

Update on the Colipa research programme for development of *in vitro* alternative methods for eye irritation

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Abstract

The COLIPA eye irritation programme for development of *in vitro* eye irritation assays incorporates integrated research projects and collaborative activities with external partners. The research projects focus on understanding mechanisms of eye injury and identification of new *in vitro* endpoints more predictive of the *in vivo* human response to chemical injury resulting in new or improved *in vitro* methods that would proceed to formal validation. There are three projects: 1) investigation of whether kinetics/patterns of change in physiological function/signals of injury released from the cornea *in vitro* can predict a chemical's potential to damage the eye with a focus on recovery; 2) identification of endpoints related to magnitude of injury and repair in 3-dimensional human corneal constructs and 3) a genomics project using a pattern recognition approach to identify new endpoints for injury and repair for potential use in current/future *in vitro* assays. Equally important to achieve validated *in vitro* methods are other activities such as continued development/optimisation of currently existing models and industry collaboration with academia, external scientific organisations and regulators. COLIPA is working with producers of Human Reconstructed Tissue (HRT) eye irritation models to further develop/optimize these models and with ECVAM through participation in its Eye Irritation Task Force and provision of an independent bio-statistician for post-hoc analysis of current *in vitro* methods.

Keywords: *in vitro*, eye irritation, replacement, COLIPA, mechanisms

Introduction

The eye can be exposed to cosmetic products and their ingredients either through use of products such as those meant to be used around the eyes (e.g. mascaras, eye creams) and through accidental exposure to products that may enter the eye in diluted form during normal use but are not meant to come into contact with the eye undiluted e.g. shampoos. As such, the evaluation of eye irritation potential for a cosmetic product and its ingredients is essential to provide reassurance that a product is safe for consumers to use through intended and foreseeable uses and accidental exposures to the eye.

For decades, the Draize rabbit eye irritation test (OECD Guideline 405 - Acute Eye Irritation/Corrosion) has been the globally accepted regulatory

method for assessing eye irritation potential of chemicals. However, success in developing and validating alternative tests to replace the Draize rabbit eye irritation test has remained elusive despite major efforts by external validation organisations, industry trade associations, individual companies and academia. An extensive list of *in vitro* models that have been developed and proposed as alternatives to the Draize test is published (Eskes *et al.*, 2005). Although some of the many alternatives assays developed have received limited attention, substantial effort has been invested in evaluating a significant number of the assays. Six major validation or evaluation studies took place between 1991 and 1997. These were the EC/HO study (Balls *et al.*, 1995), COLIPA study (Brantom *et al.*, 1997), BGA/BMBF

study (Spielmann *et al.*, 1993, Spielmann *et al.*, 1996), CTFA study (Gettings *et al.*, 1991, Gettings *et al.*, 1994, Gettings *et al.*, 1996), IRAG study (Bradlaw *et al.*, 1997) and the MHW/JCIA study (Ohno *et al.*, 1994). Unfortunately, none of the methods included in these validation/evaluation studies was found to meet all the formal validation requirements of the regulatory authorities for replacing the current animal test accepted by OECD (Guideline 405 - Acute Eye Irritation/Corrosion). As such, whilst a large number of these *in vitro* methods find applications in industry, none of them can be used today as a stand alone method that can fully replace *in vivo* testing.

More recently, two organotypic assays, the Bovine Corneal Opacity Test (BCOP) and the Isolated Chicken Eye Test (ICE) have been validated by the European Centre for the Validation of Alternative Methods (ECVAM) as partial replacements for the Draize rabbit eye test to be used as screening tests for identification of substances as ocular corrosives and severe eye irritants in a tiered-testing strategy, as part of a weight-of evidence approach (ECVAM, 2007). This is an endorsement of the outcome of the Interagency Co-ordinating Committee for the Validation of Alternative Methods (ICCVAM) Background Review Document (BRD) activity for these organotypic assays (ICCVAM, 2006).

Clearly, reduction and refinement methods/approaches and partial replacements methods for the evaluation of eye irritation are available today but validated full replacement method(s) have not yet been achieved. There remains a clearly identified need to define alternatives methods that reliably predict the human eye response to chemicals exposure and can replace the *in vivo* test. As such, a fundamental understanding of what is needed to fill the knowledge gaps is essential to continued progress.

Following the formal validation studies of the mid-1990s, workshops conducted by COLIPA (Mechanisms of Eye irritation, 1997 (Bruner *et al.*, 1998)) and ECVAM (Eye Irritation Testing: The Way Forward, 1998 (Balls *et al.*, 1999)) concluded that the reasons for this lack of success are multiple and include a lack of understanding for the underlying physiological mechanisms of eye irritation, the variability of the *in vivo* Draize test data and the ability of the Draize test to reliably predict the human response. These workshops recommended additional research to aid the development of well characterised, mechanistically-based *in vitro* eye irritation tests.

