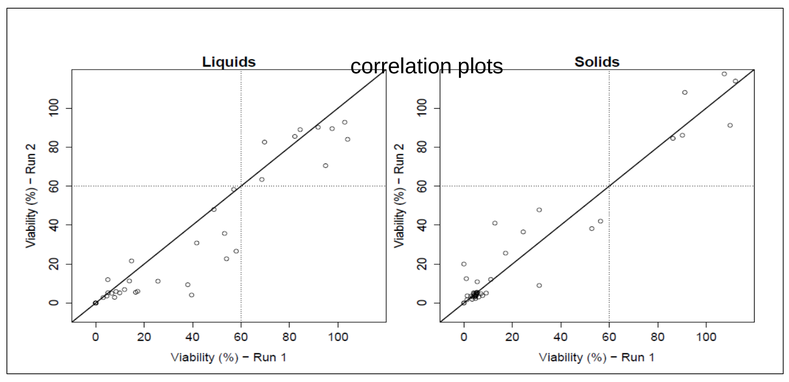


In vitro Result Classification (In vivo Prediction)
Mean tissue viability > 60% Non-Irritant (NI), No category – NO FURTHER TESTING REQUIRED
Mean tissue viability ≤ 60% Likely Irritant (I), Category 1 or Category 2 – FURTHER TESTING REQUIRED

Results

Concordance in predictions: For the reproducibility in terms of the classification I versus NI, 100% agreement in prediction between run 1 and run 2 was obtained.

Reproducibility of the viability:
The variability of the viability between the two independent runs was assessed descriptively. The high reproducibility between the runs was reflected in the correlation plots. The plot illustrates the two-by-two relation between the experiments for liquids and solids separately. Dots close to the line of equality indicate similar levels of viability for the independent experiments.



correlation plots

Predictive capacity: The predictive capacity was calculated for each run and for the cumulative results of the two runs.
A sensitivity of 96.9% and specificity of 86.7% with an accuracy of 95% was obtained overall and for both runs separately.

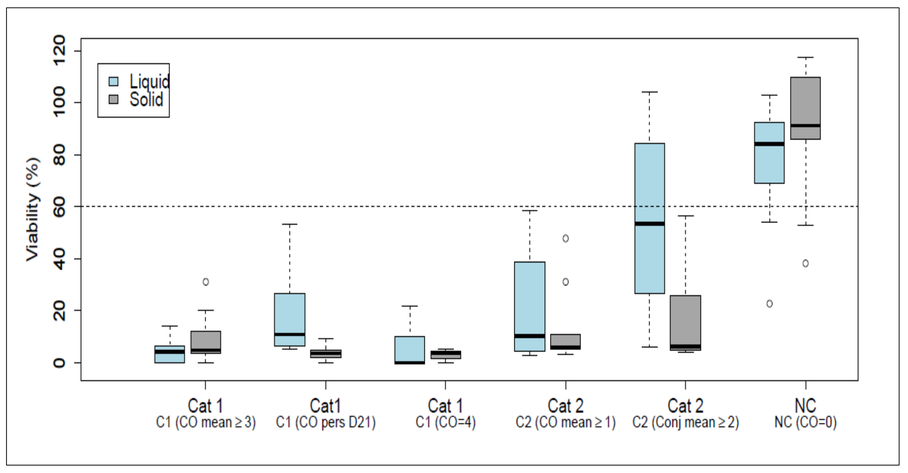


Figure 3. Boxplot showing the distribution of the viability in function of GHS Driver of classification. The dotted lines correspond with the cut-off (60%) that distinguishes I from NI.

Overall, four false negatives were reported, the following in vivo Cat 2 chemicals (both liquids) were consistently predicted NI in the two runs: Methyl cyanoacetate (No. 53, in vivo Cat 2A with Conj mean 2 as driver) and 2-Psuedoionone (No. 58, in vivo Cat 2B with Conj mean 2 as driver).

In addition, four false positives were reported, one liquid chemical (No. 66: 1,2,6-Hexanetriol) and one solid chemical (No. 74: 1-(9H-Carbazol-4-yloxy)-3-[[2-(2-methoxyphenoxy)ethyl]amino]propan-2-ol) were consistently predicted I in the two runs.

Summary

This report describes the results obtained with the EpiOcular EIT method (OECD TG 492) as a part of the CON4EI LRMI project where a set of 80 reference chemicals was tested with 8 different alternative methods.

- For the EpiOcular EIT method, 100% concordance in predictions (I versus NI) between the two runs was obtained.
- The accuracy of the EpiOcularTM EIT method was 95% with 96.9% sensitivity and 86.7% specificity.

The results of this study seem promising with regard to the evaluation of inclusion of this test method in an integrated testing strategy (ITS) for eye irritation assessment.

CON4EI LRMI project has been supported by Cefic.
This project aimed into assessment of reliability of **8 *in vitro* test systems**:

1) BCOP (Bovine Corneal Opacity and Permeability
2) BCOP-LLBO (BCOP-laser light-based opacitometer),
3) ICE (Isolated Chicken Eye),
4) EpiOcular-EIT (EpiOcular Eye Irritation Test)
5) EpiOcular-ET50,
6) SkinEthic™ HCE (Human Corneal Epithelial),
7) STE (Short Term Exposure), and
8) SMI (Slug Mucosal Irritation) to define eye irritation potential.

The Project defined applicability domains, strengths and limitations of the selected in vitro test systems. It will help to identify a tiered-testing strategy for eye irritation assessment.

Timeline: March 2015 - March 2016
LRI funding of the whole project: € 548.000