

Poster: skin models as alternatives to animal testing

Reconstructed human epidermis (RHE): an *in vitro* skin irritation model for full replacement of the Draize test

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Efforts to fully replace the Draize skin irritation test on rabbit *in vivo*, according to Method B.4 of Annex V to Directive 67/548/ECC or OECD TG 404 have been expended for many years in Europe. The issue has become still more critical as a result of the 7th Amendment of EU Cosmetic Directive and EU regulation for the Registration, Evaluation and Authorisation of Chemicals (REACH).

To date; following an ECVAM-managed skin irritation validation study, an *in vitro* test method using reconstructed human epidermis model EpiSkin has been scientifically validated as the only stand alone method to discriminate skin irritants (I) from non-irritants (NI) according to the EU 67/548/ECC classification. Furthermore a performance standard document has defined the procedure whereby the accuracy and reliability of a Me-too new test method could be evaluated (ECVAM SIVS, 2007).

The purpose of the present study was to assess an *in vitro* reconstructed Human Epidermis (RHE) model from SkinEthic Laboratories (Nice, France).

A multicentre study was performed on 20 reference chemicals under blind conditions (chemicals coded by Vitroscreen) in three independent laboratories (L'Oréal, Coty and Oroxcell) using three different batches of RHE model. The reference test chemicals were selected so as to cover a broad range of physico-chemical properties and of *in vivo* Draize test irritancy scores according to the performance standard document. RHE skin irritation test method consisted in applying topically test chemical to RHE samples and leaving on for 42 minutes before rinsing then incubate the tissues for 42 hours at 37°C before endpoint measurement. The main endpoint was cell viability (MTT reduction), with a threshold of 50% viability. Interleukin 1 alpha (IL-1 α) was also measured to determine whether predictive ability of the assay was improved by additional endpoint.

An independent statistical analysis was performed to assess intra and inter-laboratory reproducibility using standard deviation, coefficient variation, 1-way Anova, Bravais-Pearson' correlation, and identical run classification approaches. Good intra and inter-laboratories reproducibility were achieved. Correct predictions of skin irritation potential for the 20 test chemicals were obtained with 90% sensitivity and 80% specificity (MTT endpoint only). Overall accuracy was 85% and was not improved by IL-1 α data. Similar predictions levels on those 20 test chemicals were already described with the validated EpiSkin system, using the two combined endpoints.

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

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