

# CLASSIFICATION OF CHEMICALS FOR THEIR IRRITATION POTENTIAL USING AN IN-VITRO METHOD OF RECONSTRUCTED HUMAN CORNEA-LIKE EPITHELIUM (RhCE)

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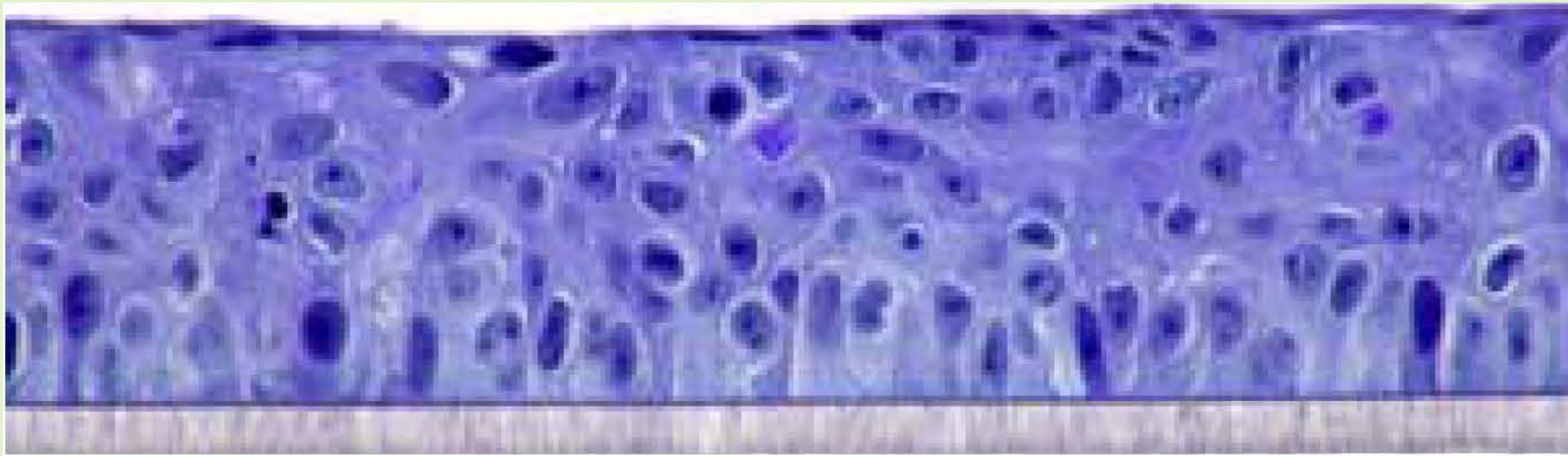
## BACKGROUND

Consumer products such as household cleaners and cosmetics may induce serious damage upon contact with the eyes, so assessment of ocular toxicity is important to ensure the safety of these types of products. The Draize rabbit test has been criticized for its lack of reproducibility, overestimation, and the use of live animals. The reconstructed cornea-like tissue model is been widely used by the cosmetic industry to evaluate the irritation potential of raw materials, as an alternative to Draize rabbit test and has been evaluated by ECVAM.

The SkinEthic™ human corneal epithelium (HCE) model uses immortalised human corneal epithelial mucosa cells that is structurally very similar to the corneal mucosa of the human eye. The reconstructed epithelial tissue morphology is similar to that of human corneal epithelium.

## METHOD

On receipt of tissue, the tissues are transfer maintenance medium in 6-well plate and incubate at least overnight (37°C)



Treatment: 2 tissues with 30 µL PBS- (Negative Control)  
2 tissues with 30 µL methyl acetate (Positive Control)  
2 tissues with 30 µL PBS- + 30 mg test chemical (test treatment)

Transfer tissues to fresh maintenance medium in the same 6-well plates

Incubate tissues for 4 h ± 5 min (37°C)

Rinse with PBS

Immerse the tissue in 4 mL fresh maintenance medium, in 12-well plate

Post-soak Period: Incubate for 30 min ± 2 min at Room temp.

Transfer tissues to fresh maintenance medium in the same 6-well plates

Incubate for 18 h (37°C)

Transfer tissues into MTT solution (1 mg/mL)

