PROTOCOL REFINEMENT OF THE BOVINE CORNEAL AND OPACITY PERMEABILITY (BCOP) TEST FOR EYE IRRITATION

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ABSTRACT

Improvement of ocular irritancy prediction using a modified (shortened) 3-minute exposure in the BCOP assay was proposed for alcohols and ketones, which have been identified by ICCVAM as responsible for the most over-predictions in the BCOP. Eight alcohols and six ketones were tested using both the modified and the standard 10-minute exposure, and the data were compared with the GHS categories from the ICCVAM database. The evaluation of the 3-minute exposure data revealed that five of the 6 over-predicted alcohols showed an improved prediction, and of the 2 correctly predicted alcohols, one became an under-prediction and one remained the same. Two of the five over-predicted ketones showed an improved prediction, with the three other remaining the same. The one correctly predicted ketone remained the same. The results of the evaluation of the modified BCOP assay using the 3-minute exposures for alcohols and ketones suggest that improvements in the predictive capacity of the assay can be achieved by reducing the over-prediction of these small molecule, solvent-type chemicals, without an adverse impact upon the rate of under-prediction of similar chemicals. It is our recommendation that a) additional small molecule alcohols and ketones exhibiting solvent-like physical characteristics should be tested in the BCOP assay using the 3-minute (or shorter) and 10-minute exposures, and b) prior to any additional testing a more thorough evaluation of the supporting rabbit ocular irritation data be conducted to ascertain whether the correct standards are being used to calibrate the assay.

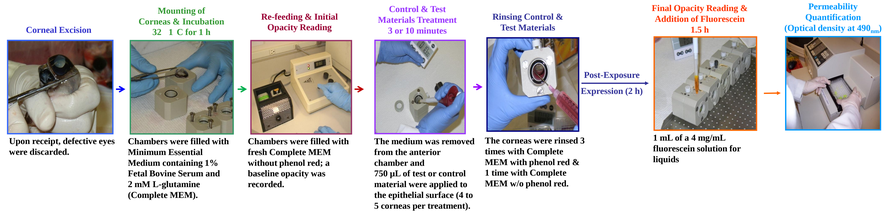
Note: Currently, 6 of 7 over-predicted alcohols (ICCVAM BCOP Database) showed improvement, and of the 2 correctly predicted, one became an under-prediction.

INTRODUCTION

The purpose of this study was to refine the BCOP assay for its use with alcohols and ketones with the goal of assigning hazard categories defined by the Globally Harmonized System (GHS) or European Union (EU) eye classification systems. The study re-analyzed a large database of BCOP study results originally reviewed by the U.S. Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM). We revised the GHS irritation prediction to include a GHS non-irritant range based upon the observation that a preponderance of non-irritant test chemicals resulted in BCOP *In Vitro* Scores of 3 or less. This proposed new prediction model helped determine which of the previously cited (by ICCVAM) chemicals representing alcohols and ketones were, in fact, inappropriately predicted.

MATERIALS & METHODS

- Bovine eyes were obtained fresh as a by-product from the abattoir
- Eyes were transported in Hanks' Balanced Salt Solution (HBSS), containing Penicillin/Streptomycin
- The alcohols and ketones were tested neat
- *In Vitro* Score = Mean Opacity Value + (15 x Mean OD_{490} Value)



CONCLUSIONS

- The results of the evaluation of the modified BCOP assay using the 3-minute exposures for alcohols and ketones, suggest that improvements in the predictive capacity of the assay can be achieved by reducing the over-prediction of these small molecule solvent chemicals, without an adverse impact upon the rate of under-prediction of similar chemicals.
- A rationale for reducing the exposure kinetics for these materials may be that dose retention (i.e., infinite dose conditions) in the *in vivo* setting is difficult to maintain, and for these classes of materials irritant effects may readily be dose volume dependent.
- For some of these materials (e.g., ethanol, methanol, acetone) the irritant effects of small doses can be rapidly reduced by dilution in aqueous conditions, such as those encountered by tear production and upon dilution in the upper epithelium in ocular exposures.
- In contrast, in the BCOP assay the infinite dose typically utilized is precisely maintained throughout the defined exposure times, and may not accurately model the exposure and dilution scenarios likely encountered *in vivo*.
- It is our recommendation that additional small molecular weight alcohols and ketones exhibiting solvent-like physical characteristics shall be tested in the BCOP assay using the 3-minute (or shorter) and 10-minute exposures. Of particular interest are the chemicals which were predicted as Category 1 irritants in the rabbit model and which should be tested to assure they retain correct categorization by the BCOP assay.

