ABSTRACT

With the ongoing implementation of the Globally Harmonized System of Classification and Labeling of Chemicals (GHS) in various forms throughout the world and a global movement towards a reduction in animal testing, more emphasis is placed on utilizing the inherent hazards of chemicals for classification and labeling; however, the assessment of the toxicity of chemical mixtures, particularly ocular irritation, can be complex. The ability to formulate mixtures to be less irritating with minor modifications to the physical form would be very beneficial. The present study used the Bovine Cornea Opacity and Permeability (BCOP) assay, which is an OECD-approved in vitro method to assess ocular irritation, to investigate how physical properties (e.g., viscosity) affect ocular irritation. We found that the BCOP in vitro scores of irritating chemicals from several classes, including strong bases, were diminished by altering the viscosity of the aqueous medium or by dosing the solution as a foam. Our data show that a 1% NaOH solution in water produced an in vitro score of 165; however, when the medium was thickened with 1% Carbopol® in the in vitro score dropped to 67.6. A change of this nature is significant, and if this were an EPA-registered antimicrobial cleaner, for example, this reduction in the BCOP score would lower the hazard category. Product form and usage can clearly impact exposure, and the present results suggest that modifications to the physical properties of chemical mixtures can alter their ocular irritation potential; perhaps by affecting exposure to the eye. Although no formal comparisons were performed in animals, the BCOP assay is an OECD-validated method to assess ocular irritation, and studies have shown that the BCOP assay does not under-predict the results of traditional animal tests; thus, there are no obvious reasons to suggest that the present results would not correlate to animals or humans.

MATERIALS AND METHODS

RESULTS

Table 1. The US EPA and OECD classification criteria for ocular irritation using the BCOP assay. According to current EPA guidelines (31 May 2013), test substances resulting in an In Vitro score ≥ 75 would receive a Category I classification indicative of irreversible ocular damage, and those resulting in an In Vitro score < 75 would receive a Category II classification indicating substantial but temporary ocular injury. OECD test guideline 437 (26 July 2013) classifies test substances with an In Vitro score > 55 as Category I indicating severe irritation or corrosive eye damage.

Figure 2: Altering the viscosity of cleaning prototypes can also attenuate ocular irritation. (A) The OECD protocol (TG 437) specifies that surfactant materials should be tested at a 1% w/v dilution in the BCOP assay. An alcohol ethoxylate surfactant that is classified as corrosive to the eye by the supplier did not meet the threshold for corrosivity. Under GHS, if a Category 1 eye irritant is found in a formula at a concentration ≥ 3%, then the formula is considered corrosive unless tested. In the present thickened cleaning prototype, which is a surfactant-based formula with < 3% of the alcohol ethoxylate, the BCOP results met the GHS criteria for “No classification” (i.e., in vitro score 0-3). Although 0.3 is a very small range, this result was reproducible in a second trial several months after the first (in vitro scores 1.0 and 2.6 in the first and second trials, respectively). (B) A different cleaning prototype composed of surfactants and solvents was tested in the BCOP assay and met the EPA criteria for a Category I corrosive formula. When the formula was thickened slightly (still remained a free-flowing liquid), the BCOP results were below the threshold for EPA Category I.

Figure 3. Histopathology Evaluation. In 1% Carbopol® treated corneas, only upper epithelial damage was observed and stromal and endothelial observations were similar to the negative control treated corneas (A-B). As Carbopol® concentration decreased in the 1% NaOH mixture, an increase in the degree of damage to the corneas was observed compared to Carbopol® only treated corneas (B-F); epithelial changes including sloughing and detachment from the basement membrane, denaturation of the epithelial proteins, and blanching of the nuclei in the epithelium were observed. The stroma and endothelium exhibited progressive damage with increasing stromal swelling and eventual loss of endothelial function (C-F).

CONCLUSIONS

The BCOP assay is a rapid, effective non-animal method to evaluate the ocular irritation potential of cleaning formulations.

Alterations to physical characteristics of formulations can impact ocular irritation predictions. Under the EPA classification criteria, for example, increasing the viscosity of cleaning prototype 2 changed the eye irritation classification from Category I to Category II.

Histology is able to provide useful information regarding the degree and depth of injury including identifying specific changes in the epithelium, stroma, and endothelium.

ACKNOWLEDGMENTS

The authors would like to acknowledge Caitlin O’Gara for her assistance and expertise in generating formulations for testing and for determining the viscosity measurements and Jennifer Nash for histopathology evaluation.