

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

***In vitro* skin irritation assessment of colored and colorant-like chemicals using the ECVAM validated EpiSkin™ assay: the need of appropriate and relevant controls**

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The use of *in vitro* reconstructed epidermis as a replacement model for the rabbit skin irritation method has been scientifically validated in April 2007 (ESAC statement, 2007). The test is based on tissue viability assessment by using the MTT test (Mosmann et al., 1983) (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and allows the discrimination between irritant chemicals (R38) and non-irritant (NC) chemicals. The MTT assay is a standard colorimetric method: Yellow MTT is reduced to purple formazan by mitochondrial dehydrogenases of the epidermis living cells. The colored solution obtained after formazan extraction is quantified by absorbance measurement at 570 nm. Therefore, due to this measurement method, any chemical able to strongly color the tissue or induce indirect and non specific coloration absorbing closely to formazan can result in a possible viability overestimation.

The purpose of this study was to investigate the use of the validated EpiSkin™ protocol (colorimetric assay) for the *in vitro* evaluation of colored chemicals irritancy potential (e.g. hair dyes). The assay consists mainly in a standardized topical application and a post-treatment incubation period (Spielmann et al., 2007; Cotovio et al., 2005). Colored chemicals as dyes can be retained in the epidermis or difficult to wash-off and induce a possible residual staining. Consequently, after extraction, non specific remaining color is able to modify final readings. In order to eliminate colorimetric interference, we introduced specific controls fitting to the validated protocol course. Control tissues followed the same treatment steps than treated ones, MTT step excepted. These controls enabled the quantification of non specific optical density (OD). Therefore calculations take into account only the true OD related to mitochondrial activity of living cells. By introducing these adapted controls, we showed that the validated EpiSkin™ MTT assay is a suitable and reliable method for *in vitro* skin irritation prediction of dyes and chemicals likely to color tissues. The applicability domain can therefore be extended to these chemical families.

References

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