In vitro 3T3 NRU Phototoxicity test

1. Objective

The *in vitro* 3T3 NRU phototoxicity test is used to identify the phototoxic potential of a test substance induced by the excited chemical after exposure to light.

2. Introduction

Phototoxicity is defined as a toxic response from a substance applied to the body which is either elicited or increased (apparent at lower dose levels) after subsequent exposure to light, or that is induced by skin irradiation after systemic administration of a substance.

The test evaluates photocytotoxicity by the relative reduction in viability of cells exposed to the chemical in the presence versus absence of light. Substances identified by this test are likely to be phototoxic *in vivo,* following systemic application and distribution to the skin, or after topical application.

3. Method

Balb/c 3T3 cells are maintained in culture for 24 h for formation of monolayers. Two 96-well plates per test chemical are pre-incubated with eight different concentrations of the test substance and with positive control and negative control for 1 h. Thereafter one of the two plates is irradiated (+UV) for 50 min. with 1.7 mW/cm^2 whereas the other plate is kept in the dark. In both plates the treatment medium is then replaced

by culture medium and after another 24 h of incubation cell viability is determined by Neutral Red uptake.

Cell viability is expressed as percentage of untreated solvent controls and is calculated for each test concentration. To predict the phototoxic potential, the concentration responses obtained in the presence and in the absence of irradiation are compared, usually at the EC_{50} level, i.e., the concentration reducing cell viability to 50 % compared to the untreated controls.

To enable evaluation of the data, a Photo-Irritation-Factor (PIF) may be calculated:

(1) $$PIF = \frac{EC_{50}(-UV)}{EC_{50}(+UV)}$$

The PIF can only be calculated if the concentration – response curves obtained in the presence and absence of UV-light drop below 50 % of the controls, because only in these cases two EC_{50} values (-UV and +UV) can be determined.

Therefore, the prediction model takes into account two additional classification rules:

If a chemical is only cytotoxic in the presence of UV (+UV) and not cytotoxic when tested without irradiation (-UV), the PIF cannot be calculate, although this is a result indicating phototoxic potential. In this case a >PIF is calculated using the highest concentration (c_{max}) of –UV:

(2) $$> PIF = \frac{c_{max}(-UV)}{EC_{50}(+UV)}$$

If both, EC_{50} (-UV) and EC_{50} (+UV) cannot be calculated due to the fact that a chemical does not show any cytotoxicity up to the highest tested concentration, this indicates no phototoxic potential. In this case a formal “PIF=*1” is used to characterise the result:

(3) $$PIF = *1 = \frac{c_{max}(-UV)}{c_{max}(+UV)}$$

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4. Results

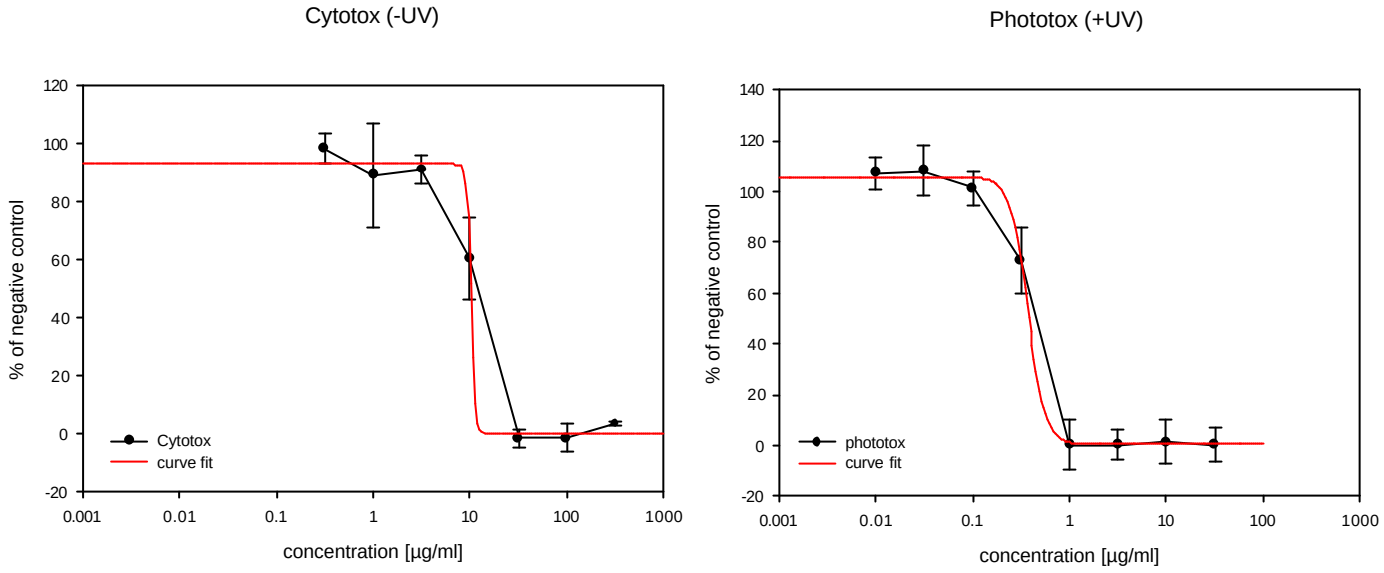

Figure 1: Example (-UV)- and (+UV)-curves of mean values of the cell viability in % of the respective negative control and calculated curve fit

$$PIF = \frac{EC_{50}(-UV)}{EC_{50}(+UV)} = \frac{10.29\,mg/ml}{0.37\,mg/ml} = 27.81$$

For more information please contact us!

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