BOVINE CORNEAL OPACITY AND PERMEABILITY ASSAY - RESULTS OF A TWO LABORATORY REPRODUCIBILITY STUDY

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Abstract

The Bovine Corneal Opacity and Permeability assay (BCOP), an internationally recognized alternative to the Draize eye irritation test, uses excised bovine corneas to predict ocular irritation. Originally developed by Gautheron (1992) and utilizing the irritation class prediction established by Sina (1994), BCOP has been used independently at MB Research and at the Institute for in Vitro Sciences (IIVS) for over fifteen years for product development, worker safety, and safety claims substantiation. The assay has recently been included in an 18-month pilot evaluation program for use for eye irritation labeling of cleaning products with antimicrobial claims (EPA Office of Pesticide Programs, May 2009). In addition, the Organization for Economic Co-Operation and Development (OECD) adopted Test Guideline 437 describing the use of the assay for identifying ocular corrosives and severe irritants (Sept. 2009). Since MB Research and IIVS have extensive experience performing the BCOP assay utilizing a variety of protocols, they agreed to develop and evaluate the reproducibility of a standard harmonized protocol for regulatory labeling. Nine blind-coded chemicals, primarily comprised of surfactant dilutions, as well as imidazole and pyridine, were tested in three independent GLP-compliant trials using exactly the same protocol. The resulting In Vitro Scores were compared to Draize MMAS results (ECETOC, 1998). Intra-laboratory and inter-laboratory reproducibility evaluations showed that both laboratories obtained the same irritation class predictions (except for cetyl pyridinium bromide). Some of the surfactant dilutions (sodium dodecyl sulfate, cetyl pyridinium bromide) were found to be under-predicted using the standard BCOP protocol for liquid test chemicals. Accordingly, the testing of certain classes of surfactants for regulatory safety using extended exposure times may be scientifically justified.

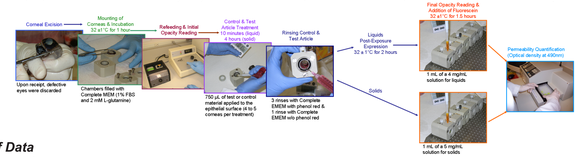
Introduction

In June 2008, the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) announced that U.S. federal regulatory agencies have accepted recommendations from ICCVAM that the Bovine Corneal Opacity and Permeability Assay (BCOP) can be used to screen and label for severe/corrosive ocular irritants and can thus reduce live animal use for ocular safety testing. Subsequently, an Office of Economic Cooperation and Development (OECD) Test Guideline describing the BCOP assay has been approved (OECD TG437). The BCOP is used to assess the potential ocular irritancy of test chemicals based on the induction of opacity and permeability (to fluorescein) in freshly isolated bovine corneas. Although ICCVAM has recommended the BCOP assay for screening of severe/corrosive ocular irritants, routine use of the assay in non-regulatory industry screening programs suggests that the test system may be used to discriminate between mild, moderate and severe eye irritants, particularly within specific chemical classes.

MB Research Laboratories and the Institute for In Vitro Sciences (IIVS) have extensive experience applying a variety of specific BCOP protocols for industrial hygiene and worker safety, raw material screening, and product development and safety applications. To meet the needs for regulatory labeling, MB Research Laboratories and IIVS developed and evaluated the reproducibility of a standardized BCOP protocol. Test chemicals were purchased commercially and provided to The Dial Corporation, A Henkel Company for repackaging, blind coding, and distribution to the labs. Four to five corneas were treated with each test chemical in at least three trials under full GLP compliance in both labs. Based on changes in corneal opacity and permeability (relative to the control corneas), in vitro scores were determined. Mean in vitro scores for the three trials were calculated and used to determine the eye irritation classifications, according to the classification system established by Sina,1994.

