Comparative evaluation of cosmetic formulations with different alternative methods for eye irritation

Andreas Heppenheimer¹, Albrecht Poth¹, Rolf Fautz² and Anne Fuchs²

¹RCC Cytotest Cell Research GmbH,
²KPSS - KAO Professional Salon Services GmbH

Corresponding author: Andreas Heppenheimer
RCC Cytotest Cell Research GmbH
In den Leppsteinswiesen 19, D-64380 Rossdorf, Germany
Phone: +(49)-6154-807-0, Fax: +(49)-6154-83399, heppenheimer@rcc-ccr.de

Abstract
The eye irritating potentials of 5 cosmetic formulations were determined by 4 different alternative methods for eye irritation. In particular the test methods were: Hen's Egg-Chorio-Allantoic Membrane (HET-CAM), Bovine Cornea Opacitiy and Permeability Test (BCOP), Rabbit Enucleated Eye Test (REET) and an in vitro method using a human cornea model (EpiOcular™ from MatTek). The cosmetic formulations were chosen to cover the whole range of irritating effects: non, mild, moderate and severe/very severe. The results of this study indicate that the eye irritation models are able to discriminate between the above mentioned irritating effects. This study shows that the existing alternative methods are able to substitute the in vivo Draize Rabbit's Eye Irritation test.

Keywords: eye irritation, alternative methods, HET CAM, BCOP, REET

Introduction
Ocular contact is one of the probable routes of human exposure. Therefore the determination of the eye irritating potential is a rational basis for risk assessment in man. The common method of determining an eye irritant potential is the in vivo Draize Rabbit Eye Test. Due to animal welfare reasons alternative methods were developed using cells, chicken eggs or eyes from rabbits and bovines by using slaughterhouse material.

This study compares four established alternative methods, in particular the Hen's Egg Chorioallantoic Membrane Test (HET-CAM), Bovine Cornea Opacitiy and Permeability Test (BCOP), Rabbit Enucleated Eye Test (REET) and an in vitro method using a human cornea model (EpiOcular™ from MatTek). All test methods are able to classify the irritating potential in non, mild, moderate and severe.

As test items 5 cosmetic formulations provided by KPSS were used. KPSS currently mainly uses the HET CAM system for the detection of eye irritating potential of cosmetic formulations.

Material and methods
HET CAM: effects of the test item on the Choriallantoic membrane of 9 day bred chicken eggs are assessed within a treatment period of 5 minutes for non coloured liquid test items. For coloured non transparent liquid test items the treatment procedure is as follows. The CAM is treated with the test item for 30 s and then the test item is washed off the CAM. Afterwards effects are assessed within 5 minutes. For solid test items at least 25 % of the CAM needs to be covered with the test item. Effects are assessed in the near surroundings of the test item within 5 minutes. The time point is noted when one of the following effects occurs: haemorrhage, lysis and coagulation. An irritation score is calculated and the test item is classified with this score. Test items can be tested neat or in dilution with the HET CAM Assay.

BCOP: freshly prepared cornea of bovine eyes are treated with the test item for 240 min (solid test items) or 10 min (liquid test items, additional 120 min incubation after treatment). Opacity and permeability of the cornea is determined. An irritation score is calculated and the test item is classified with this score. Test items can be tested neat or in dilution with the BCOP Assay.

REET: freshly isolated rabbit eyes are treated with the test item for 60 s. Changes in opacity, permeability, thickness and macroscopic or microscopic damage are assessed over a period of 4 hours (no difference between liquid or solid test items). Test items can be tested neat or in dilution with the REET Assay.
In vitro method using the cornea model EpiOcular™: a cornea model derived from human keratinocytes is treated with the test item for 3, 30 and 60 min. The cell viability is measured by dehydrogenase conversion of MTT into a blue formazan salt that is quantitatively measured after extraction from tissues. The time point when 50 % of the cells are dead (ET₅₀) is calculated. The ET₅₀ allows the classification of the test item. Test items can be tested neat or in dilution with the EpiOcular™ Assay.

Results

5 cosmetic formulations provided by KPSS were used to compare the test systems. The formulations were chosen to cover the whole range of possible irritating effects: non, mild, moderate und severe effects. Three of the test items were classified identically by all of the four alternative methods. Test item 3 was classified as moderate irritant in the BCOP, the REET and with the EpiOcular™ model (Table 3, 4, 5). Only in the HET CAM test it was classified as severe (Table 2). Test item 5 was classified as severe in the EpiOcular™ (Table 5), moderate/severe in the HET CAM (Table 2) and moderate in the BCOP Test (Table 3) and in the REET (Table 4). The results of the EpiOcular™ model should be considered with care because the test item reacted with the MTT agent and...
produced false results. In the HET CAM test system the scores differed between moderate and severe. The irritating potential increased with increasing reaction time after preparation of the formulated product.

Conclusions

• The test methods are suited to identify all irritation categories.
• Beneath some differences the test methods are producing comparable results.
• In the EpiOcular test formulations reacting with the MTT agent may lead to false results.
• The HET CAM system was the most sensitive, which confirms the current KPSS strategy for eye irritation. However, in specific cases (e.g. corrosive formulations), one of the other test systems can give supportive information in a test battery used for risk assessment.

• When testing highly reactive formulations in the HET CAM system a standardisation of the reaction time of the formulation is essential.

References

INVITTOX (UK) protocol no. 98 "The Bovine Corneal Opacity and Permeability Assay", dated February 1994
REET Protocol, Unilever Safety & Environmental Assurance Centre (SEAC)