STANDARD BCOP PROTOCOL

REFINED BCOP PROTOCOL

RESULTS

ALCOHOLS

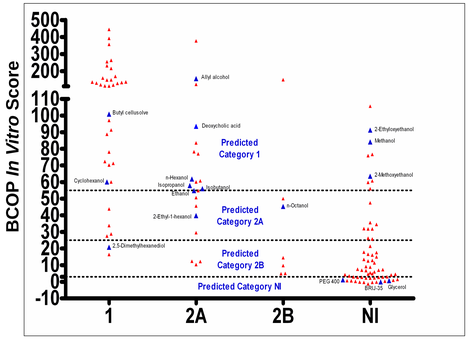


Table 1. Preliminary analysis of BCOP predictive capacity for alcohols

Alcohols (17 total)		
Correctly predicted	7	41% √
Over-predicted	9	53% ↑
Under-predicted	1	6% ↓

Figure 1. Graph of BCOP *In Vitro* Score versus *in vivo* GHS categories for all chemicals (▲) in the ICCVAM BCOP database. Materials designated by ICCVAM as alcohols are depicted by blue symbols.

Eight of the 17 alcohols were selected for testing in the 3-minute protocol:

7 over-predicted: 2-Ethoxyethanol, 2-Methoxyethanol, Allyl Alcohol, Isobutanol, Methanol, n-Hexanol, n-Octanol

1 correctly predicted: 2-Ethyl-1-hexanol

In Vitro Score	GHS Prediction
≤ 3	NI (Non Irritant; No Cat.)
3.1 to 25	Category 2B
25.1 to 55	Category 2A
≥ 55.1	Category 1

KETONES

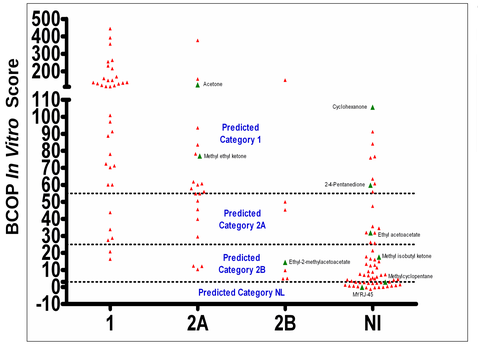


Table 2. Preliminary analysis of BCOP predictive capacity for ketones

Ketones (9 total)		
Correctly predicted	3	33.33% √
Over-predicted	6	66.67% ↑
Under-predicted	0	0% ↓

Figure 2. Graph of BCOP *In Vitro* Score versus *in vivo* GHS categories for all chemicals (▲) in the ICCVAM BCOP database. Materials designated by ICCVAM as ketones are depicted by green symbols.

Six of the 9 ketones were selected for testing in the 3-minute protocol:

5 over-predicted: Acetone, Cyclohexanone, Ethyl acetoacetate, Methyl Ethyl Ketone, Methyl Isobutyl Ketone

1 correctly predicted: Ethyl-2-methylacetoacetate

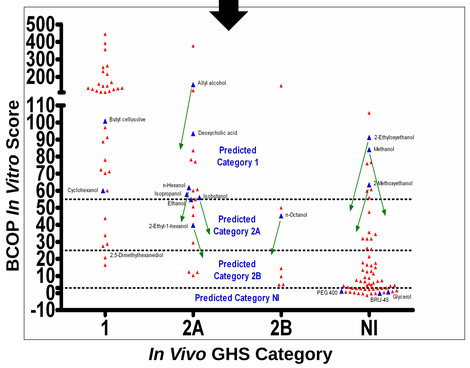


Figure 3. Evaluation of the results of the modified BCOP assay for alcohols. The changes in the *In Vitro* Scores from the 10-minute exposure to the 3-minute exposure are presented as green arrows.

