



Ocular Irritation and Corrosion Services



Leading the Field of *In Vitro* Toxicology

CeeTox can help you achieve your regulatory, product safety, EU cosmetic directive, Amendment 7 and REACH directives plus help you reduce the need for *in vivo* testing with its *in vitro* Ocular Corrosion and Irritation services.

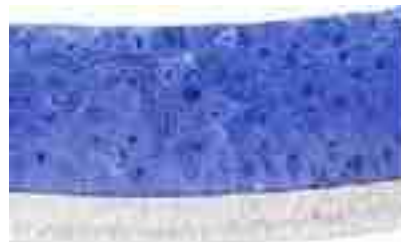
Eye Corrosion [serious eye damage] is defined as "the production of tissue damage in the eye, or serious physical decay of vision, following application of a test substance to the anterior surface of the eye, which is *not* fully reversible within 21 days of application" (UNECE, 2004).

Eye Irritation is defined as "the production of changes in the eye following the application of test substance to the anterior surface of the eye, which are fully reversible within 21 days of application" (UNECE, 2004).

CeeTox will evaluate and characterize compounds for their potential to cause corrosion or irritation to the eye using an *in vitro* 3-D human corneal epithelial model. Peer-reviewed validation studies show greater than 80% concordance of *in vitro* results to the Draize eye irritation test.

Reconstructed Human Corneal Epithelial models result from fully differentiated three-dimensional reconstituted human corneal culture grown on the air-liquid interface for 7 days in defined growth medium. The system consists of transformed human corneal epithelial cells cultivated on a polycarbonate filter which forms corneal epithelial tissue (mucosa) devoid of stratum corneum. The HCE ultra-structurally resembles in tissue morphology and thickness the corneal mucosa of the human eye. The reconstructed human corneal epithelium secretes the same mucins that are being found in the human cornea *in vivo*. Ultra-structural results show the typical presence of a columnar basal cell layer, 2-3 layers of transitional wing cells, and 2-3 layers of superficial squamous cells. CeeTox routinely uses corneal epithelial models produced by SkinEthic and MatTek which are available in multiple formats including various size culture inserts (0.38, 0.5, 1, and 4 cm²) and plates for high-throughput screening.

Human Corneal Epithelium Model



In Vitro (semi-thin)



In Vitro (light microscopy)

Viability Measurement

The magnitude of viability is quantified by using MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, thiazolyl blue) or other metabolically converted vital dyes.

Irritancy Classification

Irritancy classification is determined by the time (minutes) of exposure to test article for cells to reach 50% reduction in viability versus control:

$$\% \text{ viability} = 100 \times [\text{OD (sample)}/\text{OD (negative control)}]$$

Fig.1 Irritancy classifications of Draize MAS and EpiOcular ET₅₀ (Stern, 1998)

Draize		
Classification		Score Range
Non	A	0
Minimal	B	0.1 - 15.0
Mild	C	15.1 - 25.0
Moderate	D	25.1 - 50.0
Severe	E	50.1 - 80.0
Extreme	F	80.1 - 110.0

<i>In Vitro</i> Corneal Epithelium		
Classification		ET ₅₀ Range (mins of exposure)
Non / Minimal	A/B	> 60
Mild	C	31 - 60
Moderate	D	3 - 30
Severe / Extreme	E/F	< 3

Cytokine Release

In order to rank test articles which fall within the non-to mild irritant classification, CeeTox recommends measuring Interleukin-1 α release into the culture media by ELISA at each time point. Interleukin-1 α release into the culture media will be assayed by ELISA at each time point as a measurement of corneal irritation.

Standard Panel

The standard panel includes a single concentration (dose), two time-points (10 minutes and 60 minutes), three replicates, positive and negative controls. Histology and IL-1 α are optional. Additional doses, time-points and assays can be added depending on study objectives.

Additional Assays Available

Cytokine Release (IL-6- α , IL-8- α , TNF- α)
 Oxidative Stress
 Glutathione Levels
 Mitochondrial Function

Experimental Outline

The test consists of a topical exposure of the neat test chemical onto the reconstructed human corneal epithelium model HCE, followed by cell viability and IL-1 α assessment. Viability decrease in chemical treated tissues is expressed comparatively to negative controls (PBS treated). Cell direct or indirect toxicity can contribute to induce or initiate inflammatory processes in tissues leading to possible irritation signs.

Percent (%) viability is used to predict and classify eye irritation. Each exposure concentration and exposure time is done in replicates of three (3) wells. Following each exposure time period the cells are assayed for MTT and IL-1 α release.

Specific optical properties (color) of the test substance or its particular interaction with the reagent (MTT reducers) or the tissue (tissue penetration) may lead to some false estimates of the viability. In these cases additional controls must be used to detect and correct the test substance interference with the measurement.

When applicable, solubility of the final test solutions will be determined at the start of the experiment and at the end. Solubility is measured by nephelometry, a system that measures light scattering in the solutions.

Reports

Reporting may take three basic forms. Our standard reporting includes data charts and graphs detailing results of the assays run. A detailed report is optionally available as well. This report includes:

- Executive Summary
- Objective
- Experimental Design
- Results
- Tables and Figures
- Materials and Methods
- Appendix (if necessary)

Finally, a report complying with GLP requirements is available for those studies performed according to GLP regulations.

Ocular Corrosion & Irritation	
Purpose of Assay	Evaluation and characterization of compounds for their potential to cause toxicity or irritation to the eye.
Cell Model	Reconstructed Human Corneal Epithelial (HCE) Model
Assays Performed	MTT Reduction, IL-1 α release (optional), Histology (optional)
Controls	Vehicle, positive, negative and no compound controls
Number of Concentrations	1
Number of Replicates	3
Number of Time Points	2 (10 min, 60 min)
Standard Turn Around Time	3 weeks from sample and tissue receipt

References:

Human corneal epithelium model from <http://www.skinethic.com/HCE.asp>

Stern M., Klausner M., Alvarado R., Renskers K., and Dickens M. (1998). Evaluation of the EpiOcular Tissue Model as an Alternative to the Draize Eye Irritation Test. *Toxicology in Vitro* 12: 455-461

UNECE Globally Harmonized System (GHS) for Classification and Labeling of Chemicals, (2004), 137