Materials and Methods

- Bovine eyes were obtained fresh as a by-product from the abattoir
- Eyes were transported in Hanks' Balanced Salt Solution, containing Penicillin/Streptomycin (HBSS)
- Liquid test chemicals, A, B, C, E, F, G, H, and I, were tested neat using a standard 10-minute exposure
- Solid test chemical, D, was tested as a 20% (w/v) dilution in sterile, deionized water, for a standard 4-hour exposure



Presentation of Data

Opacity Measurement

- *Corrected Change in Opacity = (Final Opacity – Initial Opacity) - Mean Negative Control Opacity*
- Mean opacity values of each treatment group were calculated

Permeability Measurement

- *Blank Corrected* OD_{490} = *Raw* OD_{490} – *Mean Blank* OD_{490}

- Negative control corrected OD_{490} values for the test chemicals and the positive control were calculated
  Final Corrected OD_{490} = *Blank Corrected* OD_{490} – *Mean Negative Control* OD_{490}

- Mean OD_{490} values of each treatment group were calculated

In Vitro Score

- In Vitro *Score = Mean Opacity Value + (15 x Mean* OD_{490} *Value)*

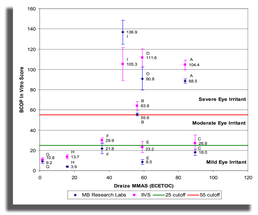
In Vitro Score		In Vitro Classification [1]
< 25	=	mild irritant
25.1 to 55	=	moderate irritant
>55	=	severe irritant

[1]In Vitro Classification - BCOP classification scheme proposed by Sina, 1994.

Results

- Mean In Vitro Scores (± 1 std. dev.) from both labs are presented relative to Draize Modified Maximum Average Score (MMAS) values (Fig. 1).
- The mean opacity and permeability values from both labs for each chemical are presented in Figures 2 through 10.
- Comparisons of the BCOP In Vitro Score classification and classifications using the in vivo Draize MMAS, GHS, and EPA systems are presented in Table 1.

Figure 1 Mean In Vitro Scores



Both laboratories obtained the same in vitro classifications, except for the two concentrations of cetylpyridinium bromide, where the IIVS predictions were just above the mild/moderate cut-off of 25, and the MB Research predictions were just below the cut-off.

The In Vitro Scores obtained at IIVS were typically slightly higher (except for Pyridine) than those obtained at MB Research, and are attributable to both higher opacity and permeability values obtained at IIVS.

The rank order of irritation determined by Draize MMAS, from highest to lowest score, was C > A > D > E > B > I > F > H > G

In comparison, the rank order determined at:
- IIVS was D > I > A > B > F > C > E > H > G
- MB Research was I > D > A > B > F > C > G > E > H

- Benzalkonium chloride induced dose-related increases in the In Vitro Score, particularly as a result of notable increases in opacity.

- Two chemicals, cetylpyridinium bromide 10% and sodium lauryl sulphate 15%, were notably under predicted relative to the in vivo classifications

- Interestingly, no dose response was observed in the BCOP assay between the 10% and 1% concentrations of cetylpyridinium bromide, in either lab. Unlike other cationic surfactants (e.g. benzalkonium chloride), the opacities induced by cetylpyridinium bromide were relatively low.

- Sodium lauryl sulphate resulted in very low opacity values at both the 15% and 3% concentrations. Dose response was essentially determined from the fluorescein permeability (OD_{490}) values. In vivo, sodium lauryl sulphate 15% resulted in the highest scores within the first day, typically with only grade 2 opacities. Complete recovery was determined in 5 of 6 test animals in 7 to 10 days (ECETOC, 1998).

For each test chemical, the irritancy predictions for the three trials within each lab were the same, except for the following, where the in vitro scores were very close to a classification cut-off:

IIVS
- Cetylpyridinium bromide 10%: 1 mild and 2 moderate predictions
- Sodium lauryl sulphate 15%: 2 mild and 1 moderate prediction

MB Research Labs
- Benzalkonium chloride 1%: 2 moderate and 1 severe prediction
- Cetylpyridinium bromide 1%: 2 mild and 1 moderate prediction

Table 1 Comparisons of the BCOP In vitro Score classification and in vivo classifications