The evaluation of the 3-minute exposures revealed the following:

3 over-predicted became correctly predicted: Isobuthanol, n-Hexanol, n-Octanol

4 remained over-predicted: 2-Ethoxyethanol, 2-Methoxyethanol, Allyl Alcohol, Methanol

1 correctly predicted became under-predicted: 2-Ethyl-1-hexanol

In Vitro Score	GHS Prediction
≤ 3	NI (Non Irritant; No Cat.)
3.1 to 25	Category 2B
25.1 to 55	Category 2A
≥ 55.1	Category 1

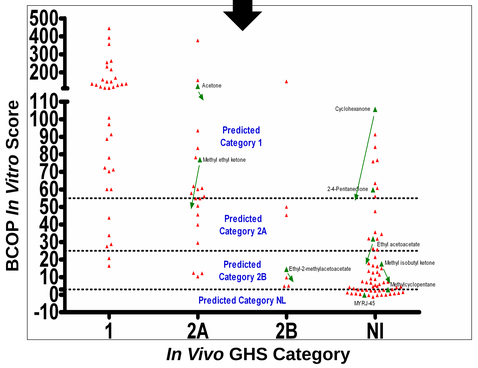


Figure 4. Evaluation of the results of the modified BCOP assay for ketones. The changes in the *In Vitro* Scores from the 10-minute exposure to the 3-minute exposure are presented as green arrows.

The evaluation of the 3-minute exposures revealed the following:

1 over-predicted became correctly predicted: Methyl Ethyl Ketone

4 remained over-predicted: Acetone, Cyclohexanone, Ethyl acetoacetate, Methyl Isobutyl Ketone

1 remained correctly predicted: Ethyl-2-methylacetoacetate

Table 3. Evaluation of the results of the modified BCOP assay of alcohols. Summary of the BCOP *In Vitro* Scores and *in vivo* GHS predictions.

Chemical name	Rabbit GHS	ICCVAM BCOP Score	BCOP GHS	IIVS 3 min Score	BCOP GHS	MMAS	MMAS Source	MW
2-Ethoxyethanol	NI	91.3 ↑	1	49.9 ↑	2A	18	Gautheron, 1994	90.12
2-Ethyl-1-hexanol	2A	39.8 √	2A	21.8 ↓	2B	51.3	ECETOC	130.23
2-Methoxyethanol	NI	63.5 ↑	1	38.0 ↑	2A	15.3	Gautheron, 1994	76.09
Allyl Alcohol	2A	156.3 ↑	1	85.2 ↑	1	31.3	Gautheron, 1994	58.08
Isobuthanol	2A	56.0 ↑	1	34.1 √	2A	60.3	ECETOC	74.12
Methanol	NI	84.2 ↑	1	44.5 ↑	2A	17	Gautheron, 1994	32
n-Octanol	2B	45.4 ↑	2A	24.2 √	2B	41	ECETOC	130
n-Hexanol	2A	61.9 ↑	1	40.3 √	2A	64.8	ECETOC	102.18

6	= improvement in GHS category at 3 minute exposure
1	= worse GHS category prediction at 3 minute exposure
1	= remained the same

ECETOC = European Centre for Ecotoxicology and Toxicology of Chemicals
ECVAM = European Centre for the Validation of Alternative Methods
MMAS = Modified Maximum Average Score
MW = Molecular Weight

Table 4. Evaluation of the results of the modified BCOP assay of ketones. Summary of the BCOP *In Vitro* Scores and *in vivo* GHS predictions.

Chemical name	Rabbit GHS	ICCVAM BCOP Score	BCOP GHS	IIVS 3 min Score	BCOP GHS	MMAS	MMAS Source	MW
Acetone	2A	123.0 ↑	1	114.2 ↑	1	65.8	ECETOC	58.08
Cyclohexanone	NI	105.6 ↑	1	55.1 ↑	1	26.7	Gautheron, 1994	98.14
Methyl Ethyl Ketone	2A	77.0 ↑	1	49.3 √	2A	50.0	ECETOC	72.11
Methyl Isobutyl Ketone	NI	17.6 ↑	2B	7.2 ↑	2B	4.8	ECETOC	100.16
Ethyl acetoacetate	NI	31.8 ↑	2A	19.4 ↑	2B	21.3	Gautheron, 1994	130.14
Ethyl-2-methyl acetoacetate	2B	14.4 √	2B	10.0 √	2B	18.0	ECETOC	202.2

2	= improvement in GHS category at 3 minute exposure
4	= remained the same

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