Abstract # 975

The inter-laboratory reproducibility of the STE test for assessing eye irritation of cosmetic products

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Introduction

STE test is an *in vitro* eye irritation test using cell viability as an end point in SIRC cells following just a 5 minute treatment, and the good correspondence has been confirmed between the STE irritation categories (non irritant [NI] and irritant [I]) and GHS categories (NC and category 1 [Cat. 1]/category 2 [Cat. 2]). Generally, cytotoxicity tests using cultured cells have an advantage of being simple, a quick procedure, and a low evaluation cost. The STE test has the advantages not only easy-to-use but also evaluable the eye irritation potential of water insoluble substances by using mineral oil as test vehicle. The STE test is planned for peer review in 2013 and may be accepted as an OECD test guideline for classifying ocular irritation. In this study, the technical transferability and inter-laboratory reproducibility of the STE test were evaluated in 3 contract research laboratories as a naive laboratory.

Study design

Step 1: Transferability

Five representative chemicals, which have a lot of background data in Kao Corporation, were tested in three naive laboratories at first. Three of five chemicals were water-soluble chemicals (Sodium lauryl sulfate, Calcium thioglycolate, and Tween 80). Two of five chemicals were water-insoluble chemicals (1-Octanol, and Dodecane). Concordance of STE rank between each laboratory was evaluated.

Step 2: Inter-laboratory reproducibility

Twenty cosmetic products (Shampoos, Conditioners, Hair coloring products, Skin cleansers, Hair stylers, Deodorants, and Moisturizers) were tested in each laboratory. Concordance of STE category (irritant or non irritant) and STE rank between each laboratory was evaluated.

Laboratories

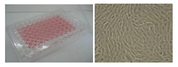
1. Institute for In Vitro Sciences, Inc. (US): IIVS
2. Harlan Cytotest Cell Research, GmbH. (Germany): Harlan
3. Food and Drug Safety Center (Japan): FDSC
*4. Kao Corporation (Japan): Kao *lead laboratory*

Method; Short Time Exposure (STE) Test

Takahashi et al., *Toxicol. In Vitro*, 2008

Cell culture → **Exposure** → **Measurement of viability**

SIRC cells
Rabbit corneal cell line



5 minutes
Test concentration : 5, 0.05%
Vehicle : Saline, 5% DMSO in saline, or mineral oil
(Vehicle selected based on solubility of chemical)

Measure of Formazan formation (MTT assay)

$$\text{Cell Viability (CV, \%)} = \frac{\text{OD570 of Test material}}{\text{OD570 of control}} \times 100$$

CV is calculated from three independent tests

Category classification

Based on viabilities at 5% test conc.

	Viability (cutoff value)
non irritant	> 70 %
irritant	≤ 70 %

Rank classification

Based on total score obtained from viabilities at 5% and 0.05% test conc.

5%	score	0.05%	score
viability > 70	0	viability > 70	1
viability ≤ 70	1	viability ≤ 70	2

5% score + 0.05% score= STE rank

1 : Minimal irritant 2 : Moderate irritant
3 : Severe irritant

Results

Step 1: Transferability

Table 1 The result of STE test in four laboratories for 5 reference chemicals

Test product	STE Solvent	IIVS 5% Viability (%)	IIVS 5% SD	IIVS 0.05% Viability (%)	IIVS 0.05% SD	IIVS Judgement Rank	Harlan 5% Viability (%)	Harlan 5% SD	Harlan 0.05% Viability (%)	Harlan 0.05% SD	Harlan Judgement Rank	FDSC 5% Viability (%)	FDSC 5% SD	FDSC 0.05% Viability (%)	FDSC 0.05% SD	FDSC Judgement Rank	Kao (Background data) 5% Viability (%)	Kao 5% SD	Kao 0.05% Viability (%)	Kao 0.05% SD	Kao Judgement Rank
Sodium lauryl sulfate	saline	1.2	1.3	1.0	0.9	3	1.5	1.0	0.0	3.7	3	0.0	0.4	2.2	0.6	3	0.0	1.2	0.4	0.9	3
Calcium thioglycolate	saline	12.5	3.5	100.4	10	2	9.4	4.4	101.8	13.7	2	33.5	11.9	103.8	1.3	2	11.3	3.2	99.2	6.4	2
Tween 80	saline	95.0	8.0	97.0	9.4	1	98.2	2.9	95.7	7.7	1	111.0	4.8	90.0	12.8	1	113.2	11.1	97.8	4.6	1
1-Octanol	Mineral oil	3.4	2.1	87.6	3.9	2	9.0	8.9	98.9	6.8	2	3.8	2.4	90.7	1.0	2	5.0	2.0	95.3	4.7	2
Dodecane	Mineral oil	75.9	9.4	90.2	5.4	1	89.4	13.1	91.1	5.6	1	97.2	6.4	87.5	3.2	1	93.3	1	1	1	1

