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Performance of the Bovine Corneal Opacity and Permeability (BCOP) Test Method in Detecting Ocular Corrosives and Severe Irritants

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Introduction

The ocular irritation or corrosion potential of substances, to which humans may be exposed, has been evaluated since 1944 by the Draize rabbit eye test (Draize et al. 1944). There have been widespread efforts to develop and validate *in vitro* alternatives that might reduce or replace the use of animals for ocular irritancy assessments. The U.S. EPA[1] formally nominated to ICCVAM[1] four *in vitro* test methods, including the BCOP[1] assay, for evaluation of their ability to identify ocular corrosives and severe irritants in a tiered testing strategy.

NICEATM[1], in conjunction with the ICCVAM Ocular Toxicity Working Group, prepared a comprehensive BRD[1] reviewing the available data and information on the BCOP test method[2]. NICEATM released the draft BCOP BRD for public comment on November 1, 2004. On January 11-12, 2005, ICCVAM convened an Expert Panel to independently evaluate the validation status of BCOP and three other *in vitro* test methods for identifying ocular corrosives or severe irritants[3]. Public comments at that meeting indicated that additional data was available. The Expert Panel recommended that any additional data that could be obtained should be considered for a reanalysis of the accuracy and reliability of each test method.

In response to the Expert Panel recommendation, an *FR*[1] notice requesting the submission of all available *in vitro* BCOP test data and corresponding *in vivo* rabbit eye test data was reissued on February 28, 2005 (*FR* Vol. 70, No. 38, pp. 9661-9662). In addition to considering any BCOP data received in response to the *FR* notice, the reanalysis of the accuracy and reliability of this test method took into account (1) changes that occurred in the ocular irritancy classification of a few substances in response to clarification of the EU[1] (2001) and UN GHS[1] (UN 2003) ocular irritation classification rules, (2) a decision to use classifications based on *in vivo* rabbit eye test data only, and (3) revised chemical class assignments for some substances. Additional information on the reanalysis can be obtained at http://iccvam.niehs.nih.gov/methods/ocudocs/reanalysis.htm

[1] BCOP = Bovine Corneal Opacity and Permeability; BRD = Background Review Document; *FR* = Federal Register; GHS = Globally Harmonized System; ICCVAM = Interagency Coordinating Committee on the Validation of Alternative Methods; NICEATM = National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods; UN = United Nations; U.S. EPA = U.S. Environmental Protection Agency.

[2] The draft BCOP BRD can be obtained at http://iccvam.niehs.nih.gov/methods/ocudocs/ocu_brd.htm

[3] The Expert Panel Report can be obtained at http://iccvam.niehs.nih.gov/methods/ocudocs/EPreport/ocureport.htm

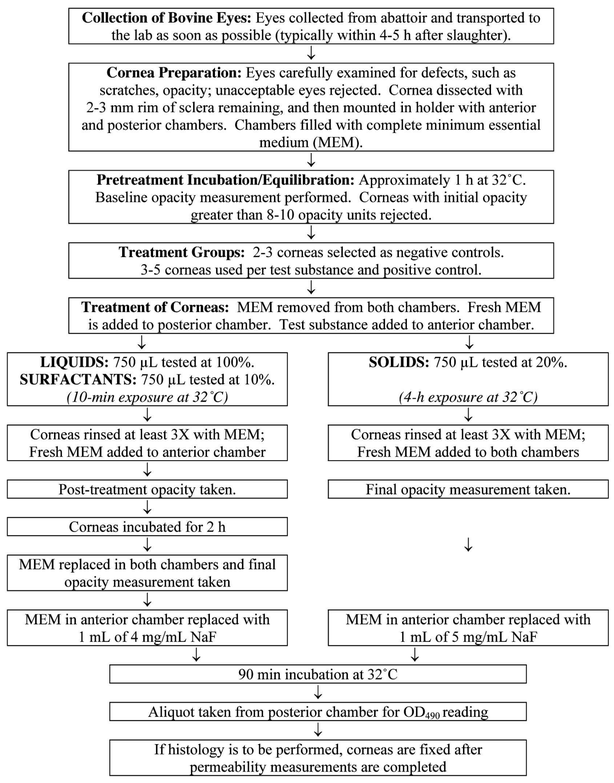
Test Method Overview

The basic procedure for the BCOP test method is provided in **Figure 1**. Historically, negative control corneas have been used to correct opacity and permeability values measured on treated corneas. Mean corrected opacity and mean corrected permeability values are calculated for each treatment group. An *In Vitro* Irritancy Score (IVIS) is calculated using the following empirically-derived formula (Sina et al. 1995): IVIS = Opacity value + (15 x optical density at 490 nm [OD_{490}] value). An *In Vitro* Irritancy Score > 55.1 is considered a severe eye irritant.

