CON4EI: Short Time Exposure (STE) Model.

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ABSTRACT

Assessment of ocular irritancy is an international regulatory requirement in the safety evaluation of industrial and consumer products. The objective of the CON4EI (CONsortium for *in vitro* Eye Irritation testing strategy) project is to develop tiered testing strategies for eye irritation assessment for all drivers of classification. For this, a set of 80 reference chemicals (38 liquids and 42 solids) was tested in eight *in vitro* test methods. Here, the results obtained with Short Time Exposure (STE) model are shown. The primary aim of this study was an evaluation of the performance of the test method to discriminate chemicals not requiring classification for serious eye damage/eye irritancy (No Category) from chemicals requiring classification and labelling for inducing serious eye damage (Category 1). In addition, the predictive capacity in terms of *in vivo* driver of classification was investigated. In a second step, it was investigated if STE can be used as part of a tiered-testing strategy for eye irritation assessment when assessing chemicals that fit the applicability domain.

For the STE method, the accuracy in identifying Cat 1 chemicals was 61.3% with 23.7% sensitivity and 95.2% specificity. Excluding non-qualified results did not affect the ability to correctly identify Cat 1 chemicals (accuracy 61.2% with 26.9% sensitivity and 100% specificity). The accuracy of the STE test method to identify No Cat chemicals was 72.5% with 66.2% sensitivity and 100% specificity. Excluding non-qualified results improved the predictivity (accuracy 87.8% with 85.4% sensitivity and 100% specificity).

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INTRODUCTION

Ocular irritation testing has historically been performed using the Draize rabbit eye test (Draize et al.). However, the Cosmetics Testing Ban enacted by the EU and REACH have prompted the development of *in vitro* ocular irritation alternatives. Recently a number of *in vitro* testing strategies have been validated as replacements for the Draize rabbit eye test. These include the Bovine Corneal Opacity and Permeability (BCOP) test method (OECD TG 437), Isolated Chicken Eye (ICE) test method (OECD TG 438), the Short Time Exposure (STE) test method (OECD TG 491) and the Reconstructed human Cornea-like Epithelium (RhCE) test method (OECD TG 492), among others. Many of these assays are able to define Category 1 (severe irritation) versus No Category (no irritation) like the BCOP assay and the STE assay. Other assays like the RhCE, or Eye Irritation Test (EIT) assay, using MatTek EpiOcular™ tissues can predict irritation (Category 1 and 2) or no irritation (No Category) but are unable to definitively separate Category 1 from Category 2 compounds. This has prompted a search for the optimal tiered testing strategy (either "top-down" or "bottom up") where two or more assays are combined in order to correctly categorize test substances.

The objective of this study was to evaluate the reliability of the Short Time Exposure (STE) test method (OECD TG 491) to properly identify the ocular irritation categorization of a set of 80 blinded test chemicals. In addition, we set out to define the applicability domains, strengths and limitations of the STE test system.

MATERIALS AND METHODS

Cell Culture
SIRC (Statens Seruminstitut Rabbit Cornea) cells were purchased from ATCC (Manassas, VA) and maintained in Eagle's Minimum Essential Medium supplemented with 10% fetal bovine serum using standard culture conditions.

Test Chemicals
The 80 blinded test chemicals assessed in this study were identified based on existing *in vivo* data across various drivers of classification. Each test material was initially assessed for solubility in physiological saline at 5% (w/v for solids or v/v for liquids). If the solubility was poor or could not be dissolved or suspended uniformly for at least five minutes in physiological saline, 5% DMSO in physiological saline was used as a second solvent option. For test chemicals that could not be dissolved or suspended uniformly for at least five minutes in either physiological saline or 5% DMSO in physiological saline, then pure mineral oil was used as a third solvent choice

Exposures

The SIRC cells were plated at 6 x 10^3 cells per well in 96-well plates and incubated at 37C, 5% CO_2 for 4 days prior to exposures. The test materials were to 0.05% and 5% in the appropriate. The media was removed from the cells, and the solvent containing the test material was applied to the cells for 5 minutes. After the exposure, the cells were rinsed and viability was assessed using the MTT assay.

