Combining In Vitro Tests as an Alternative to In Vivo Eye Irritation Tests

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Summary — Accurate methods that test the eye irritation potential of chemicals, which do not involve the use of animals, are needed to meet new regulatory standards. We evaluated the applicability and predictive capacity of five in vitro tests for eye irritation: the Hen’s Egg Test-Chorioallantoic Membrane (HET-CAM) assay; the Chorioallantoic Membrane-Trypan Blue Staining (CAM-TBS) assay; the Fluorescein Leakage Test (FLT); the 3T3-Neutral Red Uptake (3T3-NRU) cytotoxicity assay; and the red blood cell (RBC) haemolysis assay. A panel of 16 chemicals (some at multiple concentrations) was assessed by using the five tests, and the results were compared with historical in vivo Draize test data. The results showed rank correlation and class concordance between the five alternative methods and the Draize test for the 16 chemicals. These in vitro assays had good predictive capacity, reproducibility and reliability when compared to the Draize test. The best relationship was between the HET-CAM, CAM-TBS and FLT results, and the modified maximum average score(s) (MMAS). A prediction model (PM) was developed, based on the maximum possible correlation between the MMAS and the HET-CAM, CAM-TBS and FLT results. The PM had a good predictive capacity when compared to the results of animal tests, indicating its potential value for the in vitro screening of chemicals for eye irritation effects.

Key words: alternative methods, eye irritation test, in vitro methods, partial least squares.

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Introduction

The Draize test is an animal method for investigating chemical hazard, specifically acute eye irritation. However, recent public attention concerning test animals has led to widespread calls for abolishing in vivo testing (1). In 2007, the European Union (EU) issued the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) regulations, to better understand the hazards represented by chemicals used in commerce. Although only traditional animal-based methods were originally considered by the REACH system, the final policy now promotes the active reduction or elimination of unnecessary experimentation on animals (2). The EU Cosmetic Products Directive 2003/15/EC (7th Amendment to Directive 76/768/EEC) banned the use of animals in acute and topical toxicity testing (including eye irritation) of ingredients in EU countries from March 2009. One of its goals includes a step-wise ban on the import and sale, in the Member States, of cosmetic ingredients evaluated by using animal-based assessment methods (3). This directive provides an imperative for reducing, refining and replacing animal use in the toxicological safety evaluation of chemicals.

Although research on alternatives to animal-based eye irritation tests has been in progress for many years, no single alternative method has been able to detect all levels of eye irritation for all types of substances and products. This is probably due to the complexity of reactions that cause eye irritation, and the wide variety of chemicals and ingredients to be tested. Nonetheless, many methods have potential as safety screening assays, and their applicability can be enhanced by devising appropriate in vitro predictive models or detection strategies (4).

Partial least squares (PLS) regression is a technique widely used for dealing with numerous correlated explanatory variables (5). PLS regression also aims to identify components to explain, as far as possible, the variance of the predictor variables. These components are simultaneously correlated with the response variable. In this work, matrix data were divided into two sets of in vitro X (independent) and in vivo Y (dependent) variables, evaluated for the same set of objects. The PLS technique summarises the information contained in a data set, and finds the relationship between Y and X that makes it possible to predict Y, given X. When Y and X are highly correlated in sets, the