Alternative *in-vitro* methods for the replacement of *in-vivo* eye irritation testing

Eye is used for the visual perception of one's surroundings. The surface of the eye is protected with a thin transparent tissue, i.e., cornea [1]. The location, physiology, and sensitivity of the corneal surface tend cornea to expose to a variety of potentially hazardous chemicals on a regular basis. Various chemicals can damage cornea varying from irritation to tissue corrosion resulting in an irreversible blindness [2]. For the evaluation of these chemicals and manufactured end products, the Food and Drugs Administration came up with a standard assay, for acute ocular toxicity in the rabbit, i.e., *in-vivo* Draize eye test method [3]. This method is government endorsed protocol, accepted by the Organization for Economic Co-operation and Countries Development [4].

New Zealand white rabbits are commonly used in this test. A maximum of three rabbits of either sexes are exposed to a dosage volume of 0.1 mL (or 0.1 g solid) of the test substance.

Based on results obtained, using Draize eye test, the test item can be classified either as severely irritant or corrosive substance. Moreover, the test item can be differentiated as mild irritant or severe irritant [4].

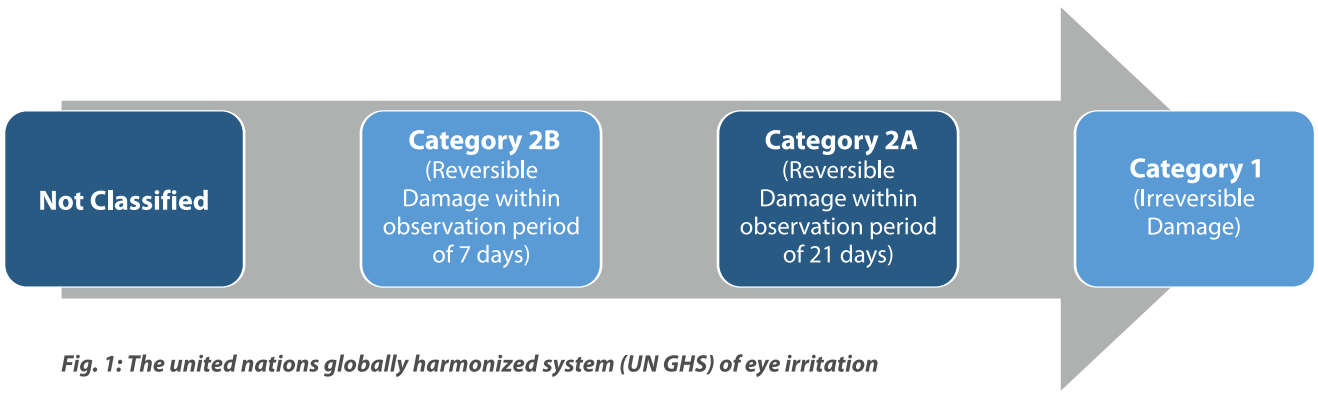

Fig. 1: The united nations globally harmonized system (UN GHS) of eye irritation

However, this test has drawn scientific and ethical criticism. Points of criticism include not only the methodology but also species-specific differences regarding eye's physiology, use, and interpretation of scores, reproducibility, relevance of findings, and ethical issues. In addition, recent legislative changes, i.e., the REACH Regulation, intensified the demand for alternative test systems, as the animals involved in such testing needs to be reduced. At the same time, toxicity assessments of test items are continual process [3].

The alternatives available for Draize eye test are organotypic model and *in-vitro* cell based assay.

Bovine Corneal Opacity and Permeability test (BCOP)

The BCOP uses bovine eyes from abattoir animals. Eyes are dissected and corneas are collected and mounted on to cornea holders. These corneas are exposed to undiluted liquid and surfactants for 10 minutes, followed by a 2 hour incubation. In case of a solid test item, corneas are exposed with the test item for 4 hours at 20% concentration in a suitable vehicle. The effect of the test item on cornea is measured by its ability to induce opacity and corneal permeability. Corneal opacity is measured by the amount of light passing through the cornea while the permeability is measured by the amount of sodium fluorescein passing through cornea to reach the lower chamber.

Based on these results, the test item is classified in GHS Category 1, i.e., severe irritant, GHS category 2, i.e., irritant or 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[5].

Three-dimensional corneal epithelial model

The SkinEthic™ human corneal epithelium (HCE) model uses immortalised human corneal epithelial mucosa cells that is structurally very similar to the corneal mucosa of the human eye. The reconstructed epithelial tissue morphology is similar to that of human corneal epithelium. The SkinEthic™ HCE protocol differs between solids and liquids in the used exposure time and post-exposure period allowing for development of cytotoxic effects (for liquids 30 minutes exposure and for solids 4 hour exposure followed by 18 hour post-exposure). Tissue viability is determined by the MTT assay. According to the prediction model, solid test items, which reduce the relative tissue viability to at least 50 % are classed as potential irritants, while those inducing a viability of less than 50 % are categorised as non-irritants while for liquid test items, which reduce the relative tissue viability to at least 60 % are classed as potential irritants, while those inducing a viability of less than 60 % are categorised as non-irritants.

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[6].

Only under certain circumstances when the test item is further needed to be classified under GHS category 2A or 2B, further testing using *in-vivo* method is required, as no single in-vitro test method will be able to fully replace the *in-vivo* Draize eye test. However, using the combination of these *in-vitro* assays, test items can be classified under GHS category 1 or GHS No category.[4, 5,6]

Disclaimer: Only a few *in-vitro* methods, accepted by the OECD under various Test Guidelines, are suggested in this article. However, there are numerous other available *in-vitro* methods also.

References:

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