TEST CODE:
CT-033

Eye Irritation Test

RECONSTRUCTED HUMAN CORNEA-LIKE EPITHELIUM METHOD (OECD TEST GUIDELINE 492)

OVERVIEW

Eye irritation is defined as changes in the eye following the application of a test chemical to the surface, which are fully reversible within 21 days of application.[1]

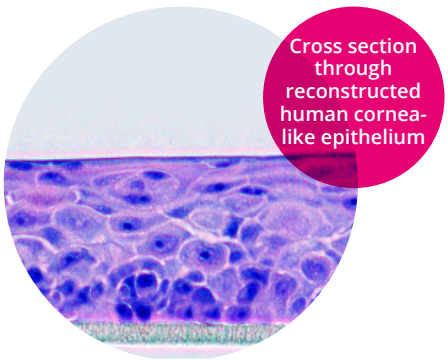
The test described here is fully accepted at a regulatory level for the hazard identification of chemicals not requiring classification and labelling for eye irritation or serious eye damage. According to UN GHS, these are defined as chemicals that do not decrease tissue viability below a defined threshold (i.e., tissue viability >60% for UN GHS No Category). It can be used for single substances and for mixtures including finished products. It is appropriate for compliance with a range of legislation including REACH (Registration, Evaluation, Authorisation and restriction of CHemicals) and the EU Cosmetics Regulation 1223/2009. The test is based on the depth of injury model or Maurer and Jester, using the rationale that the degree of irritation caused by a test substance correlates with the degree of penetration into cell layers and subsequent impact on cell viability.

The method utilises reconstructed human cornea-like epithelium (RhCE), which in its overall design mimics the biochemical and physiological properties of the corneal epithelium of the human eye. The test item is applied directly to the cornea surface, providing a good model of "real life" exposure.

Cell viability is measured by enzymatic conversion of the vital dye MTT into a blue formazan salt that is quantitatively measured after extraction from the skin tissues. If the viability is greater than 60%, the test item is classified as Non-Irritant ("no-label", or UN GHS No Category). If the viability is 60% or lower, then no prediction can be made and further testing may be required.

TEST SYSTEM: RECONSTRUCTED HUMAN CORNEA-LIKE EPITHELIUM

Reconstructed human cornea-like epithelium is a corneal model composed of living human cells which have been cultured to form a multi-layered, differentiated corneal epithelium. The levels of differentiation obtained are at the cutting edge of *in vitro* tissue technology. The model consists of highly organized basal cells which progressively flatten out as the apical surface of the tissue is approached, analogous to the normal human *in vivo* corneal epithelium. The profiles of key differentiation markers also mirror those seen in *vivo.* The cells are both metabolically and mitotically active, and release many of the pro-inflammatory agents (cytokines) known to be important in eye irritation and inflammation. Reconstructed human cornea-like epithelium is grown on special platforms at the air-liquid interface, allowing for direct application of test items in a way that accurately models "real life" eye exposure.



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SUMMARY OF THE TEST METHOD

- Corneal models are pre-warmed in a cell culture incubator (37°C / 5% CO_2) for 60 minutes or overnight. The culture medium is replaced prior to applying treatment.
- The test item is applied to the surface of the corneal models: triplicate models are dosed at the apical surface with 50µl (liquid) or 50mg (solid).
- Controls consist of ultrapure water (negative control) and methyl acetate (positive control).
- The dosed cornea models are placed into a cell culture incubator for 30 minutes (liquids) or 6 hours (solids).
- Test items and control substances are removed from the cornea models' surface by washing.
- Following a post-exposure soak in culture medium, the viability of the corneal models is assessed by MTT conversion. MTT solution is applied to the surface of the models and placed into a cell culture incubator for 3 hours. The blue formazan metabolite produced by viable cells is then extracted into isopropanol by incubation at room temperature for 2 hours.
- Triplicate samples of the extracted formazan solution are transferred to a microplate and the formazan product is quantified by absorbance spectrophotometry (wavelength 570nm).
- Absorbance readings of the formazan product from corneal models incubated with test items are compared with those of negative controls to calculate percentage viability.
- A range of acceptance criteria must be satisfied in accordance with the OECD Test Guideline.
- If the viability is greater than 60%, the test item is classified as Non-Irritant ("no-label" or UN GHS No Category). If the viability is 60% or lower, then no prediction can be made and further testing may be required.

TURNAROUND TIME	*5 – 8 weeks*
AMOUNT OF SAMPLE REQUIRED	*10ml (liquids) / 10g (solids) Please enquire if sample availability is limited.*
PRICE	*Our test prices are dependent on the quantity of test items. Please enquire for a quote using the contact information shown below, or the contact form on our website.*
FURTHER DOWNLOADS	OECD Test Guideline 492 XCellR8 Good Laboratory Practice (GLP) Compliance Certificate.
REFERENCES	[1]*UN (2009), United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS), Third revised edition, UN New York and Geneva.*

QUALITY STATEMENT

XCellR8 is accredited by the UK Medicines and Healthcare Products Regulatory Authority (MHRA) for the conduct of *in vitro* safety testing in compliance with Good Laboratory Practice (GLP). This means that we are able to provide our clients with test results that may be used at a regulatory level to demonstrate product safety, where the test is an approved regulatory method. The regulatory status is applicable in all global territories participating in the OECD's Mutual Acceptance of Data.

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