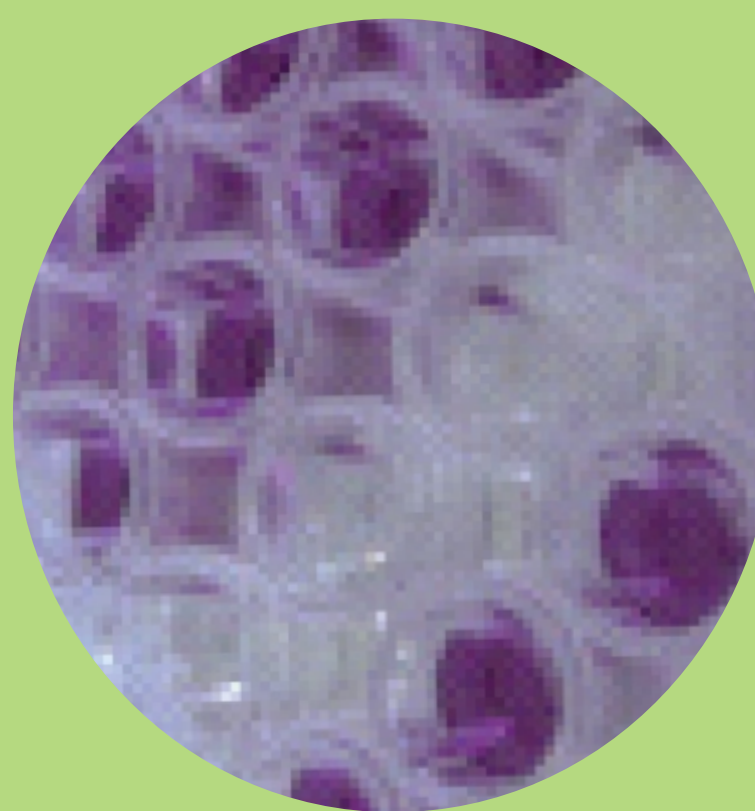
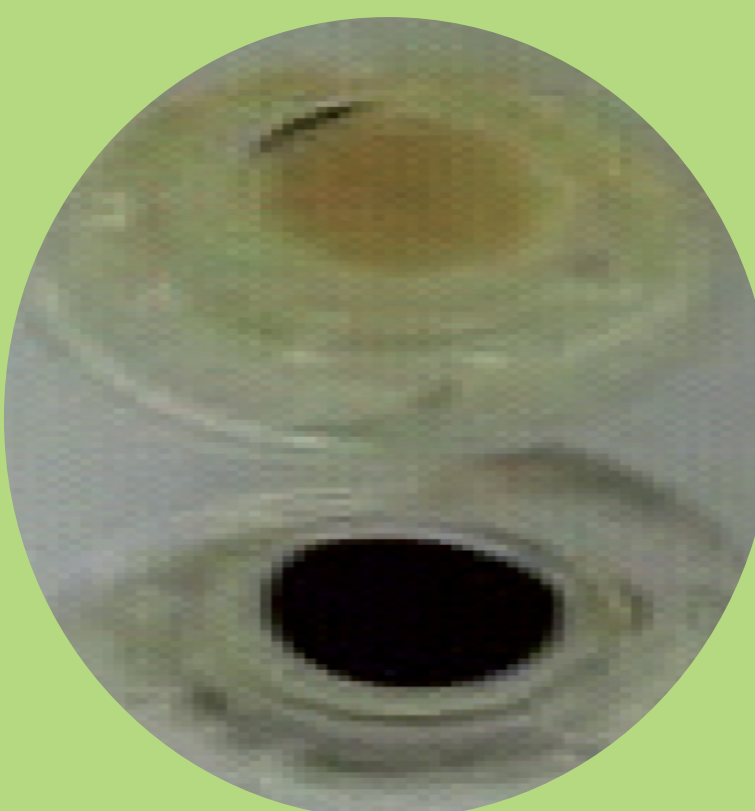
Incubate tissues for 3h (37°C)

Place the inserts on 1.5 mL isopropanol in 12-well plate

Extract formazan for 2 h (RT)

Remove the insert and homogenize formazan extract

Read OD with microplate spectrophotometer at 570 nm



## DECISION CRITERIA

Result	Classification
Mean tissue viability > 50%	Non-irritant (No Category)
Mean tissue viability < 50%	Irritant (Category 1 / Category 2)

## RESULTS

Name of Test Item	Percent Viability	Category Obtained
2,5-Dimethyl-2,5-hexanediol	0.45	Irritant
Sodium oxalate	15.56	Irritant
Sodium benzoate	0.86	Irritant
(±)-Camphene (contains ca.20% Tricyclene)	0.45	Irritant
3,4,4'-Trichlorocarbanilide	108.17	Non-Irritant
2,2'-Methylenebis[6-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)phenol]	97.18	Non-Irritant
Potassium tetrafluoroborate	95.61	Non-Irritant

## CONCULSION

Percent mean viability of <50 was observed in 2,5-dimethyl-2,5-hexanediol, sodium oxalate, sodium benzoate and camphene which were classified as “Irritant” as per the test guideline OECD 492. For classification of these test item further testing was required. Percent mean viability of >60 was observed in 3,4,4'-trichlorocarbanilide, 2,2'-Methylenebis[6-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)phenol] and potassium tetrafluoroborate and were classified as “Non-Irritant” and no further testing was required.

## REFERENCES

- OECD, 1998: OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring, Number 1, “OECD Principles on Good Laboratory Practice” ENV/MC/CHEM(98)17 (as revised in 1997)..
- OECD, 2017: The Organisation for Economic Co-operation and Development (OECD) Guideline for the Testing of Chemicals, OECD 492, “Reconstructed human Cornea-like Epithelium (RhCE) test method for identifying chemicals not requiring classification and labelling for eye irritation or serious eye damage”, adopted by the Council on October 09, 2017.
- SkinEthic™ HCE EIT SOP, Version 3. (June 07, 2015). SkinEthic™ HCE Eye Irritation Test (EITS for Solids) for the Prediction of Acute Ocular Irritation of Chemicals..
- JRF/TOX/SOP-2206, Issue No A, 2018: Reconstructed Human Cornea-like Epithelium (RhCE) Test Method.