Current Status of the Cosmetics Europe Animal-Free Genotoxicity Projects

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Introduction

The Cosmetics Europe (formerly COLIPA) Genotoxicity Task Force has led 3 projects to help improve the specificity of current *in vitro* genotoxicity assays. Here, we present an up-date on the projects. The program has already helped improve the initial test battery predictivity and the reconstructed skin (RS) projects have provided sound support for their use as alternative models to human skin in the absence of *in vivo* data.

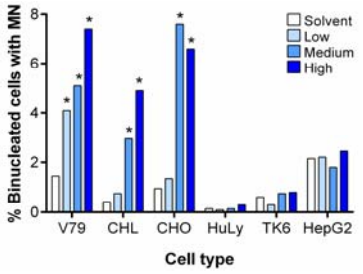
False Positives Project

The now complete "False Positives" project optimized current mammalian cell assays in order to improve specificity. This project addressed two aspects of the *in vitro* micronucleus (MN) assay:

1) Revised Cell Selection[1]

The rodent cell lines, V79 and CHL, were the least predictive and gave the most false positive results, closely followed by CHO. The human cell line, TK6, was less likely to give false positives and HuLy and HepG2 were least likely to give false positive responses. An example of the differences in MN formation between cell types is shown in Figure 1.

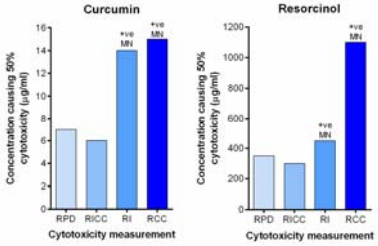
Figure 1. %MN in different cell types treated with different concentration of resorcinol. * denotes P<0.05



2) Genotoxicity Tests at Lower Cytotoxicity[2]

Different measures of cytotoxicity were compared to determine whether they affect the top dose. In most cases, Relative Increase in Cell Counts (RICC) was the most sensitive endpoint, leading to selection of a lower dose range. By contrast, Replication Index (RI) and Relative Cell Counts (RCC) underestimated toxicity and led to selection of a higher dose range. A collaborative trial (OECD guideline validation) shows "true positives" are detected using Relative Population Doubling or RICC.

Figure 2. Comparison of the sensitivity of different cytotoxicity endpoints. A lower sensitivity results in higher top concentrations and positive MN responses (+ve MN).



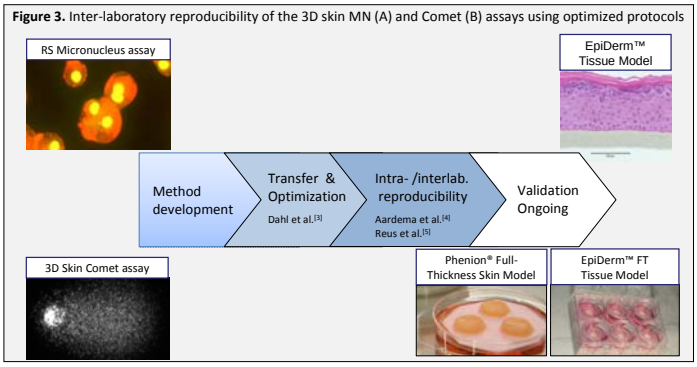
Conclusion

These data suggest that the selection of more relevant cells (e.g. human lymphocytes) and more sensitive toxicity measures (e.g. RPD and RICC) can prevent >60% false positive findings. In parallel, the sensitivity of this assay remained consistently high (Fowler et al., *submitted*).

"3D Skin Model" Project

The ongoing "3D skin model" project continues to focus on developing and validating the use of human reconstructed skin (RS) models in order to better reflect exposure conditions of dermally applied products, such as cosmetics.

Figure 3. Inter-laboratory reproducibility of the 3D skin MN (A) and Comet (B) assays using optimized protocols



Optimization and transferability – Completed[3].

Intra- and inter-laboratory reproducibility – Completed[4]. The 3D Skin Comet and MN assays have demonstrated good inter- and intra-laboratory reproducibility using coded chemicals.

Validation – Ongoing:

To investigate the predictive capacity and to evaluate further the reproducibility of the 3D Skin MN assay, the domain of chemicals tested is being increased, of which 29 have already been evaluated.

