A TIERED *IN VITRO* IRRITATION/CORROSION TESTING STRATEGY FOR GHS CLASSIFICATION OF PHARMACEUTICAL COMPOUNDS

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ABSTRACT

Irritation reactions are a frequently reported occupational health hazard. To reduce animal testing, BMS and IIVS have developed a testing strategy using three *in vitro* assays to assess the irritation/corrosive potential of pharmaceutical compounds (PC) for worker safety. The strategy allows for GHS classification by utilizing the Corrositex® assay for corrosivity (OECD TG 435), the Bovine Corneal Opacity and Permeability (BCOP) assay for ocular irritation (OECD TG 437), and the EpiDerm™ skin irritation test (SIT) for dermal irritation (OECD TG 439). Twenty-five solid PCs were evaluated in this tiered testing strategy. First the pH of each substance was determined. If the pH was ≥11 or ≤2, a Corrositex® assay was conducted. If the compound was negative in the Corrositex® assay or the pH was between 2 - 11, a BCOP assay was performed followed by a SIT assay. Based on their extreme pH, 4 compounds were tested in the Corrositex® assay, which resulted in corrosive predictions (packing group II or III) and thus no further testing was needed. Twenty-two compounds were evaluated in the BCOP assay (both neat and as a 20% dilution), with the higher response used for classification. The results were 5 Category 1 (score>55), and 8 non-irritants (score<3). There were 9 compounds with scores between 3 - 25, which were described as mild irritants on internal BMS hazard communications. Twenty-five compounds were evaluated using the SIT assay and were classified as non-irritants to skin. This is consistent with the BMS historical animal model results showing very low number of PCs as skin irritants. The comparison also confirmed 50% viability as an acceptable cut off for GHS dermal irritation classification. This tiered testing strategy, which replaces the use of animal studies, represents a rational platform that can be utilized for the prediction of ocular and dermal irritation/corrosive potential of PCs.

INTRODUCTION

BMS and IIVS utilized three *in vitro* assays to develop a tiered testing strategy for the assessment of ocular and dermal hazards posed to workers handling pharmaceutical compounds. Each assay used in the testing strategy has been accepted by regulatory agencies, and has an OECD guideline associated with it. For the four materials that were found to be corrosive in the Corrositex® assay, additional testing was generally not performed since it was assumed that they would likely yield positive results in the SIT and BCOP assays. Testing procedures followed the OECD guidelines for all three assays; however an additional exposure method and evaluations were also utilized in the BCOP assay to further classify the test material. The majority of the PCs tested were solids, and it is documented that solid materials can have a higher rate of under-prediction in the BCOP assay. Therefore, we took a more conservative approach of testing a 20% dilution of the PC (OECD method), as well as the neat material (~150 mg per cornea), and utilized the highest score for classification. BMS also developed its own hazard category scale in order to provide classification for all *In Vitro* scores obtained in the assay, in addition to those outlined in the test guideline.

