

Lecture: skin models as alternatives to animal testing

The use of the SkinEthic Human Corneal Epithelial (HCE) model to predict ocular irritancy: optimized 1H/16H protocol applied to a large set of 400 industrial chemicals

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The 7th amendment of the EU Cosmetic Directive will lead to the ban of animal testing for cosmetic ingredients in March 2009. Thus alternative strategies and tests are urgently required in order to evaluate eye damage potential of chemicals. Tissue engineering progresses ended up to a better availability of 3D *in vitro* tissues thus participating to efforts that have been done and are still currently made in order to find reliable and relevant alternative methods.

The HCE model from SkinEthic laboratories is a standardized epithelium reconstructed with immortalized human corneal epithelial cells. Its specific structure allows chemicals to be tested in conditions similar to *in vivo* use. We have used the HCE model to evaluate *in vitro* eye irritation potential of chemicals. Because intrinsic cytotoxicity potential of chemicals is decisive factor affecting cornea after contact, we have used cell viability (MTT test) assessment as the main endpoint to classify chemicals.

Adapted protocols were based on specific contact times, adapted applied volume and a specific post-incubation period. The “long” 1 hour/16hours optimized HCE protocol established on a set of 102 chemicals (Cotovio et al., 2008), was applied to an larger set of industrial “real life” chemicals (>400). The previously defined Prediction Model (PM) based on a 50% viability cut off, allowed the drawing up of 2 chemicals classes (irritants and non irritants). Inter-batch and intra-batch reproducibility was assessed by using a specific batch control (Triton X100), positive control (ethanol) and negative control (Phosphate buffer). Predictive capacities based on these data were analyzed and discussed. The overall performances were good (concordance >80%) and well balanced sensitivity and specificity, thus demonstrating the robustness of the protocol when applied to significant very large set of chemicals representative of industrial needs.

Based on these results, the HCE protocol proved to be an efficient *in vitro* tool for the *in vitro* assessment of ocular irritancy potential. Additional work is currently done in order to evaluate the scope of application of the PM in a larger context of integrated test strategies for ocular irritancy

References

Cotovio, J. et al. (2008). *AATEX Spec. Issue*, 343-350 procs. WC6

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