VALIDATION OF THE RECONSTRUCTED HUMAN EPIDERMIS (RHE) SKIN IRRITATION ASSAY FOR FULL REPLACEMENT OF THE DRAIZE TEST


E-mail: jean.pachot@oroxcell.com

INTRODUCTION

The Directive 67/548/EEC or OECD TG 404 to fully replace the in vivo Draize skin irritation test, was reinforced with the 7th Amendment of the Cosmetic Directive and the REACH regulation. Two reconstructed human epidermis models, Epiderm and Episkin, were scientifically validated with the reliability, as for Episkin, to discriminate skin irritants (R38) from non-irritants (no label) as defined with EU risk phrases. The aim of this study was to assess weather the in vitro Reconstructed Human Epidermis (RHE) model, commercialized by SkinEthic Laboratories could be an alternative to the historical Draize rabbit test.

MATERIALS AND METHODS

General model characteristics

- Fully differentiated Reconstructed Human Epidermis
- Grown for 17 days at the air-liquid interface in a chemically defined medium
- 4 to 7 viable layers: at least basal, suprabasal, spinous and granular cell layers
- Presence of a barrier function: stratum corneum
- Epidermis differentiation markers expressed
- Commercialized by SkinEthic Laboratories. Certified ISO9001

RESULTS AND DISCUSSION

Test substances

20 reference test substances, 10 non-irritants and 10 irritants, were selected from the list of 98 chemicals used in the ECVAM SIVS (2007). Two false positive (1-bromo-4-chlorobutane and 4-methyl-thio-benzaldehyde) and three false negative compounds (hexyl salicylate, terpinyl acetate and dipropyl disulfide) using Episkin method were included in order to assess performance improvements with the new skin model or modified test protocol. Test substances were coded by Vitroscreen.

Model quality control

- Viability (MTT) test: OD > 0.7
- OD > 20x NC < 50µg/mL

Cell viability (MTT) release

MTT – viability endpoint: reproducibility and correlation

 statistical analysis

Data were analysed by Effi-Stat. Means, standard deviations and coefficients of variation were determined. In addition, 1-way ANOVA and correlation according to Bravais-Pearson were performed. Contingency table statistics were used for evaluating the reproducibility and predictive capacity of RHE model.

Prediction models

A) MTT endpoint: the prediction model was based on relative tissue viability of the test substance-treated tissue compared to the negative control-treated tissue.

B) MTT + IL-1α endpoints: in addition IL-1α endpoint measurement was evaluated to determine its potential added value compared to the MTT endpoint only. Three types of IL-1α analyses were performed.

Acceptance criteria

Quality criteria Accepted values
NC: Absolute response OD ≥ 1.2
PC: Mean viability Viability ≤ 40%
Variability SD ≤ 18
PC: Viability SD ≤ 18
Test substances: Variability SD ≤ 18
NC: Negative Control, PBS
PC: Positive Control, 0% SDS

CONCLUSIONS

The present RHE assay appears to be a promising in vitro test method to fully replace Draize skin irritation on rabbits.

REFERENCES


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