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

Commonly used acronym: *RhCE test method, OECD TG 492*

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Organisation

Name of the organisation Vlaamse Instelling voor Technologisch Onderzoek (VITO)
Department Health
Country Belgium
Geographical Area Flemish Region

SCOPE OF THE METHOD

The Method relates to	Human health
The Method is situated in	Regulatory use - Routine production
Type of method	In vitro - Ex vivo
Specify the type of cells/tissues/organs	Human immortalized corneal epithelial cells for SkinEthic™ HCE EIT test method

DESCRIPTION

Method keywords

RhCE
HCE EIT
Epidermal model
3D model
cytotoxicity
Tissue viability
MTT assayRhCE
MTT assay
eye irritation

Scientific area keywords

Chemical testing
toxicity testing
hazard assessment
Ocular irritation

Serious Eye Damage

Method description

Four validated test methods using commercially available RhCE models are included in this Test Guideline 492. Here we focus on the SkinEthic Human Corneal Epithelium (HCE) Eye Irritation Test (EIT). It is an *in vitro* procedure allowing the identification of chemicals (substances and mixtures) not requiring classification and labelling for eye irritation or serious eye damage in accordance with UN GHS. It makes use of reconstructed human cornea-like epithelium (RhCE) which closely mimics the histological, morphological, biochemical and physiological properties of the human corneal epithelium. The purpose of this Test Guideline is to describe the procedure used to evaluate the eye hazard potential of a test chemical based on its ability to induce cytotoxicity in a RhCE tissue construct, as measured by the tetrazolium dye, i.e. MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide. RhCE tissue viability following exposure to a test chemical is measured by enzymatic conversion of the vital dye MTT by the viable cells of the tissue into a blue MTT formazan salt that is quantitatively measured after extraction from tissues. The viability of the RhCE tissue following exposure to a test chemical is determined in comparison to tissues treated with the negative control substance (%viability), and is then used to predict the eye hazard potential of the test chemical. Test chemicals absorbing light in the same range as formazan dye and test chemicals able to directly reduce the vital dye may interfere with the tissue viability measurements and need the use of adapted controls for corrections. The test chemical is applied topically to a minimum of two three-dimensional RhCE tissue constructs and tissue viability is measured following exposure and a posttreatment incubation period. The RhCE tissues have been cultured for several days to form a stratified, highly differentiated squamous epithelium morphologically similar to that found in the human cornea. The SkinEthic™ HCE RhCE tissue construct consists of at least 4 viable layers of cells including columnar basal cells, transitional wing cells, and superficial squamous cells similar to that of the normal human corneal epithelium.

Lab equipment

- Standard equipment for working with cell cultures;
- Microplate reader (OD) or HPLC/UPLC-spectrophotometer.

Method status

Validated by an external party (e.g. OECD, EURL ECVAM,…)

PROS, CONS & FUTURE POTENTIAL

Advantages

- Use of commercially available RhCE tissue constructs.
- Recommended method to identify chemicals that do not require classification for eye irritation or serious eye damage according to UN GHS.
- Similar to the *in vivo* corneal epithelium three-dimensional structure and are produced using cells from the species of interest.
- Directly measures cytotoxicity resulting from penetration of the chemical through the cornea and production of cell and tissue damage following chemical exposure, which determines the overall *in vivo* serious eye damage/eye irritation response.
- Can be used on a variety of chemical types, chemical classes, molecular weights, LogPs, chemical structures, etc.

- Mainly for mono-constituent substances, but also for several multi-constituent substances.
- Applicable to substances and mixtures, and to solids, liquids, semi-solids and waxes. The liquids may be aqueous or non-aqueous; solids may be soluble or insoluble in water. Whenever possible, solids should be ground to a fine powder before application; no other pre-treatment of the sample is required.
- High level of reproducibility within- and between laboratories.
- High level of accuracy.

For more information: see OECD TG nr. 492

Challenges

- This method does not allow discrimination between eye irritation/reversible effects on the eye and serious eye damage/irreversible effects on the eye, nor between eye irritants and mild eye irritants.
- Gases and aerosols have not been assessed in a validation study.
- Test chemicals absorbing light in the same range as formazan dye (FD, naturally or after treatment) and test chemicals able to directly reduce the vital dye TD (to FD) may interfere with the tissue viability measurements and need the use of adapted controls for corrections.
- It is currently generally accepted that, in the foreseeable future, no single *in vitro* test method will be able to fully replace the *in vivo* Draize eye test to predict across the full range of serious eye damage/eye irritation responses for different chemical classes. However, strategic combinations of several alternative test methods within (tiered) testing strategies such as the Bottom-Up/Top-Down approach may be able to fully replace the Draize eye test.

For more information: see OECD TG nr. 492

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

References

Van Rompay AR, Alépée N, Nardelli L, Hollanders K, Leblanc V, Drzewiecka A, Gruszka K, Guest R, Kandarova H, Willoughby Sr JA, Verstraelen S, Adriaens E, 2018. “CON4EI: SkinEthic™ Human Corneal Epithelium Eye Irritation Test (SkinEthic™ HCE EIT) for hazard identification and labelling of eye irritating chemicals.” Toxicol *In Vitro*. 49, 11-20. DOI: 10.1016/j.tiv.2017.06.012

Associated documents

OECD Test No. 492.pdf

Links

Reconstructed human Cornea-like Epithelium (RhCE) test method for identifying c…

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