Investigations on Reducing Ocular Irritation Associated with Harsh Ingredients by Altering Physicochemical Properties of the Formulation

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ABSTRACT

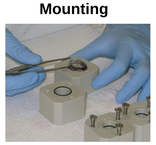
With the upcoming implementation of the Globally Harmonized System of Classification and Labeling of Chemicals (GHS) in various forms throughout the world and a global movement towards a reduction in animal testing, more emphasis is placed on utilizing the inherent hazards of chemicals for classification 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®, 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

Corneal Excision

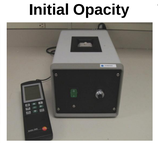
Step 1: Upon receipt, eyes were examined, and the corneas that were free of defects were excised.

Mounting



Step 2: Corneas were mounted into chambers and incubated for 1 h at 32 ± 1°C in complete minimum essential medium (cMEM).

Initial Opacity

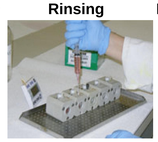


Step 3: cMEM was removed and refilled, and the initial opacity was read on an opacitometer

Test Article Exposure

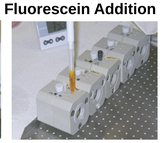
Step 4: 750 µL of test substance was applied to the anterior chamber for 10 min at 32 ± 1°C.

Rinsing



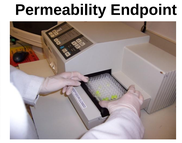
Step 5: Corneas were rinsed thoroughly to remove the test substance and incubated for 2 h before a final opacity reading.

Fluorescein Addition



Step 6: 1 mL of a 4 mg/mL fluorescein solution was added to the epithelial side of the corneas and incubated at 32 ± 1°C for 90 min.

Permeability Endpoint



Step 7: Media was sampled from the posterior chamber, and the optical density at 490 nm was quantified using a microplate reader.

Fixing the Corneas

Step 8: Treated corneas were saved from the assay and fixed in formalin for histological evaluation.

RESULTS

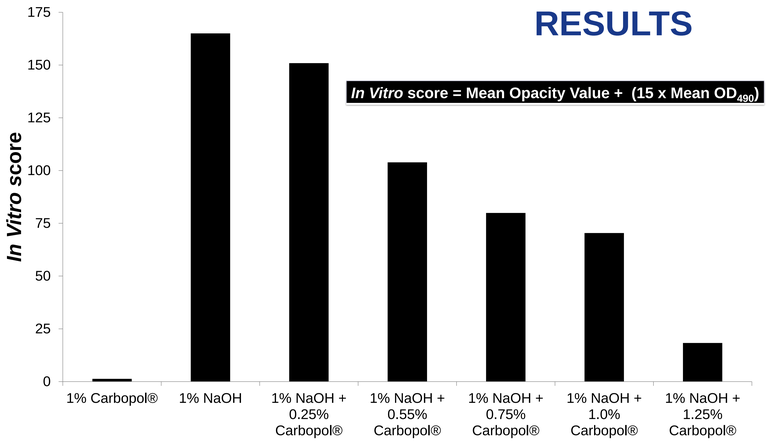


Figure 1: Ocular irritation diminishes as viscosity increases. The BCOP assay (n=3 corneas per sample) was performed for 1% NaOH solutions thickened with an increasing amount of Carbopol® to modify the viscosity of the solution (the viscosities of the 0.25%, 0.55%, 0.75%, 1%, and 1.25% Carbopol® solutions are 4 cps, 10 cps, 195 cps, 8400 cps, and 18700 cps, respectively). The 1% Carbopol® control solution demonstrates that the Carbopol® is not contributing to the irritation, whereas the 1% NaOH positive control solution indicates that the solution is corrosive based on the BCOP classification criteria (shown in Table 1).

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 1 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 1 indicating severe irritation or corrosive eye damage.

EPA Use of BCOP	OECD / GHS Use of BCOP
In Vitro score ≥ 75 = Cat I	*In Vitro* score > 55 = Cat 1
In Vitro score < 75 = Cat II	*In Vitro* score ≤ 3 = Not Classified
Not used for Cat III or IV prediction	*In vitro* score > 3 but ≤ 55 = No prediction; perform another test

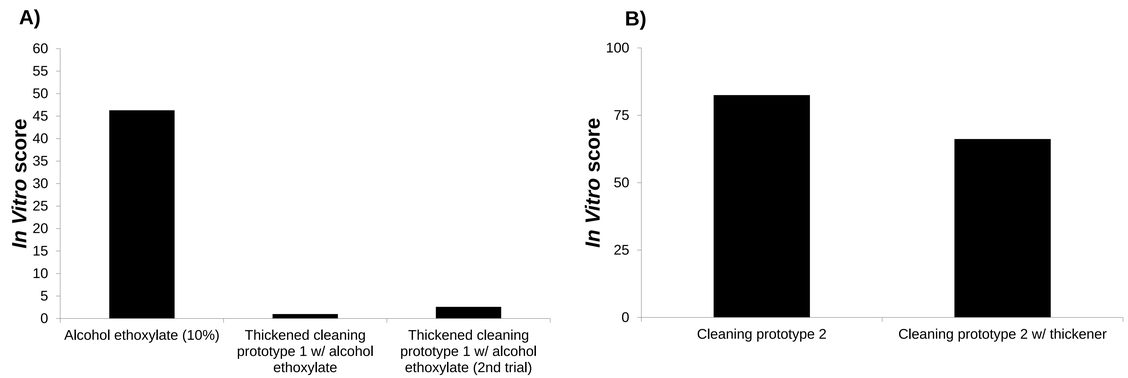


Figure 2: Altering the viscosity of cleaning prototypes can also attenuate ocular irritation. (A) The OECD protocol (TG 437) specifies that surfactant materials should be tested at a 10% w/v dilution in the BCOP assay. An alcohol ethoxylate surfactant that is classified as corrosive to the eye by the 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 were 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.

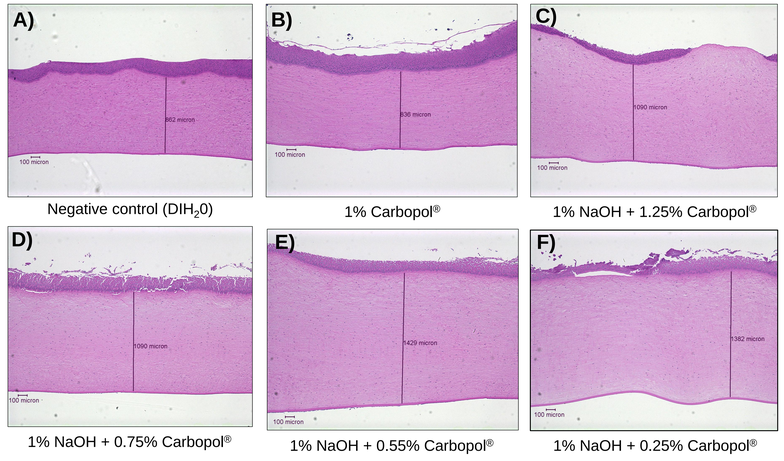


Figure 3. Histopathology Evaluation. In 1% Carbopol® treated corneas, only upper epithelial damage was observed and stromal and endothelial observations were similar to the negative control treated corneas (A-B). As Carbopol® concentration decreased in the 1% NaOH mixture, an increase in the degree of damage to the corneas was observed as 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, stoma, and endothelium.

ACKNOWLEDGMENTS

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