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3T3 Neutral Red Uptake *in vitro* Phototoxicity Test

Regulatory Acceptance

Phototoxicity (photo-irritation) is an acute light induced, non-immunologic skin response to a photo-reactive chemical. A thoroughly validated *in vitro* method is available for phototoxicity testing. The 3T3 NRU Phototoxicity test has been officially accepted by the EU Commission and the EU member states *Annex V to Directive 67/548/EEC on the Classification, Packaging and Labelling of Dangerous Substances: Testing Method B.41 Phototoxicity – in vitro 3T3 NRU Phototoxicity Test* and is also available as OECD guideline 432, *In Vitro* 3T3 NRU Phototoxicity Test, published November, 23, 2004.

Test principle

The test is based on a comparison of the cytotoxicity of a chemical when tested in the presence and in the absence of exposure to a non-cytotoxic dose of UVA/vis light. The use of the permanent mouse fibroblast cell line Balb/c 3T3 is recommended in the standard protocol of the EU and the OECD guideline. Cytotoxicity is expressed as a concentration dependent reduction of the uptake of the vital dye, Neutral Red. In most cases data from this in vitro test may provide sufficient information for the preclinical assessment of the phototoxic potential of a drug product and thus *in vivo* non-clinical studies are normally not warranted.

Advantages of the 3T3 NRU Phototoxicity test include: regulatory approval for in vitro assessment of phototoxic potential, relatively simple technical procedures, excellent reproducibility of results and cost efficiency.

Test procedure

Balb/c 3T3 cells are cultured in 96well plates to form monolayer. First, the 96well plates are incubated with different concentrations of the test substance. Some plates are exposed to a dose of light, while control plates are kept in the dark. Then, the assay medium is replaced with fresh culture medium and the cells are incubated for 18-20 hours. Afterwards, cell viability is determined with the vital dye neutral red. The optical density is determined using a microplate reader. Cell viability at the tested chemical concentration with and without light exposure is compared to determine the photo-irritation potential.

An UV lamp is used as a light source. A dose of UVA equivalent to 5 J/cm^2 is delivered to the 96well plate.

The IC_{50} concentrations (i.e. the concentrations by which cellular viability is reduced by 50%) are determined for the plates incubated in the dark (Cytotoxicity) and under UVA (Phototoxicity), Then, the phototoxic potential of a test chemical or photo-irritancy factor (PIF) is determined by comparison of the IC_{50} without and with UVA exposure:

$$PIF = \frac{IC_{50}\ (-UVA)}{IC_{50}\ (+UVA)}$$

In addition, the mean photo effect (MPE) is calculated. The MPE is based on the comparison of the complete concentration response curves. It is defined as the weighted average across a representative set of photo effect values. The MPE is a useful measure of phototoxic potential for cases in which to equally effective concentrations (IC_{50}) in the dark (-UVA) and light (+UVA) cannot be determined.

Base on the guideline, the Phototoxicity potential of the test substance is interpreted as follows:

Mean Photo Effect (MPE)	Photo-irritancy Factor (PIF)	Phototoxic Potential
< 0.1	< 2	Non-phototoxic
> 0.1 and < 0.15	> 2 and < 5	Probably Phototoxic
> 0.15	> 5	Phototoxic

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