TEST CODE
105

Eye Hazard Identification Test

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

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. Serious eye damage refers to the production of tissue damage in the eye, or serious physical decay of vision, which is not fully reversible.

The test described here is fully accepted at a regulatory level for the hazard identification of chemicals by discriminating between the three categories for serious eye damage/eye irritation as defined by UN GHS. According to UN GHS, chemicals inducing serious eye damage are classified as Category 1, those inducing eye irritation are classified as Category 2 (A or B), and chemicals that do not meet the criteria for Categories 1 or 2 are referred to as No Category. The test is recommended as a full replacement to the *in vivo* Draize acute eye irritation test. 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 time-to-toxicity model, using the rationale that the degree of irritation caused by a test substance correlates with the time required to affect 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 tissues. Test items are classified by their ability to decrease cell viability below defined threshold levels.

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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.

SUMMARY OF THE TEST METHOD

- Corneal models are pre-warmed in a cell culture incubator (37°C / 5% CO_2) for at least 30 minutes or overnight. The culture medium is replaced prior to applying treatment.
- **The test item is applied to the surface of the corneal models:**
  For liquids: Two tissues per timepoint (6 total for liquids, 4 total for solids) are dosed at the apical surface with 80µl. Neat test item is applied for 5 minutes, while 20% (w/v) dilutions in water are applied for 16 and 120 minutes. For solids: Two tissues per timepoint are dosed at the apical surface with 80mg, followed by 80µl sterile water. Exposure times are 30 minutes and 120 minutes.
- Controls consist of Dulbecco's Phosphate Buffered Saline as a negative control, methyl acetate as the positive control for liquids, and 1% lactic acid solution as the positive control for solids.
- Test items and control substances are removed from the cornea models' surface by washing with 25ml sterile DPBS.
- Following a post-exposure soak in culture medium (10 ± 1 minutes for liquids, 30 ± 2 minutes for solids), 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 at least 2 hours.

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- Duplicate 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.

GHS/OECD CLASSIFICATION CRITERIA FOR TISSUE VIABILITY

Physical State	Category 1 (Corrosive)	No Category (Non-Corrosive)	Category 2 (Irritant)
Liquids	Mean viability ≤ 50% at 5, 16, and 120 minutes	Mean viability > 50% at 5, 16, and 120 minutes	Any other combination
Solids	Mean viability ≤ 40% at 30 min AND ≤ 60% at 120 min	Mean viability > 40% at 30 min AND > 60% at 120 min	Any other combination

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 above, or the contact form on our website.

REFERENCES

OECD (2022), Test Guideline No. 492B: Reconstructed Human Cornea-like Epithelium (RhCE) Test Method for Eye Hazard Identification, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris.

DB-ALM Protocol: In *vitro* eye hazard identification time-to-toxicity test methods on liquids (TTL) and solids (TTS) using SkinEthic™ human corneal epithelium model (HCE) (June 2020).

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