PROPOSED TIERED TESTING STRATEGY

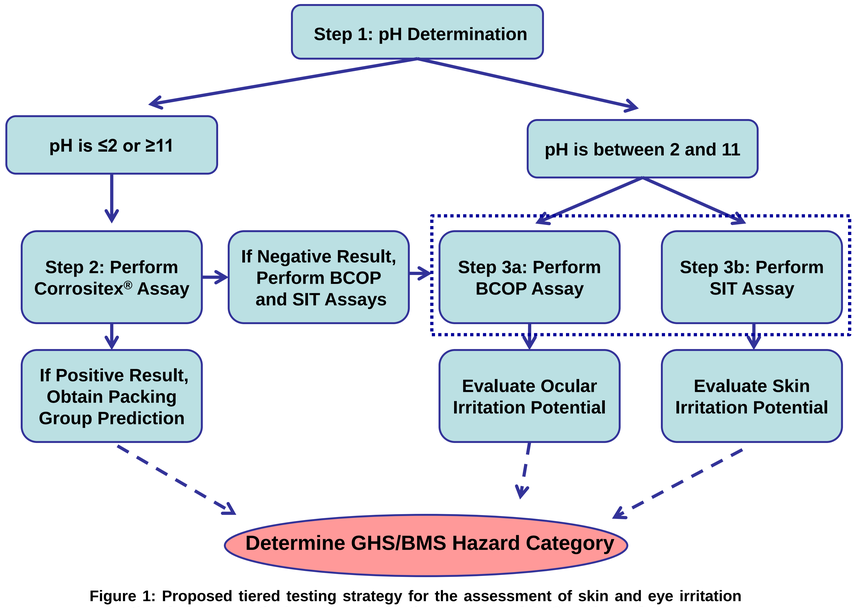


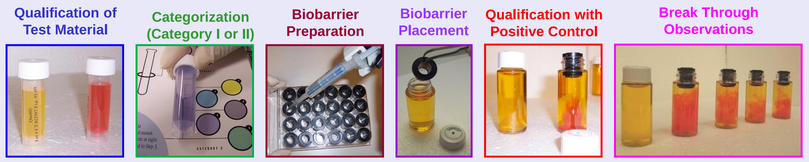
Figure 1: Proposed tiered testing strategy for the assessment of skin and eye irritation potential of pharmaceutical compounds for the purposes of BMS worker safety.

MATERIALS & METHODS

Step 1: pH Determination

The pH values of 25 pharmaceutical compounds (provided by BMS) were tested as 20% w/v dilutions in saline using pH strips (Figure 1, Step 1). PCs with a pH ≥11 or ≤2 were subsequently tested in the Corrositex® assay (Figure 1, Step 2), while PCs with a pH between 2 and 11 were tested in both the BCOP and SIT assays (Figure 1, Steps 3a and 3b).

Step 2: Corrositex® assay



The Corrositex® assay, as outlined above, was performed in accordance with OECD TG 435 using PCs with an identified pH ≥11 or ≤2 . After categorization of the test material (Category I or II), the following tables were used to evaluate the test results.

Category I Classification

Mean Time to Produce a Change in Chemical Detection System	Packing Group
≤ 3 Minutes	I
> 3 Minutes - 1 Hour	II
> 1 - 4 Hours	III
> 4 Hours	Not Applicable

Corrosivity
Corrosive
Corrosive
Corrosive
Non-corrosive

Category II Classification

Mean Time to Produce a Change in Chemical Detection System	Packing Group
≤ 3 Minutes	I
> 3 Minutes - 30 minutes	II
> 30 - 60 minutes	III
> 60 minutes	Not Applicable

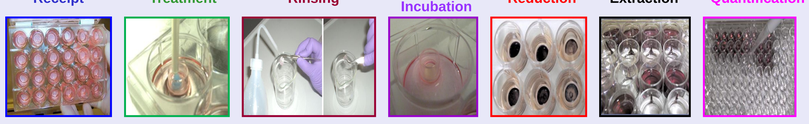
Step 3a: Bovine Corneal and Opacity (BCOP) Assay

The BCOP assay, as outlined above, was performed in accordance with OECD TG 437 using PCs with an identified pH between 2 and 11. PCs were exposed to the corneas both neat and as 20% w/v dilutions in saline. A GHS (highlighted in Purple) or BMS hazard category (highlighted in Yellow) was determined based on the calculated *In Vitro* Score. The following classification system, based on and further modified from Gautheron et al., 1992 (Sina scale), was used to evaluate the eye irritation potential of each PC. Between the two testing concentrations, the concentration yielding the higher *In Vitro* score was utilized for classification.

In Vitro Score	GHS/BMS Hazard Category
> 55	Category 1 (Severe Irritant)
>25 to ≤55	Moderate Irritant
>3 to ≤25	Mild Irritant
≤ 3	No Category (Non-Irritant)

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

Step 3b: Skin Irritation (SIT) Assay



Mean Tissue Viability	Prediction to be considered UN GHS Category	
< 50%	Irritant	Category 2
≥ 50%	Non-irritant	No Category

The SIT assay, as outlined above, was performed in accordance with OECD TG 439 using PCs with an identified pH between 2 and 11. The following table was used for classification of skin irritation.

