

Poster: 7th cosmetics amendment – can all goals be achieved in time?

The comet assay in the Phenion[®] full thickness model as a putative tool for improved *in vitro* genotoxicity testing

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Genotoxicity is one of the first toxicological endpoints to be tested within the course of safety assessment e.g. for cosmetic ingredients, pharmaceuticals, or agrochemicals. Regulation requires that cosmetic ingredients are subjected to an *in vitro* test battery first. The currently accepted *in vitro* tests exhibit high sensitivity but unfortunately low specificity. Therefore, *in vivo* tests are often needed to clarify the *in vitro* data. However, due to the 7th Amendment to the European Cosmetics Directive *in vivo* tests for several toxicological endpoints, e.g. genotoxicity, will be banned starting in 2009. In addition, the new EU regulation on chemicals REACH requires safety tests for nearly 30,000 cosmetic or other raw materials during the next decades which is expected to trigger high numbers of animal testing.

Therefore, it is a challenging task to develop new alternative methods which have a higher biological relevance than the already validated genotoxicity methods in order to supplement existing test batteries and to finally replace current *in vivo* genotoxicity assays.

The high rate of false positive results of the currently used *in vitro* tests might be caused by a wide-spread use of cell lines often of non-human origin which are partly transformed and which lack normal metabolism. To overcome these limitations we established a Comet Assay with the Phenion[®] Full Thickness Skin Model. Thus, the skin as the first site of contact for most cosmetics and many environmental stimuli and as the organ with maximum exposure is introduced into *in vitro* genotoxicity testing. The tissue enables realistic application of test substances and by the use of primary human cells an organ- and species-specific metabolism is provided.

The Comet Assay as a widely used and scientifically well accepted method was chosen because it detects several types of permanent or transient DNA damages and allows the analysis of both keratinocytes and fibroblast. Recommendations for methodological standards of international expert groups are available and adopted for the presented protocol.

Several direct acting mutagens (Methylmethane Sulfonate, 4-Nitroquinolineoxide) or pro-mutagens (e.g. Benzopyrene) in different concentrations were applied topically to the tissues. After defined time points keratinocytes or fibroblast respectively were isolated and analyzed separately regarding the fluorescence intensity in the tail. The tested compounds induced a dose-dependent increase of %tail DNA.

In conclusion, this model represents a system close to the *in vivo* situation and allows analyzing the *in vivo* relevance of *in vitro* positive substances in more detail.

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