In Vitro Ocular Irritation Testing Strategy for Prototype and Final Cleaning Products

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ABSTRACT

The Bovine Corneal opacity and Permeability (BCOP) assay can be used for predicting mild, moderate, and severe ocular irritation through quantitative assessment of the changes in opacity and permeability of the bovine cornea. In addition, histological evaluation of the corneas can be performed to assess the depth of damage. The BCOP assay with histology was used to determine the ocular irritation potential of prototype cleaning products with antimicrobial claims according to the guidance provided by the EPA-Office of Pesticide Program (OPP).

Several prototype cleaners with similar formulation were evaluated along with a reference material. The results of the BCOP assay showed noticeable differences among the products. The in vitro score, determined by changes in opacity and permeability, of the corneas treated with products ranged from ~15 to 80. These scores indicate mild, moderate, and severe irritation according to the guideline provided in the EPA-OPP document. In addition, the histological evaluation of the corneas showed differences in the depth of damage between moderate and severe category products, confirming the in vitro score.

The assay distinguished ocular irritation potential among similar prototypes demonstrating its effectiveness during product development. Additionally, the results demonstrate the utility of the BCOP assay with histology as a stand-alone assay for eye irritancy evaluation in the EPA-OPP program.

TESTING STRATEGY

- Prototype cleaning products were testing in a top-down approach according to the non-animal testing approach for cleaning products with antimicrobial claims[1] (Figure 1) using the BCOP assay (Table 1) and histopathology (Table 2).
- This program was established to evaluate the use of *in vitro/ex vivo* assays to replace the Draize rabbit eye test for ocular irritation prediction and hazard labeling of antimicrobial products with cleaning claims. Voluntary pilot project initiated on 5/11/2009; anticipated guideline June 2013.

Table 1. BCOP data (In Vitro Score) Criteria

In Vitro Score	EPA Category	Histology
≥ 75	I	recommended
<75 and ≥25	II	required
<25	III	required

Table 2. Histopathology Decision Criteria

Extent of Cellular Damage or Collagen Matrix damage	Suggested EPA Category
Extending into the lower third of stroma and/or damage to endothial cells	I
No further than two-thirds of the way through the stroma	II
No further than the upper third of stroma	III

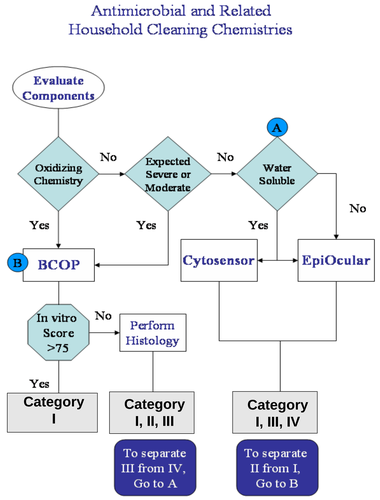

Figure 1. Decision tree for non-animal testing approach to EPA labeling for eye irritation of antimicrobial products with cleaning claims.

MATERIALS & METHODS

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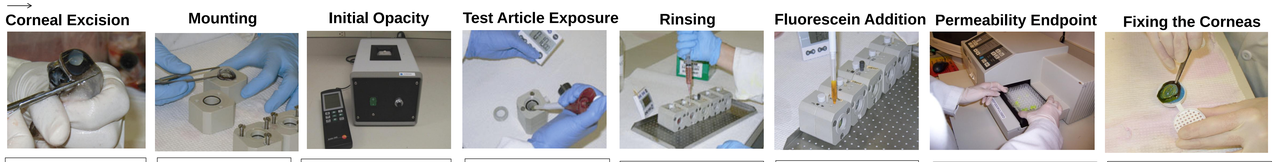

Corneal Excision	Mounting	Initial Opacity	Test Article Exposure	Rinsing	Fluorescein Addition	Permeability Endpoint	Fixing the Corneas
Upon receipt, eyes were examined and corneas free of defects were excised.	Corneas were mounted into chambers, and incubated for 1 hr. at 32 ± 1°C in cMEM .	cMEM was removed and refilled and the initial opacity was read on an opacitometer	750 µL of test substance was applied to the anterior chamber for 10 minutes at 32 ± 1°C.	Corneas were rinsed thoroughly to remove test substance, corneas incubated for 2 hours then a final opacity taken.	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 minutes.	Media was sampled from the posterior chamber and the optical density at 490 nm was quantified using a microplate reader.	Treated corneas were saved from the assay and fixed in formalin for optional histological evaluation.

