The BCOP assay is well suited to evaluate surfactants and surfactant formulations because it can be used to identify chemicals which induce severe/conjunctival eye irritation and those that do not require classification. However, BCOP has historically under predicted certain anionic surfactants, when tested according to the standard liquid protocol. TG 437 specifies that liquid and solid surfactants may be tested at 10% aqueous dilutions for 10 minutes (although alternate dilutions and exposure times may be conducted with scientific rationale). The relevant guidance document (GD) No. 161 suggests that solid and concentrated liquid surfactants may be diluted to 10% for testing. However, GD No. 160 further directs that surfactant-based formulations are usually tested neat, but could be diluted with justification, imparting some confusion in identifying the most appropriate test methods. Additionally, as part of the EPA classification of ocular irritation, the BCOP assay may be used to assess anti-microbial products with cleaning claims. Such products may contain surfactants and are generally tested neat for classification purposes.

Since neither the basis for selecting the appropriate surfactant test methods, nor the justification for modifications are clearly presented in TG 437 or GD No. 160, we present on the testing of a few common surfactant ingredients, including sodium laurel sulfate (SLS), Triton X-100, and benzalkonium chloride, and surfactant based formulations in the BCOP assay using standard and modified dilutions and exposures to elucidate the impact of these variables on eye irritation prediction.

As examples, in vitro scores of 20.7, 28.4, and 28.3 were obtained when testing SLS at concentrations of 50, 20, and 10% for 10 minutes, showing that irritation responses were not fully concentration-dependent. As a complement to the BCOP assay, histopathology was performed to assess the surfactant-induced corneal changes. Based upon these results, a framework for testing surfactant ingredients and surfactant based formulations is proposed.

**INTRODUCTION**

In this study we investigated the BCOP assay for evaluation of the ocular irritancy potential of surfactants. There are several key considerations when evaluating surfactants in the BCOP assay.

**KEY CONSIDERATIONS:**

- Is the sample to be tested for regulatory classification and labeling?
- If so, what is the appropriate regulatory protocol per OECD TG 437?
- Is the assay being conducted to support product development? Alternate protocols may be used to enhance resolution and rank ordering of prototypes.
- What are the physicochemical properties of the sample (liquid/solid, viscosity, charge, pH)?
- Is the sample an ingredient or formulation?
- What exposure conditions are being modeled (industrial hygiene, transport, end use)?
- Is the sample for professional or home use?
- Is the formulation a concentrate or at end use?
- If the formulation is a concentrate or at end use, does the sample need to be tested neat or as 10% aqueous dilutions for 10 minutes (although alternate dilutions and exposure times may be conducted with scientific rationale)?
- If so, what is the appropriate regulatory protocol per OECD TG 437?
- Fixing the corneas: Corneal excision, mounting, initial opacity, test article exposure, permeability endpoint, fixing the corneas.
- Rinsing: Corneas were rinsed thoroughly to remove test substance, corneas incubated for 2 hours then a final opacity taken.
- Fluorescein addition: 0.1 mL of 4 mg/mL fluorescein solution was added to the epithelial side of the corneas and incubated (32 ± 1°C) for 90 minutes.
- Media was sampled from the posterior chamber and the optical density at 490 nm was quantified using a microplate reader.
- Fixing the corneas: treated corneas were fixed in the assay buffer and fixed in formalin for histological analysis.

**MATERIALS AND METHODS**

**RESULTS**

- **Figure 2.** Fluorescein Permeability values (OD_{490} of SLS tested at various exposure times and various concentrations. The results were exposure time-dependent, however, SLS showed an optimal activity at the lower 10% dilution.
- **Figure 3.** Anionic (SLS), cationic (Benzalkonium Chloride) and non-ionic (Triton X-100) surfactants evaluated at various concentrations for an exposure time of 10 minutes. Three corneas evaluated at each treatment condition.Opacity represented by grey bars and OD_{490} represented by bright green bars. SLS and Benzalkonium Chloride were not tested neat since they are solids.
- **Figure 4.** Various surfactant based formulations evaluated neat and at 10% w/v in sterile water for an exposure time of 10 minutes. Three corneas were evaluated at each treatment condition.Opacity represented by grey bars and OD_{490} represented by bright green bars.
- **Figure 5.** Histopathology Evaluation. 5a. Negative Control showing intact epithelium and organized upper stroma.
- **Figure 5.** Positive Control showing complete loss of epithelium, represented in red. Marked stromal edema and disorganization results in modest opacity.

**CONCLUSIONS**

- The BCOP assay is well suited to evaluate surfactants and surfactant formulations because it can detect a wide range of irritancy potential (mild, moderate, severe).
- When evaluating surfactants in the BCOP assay, key points (listed in Introduction) should be considered to determine the most appropriate protocols to meet your project goals.
- When evaluating anionic or non-ionic surfactants in the BCOP, the permeability endpoint should be considered independently of the opacity and In Vitro Score, because the opacity may be artificially low potential for under prediction.
- Surfactant-induced loss of corneal barrier function is measured objectively by the fluorescein permeability endpoint.
- Histological observation supports that the permeability endpoint may be more reflective of corneal damage and therefore a more relevant measurement for eye irritation prediction for certain surfactants than the opacity endpoint.

**REFERENCE**

*Organisation for Economic Co-operation and Development (OECD) Test Guideline: “Bovine Corneal Opacity and Permeability Test Method for Identifying Chemicals Inducing Serious Eye Damage and/or Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage” (TG 437), adopted 26 July 2013.*