MTT Assay
Cell viability was determined by measuring the reduction of 3-[4,5-dimethylthiazol-2-yl] 2,5 diphenyltetrazolium bromide (MTT). After the 5 minute exposure the plates were aspirated and rinsed with DPBS and 0.5 mg/mL MTT solution in media was placed in each well of the 96-well plates. Plates were incubated for 2 hours at 37°C with 5% CO_2. The MTT media was decanted and formazan was extracted by addition of acidified isopropanol. Sample absorbance was read at 570 nm and reference absorbance at 650 nm with a BioTek Synergy H4 plate reader.

PREDICTION MODEL

TABLE 1. Prediction model for the STE Assay.

Cell Viability		UN GHS Classification
At 5%	At 0.05%	
> 70%	> 70%	No Category
≤ 70%	> 70%	No prediction can be made
≤ 70%	≤ 70%	Category 1

RESULTS

FIGURE 1. Distribution of Liquid Chemicals According to Drivers of Classification

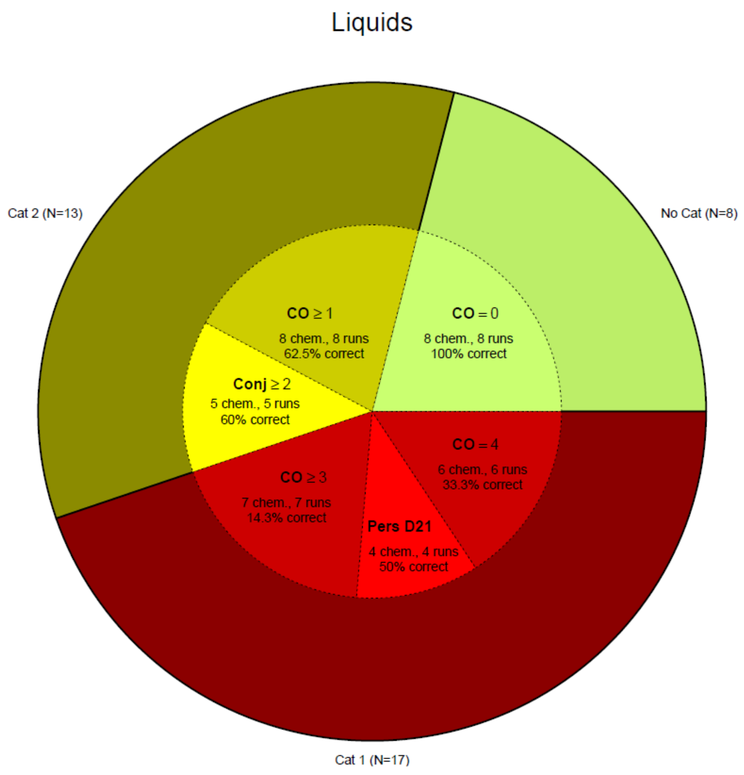

FIGURE 2. Distribution of Solid Chemicals According to Drivers of Classification

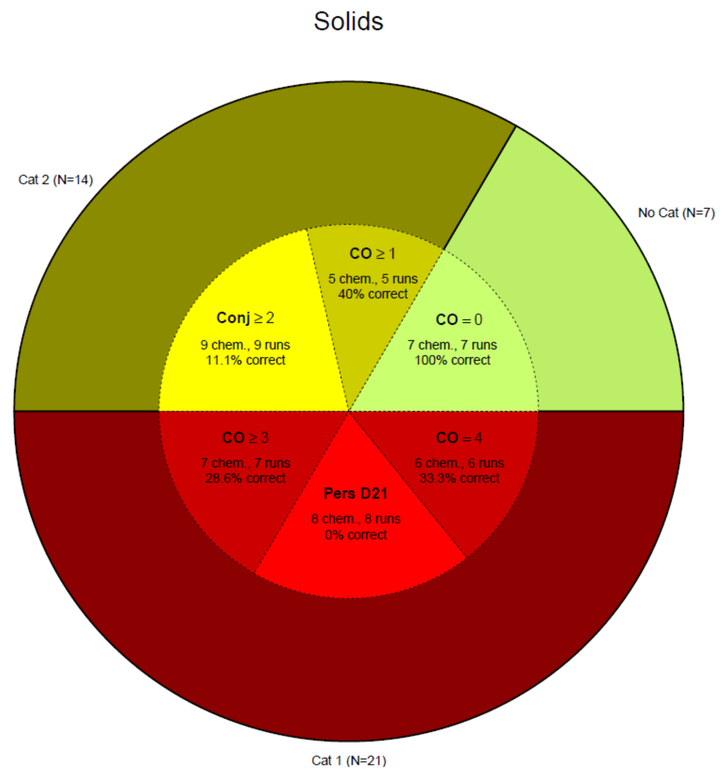

TABLE 2. Agreement Between the In Vitro Classification and UN GHS Driver of All 80 Chemicals

Driver	STE - Overall			STE - Liquids			STE - Solids		
	Cat I	No Pred	No Cat	Cat I	No Pred	No Cat	Cat I	No Pred	No Cat
CO mean = 3	**21.4% (3/14)**	64.3% (9/14)	14.3% (2/14)	**14.3% (1/7)**	85.7% (6/7)	0% (0/7)	**28.6% (2/7)**	42.9% (3/7)	28.6% (2/7)
CO pers D21	**16.7% (2/12)**	66.7% (8/12)	16.7% (2/12)	**50% (2/4)**	25% (1/4)	25% (1/4)	**0% (0/8)**	87.5% (7/8)	12.5% (1/8)
