Ocular Irritation Risk Assessments Using *In Vitro* BCOP (OECD 437) and 3D Assays (OECD 492)

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1 Introduction

Ocular safety testing classifies compounds according to the United Nations Global Harmonized System of Classification and Labeling of Chemicals (UN GHS) as No Category (non irritant), Category 2 (irritant) or Category 1 (corrosive/ irreversible damage). A number of alternatives to the rabbit eye irritation test (OECD 405[a]; Draize) are now in use, based on the principles of reduction, refinement and replacement (3Rs). The most frequently used models are the Bovine Corneal Opacity and Permeability (BCOP) assay (OECD 437[b]) and the 3D reconstructed tissue models; MatTek EpiOcular™ and SkinEthic™ HCE (OECD 492[c]).

Each of the *in vitro* assays predicts some of the UN GHS categories, however, none of the available validated non-animal alternatives are universally applicable. BCOP assigns classifications of "No Category", "No prediction can be made" and "Category 1". The 3D models assign classifications of "No Category" and "Category 1/2". Together, these *in vitro* models are being used in top-down and bottom-up (Figure 1) approaches for risk assessment. A cross section through the EpiOcular™ tissue model is presented in Figure 2. The BCOP equipment is presented in Figure 3.

2 Figure 1: Ocular Irritation Risk Assessment Strategy

3 Figure 2: EpiOcular™ Model

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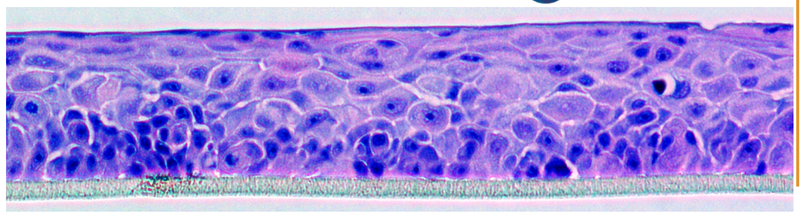

4 Figure 3: BCOP Equipment

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5 Methods and Results

The OECD test guidelines require that testing laboratories demonstrate technical competence with a proficiency panel of solid and liquid test chemicals. The test guidelines and any relevant manufacturers' instructions were followed.

For BCOP, the proficiency panel contained 5 "No Category", 3 "No prediction can be made" and 4 "Category 1" chemicals. All chemicals were correctly assigned based on the resultant *In Vitro* Irritation Score (IVIS). Results are presented in Table 1.

For EpiOcular™, the proficiency panel contained 7 "No Category" and 8 "Category 1/2" chemicals. All chemicals were correctly assigned based on viability measured by an MTT assay. Results are presented in Table 2.

For HCE, Charles River participated in a multisite ECVAM style validation. A total of 60 chemicals (30 solid and 30 liquid) were blind tested on 3 occasions each by the participating laboratories. The chemicals covered all GHS classifications. The defined acceptance criteria were achieved for both solids and liquids. Results have been published (Alépée *et al*; 2016)[d].

6 Table 1: BCOP Results

Test Substance	Test Method	IVIS*	UN GHS Category Prediction
EDTA-di-potassium salt	Solid	0.00	No Category
Tween 20	Liquid	0.00	No Category
Polyoxyethylene 23 lauryl ether	Liquid	0.30	No Category
2-Mercapto-pyramidine	Solid	1.99	No Category
Phenylbutazone	Solid	2.99	No Category
Ethyl-2-methylacetoacetate	Liquid	6.72	No prediction can be made
Ammonium nitrate	Solid	9.54	No prediction can be made
2,6-Dichlorobenzoyl chloride	Liquid	12.00	No prediction can be made
Chlorhexidine	Solid	95.21	Category 1
Benzalkonium chloride	Liquid	126.46	Category 1
Dibenzoyl- L-tartaric acid	Solid	128.27	Category 1
Trichloracetic acid	Liquid	244.12	Category 1

7 Table 2: EpiOcular™ EIT Results

Test Substance	Test Method	Viability (%)	UN GHS Category Prediction
Sodium oxalate	Solid	4.27	Category 1/2
Methylthioglycolate	Liquid	8.16	Category 1/2
2,5-Dimethyl-2,5-hexanediol	Solid	8.84	Category 1/2
Chlorhexidine digluconate	Liquid	10.25	Category 1/2
Diethyl toluamide	Liquid	16.96	Category 1/2
Camphene	Solid	25.07	Category 1/2
1,5-Naphthalenediol	Solid	39.25	Category 1/2
Tetraethylene glycol diacrylate	Liquid	57.94	Category 1/2
Kolliphor® RH40	Liquid	71.85	No Category
1-Ethyl-3-methylimidazolium ethylsulphate	Liquid	84.56	No Category
Dipropyl disulphide	Liquid	94.16	No Category
Piperonyl butoxide	Liquid	100.85	No Category
2,2'-Methylene-bis-(6-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethyl-butyl)-phenol)	Solid	110.33	No Category
Potassium tetrafluoroborate	Solid	110.73	No Category
3,4,4'-Trichloro-carbanilide	Solid	135.27	No Category

8 Conclusions

In conclusion, Charles River have demonstrated technical proficiency in BCOP, EpiOcular™ and HCE assays. In-house fit-for-purpose validation data confirmed that BCOP and EpiOcular™ assays correctly assigned ocular irritation potential of the proficiency panels of chemicals. While neither method alone is capable of fully classifying substances in UN GHS categories, a strategic combination of these tests in a tiered approach can be used to generate UN GHS ocular irritation categorization.

9 References

[a] OECD (2012). OECD Guideline for the Testing of Chemicals No. 405: Acute Eye Irritation / Corrosion.
[b] OECD (2013). OECD Guideline for the Testing of Chemicals No. 437: Bovine Corneal Opacity and Permeability Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage.
[c] OECD (2015). OECD Guideline for the Testing of Chemicals No. 437 Reconstructed Human Cornea-like Epithelium (RhCE) Test Method for Identifying Chemicals Not Requiring Classification and Labelling for Eye Irritation or Serious Eye Damage.
[d] Alépée N, Leblanc V, Adriaens E, Grandidier MH, Lelièvre D, Meloni M, Nardelli L, Roper CS, Santirocco E, Toner F, Van Rompay A, Vinall J and Cotovio J (2016). Multi-laboratory validation of SkinEthic HCE test method for testing serious eye damage/eye irritation using liquid chemicals. Toxicology *In Vitro* 31: 43-53.