Some substances, such as anionic and nonionic surfactants, increase permeability without significant opacity; thus, only permeability values are used for certain chemical classes. In such situations, a test substance that increase permeability (OD_{490} > 0.600) is considered a severe irritant.

In addition, histopathological evaluation of the treated cornea (conducted after permeability is assessed) has been used on a case-by-case basis (Curren et al. 2000). However, a standardized histopathology test method protocol and data is not available, and therefore not included in this evaluation.

Figure 1. Basic Procedures for the BCOP Assay



Test Method Database

The following studies were used for the various reanalyses

- Gautheron et al. (1994)
- Balls et al. (1995)
- Swanson et al. (1995)
- Gettings et al. (1996)
- Casterton et al. (1996)
- Southee (1998)
- Swanson and Harbell (2000)
- Bailey et al. (2004)
- Submission from Dr. Joseph Sina

Test Method Protocols

The BCOP test method protocols used in these studies were similar to each other, but not identical (differences included number of corneas used [n=3-5], storage conditions of bovine eyes during transport, different negative controls).

BCOP Test Method Accuracy Analysis

The accuracy of the BCOP test method for the various data analysis methods, when compared to *in vivo* rabbit eye test classifications using the EPA (1996), EU (2001), and UN GHS (UN 2003) classification systems are provided in **Table 1**. The overall BCOP test method accuracy with regard to each of the three classification systems ranged from 79% to 81%, while the false positive and false negative rates ranged from 19% to 21% and 16% to 25%, respectively. The small number of substances representing most chemical classes allows for only limited conclusions with respect to the accuracy of BCOP by chemical class or property of interest (e.g., solids vs. liquids, basic vs. acidic pH, surfactants). However, among classes with at least six substances for analysis, alcohols, carboxyclic acids, heterocycles, and ketones tend to be overpredicted, while solids tend to be underpredicted (**Table 2**). The underprediction rate was independent of whether substances were classified *in vivo* as ocular corrosives/severe irritants based on ocular lesion severity or lesion persistence (data not shown).

Table 1. BCOP Test Method Accuracy[1]

Statistic	Ocular Hazard Classification System		
	GHS (n=147)	EPA (n=143)	EU (n=143)
Accuracy	81% (119/147)	79% (113/143)	80% (114/143)
Sensitivity	84% (36/43)	75% (30/40)	82% (33/40)
Specificity	80% (83/104)	81% (83/103)	79% (81/103)
False Positive Rate	20% (21/104)	19% (20/103)	21% (22/103)
False Negative Rate	16% (7/43)	25% (10/40)	18% (7/40)

Abbreviations: EPA = U.S. Environmental Protection Agency; EU = European Union; GHS = United Nations Globally Harmonized System for Classification and Labeling of Chemicals; n = number of substances in the database. The numbers in parenthesis indicate the data on which the % value is based.
[1]BCOP data from the following studies were pooled for this analysis: Gautheron et al. (1994), Balls et al. (1995), Swanson et al. (1995), Gettings et al. (1996), Southee (1998), Swanson and Harbell (2000), Bailey et al. (2004). Performance calculated using the overall *in vitro* classification based on the majority and/or most severe classification among the multiple testing laboratories and tests (for substances tested multiple times in a laboratory).

Table 2. False Negative and False Positive Rates of the BCOP Test Method, by Chemical Class and Properties of Interest, for the GHS[1] Classification System

Category	N[2]	False Positive Rate[3]	False Negative Rate[3]
Overall	147	20% (21/104)	16% (7/43)
Chemical Class			
Alcohols	21	50% (9/18)	67% (2/3)
Amine/Amidine	8	0% (0/4)	0% (0/4)
Carboxylic acids	16	33% (3/9)	14% (1/7)
Esters	12	12% (1/8)	0% (0/4)
Ether/Polyether	6	0% (0/5)	0% (0/1)
Heterocycles	12	33% (2/6)	17% (1/6)
Hydrocarbons	11	9% (1/11)	- (0/0)
Ketones	9	33% (3/9)	- (0/0)
Onium compounds	11	0 % (0/3)	0% (0/8)
Properties of Interest			
Liquids	93	2% (18/69)	4% (1/24)
Solids	34	10% (2/20)	43% (6/14)
Pesticide	8	33% (1/3)	40% (2/5)
Surfactants[4]	35	5% (1/21)	7% (1/14)

[1]GHS = Globally Harmonized System (UN 2003).
[2]N = number of substances.
[3]False Positive Rate = the proportion of all negative substances that are falsely identified as positive *in vitro;* False Negative Rate = the proportion of all positive substances that are falsely identified as negative *in vitro*. The data used to calculate the percentage are provided in parenthesis.
[4]Combines single chemicals labeled as surfactants along with surfactant-containing formulations.
Highlighted cells indicate those chemical classes and properties of interest where the rate of misclassification is (a) greater than the overall rate, (b) is based on a sufficient number of substances (N > 6 substances), and (c) would be expected to have an appreciable effect on the overall rate, if excluded from the database when conducting an accuracy analysis.