Rank3: Severe irritant Rank2: Moderate irritant Rank1: Minimal irritant The column, which cell viability was below 70%, is colored orange Mean viability was shown in the table. SD means standard deviation from three independent assays.

STE ranks of 5 chemicals in four laboratories were identical.

Transferability was successfully confirmed.

Step 2: Inter-laboratory reproducibility

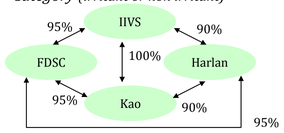
Table 2 The result of STE test in four laboratories for 20 cosmetic products

Code	Test product	STE Solvent	IIVS Viability (%) 5%	IIVS Viability (%) 0.05%	IIVS Judgement NI/I	IIVS Judgement Rank	Harlan Viability (%) 5%	Harlan Viability (%) 0.05%	Harlan Judgement NI/I	Harlan Judgement Rank	FDSC Viability (%) 5%	FDSC Viability (%) 0.05%	FDSC Judgement NI/I	FDSC Judgement Rank	Kao Viability (%) 5%	Kao Viability (%) 0.05%	Kao Judgement NI/I	Kao Judgement Rank
A	Shampoo	saline	1.0	87.2	I	2	9.0	65.7	I	3	1.7	52.1	I	3	1.3	91.7	I	2
B	Shampoo	saline	1.1	84.0	I	2	0.0	88.7	I	2	0.7	84.3	I	2	1.2	108.3	I	2
C	Conditioner (Rince-Off)	saline	83.6	101.8	NI	1	87.1	108.5	NI	1	102.6	100.3	NI	1	90.6	95.8	NI	1
D	Conditioner (Rince-Off)	saline	98.6	98.6	NI	1	58.8	90.6	I	2	98.0	99.5	NI	1	83.5	97.6	NI	1
E	Color glaze	saline	97.4	95.9	NI	1	94.4	95.9	NI	1	94.4	99.9	NI	1	92.4	104.9	NI	1
F	Color glaze	saline	22.5	102.1	I	2	36.9	101.0	I	2	17.4	100.5	I	2	18.6	101.2	I	2
G	Hair color	saline	75.4	99.9	NI	1	47.0	105.3	I	2	64.2	96.2	I	2	91.9	98.9	NI	1
H	Hair color	saline	1.4	101.1	I	2	4.5	110.3	I	2	2.0	104.2	I	2	0.7	107.4	I	2
I	Face cleanser	saline	0.4	94.3	I	2	8.8	107.4	I	2	3.7	100.6	I	2	1.6	100.6	I	2
J	Face cleanser	saline	1.8	41.3	I	3	1.5	54.3	I	3	3.1	96.2	I	2	0.1	65.0	I	3
K	Body cleanser	saline	0.9	80.3	I	2	12.8	60.0	I	3	4.7	69.1	I	3	0.0	93.1	I	2
L	Hand soap	saline	0.2	94.7	I	2	3.4	77.7	I	2	4.7	95.6	I	2	0.6	102.3	I	2
M	Soap	saline	0.5	31.0	I	3	0.0	57.0	I	3	0.0	20.8	I	3	0.5	49.8	I	3
N	Conditioner (Leave-On)	saline	16.4	85.6	I	2	9.5	97.6	I	2	9.5	87.8	I	2	13.2	88.8	I	2
O	Conditioner (Leave-On)	saline	52.5	98.5	I	2	34.8	92.0	I	2	57.7	96.3	I	2	47.0	96.9	I	2
P	Hair styler	saline	51.0	103.5	I	2	49.9	97.8	I	2	24.5	91.4	I	2	16.6	100.2	I	2
Q	Hair spray	saline	94.6	87.7	NI	1	109.1	109.3	NI	1	83.8	97.2	NI	1	101.7	97.6	NI	1
R	Hair styler	Mineral oil	68.1	63.4	I	3	66.9	83.2	I	2	66.4	91.3	I	2	63.0	93.1	I	2
S	Deodorant	Mineral oil	123.7	96.8	NI	1	101.6	107.1	NI	1	103.3	98.4	NI	1	114.6	107.6	NI	1
T	Moisturizer	saline	100.6	97.8	NI	1	101.3	107.2	NI	1	108.5	95.1	NI	1	108.7	98.7	NI	1

