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Review

A proposed eye irritation testing strategy to reduce and replace *in vivo* studies using Bottom–Up and Top–Down approaches ☆

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ABSTRACT

In spite of over 20 years of effort, no single *in vitro* assay has been developed and validated as a full regulatory replacement for the Draize Eye Irritation test. However, companies have been using *in vitro* methods to screen new formulations and in some cases as their primary assessment of eye irritation potential for many years. The present report shows the outcome of an Expert Meeting convened by the European Centre for the Validation of Alternative Methods in February 2005 to identify test strategies for eye irritation. In this workshop test developers/users were requested to nominate methods to be considered as a basis for the identification of such testing strategies. Assays were evaluated and categorized based on their proposed applicability domains (e.g., categories of irritation severity, modes of action, chemical class, physicochemical compatibility). The analyses were based on the data developed from current practice and published studies, the ability to predict depth of injury (within the applicable range of severity), modes of action that could be addressed and compatibility with different physiochemical forms. The difficulty in predicting the middle category of irritancy (e.g. R36, GHS Categories 2A and 2B) was recognized. The testing scheme proposes using a Bottom–Up (begin with using test methods that can accurately identify non-irritants) or Top–Down (begin with using test methods that can accurately identify severe

☆ Disclaimer: The authors of this document participated as individuals, and the opinions expressed do not represent the official positions of any government agency or other organization. Affiliations given were those current at the time of the Expert Meeting.

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irritants) progression of *in vitro* tests (based on expected irritancy). Irrespective of the starting point, the approach would identify non-irritants and severe irritants, leaving all others to the (mild/moderate) irritant GHS 2/R36 categories.

Contents

1. Introduction

The Draize eye irritation test continues to be the primary method accepted by regulatory agencies worldwide. Most regulatory agencies divide responses into categories of irritation based on specific tissue scores and the duration over which the lesions persist. While scoring of the specific tissue lesions is similar across most agencies, the various hazard categorization schemes have distinct differences (EPA,1998; ECC, 1967; EU, 2001, 2004; OECD, 2002; UN/ECE, 2003). Over the past decades, there have been substantial investments into both the development of *in vitro* methods and execution of validation/evaluation studies to assess the reliability and reproducibility of these test methods to predict the eye irritation responses in the Draize Test (Bruner et al., 1991; Balls et al., 1995; Brantom et al., 1997; Gettings et al., 1991, 1992, 1994, 1996; Spielmann et al., 1993, 1996; Bradlaw et al., 1997; Ohno et al., 1999; ICCVAM, 2007, 2006a,b,c,d). Despite these efforts, no *in vitro* test has successfully been validated to fully replace the Draize eye irritation test for regulatory purposes.

Despite the lack of formally validated in vitro eye irritation test methods for regulatory purposes, in vitro eye irritation tests have a long history of use and acceptance by industry for specific purposes (Harbell and Curren, 2001; Curren and Harbell, 2002; Eskes et al., 2005). More recently, there has been limited acceptance by regulatory agencies for the prediction of severe eye irritants (EC, 2004; NIEHS, 2008). In February 2005, an Expert Meeting was convened by ECVAM to critically evaluate the limitations and advantages of selected in vitro test methods with a view to identify proposed applicability domains where reliable and relevant results may be obtained, based on expertise from in-house experience and from the various evaluation studies which took place in the last decades. From these analyses, it was envisioned that an in vitro eye irritation testing strategy could be developed to reduce, and ultimately replace, animal use (Goldberg and Silber, 1992; Rougier et al., 1992; Balls et al., 1999; Eskes et al., 2005).

2. Eye irritation expert meeting objectives

To progress this concept, ECVAM invited test method developers and users to nominate in vitro eye irritation test methods that could be considered as a basis for a testing strategy. Specifically, participants were asked to provide parallel in vivo and in vitro data to support the usefulness of the nominated in vitro test method for a specific applicability domain (e.g., range of eye irritation severity, chemical class, mechanisms of irritation).

On the 8th–11th of February 2005, over thirty scientists from academia, government, non-profit organizations, private industry (including contract test laboratories), as well as international validation experts and regulators met in Ispra, Italy to share data in support of in vitro eye irritation test method(s) nominated for consideration.

The objectives of this activity were to:

1. Obtain in vitro eye irritation test method nominations, with supporting data, for a specific applicability domain(s).
2. Clarify how each nominated method is currently being used (e.g., screening to guide Research & Development (R&D) efforts, hazard classification, risk assessment).
3. Identify partners to progress promising testing methods into validation.
4. Identify gaps where to focus future research and method development efforts.
5. Propose in vitro testing strategies or approaches that could be further developed to validation by ECVAM in their overall efforts to reduce, and ultimately replace, animal use for eye irritation hazard identification and classification in accordance with Annex V of Directive 67/548/EEC (the "Dangerous Substances Directive") (ECC, 1967; EU, 2001, 2004).

Fourteen test methods were nominated for which supporting data was submitted to ECVAM for review (Fig. 1). Each participant provided an overview of the optimized test method(s) used within their organizations, described how each method was currently used for specific purposes and submitted data to support their proposed applicability domain. Based on the knowledge acquired and the expert's experience, a testing strategy approach to reduce, and hopefully replace, animal use for eye irritation was proposed.

3. Results

A large variety of test methods was nominated for use within a testing strategy (Fig. 1) including isolated corneas/eyes (bovine, porcine, rabbit, chicken), chorioallantoic membrane methods (HET-CAM and CAM-TBS), reconstructed human tissues (RHT) engineered with either transformed human corneal epithelial cells (Human Corneal Epithelium (HCE or SkinEthic™HCE) or normal human foreskin keratinocytes (EpiOcular™), cytotoxicity assays (Neutral Red Release & Red Blood Cell Lysis), cell function-based assays (Fluorescein Leakage & Cytosensor™ microphysiometer), simulated corneal opacity models (Irritection®) and the slug muco-