To address development of alternative methods based on mechanistically relevant biological events, the European Cosmetics Industry Trade Association (Colipa) through its Steering Committee on Alternatives to Animal Testing (SCAAT) programme for development of alternatives to animal testing has in place a programme for the development of *in vitro*

assays for eye irritation that is managed by the project team PT-SCAAT Eye Irritation. This programme is composed of: 1) integrated basic research projects that are conducted in collaboration with academia for which the scope is to identify new *in vitro* endpoints predictive of the *in vivo* response to chemical injury; 2) method development/optimisation of existing models and 3) collaborative activities with external partners such as ECVAM.

A brief update on each element of the Colipa PT-SCAAT Eye Irritation programme is provided below.

Colipa PT-SCAAT eye irritation programme

1) Research Programme

Building on the experience of the earlier validation studies and scientific workshops, the strategy and objective of the research programme is to gain an understanding of cellular and molecular mechanisms of chemically induced eye irritation, with focus on corneal injury and recovery. Through this understanding, the expected outcome is the identification of *in vitro* endpoints related to the dynamics of injury and recovery that are more predictive of the *in vivo* human response to chemical injury. This will enable the development of prediction models for pre-validation of new or improved *in vitro* methods that would proceed to formal validation.

There are three integrated research projects: 1) development of an *in vitro* model of excised corneas maintained in culture to allow observation of injury/recovery after chemical exposure to investigate whether kinetics/patterns of change in physiological function and signals of injury released from the cornea *in vitro* can predict a chemical's potential to damage the eye with a focus on recovery; 2) development of sequentially built 3-dimensional, multi-layer bioengineered human corneal constructs consisting of epithelium, stroma and endothelium to better understand underlying mechanisms of action to enable identification of endpoints related to magnitude of injury and quality of repair and 3) a genomics project using a pattern recognition approach to identify new endpoints for injury and repair that builds on corneal models for potential use in current/future *in vitro* assays. Brief update details of each project are given below and key outcomes where appropriate are outlined.

Project 1: *In vitro* corneal culture eye irritation assay (Schrage *et al.*, 2005)

This project was conducted by Professor Norbert Schrage and Markus Frenz of the University of Aachen, Germany and is now ended. The aims were to: 1) develop an *in vitro* model of excised corneas maintained in culture to allow observation of injury and recovery following chemical exposure and 2) investigate whether kinetics/patterns of change in

physiological function and signals of injury released from the perfused cornea *in vitro* can predict a chemicals potential to damage the eye, with a focus on recovery.

A stepwise approach was adopted to develop a new isolated perfused corneal culture model that could be maintained for a period of time under steady state culture conditions that resulted in:

- Development of a new isolated perfused corneal culture model maintained in steady state culture conditions for a period of time i.e. maintained for 21 days (based on glucose/lactate turnover) and stable for up to 7 days (based on no increase in corneal thickness in that time)
- Investigation of viability and stability of the isolated perfused corneal culture system both morphologically and metabolically for definition of the parameters to be used routinely to confirm system viability and stability. Evaluation methods included biomicroscopy, pachymetry and glucose/lactate turnover
- Investigation of suitability of the model to investigate wound healing by mechanical abrasion i.e. self healing after mechanical abrasion
- Investigation of evaluation methods such as: 1) morphology (microscopic changes to the epithelium, stroma and endothelium) and 2) biochemical analysis of LDH, cytokines (e.g. IL-1 α , IL-2, IL-6 IL-8, MIP1) and growth factors (e.g. FGF, VEGF) via access to mediator production in the perfusion medium and the surface flushing medium to evaluate initial injury, inflammation and late changes related to understanding the dynamics of injury and recovery after mechanical trauma or toxicant exposure
- Investigation of a preliminary exposure system for the isolated perfused corneal system to model toxicants i.e. system showed some reactivity to different types of chemicals
- Preliminary investigation of possible endpoints (e.g. IL8 and VEGF) for further development in other models evaluating chemically induced eye injury

Project 2: Cell culture models for ocular toxicity studies (Berry *et al.*, 2005)

This project was undertaken by Dr. Monica Berry and Marcus Radburn-Smith at the University of Bristol, UK. The aims of this project were to: 1) sequentially build 3-dimensional, multi-layer, bioengineered human corneal constructs consisting of epithelium, stroma and endothelium in order to

better understand underlying mechanisms of action of eye irritation and 2) identify new end points related to magnitude of injury and quality of repair in human corneal models that will enable prediction of the severity of toxicant effects.