RESULTS

PREDICTED HAZARD CATEGORIES BASED ON PROPOSED TIERED TESTING

PC ID	pH	Dermal Irritation			Ocular Irritation	
		Corrositex® Assay	SIT Assay	GHS/BMS Skin Hazard Category	BCOP Assay *In vitro* Score[a]	GHS/BMS Eye Hazard Category[b]
A	1.5	Packing Group III		Corrosive (Category 1C)		
B	1.5	Packing Group III		Corrosive (Category 1C)		
C	0.0	Packing Group II		Corrosive (Category 1B)		
D	1.5	Packing Group II		Corrosive (Category 1B)	133.2	Category 1 (Severe Irritant)
E	13.0		Non-Irritant	No Category	222.9	Category 1 (Severe Irritant)
F	4.5		Non-Irritant	No Category	146.1[c]	Category 1 (Severe Irritant)
G	7.5		Non-Irritant	No Category	65.6[c]	Category 1 (Severe Irritant)
H	5.0		Non-Irritant	No Category	61.0[c]	Category 1 (Severe Irritant)
I	4.0		Non-Irritant	No Category	39.7	Moderate Irritant
J	3.5		Non-Irritant	No Category	20.3	Mild Irritant
K	3.0		Non-Irritant	No Category	16.6	Mild Irritant
L	5.5		Non-Irritant	No Category	15.7[c]	Mild Irritant
M	5.0		Non-Irritant	No Category	12.0	Mild Irritant
N	4.0		Non-Irritant	No Category	9.1[c]	Mild Irritant
O	6.0		Non-Irritant	No Category	7.3	Mild Irritant
P	4.0		Non-Irritant	No Category	6.0[c]	Mild Irritant
Q	5.0		Non-Irritant	No Category	4.6[c]	Mild Irritant
R	5.0		Non-Irritant	No Category	2.0	No Category (Non-Irritant)
S	3.5		Non-Irritant	No Category	1.7	No Category (Non-Irritant)
T	8.0		Non-Irritant	No Category	1.6	No Category (Non-Irritant)
U	5.5		Non-Irritant	No Category	1.5	No Category (Non-Irritant)
V	6.0		Non-Irritant	No Category	1.3	No Category (Non-Irritant)
W	4.5		Non-Irritant	No Category	0.3	No Category (Non-Irritant)
X	5.0		Non-Irritant	No Category	-1.4	No Category (Non-Irritant)
Y	5.0		Non-Irritant	No Category	-1.6	No Category (Non-Irritant)

[a]-PCs were tested in the BCOP assay neat and as 20% w/v dilutions in saline. The reported *In Vitro* score and corresponding classification are from the test concentration resulting in the higher value.
[b]-Hazard categorizations adhering to GHS guidelines (≤3 or >55) are highlighted in Purple while hazard categorizations further classified by BMS (BCOP *In Vitro* Score >3 and ≤55) are highlighted in Yellow.
c-A higher *In vitro* score and hazard category were obtained using the results from the neat test article, as opposed to the 20% dilution.

CONCLUSIONS

The testing strategy developed by IIVS and BMS proved to be a useful tool in replacing animal studies for the assessment of ocular or dermal hazards of pharmaceutical compounds. The Corrositex® assay confirmed the corrosive nature of the 4 materials predicted to be corrosive by pH measurements. The Skin Irritation Test classified all 22 materials tested as non-irritant, which is supported by BMS historical in vivo data for PCs. BMS utilized a more conservative approach of using the highest score obtained from either the neat or diluted test article to classify their compounds. As a result, 7 of the 22 compounds tested in BCOP received a higher hazard category than what would have been predicted with the 20% dilution. Although the test guideline does not provide classifications for materials with *in vitro* scores of between 3 and 55, BMS utilized historical information on the BCOP assay to classify PCs as mild irritants (with *in vitro* scores of 3-25), or GHS Category 2 - moderate irritants (with *in vitro* scores of 25.1-55). Continued efforts should be made to generate data in this range, so that future classifications may gain regulatory acceptance.

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