CO = 4	**33.3% (4/12)**	33.3% (4/12)	33.3% (4/12)	**33.3% (2/6)**	33.3% (2/6)	33.3% (2/6)	**33.3% (2/6)**	33.3% (2/6)	33.3% (2/6)
CO mean = 1	15.4% (2/13)	**53.8% (7/13)**	30.8% (4/13)	12.5% (1/8)	**62.5% (5/8)**	25% (2/8)	20% (1/5)	**40% (2/5)**	40% (2/5)
Conj mean = 2	0% (0/14)	**28.6% (4/14)**	71.4% (10/14)	0% (0/5)	**60% (3/5)**	40% (2/5)	0% (0/9)	**11.1% (1/9)**	88.9% (8/9)
CO = 0	0% (0/15)	0% (0/15)	**100% (15/15)**	0% (0/8)	0% (0/8)	**100% (8/8)**	0% (0/7)	0% (0/7)	**100% (7/7)**

TABLE 3. Agreement Between the In Vitro Classification and UN GHS Driver of 49 Valid Results

Driver	STE - Overall			STE - Liquids			STE - Solids		
	Cat I	No pred	No Cat	Cat I	No pred	No Cat	Cat I	No pred	No Cat
CO mean = 3	**23.1% (3/13)**	46.2% (6/13)	30.8% (4/13)	**20% (1/5)**	80% (4/5)	0% (0/5)	**25% (2/8)**	25% (2/8)	50% (4/8)
CO pers D21	**12.5% (2/16)**	43.8% (7/16)	43.8% (7/16)	**50% (2/4)**	25% (1/4)	25% (1/4)	**0% (0/12)**	50% (6/12)	50% (6/12)
CO = 4	**22.2% (2/9)**	44.4% (4/9)	33.3% (3/9)	**40% (2/5)**	40% (2/5)	20% (1/5)	**0% (0/4)**	50% (2/4)	50% (2/4)
CO mean = 1	0% (0/11)	**63.6% (7/11)**	36.4% (4/11)	0% (0/7)	**71.4% (5/7)**	28.6% (2/7)	0% (0/4)	**50% (2/4)**	50% (2/4)
Conj mean = 2	0% (0/7)	**57.1% (4/7)**	42.9% (3/7)	0% (0/5)	**60% (3/5)**	40% (2/5)	0% (0/2)	**50% (1/2)**	50% (1/2)
CO = 0	0% (0/8)	0% (0/8)	**100% (8/8)**	0% (0/8)	0% (0/8)	**100% (8/8)**	0% (0/0)	0% (0/0)	**N/A**

TABLE 4. Accuracy of the STE Method For Identifying UN GHS Cat 1 – All 80 Chemicals