RESULTS

Table 3. BCOP data (In Vitro Score) for Trial 1.

Sample	In Vitro Score	EPA Category
A	87.9	I
R (Reference)	14.5	III

Table 4. BCOP data (In Vitro Score) for Trial 2.

Sample	In Vitro Score	EPA Category
A	77.2	I
A1 (1.9% Quat)	67.7	II
A2 (1.85% Quat)	66.7	II
R (Reference)	19.4	III

Table 5. BCOP data (In Vitro Score) and Histology data for Trial 3.

Sample	In Vitro Score	EPA Category by BCOP	EPA Category by Histopathology	EPA Category
A	80.9	I	I	I
A3	64.2 (no Quat)	II	low I/ high II	I/II
A4	52.5 (1% Quat)	II	II	II

- **Trial 1.** Sample formulation A along with a reference formulation was evaluated in the BCOP assay. Sample formulation A was an EPA Category I by In Vitro Score. The reference sample was an EPA Category III (also Category III by historical animal data on the formulation). No histology performed.
- **Trial 2.** Sample formulation A re-tested in the BCOP assay along with 2 new formulations of Sample A formulated to be milder (each re-formulation contained 20% surfactant with varying levels of quaternary ammonium compounds as indicated in Table 4) and the reference formulation. Sample formulation A was an EPA Category I by In Vitro Score (as previously shown in Trial 1). New formulations of Sample A demonstrated lower In Vitro Scores and each would be an EPA Category II. Reference sample was an EPA Category III. No histology performed.
- **Trial 3.** Sample formulation A re-tested in the BCOP assay along with 2 new formulations of Sample A (each re-formulation contained 20% surfactant with varying levels of quaternary ammonium compounds as indicated in Table 5). Each re-formulation was an EPA Category II. Histopathology performed and based on criteria in Table 2 EPA categories were assigned to each sample. Overall EPA category based on In Vitro Score and Histology.

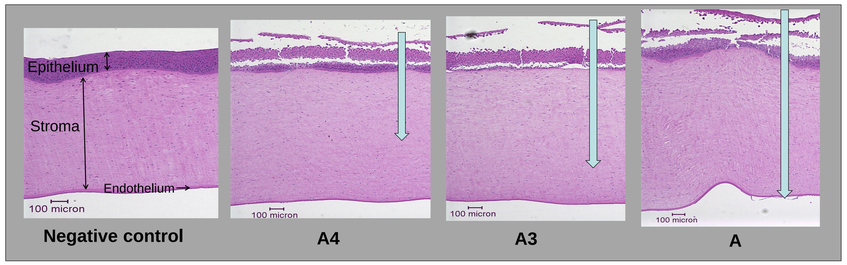

Figure 2. Histopathology Evaluation. Representative full thickness cross section of corneas treated in Trial 3. The epithelium, stroma, and endothelium was evaluated for each set of corneas and based on the criteria in Table 2 an EPA category was determined. Light blue arrows indicate depth of corneal damage.

CONCLUSIONS

- The BCOP assay proved to be a rapid, effective way to evaluate the ocular irritation potential of prototype cleaning formulations.
- The BCOP assay was able to discriminate amongst formulations with minor formulation modifications to assist in determining the formulation with the least irritation potential.
- Histopathology may be used to further evaluate the depth of injury and degree of penetration of the sample and is a critical component of the EPA non-animal eye irritation guideline.
- Using the guidelines presented by the EPA for a non-animal testing approach to EPA labeling for eye irritation, the top-down approach of BCOP combined with histology may be used during product development and for evaluation of final formulations and submission to EPA for hazard labeling.

REFERENCES

1. Redden, J, Perry, MJ, Leighton, T, Chen, J, and McMahon, T. (2009) Non-Animal Testing Approach to EPA Labeling for Eye Irritation. US Environmental Protection Agency Office of Pesticide Programs.