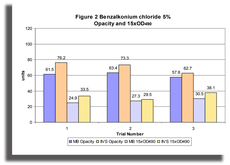
Test Chemical	Mean BCOP In vitro Score	In Vitro Classification[1]	MMAS[2]	In vivo GHS[3]	In vivo EPA[4]	In Vitro Prediction
C - Cetylpyridinium bromide 10%	IIVS: 26.9 MB Res: 18.0	IIVS: moderate MB Labs: mild	89.7	Cat. 1	Cat. I	under
A - Benzalkonium chloride 5%	IIVS: 104.4 MB Res: 88.5	severe	83.8	Cat. 1	Cat. I	OK
D - Imidazole	IIVS: 111.6 MB Res: 90.8	severe	59.3	Cat. 1	-	OK
E - Sodium lauryl sulphate 15%	IIVS: 23.2 MB Res: 8.5	mild	59.2	Cat. 1	Cat. I	under
B - Benzalkonium chloride 1%	IIVS: 63.9 MB Res: 55.6	severe	56.3 34.3	Cat. 2A	Cat. I	OK
I - Pyridine	IIVS: 105.3 MB Res: 136.9	severe	48.0	Cat. 1	-	MMAS - over GHS - OK
F - Cetylpyridinium bromide 1%	IIVS: 29.9 MB Res: 21.8	IIVS: moderate MB Labs: mild	36.0	-	-	OK, to slightly under
H - Sodium lauryl sulphate 3%	IIVS: 13.7 MB Res: 3.9	mild	16.0	No category	Cat. III	OK
G - Triton X-100 1%	IIVS: 10.8 MB Res: 9.2	mild	1.7	No category	Cat. III	OK

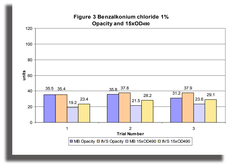
[1]In Vitro Classification - BCOP classification scheme proposed by Sina, 1994.
[2]MMAS - Modified Maximum Average Score - ECETOC Technical Report No. 48(2), 1998
[3]GHS - Globally Harmonized System (UN, 2003) [4]EPA - U.S. Environmental Protection Agency (1996)

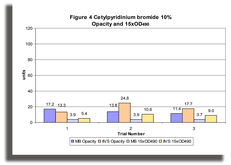
under prediction | OK prediction | over prediction

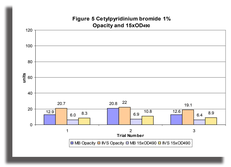
Criteria for Determination of a Valid Test

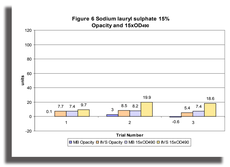
The BCOP assay was accepted when the positive control (ethanol or imidazole) caused an In Vitro Score that fell within two standard deviations of the individual lab's historical mean.

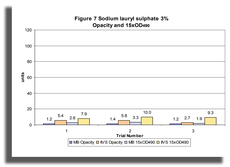


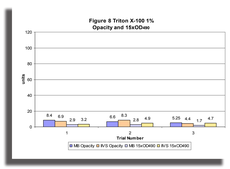


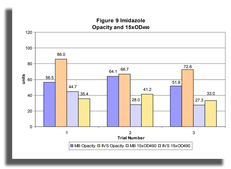


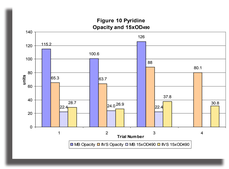












Conclusions

Intra-laboratory reproducibility evaluations showed that generally the same irritation class predictions were made in each of the three trials (51 of 55).

Inter-laboratory reproducibility evaluations showed that both laboratories generally obtained the same irritation class predictions for each chemical (7 of 9).

Overall, the BCOP assay provided similar rank order of eye irritation and similar predictions of irritation classes, relative to the available in vivo data. However, using the standard BCOP protocol for liquids, some of the surfactants were under predicted.

Some surfactants result in relatively low opacities (e.g. SLS), as a result of limited protein binding, progressive loss of epithelium, and minimal corneal swelling. Accordingly, the action of surfactants on the corneal epithelium has often been evaluated primarily based upon increases in the fluorescein permeability values (OD_{490}). Furthermore, surfactants have been tested historically using modified exposure times of 10, 30, or 60 minutes to enhance the evaluation of changes in epithelial barrier function (Cater, 2006), and to enhance the potential for progression of opacities due to stromal swelling and disorganization. These results suggest that for regulatory safety testing of certain classes of surfactants, extended exposure times may be justified.

Acknowledgments

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