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RISK ASSESSMENT AND CLASSIFICATION OF CHEMICALS FOR THEIR IRRITATION/CORROSIVE POTENTIAL USING AN IN-VITRO METHOD OF BOVINE CORNEAL OPACITY AND PERMEABILITY TEST (BCOP)

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ABSTRACT

Bovine Corneal Opacity and Permeability test is an in vitro method, which is rapidly gaining popularity due to short term maintenance of the test system, economical to conduct, provide reliable predictive data and most importantly replacing the use of animals. This test was evaluated by ICCVAM, ECVAM and JaCVAM and confirmed for its sensitivity to identify chemicals inducing severe eye damage as well as chemicals not classified for eye irritation. While it is not considered valid as a stand-alone replacement for the in-vivo rabbit eye test, the BCOP test method is recommended as an initial step within a testing strategy such as the Top-Down approach.

In compliance with Test Guideline OECD 437 (BCOP assay), non-surfactant solid materials were tested as 20% dilutions prepared in 0.9% sodium chloride solution 4 h at approximately 32°C followed by wash to remove the excess test item. Liquid test item were tested undiluted and were applied for 10 minutes on corneas then corneas were washed followed by incubation for 2 h at approximately 32°C. Change in corneal opacity was determined by the amount of light passing through the cornea using an Opacitometer. Impairment of the corneal barrier function was measured by the amount of fluorescein released through the cornea. Using the method of In-vitro Irritancy Score (IVIS Score), different chemicals were tested for their eye irritancy. Benzalkonium chloride, Chlorhexidine, Dibenzoyl-L- tartaric acid, Imidazole, Trichloroacetic acid, Ethanol (56.34) and Dimethylformamide were tested and the IVIS was found to be >55 hence all these chemicals were classified under "Category 1" whereas 2,6-Dichlorobenzoyl chloride and Ethyl-2-methylacetoacetate were tested revealed that the IVIS was in between 3 and 55 so these chemicals were classified under "No accurate/Reliable prediction". EDTA, di-potassium salt, Tween 20, 2-Mercaptopyrimidine, Phenylbutazone and Polyoxyethylene 23 lauryl ether were also tested and the IVIS was below 3 which categorised these chemicals under "Not Classified". Moreover histopathology of Imidazole and Dimethylformamide revealed sloughing of surface squamous layer and stroma thicker when compared to normal saline treated corneas.

Based on the results, it could be concluded that the chemicals used in the present study has revealed different intensity of eye damage which was identified and classified efficiently by BCOP test procedures used in this experiment.

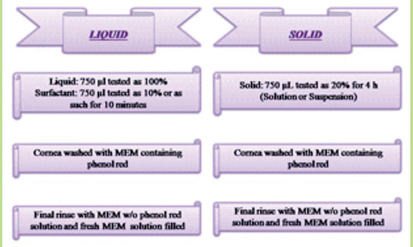
BACKGROUND

New chemicals are usually tested for their potential to cause eye irritation as an important part of the toxicology programme, e.g. for occupational safety. Eye irritation has traditionally been examined with the Draize eye irritation test by using rabbits. However, advances in ocular toxicology are challenging the validity, precision, and relevance of the Draize eye irritation test. Significant levels of variability have been observed since the test is based on a subjective scoring procedure. Moreover, the test causes considerable discomfort, distress and pain to rabbits, it is also recognized that the response in the rabbit is not always predictive of that found in humans. Taken together, both ethical and scientific reasons stimulated the development and validation of several alternative *in vitro* methods to assess eye irritation.

The Bovine Corneal Opacity and Permeability Test (BCOP) is one of the alternative *in vitro* eye irritation test method suggested by Organisation for Economic Co-operation and Development (OECD). The BCOP test is a modified organotypic model which requires short-term maintenance of normal physiological and biochemical functions of the cornea in an isolated system. The basis of the test relies on the role of the cornea as an indicator of visual impairment resulting from damage occurring during accidental eye exposure to chemicals. In addition, results obtained with the BCOP test are comparable to those obtained with the *in vivo* Draize eye test, since the corneal effects with the latter are of considerable significance in the scoring system for ocular irritancy.

The purpose of this study is to evaluate the eye hazard potential of a test chemical by measuring its ability to induce opacity and increased permeability in an isolated bovine cornea. Toxic effects to the cornea are measured by: (i) decreased light transmission (opacity) and (ii) increased passage of sodium fluorescein dye (permeability). The opacity and permeability assessments of the cornea following exposure to a test chemical are combined to derive an In Vitro Irritancy Score (IVIS), which is used to classify the irritancy level of the test chemical. The BCOP test method is an organotypic model that provides short-term maintenance of normal physiological and biochemical function of the bovine cornea *in vitro*. The method used Corneas of Bovine eyes from the abattoirs. The eyes are of no commercial value and are discarded as biological waste.

METHOD



In vitro Irritancy Score (IVIS) Calculation

In vitro irritancy score (IVIS) was calculated for each treatment group as follows:

IVIS = mean opacity value + (15 x mean permeability OD_{490} value)

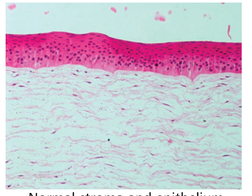
Decision Criteria

IVIS	UN GHS
≤ 3	No Category
> 3; ≤ 55	No prediction can be made
> 55	Category 1

RESULTS

Name of Test Item	Results of IVIS	Classification
Benzalkonium chloride	211.54	Category 1
Chlorhexidine	109.24	Category 1
Dibenzoyl-L- tartaric acid	73.09	Category 1
Imidazole	213.33	Category 1
Trichloroacetic acid	276.84	Category 1
Ethanol	56.34	Category 1
Dimethylformamide	103.86	Category 1
2,6-Dichlorobenzoyl chloride	35.29	No accurate/Reliable prediction
Ethyl-2-methylacetoacetate	21.32	No accurate/Reliable prediction
EDTA, di-potassium salt	2.00	Not Classified
Tween 20	-0.87	Not Classified
2-Mercaptopyrimidine	2.46	Not Classified
Phenylbutazone	2.91	Not Classified
Polyoxyethylene 23 lauryl ether (BRIJ-35) (10%)	2.10	Not Classified

Normal Saline treated Cornea

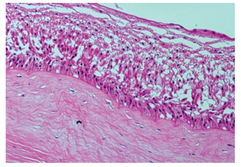


Normal stroma and epithelium

Imidazole treated cornea

Non-viable epithelium with marked chromatin condensation and degeneration

Dimethylformamide Treated Cornea



Non-viable epithelium with marked chromatin condensation, cellular vacuolation and degeneration.

CONCLUSION

IVIS > 55 was observed in corneas treated with Ethanol, Dimethylformamide, Benzalkonium chloride, Imidazole, Trichloroacetic acid, Chlorhexidine and Dibenzoyl-L-Tartaric acid.

IVIS > 3 to ⩽ 55 was observed in corneas treated with Ethyl-2-methylacetoacetate and 2,6-Dichlorobenzoyl chloride.

IVIS ⩽ 3 was observed in corneas treated with Tween 20, EDTA di-potassium salt, 2-Mercaptopyrimidine, Polyoxyethyene 23 lauryl ether and Phenylbutazone.

REFERENCE

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