JRF GLOBAL

Reconstructed Human Cornea-like Epithelium (RHCE) Test Method for Eye Hazard Identification (OECD 492B)

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INTRODUCTION

1. Serious eye damage refers to the production of tissue damage in the eye, or serious physical decay of vision, which is not fully reversible, occurring after exposure of the eye to a test chemical.

2 - Eye irritation refers to the production of changes in the eye, which are fully reversible, occurring after exposure of the eye to a test chemical.

3. Test chemicals not classified for eye irritation or serious eye damage are defined as those that do not meet the requirements for classification as UN GHS Category 1 or 2 (2A or 2B) i.e., they are referred to as UN GHS No Category (No Cat).

4. This test guideline describes a validated test method using a commercially available reconstructed human cornea-like epithelium (RhCE) model, namely the SkinEthic™ Human Corneal Epithelium (HCE) Time-to-Toxicity (TTT) test.

5. The purpose of this TG 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 MTT (CAS RN 298-93-1).

IMPORTANCE

1. This test is modified version of OECD 492
2. Animal testing is strongly discouraged by the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) regulation (No 1907/2006) and is only acceptable as a last resort when all other data sources have been exhausted.
3. There are various *in vitro* methods develop previously like BCOP, RhCE, Chicken eye test.... but all the tests are not able to identify eye irritant, it only able to classify in Category 1 (corrosive) and No category (non-irritant).
4. OECD 492b is the first *in vitro* standalone method which can predict three categories given by UNGHS (Cat 1, 2 and No cat).
5. The test method was recommended as a full replacement to the *in vivo* Draize acute eye irritation test for classification of chemicals.

PROCEDURE

- 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 post-soak incubation period
- The assay used for quantifying tissue viability is the tetrazolium dye (MTT) assay. Viable cells of the RhCE tissue construct reduce the vital dye MTT into a blue MTT formazan precipitate, which is then extracted from the tissue using isopropanol (or a similar solvent)
- The measurement of viability of the RhCE tissue construct after topical exposure to a test chemical to identify chemicals not requiring classification for serious eye damage/eye irritancy (UN GHS No Category) is based on the assumption that all chemicals inducing serious eye damage or eye irritation will induce cytotoxicity in the corneal epithelium and/or conjunctiva
- The extracted MTT formazan (from tissues) may be quantified by a standard absorbance (OD) measurement at 570 nm using microplate reader

REFERENCES

1. OECD, 2022: The Organisation for Economic Co-operation and Development (OECD) Guideline for the Testing of Chemicals, OECD 492b, " Reconstructed human Cornea-like Epithelium (RhCE) test method for identifying chemicals not requiring classification and labelling for eye irritation or serious eye damage" adopted by the Council on June 30, 2022

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