In-house Validation of the EpiOcular™ Eye Irritation Test and its Combination with the Bovine Corneal Opacity and Permeability Test for the Assessment of Ocular Irritation

Susanne N. Kolle,¹ Helena Kandárová,² Britta Wareing,¹ Bennard van Ravenzwaay,¹ and Robert Landsiedel¹

¹BASF SE, Experimental Toxicology and Ecology, Ludwigshafen, Germany; ²MatTek In Vitro Life Science Laboratories, Bratislava, Slovakia

Summary — In 2009, the Bovine Corneal Opacity and Permeability (BCOP) test was accepted by the regulatory bodies for the identification of corrosive and severe ocular irritants (Global Harmonised System [GHS] Category 1). However, no in vitro test is currently accepted for the differentiation of ocular irritants (GHS Category 2) and non-irritants (GHS No Category). Human reconstructed tissue models have been suggested for incorporation into a tiered testing strategy to ultimately replace the Draize rabbit eye irritation test (OECD TG 405). The purpose of this study was to evaluate whether the EpiOcular™ reconstructed corneal-like tissue model and the COLIPA pre-validated EpiOcular Eye Irritation Test (EpiOcular-EIT) could be used as suitable components of this testing strategy. The in-house validation of the EpiOcular-EIT was performed by using 60 test substances, including a broad variety of chemicals and formulations for which in vivo data (from the Draize rabbit eye irritation test) were available. The test substances fell into the following categories: 18 severe irritants/corrosives (Category 1), 21 irritants (Category 2), and 21 non-irritants (No Category). Test substances that decreased tissue viability to ≤ 60% (compared to the negative control tissue) were considered to be eye irritants (Category 1/2). Test substances resulting in tissue viability of > 60% were considered to be non-irritants (No Category). For the assessed dataset and the classification cut-off of 60% viability, the EpiOcular-EIT provided 98% and 84% sensitivity, 64% and 90% specificity, and 85% and 86% overall accuracy for the literature reference and BASF proprietary substances, respectively. Applying a 50% tissue viability cut-off to distinguish between irritants and non-irritants resulted in 93% and 82% sensitivity, 68% and 100% specificity, and 84% and 88% accuracy for the literature reference and BASF proprietary substances, respectively. Further, in the EpiOcular-EIT (60% cut-off), 100% of severely irritating substances under-predicted by the BCOP assay were classified as Category 1/2. The results obtained in this study, based on 60 test substances, indicate that the EpiOcular-EIT and the BCOP assay can be combined in a testing strategy to identify strong/severe eye irritants (Category 1), moderate and mild eye irritants (Category 2), and non-irritants (No Category) in routine testing. In particular, when the bottom-up strategy with the 60% viability cut-off was employed, none of the severely irritating substances (Category 1) were under-predicted to be non-irritant. Sensitivity for Category 1/2 substances was 100% for literature reference substances and 89% for BASF SE proprietary substances.

Key words: BCOP, EIT, EpiOcular eye irritation test, in vitro, in-house validation, eye irritation, reconstructed ocular tissue model.

Address for correspondence: Robert Landsiedel, BASF SE, Experimental Toxicology and Ecology, Carl-Bosch-Str. 38, 67056 Ludwigshafen, Germany. E-mail: robert.landsiedel@basf.com

Introduction

Industrial substances and formulations must be tested for their potential to cause eye irritation as part of the toxicology programme, e.g. for occupational safety of workers and safety for transport. Eye irritation has traditionally been examined by using the Draize rabbit eye irritation test (1) and modifications thereof. After assessment of the eye irritation/corrosion potential according to OECD TG 405 (2), test substances are classified according to the Globally Harmonised System (GHS; 3), as Category 1 (severe irritants causing irreversible damage to the eye), Category 2A (moderate irritants having reversible effects on the eyes) or Category 2B (mild ocular irritants). Non-irritating substances are not labelled according to the GHS system.

The Draize rabbit eye test has long been criticised for: a) differences between species (e.g. compared to human eyes, rabbit eyes have a nictitating membrane, a thinner cornea and lack significant tearing); b) subjective scoring; and c) high variability between experiments (4). Because adequate human data for the evaluation of eye irritation are rare, alternative methods are compared to the in vivo ‘gold standard’, i.e. the data from the Draize rabbit eye irritation test, despite the known drawbacks.