UN GHS Cat 1	STE		
	Overall	Liquids	Solids
Accuracy	61.3% (49/80)	65.8% (25/38)	57.1% (24/42)
Sensitivity	23.7% (9/38)	29.4% (5/17)	19% (4/21)
False negatives	76.3% (29/38)	70.6% (12/17)	81% (17/21)
Specificity	95.2% (40/42)	95.2% (20/21)	95.2% (20/21)
False positives	4.8% (2/42)	4.8% (1/21)	4.8% (1/21)

TABLE 5. Accuracy of the STE Method For Identifying UN GHS Cat 1 – 49 Valid Results

UN GHS Cat 1	STE		
	Overall	Liquids	Solids
Accuracy	61.2% (30/49)	73.5% (25/34)	33.3% (5/15)
Sensitivity	26.9% (7/26)	35.7% (5/14)	16.7% (2/12)
False negatives	73.1% (19/26)	64.3% (9/14)	83.3% (10/12)
Specificity	100% (23/23)	100% (20/20)	100% (3/3)
False positives	0% (0/23)	0% (0/20)	0% (0/3)

TABLE 6. Accuracy of the STE Method For Identifying UN GHS No Cat – All 80 Chemicals

UN GHS No Cat	STE		
	Overall	Liquids	Solids
Accuracy	72.5% (58/80)	81.6% (31/38)	64.3% (27/42)
Sensitivity	66.2% (43/65)	76.7% (23/30)	57.1% (20/35)
False negatives	33.8% (22/65)	23.3% (7/30)	42.9% (15/35)
Specificity	100% (15/15)	100% (8/8)	100% (7/7)
False positives	0% (0/15)	0% (0/8)	0% (0/7)

TABLE 7. Accuracy of the STE Method For Identifying UN GHS No Cat – 49 Valid Results

UN GHS No Cat	STE		
	Overall	Liquids	Solids
Accuracy	87.8% (43/49)	82.4% (28/34)	100% (15/15)
Sensitivity	85.4% (35/41)	76.9% (20/26)	100% (15/15)
False negatives	14.6% (6/41)	23.1% (6/26)	0% (0/15)
Specificity	100% (8/8)	100% (8/8)	N/A
False positives	0% (0/8)	0% (0/8)	N/A

SUMMARY

All of the *in vivo* No Cat liquids resulted in a viability > 70% at both concentrations and were therefore correctly identified.

Several of the *in vivo* Cat 2 liquids resulted in a viability > 70% at a 0.05% concentration and a viability < 70% at 5% concentration and were identified as "No prediction can be made".

The *in vivo* Cat 2 liquids that were under or over-predicted were not specifically related to the driver of *in vivo* classification.

Only a few *in vivo* Cat 1 liquids resulted in a viability < 70% at both concentrations and were correctly identified as Cat 1. Those that were under or over-predicted were not specifically related to the driver of *in vivo* classification.

When used to identify chemicals inducing serious eye damage (UN GHS Cat 1), the STE test method has an overall accuracy of 61.3% (49/80), a false positive rate of 4.8% (2/42) and a false negative rate of 76.3% (29/38).

When used to identify chemicals not requiring classification (UN GHS No Cat), the STE test method has an overall accuracy of 72.5% (58/80), a false positive rate of 0% (1/15) and a false negative rate of 33.8% (22/65).

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CONCLUSION

The accuracy of the STE test method to identify Cat 1 chemicals was 61.3% with 23.7% sensitivity and 95.2% specificity. Excluding non-qualified results did not have an important effect on the accuracy to identify Cat 1 chemicals (accuracy 61.2% with 26.9% sensitivity and 100% specificity).

The accuracy of the STE test method to identify No Cat chemicals was 72.5% with 66.2% sensitivity and 100% specificity. Excluding non-valid results resulted in an important improvement of the performance of the STE test method (accuracy 87.8% with 85.4% sensitivity and 100% specificity).

The results of this study show that the STE test method may be promising with regard to the bottom-up approach or second step in a top-down approach (identification of No Cat chemicals) in an integrated testing strategy (ITS) for eye irritation assessment taking into account the restriction of the method with regard to the testing of solids.

REFERENCES

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