Alternative *in-vitro* methods for the Replacement of *in-vivo* Eye Irritation Testing

Introduction

An ocular toxicity testing is required for the evaluation of risk associated with the harmful test items on our eyes. Moreover, location, physiology, and sensitivity of the corneal surface tend our cornea to expose to a variety of potentially hazardous chemicals often. Various chemicals have the potential to damage cornea, varying from irritation to tissue corrosion, resulting in irreversible blindness[1].

Traditionally, animal tests (such as Draize test) were exclusively used to determine the level of ocular irritation potential of the test item, post-exposure to the eyes of the rabbit and evaluating the biological response as a result of it[2,3]. Significant regulatory attempts are being made to replace, refine, and reduce the *in-vivo* testing to assess the risk posed by chemicals of the end-products to the human eyes. Legislation have been introduced to reduce animal testing and promote alternative techniques. These techniques include organotypic assays on deceased animals' tissues and *in-vitro* assays based on the two-dimensional and three-dimensional assays[4].

Out of numerous *in-vitro* methods, only a few have been accepted by the OECD, under various Test Guidelines. JRF conducts *in-vitro* Bovine Corneal Opacity and Permeability test (BCOP) and Three-dimensional corneal epithelial model (using the SkinEthic™ human corneal epithelium (HCE) model) tests for the determination of the eye irritation potential of a test item using and *in-vivo* assay too, under certain circumstances further to classify the test item in Category 2A or 2B. The test item can be classified by combining these methods, based on the current GHS classification system.

Three-dimensional Corneal Epithelial Model

Three-dimensional Reconstructed human Cornea-like Epithelium (RhCE) uses primary human cells, which have been cultured for several days to form a stratified, highly differentiated squamous epithelium morphologically like that found in the human cornea. The SkinEthic™ HCE tissue construct consists of, at least, 4 viable cell layers, including columnar cells and wing cells, with the presence of intermediate filaments, mature hemi-desmosomes and desmosomes, and specific human corneal cytokeratins. The test chemical is applied topically to RhCE tissue constructs. Following the exposure and post-treatment incubation periods, tissue viability is assessed by the enzymatic conversion in viable cells of the vital dye MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Thiazolyl blue tetrazolium bromide) into a blue MTT formazan salt which is extracted from the tissues and quantitatively measured. This model is intended to differentiate materials that are non-irritants from those that would require labeling either as GHS category 1 or category 2, but the assay is not able to distinguish between GHS category 1 and category 2. For these purposes, further testing with other *in-vitro* test methods is required[5].

Scope:

- human-based tissue models
- used for the testing of substances and mixtures
- can be used within the Top-Down (begin with using test methods that can accurately identify severe irritants) and Bottom-Up (begin with using test methods that can accurately identify non-irritants) approaches and to initiate the Bottom-Up approach
- test chemicals that do not require a classification for eye hazard

Limitations:

- not recommended for the identification of chemicals which should be classified for eye irritation or serious eye damage
- gases and aerosols cannot be tested[6]

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Bovine Corneal Opacity And Permeability Test (BCOP)

The BCOP test method is an organotypic *ex*-vivo assay using isolated corneas from the eyes of freshly slaughtered cattle. Test chemicals are applied to the epithelial surface of the cornea. Damage by the test item is assessed by quantitative measurements of:

I. **Corneal opacity changes, measured as the amount of light transmission through the cornea; and**

ii. **Permeability, measured as the amount of sodium fluorescein dye that passes across the full thickness of the cornea, detected with the help of a visible light spectrophotometer.**

Based on these results, the test item is classified in GHS Category 1, i.e., severe irritant, GHS category 2, i.e., irritant or 3, GHS No category, i.e., non-irritant.

This model cannot be used to classify the test item under GHS category 2A or 2B and further testing, using *in-vivo* study, will be required as no *in-vitro* test is capable of classifying test items in this category[7].

Scope:

- **used for the testing of substances and mixtures**
- **addresses corneal effects, which are one of the major drivers of classification *in-vitro***
- **can be used within the Top-Down (begin with using test methods that can accurately identify severe irritants) and Bottom-Up (begin with using test methods that can accurately identify non-irritants) approaches and to initiate the Bottom-Up approach**

Limitations:

- **potential overprediction for alcohols and ketones and false-negative predictions for solids**
- **not suitable for an assessment of the potential for systemic toxicity associated with ocular exposure**
- **gases and aerosols cannot be tested[6]**

References

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