Teil 3: Neue Entwicklungen

Development and Validation of Reconstructed Human Cornea Models for Eye Irritation Testing

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The recently implemented 7th Amendment to the EU Cosmetics Directive and the EU REACH legislation have heightened the need for in vitro ocular test methods covering broad range of irritation responses. While there are already validated and regulatory accepted methods for severe eye irritation testing (BCOP and ICE tets), the prediction of mild/moderate and non-irritating compounds and formulations by in vitro methods is not yet fully covered. To address this need, the EpiOcular™ Eye Irritation Test (EpiOcular-EIT), which utilizes primary, non transformed human cell-based EpiOcular tissue model, and SkinEthic™ Short Exposure Time/Long Exposure Time eye irritation test, which utilize immortalized, corneal cell line based SkinEhtic HCE model, have been developed.

The EpiOcular-EIT is based on evidence, that most of the eye irritating chemicals chemicals do cause corneal injury of various depth and that cytotoxicity caused to the corneal tissue should correlate well with the overall irritation effect in most cases. The EpiOcular EIT uses a single exposure time combined with post-exposure period allowing for development of cytotoxic effect. Tissue viability is determined by the MTT assay. EpiOcular EIT comprises of two test protocols: protocol for liquids (30 min exposure followed by 2 hours post-exposure) and protocol for solids (90min exposure followed by 18-hour post-exposure). A chemical is classified as an irritant, if the tissue viability is ≤ 60%, and as a non-irritant, if the viability is > 60%.

The SkinEhtic assay is based on assumption that chemicals can be divided into the two groups based on initial assessment of reactivity using Direct Peptide Reactivity Assay (DPRA). Depending on the reactivity of chemicals, either Short Exposure Time (SET) protocol (10 min exposure followed by MTT viability test) or Long Exposure Time (LET) protocol (60 min exposure followed by 16-h postincubation period) is used to assess cytotoxicity. Tissue viability is determined by the MTT assay.

The EpiOcular and SkinEthic eye irritation tests were developed and pre-validated during 2004-2008, and are currently involved in a formal, multi-laboratory validation study sponsored by the European Cosmetics Association (COLIPA) under the auspices of the European Centre for the Validation of Alternative Methods (ECVAM).

In addition to recent activities described above, in early 1990's, a protocol that utilizes EpiOcular ET-50 assay was developed by industry for testing of surfactants and formulations. Surfactants related validation study was performed with EpiOcular-ET50 protocol in 2003 and the data were

submitted to ECVAM for review in 2004 and resubmitted in 2009. In 2009, US EPA initiated investigation of the EpiOcular ET-50 protocol for assessment of antimicrobial cleaning products.

Recently EpiOcular FT model (containing epithelium and stroma layer) has been developed with the aim to enable assessment of advanced ocular toxicity studies. These would include e.g. recovery from the mild/moderate injury which was not possible to assess with currently pre-validated models.

In summary, reconstructed human corneal models belong to the most promising tools in assessment of eye irritation effects of chemicals and formulations in vitro. Ongoing validation studies performed by ECVAM should confirm high reproducibility, reliability and long-term positive industry experience with 3D models.