The 3D Skin Comet assay has entered its validation stage in collaboration with laboratories funded by a German Ministry (BMBF). The Cosmetics Europe project concentrated on the epidermal model EpiDerm™ (MatTek); whereas, the BMBF project focused on full thickness (FT) models. The Phenion® Full-Thickness Skin Model (Henkel) and EpiDerm™ FT Tissue (MatTek) were selected to enter the validation phase. The current phase concentrates on inter- and intra-laboratory reproducibility, with each of the first 8 chemicals being evaluated in 3 of the 5 participating laboratories.

Conclusion

Results so far indicate good reproducibility of the assays. In addition, for the RSMN assay which is farther along in the validation process, an improved specificity compared to standard *in vitro* tests was observed. Both 3D Skin MN and 3D Skin Comet methods were readily adapted and transferred to different laboratories. As both assays together detect all three kinds of DNA damage they are intended to provide a new approach to be used as a follow-up for positive results from the current *in vitro* genotoxicity test battery[6].

Skin Metabolism Project

The completed "Metabolism" project evaluated the enzyme capacities of human skin and 3D skin models in order to better understand the role of metabolism in bioactivation and detoxification of dermally applied chemicals.

Two endpoints were measured:

1) Proteomic profile – Imperial college, London[7,8]
2) Selected enzyme activities – IUF, Düsseldorf University[9]

Activities were measured in:

- Native human skin
- 3D skin models (EpiDerm™, EPISKIN™, Reconstructed Human Epidermis)
- Cell lines (primary and immortalized keratinocyte)

Figure 4. Potential routes of xenobiotic metabolism in skin and liver.

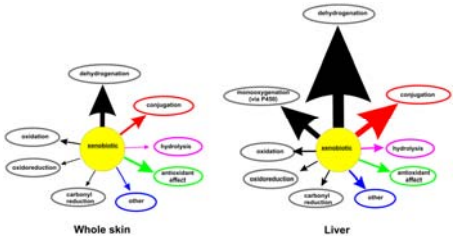


Figure 4 (taken from van Eijl et al.[6]) provides an overview of the potential routes of metabolism of xenobiotics in the skin and how these are comparable with the liver. The size of each arrow is proportional to the number of XMEs detected that may catalyze each bioconversion indicated. The known low expression and function of phase 1 enzymes in native whole skin was reflected in the *in vitro* models. Some XMEs in whole skin were not detected in *in vitro* models and *vice versa*, and some major hepatic XMEs such as cytochrome P450-monooxygenases were absent or measured only at very low levels in the skin. Conversely, levels of phase 2 enzymes, functional activity of glutathione S-transferases, *N*-acetyltransferase 1, and UDP-glucuronosyltransferases were all readily measurable in whole skin and *in vitro* skin models at activity levels similar to those measured in the liver.

Conclusion

The RS models exhibited comparable metabolic capacities to native human skin.

Overall Conclusions

The Cosmetics Europe Genotoxicity Task Force projects have helped improve predictive capacity of *in vitro* cytogenetic assays. The increase in the predictive capacity of the initial test battery of *in vitro* assays will reduce the number of chemicals de-selected due to false positives. By establishing a good predictivity of the RS MN and 3D Skin Comet assays, together with the confirmation that the RS models mimic native human skin in terms of their metabolic capacity , our results support their use in follow-up tests in the assessment of the genotoxic hazard of cosmetic ingredients in the absence of *in vivo* data.

References

[1] Fowler et al. Mutat Res. 2012, 742(1-2):11-25; [2] Fowler et al. Mutat Res. 2012, 30;747(1):104-17; [3] Dahl et al. Mutat Res. 2011, 720(1-2):42; [4] Aardema et al. 2010, Mutat Res. 2010, 701(2):123; [5] Reuss et al. Mutagenesis, 2013, *in press*; [6] Pfuhler et al. 2010, Reg Pharm Tox; [7] Götz et al. Exp Dermatol, 2012, 21(5):358-63; [8] Gözt et l. Exp Dermatol. 2012 May;21(5):364-9; [9] van Eijl et al. PLoS One. 2012;7(7):e41721.

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