A stepwise approach was adopted in this project to investigate physiological responses to ocular injury (e.g. cell activation/signaling to immune system effector cells) by evaluating responses to model toxicants in increasingly complex 3-dimensional, multi-layer bioengineered human corneal constructs that resulted in:

- Investigation and development of increasingly complex constructs (e.g. monolayer, stratified epithelia, stromal equivalents, 2 and 3 layer constructs) using human corneal and conjunctival cell lines (e.g. culture conditions, growth characteristics and suitability for use in 3-dimensional constructs)
- Development of a two layer model (epithelium and stroma) that is stable (i.e. maintains constant protein content, steady reductive state, and very low secretion of cytokines) for 7 days after optimal epithelial stratification
- Definition of a preliminary exposure system for the 3-dimensional, 2-layer bioengineered human corneal construct to model toxicants
- Preliminary investigation of physiological responses to ocular injury by evaluating responses to model toxicants in monolayers and increasingly complex constructs using different human corneal and conjunctival cell lines using evaluation methods including light and confocal microscopy, characterization of surface markers, barrier formation assessment, membrane damage, metabolic activity, profiling of cytokine secretion
- Preliminary evaluation of construction of a 3-dimensional, 3-layer bioengineered construct by the addition of an endothelium cell layer
- Availability of a 3-dimensional, 2-layer bioengineered human corneal construct composed of a 3-dimensional barrier-forming epithelium and non-activated stromal cells in a collagen matrix for further standardisation, development/optimisation and evaluation

Work is currently ongoing for the continued standardisation, development/optimisation of the 3-dimensional, 2-layer (epithelium, stroma) bioengineered human corneal construct model. This includes further standardisation of the model followed by scale-up of construct production to enable further research and development work to continue. This further research and development work is focused on

definition of the prediction model and identification of the domain of applicability through evaluation of chemicals that span the range of irritancy from none to severe and which are derived from a wide range of chemical classes.

Project 3: Development of gene expression fingerprints to identify chemicals toxic to the cornea

A genomics project was started in 2006. It remains a high priority for the Project Team to determine how best to integrate a genomics project into the work being undertaken to better understand mechanisms of eye irritation and identification of *in vitro* endpoints related to the dynamics of injury and recovery that are more predictive of the *in vivo* human response to chemical injury. As such, a genomics project is currently under review for integration into the Colipa PT-SCAAT eye irritation research programme. The aims of this project are to: 1) generate proof of concept that generic chemicals will cause differential gene expression in corneal models, 2) identify gene expression profiles in corneal models exposed to generic classes of chemicals and 3) develop a gene fingerprint directory to identify chemicals toxic to the cornea. The principal outcome should be the application of the knowledge to better understand new endpoints for eye irritation and enable further development of current and future *in vitro* methods.

2) Method Development/Optimisation of Existing Models

The activity related to method development/optimisation of current *in vitro* assays within the Colipa PT-SCAAT eye irritation programme is focused on *in vitro* Human Reconstituted Tissue (HRT) models.

Several HRT models are available with some being more advanced in both development and availability than others. Two of the most advanced models currently available are the SkinEthic Human Corneal Epithelium (HCE) model and the MatTek Epiocular™ model. Both models were submitted to ECVAM for formal validation in 2005. Subsequent protocol optimisation has been led by industry in collaboration with the owners/producers of the models to extend the domain of applicability and work is currently ongoing within industry using the optimised protocols to expand the data available for predictivity and reproducibility. It is expected that these data will be submitted for an ECVAM HRT validation workshop in which the validation studies for these assays will be defined.

Other HRT eye irritation models are available some of which have improved barrier function capability over some of the commercially available models. Colipa is engaged in understanding whether the use of barrier

function as an endpoint is useful to measure in an HRT assay for evaluation of eye irritancy at the lower (non-irritant/very mild) end of the irritancy range.

Finally, although Colipa has developed a 3-dimensional, 2-layer (epithelial/stroma) bioengineered human corneal construct that it is actively progressing to further standardisation, development/optimisation and possible external validation, there are other multi-layered corneal models identified in the scientific literature. Colipa is engaging in collaboration with such model developers to determine collaborative activities for further method development in this area.

3) External Collaboration

Equally important to achieve validated *in vitro* methods is collaboration between industry, academia, external scientific organisations and regulators. COLIPA is working with ECVAM by active mutual participation in both COLIPA and ECVAM Eye Irritation Project Teams/Task Forces. COLIPA Project Team members participated in an ECVAM Expert Meeting on *In Vitro* Eye Irritation in February 2005, contributed as co-authors to the ECVAM-led comprehensive status review of *in vitro* eye irritation methods that was published in ATLA in 2005 (Eskes *et al.*, 2005) and actively support the ECVAM current initiatives in review of *in vitro* eye irritation assays e.g. retrospective validation activity on cytotoxicity and cell function-based based *in vitro* assays. COLIPA is also providing ECVAM with support for post-hoc statistical analysis of current *in vitro* methods via the funding of an independent biostatistician. In addition, the Project Team has contributed to ICCVAM workshops and other initiatives, e.g. review of the validation status of four *in vitro* methods for ocular corrosion and severe eye irritation and the Mechanisms of Ocular Irritation Workshop in May 2005.

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