Rank3: Severe irritant Rank2: Moderate irritant Rank1: Minimal irritant The column, which cell viability was below 70%, is colored orange Mean viability was shown in the table.

Concordance

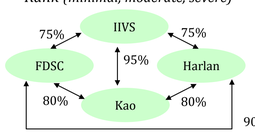
Category (irritant or non irritant)



Concordance = 90-100%

18 out of 20 products were categorized the same in 4 laboratories.

Rank (minimal, moderate, severe)



Concordance = 75-95%

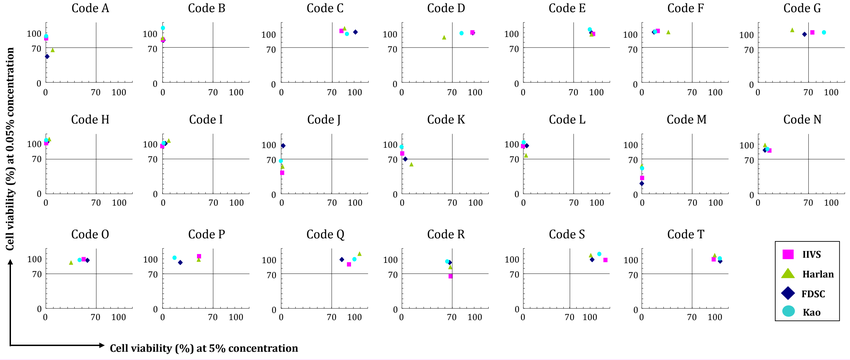
14 out of 20 products were ranked the same in 4 laboratories.

- Reference -
FL* assay (classifying severe and non-severe irritant)
Concordance = 52-84% (4 laboratories, 25 chemicals)
BCOP** assay (classifying 3 ranks, severe, moderate, and mild)
Concordance = 77-88% (5 laboratories, 60 chemicals)

* Fluorescein Leakage, FL BRD, ECVAM, 2008 <http://ecvam.jrc.ec.europa.eu/fr_doc/doc-05_FL_BRD_Report_Jan08%20cleaned.pdf>
** Bovine Cornea Opacity and Permeability, BCOP BRD, ICCVAM, 2006 <http://iccvam.niehs.nih.gov/methods/ocutox/ivocutox/ocu_brd_bcop.htm>

Transferability was successfully confirmed as well as FL assay and BCOP assay.

Distribution of cell viability for each of 20 cosmetic products



There is only small variation in some test samples. Code A, D, G, J, K, and R were distributed astride 2 ranks.

Technical alignment

Details of unmatched materials

Solvent	Saline		Mineral oil	
Soluble state	Dissolution	Uniform dispersion	Dissolution	Uniform dispersion
# of test materials	12	6	0	2
# of unmatched materials	3	2	-	1
Code	A, J, K	D, G	-	R

■ Sample preparation (Solubility)
Diluents of Code D, G, and R were clouded (uniform dispersion).
→ The soluble states of each material might be different between laboratories, and may cause difference in viability.

■ Exposure of mineral oil
In case of Code R, mineral oil was used as solvent. Mineral oil sometimes induce cell ablation.
→ It might effect the cell viability depending on the skill.

Better concordance may be obtained with technical improvement in sample preparation, exposure, and washing.

Conclusion

Transferability

■ Identical results were obtained for the 5 standard chemicals by each lab; STE is transferable.

Inter-laboratory reproducibility

From the data of 20 cosmetic products,
■ Concordance of category classification (irritant or non-irritant) was high.
■ Concordances of category classification as well as rank classification were comparable with FL assay and BCOP assay.
■ Technical improvement, especially in the step of sample preparation, may increase concordance.