BCOP Test Method Reliability Analysis

Table 3. %CV Values for *In Vitro* Irritancy Scores from Replicate Corneas

		Dr. Sina[1] (n=4)	Swanson et al. (1995)[2] (n=5)	Southee (1998)[3] (n=3)		
				Lab 1	Lab 2	Lab 3
All Data	Mean	71%	10.9%	48.3%	39.2%	30.5%
	Median	35%	9.4%	14.2%	11.8%	12.4%
	Range	1.1% - 479%	1.9% - 32.5%	0.1% - >500%[4]	2.1% - >500%[5]	4.3% - >500%[5]
Severe *In Vitro*	Mean	8.2%	8.9%	8.4%	8.4%	11.1%
	Median	8.1%	7.9%	7.1%	8.1%	9.3%
	Range	1.1% - 13%	1.9% - 25.7%	0.1% - 22%	2.1% - 21.7%	5.1% - 30.3%

Abbreviations: CV = coefficient of variation; n = number of corneas per test substance.
[1]Protocol used for study: five substances classified as severe irritants *in vitro*.
[2]Eighteen of 20 test substances evaluated were used for this analysis, since two of the substances produced negative *In Vitro* Irritancy Scores (resulted in negative CVs); 16 substances classified as severe *in vitro*.
[3]Sixteen substances were evaluated in 3 laboratories (2-5 experiments each).
[4]Sodium oxalate, which was tested twice in this laboratory, produced a mean *in vitro* score <1 and %CVs >500. Results around the baseline of the assay tended to produce higher %CVs.
[5]In this laboratory, one trial of Tween 20 produced a mean *in vitro* score <1 and a %CV >500. Results around the baseline of the assay tended to produce higher %CVs.

Intralaboratory Reproducibility[4]

Gettings et al. (1996): 25 substances, 3 trials, 1 lab

- Mean %CV and Median %CV for permeability value were 33.4 and 29.0, respectively
- Substances spanned a range of irritancy
- Surfactant-based personal care cleaning formulations

Southee (1998): 16 substances, = 2 trials, 3 labs

- Mean %CVs for IVIS ranged from 12.6 to 14.8
- Median %CVs for IVIS ranged from 6.7 to 12.4
- Substances spanned a range of irritancy and chemical classes

Interlaboratory Reproducibility[5]

Two types of interlaboratory reproducibility analyses were conducted:

- ***Qualitative analysis***: Extent of agreement among testing laboratories for classification of ocular corrosives and severe irritants
- ***Quantitative analysis***: Evaluated using a CV calculation to compare variability in IVIS values

Qualitative Analysis

Table 4. BCOP Interlaboratory Reproducibility – Percentage of GHS Classification Agreement Among Laboratories

% Interlaboratory Agreement	Gautheron et al. (1994) (11 or 12 labs)	Balls et al. (1995) (5 labs)	Southee (1998) (3 labs)
100% (all substances)	65% (34/52)	68% (41/60)	94% (15/16)
≥80% (all substances)	87% (45/52)	85% (51/60)	94% (15/16)
100% (GHS Category 1 Substances)	67% (4/6)	76% (13/17)	100% (4/4)
≥80% (GHS Category 1 Substances)	83% (5/6)	94% (16/17)	100% (4/4)

Abbreviations: GHS = Globally Harmonized. The numbers in parenthesis indicate the data on which the % value is based.

Quantitative Analysis

Table 5. BCOP Interlaboratory %CV Values for *In Vitro* Irritancy Scores

		Gautheron et al. (1994) (11 or 12 labs)	Balls et al. (1995) (5 labs)	Southee (1998) (3 labs)
All Data	Mean	168% (52)	50% (50)	32% (16)
	Median	46.9% (52)	26% (50)	23% (16)
	Range	16.5% – 1325% (52)	7.6% - 712% (50)	7.5% - 109% (16)
GHS Category 1 Substances	Mean	36% (17)	25% (32)	11.1% (5)
	Median	17% (17)	22% (32)	8.6% (5)
	Range	16.5% - 55.7% (17)	7.6% - 89.4% (32)	7.5% - 21.6% (5)

Abbreviations: CV = coefficient of variation; GHS = Globally Harmonized System. The number in the parentheses is the number of substances on which the %CV analyses are based.

[4] Intralaboratory Repeatability=The closeness of agreement between test results obtained within a single laboratory when the procedure is performed on the same substance under identical conditions within a given time period; in this case, this refers to the variability among replicate corneas. Intralaboratory Reproducibility=A determination of whether qualified people within the same laboratory can successfully replicate results using a specific test protocol at different times.

[5] Interlaboratory Reproducibility=A measure of whether different qualified laboratories using the same protocol and test substances can produce qualitatively and quantitatively similar results.

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