



In vitro Phototoxicity Validation Study using Chlorpromazine Technical

LP-08

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Purpose

The *in vitro* 3T3 Neutral red uptake (NRU) phototoxicity test is used to identify the phototoxic potential of the test item activated by exposure to light. In the context of pharmaceuticals and other chemicals, phototoxicity can be particularly relevant because some compounds become harmful only when exposed to light.

Objective

The objective of this study is to assess cytotoxicity and phototoxicity of Chlorpromazine Technical to 3T3 cells (in presence and absence of UVA light) by Neutral red uptake method.

Test System:

BALB/3T3 clone A31 (mouse fibroblast) cell line (Source: ATCC)

Equipment for photo-irradiation:

Solar Simulator (SSL), Honle, Germany

Materials and Methods

Target cell preparation in 96 well microtiter plate



Incubation with the test item (different concentrations of Chlorpromazine Technical along with the controls and eight replicate wells) at 37°C for 60 minutes



+SSL plate: cells were irradiated at room temperature for 50 minutes through the lid of 96 well plate with 1. 7 mW/cm2 (5 J/cm2)



-SSL plate: kept at room temperature in the dark for approximately 50 minutes



After irradiation, +SSL and -SSL, cells were washed and incubated in culture medium for 24 hours



Following incubation, microscopic observation of each plate for growth, morphology and integrity of the monolayer



After incubation, the uptake of the Neutral red into the lysosomes of living cells were used as a quantitative indication of cell number and viability



The absorption was measured at 540 nm



Data analysis and Interpretation using Phototox 2.0 software

%Viability = $100 \times OD_{540e} / OD_{540b}$

 OD_{540e} is the mean value of the measured optical density of the test item/positive control/negative control

OD_{540b} is the mean value of the measured optical density of the respective vehicle control PIF (Photo irradiation factor) and MPE (Mean Photo Effect) factors were calculated.

Evaluation and Interpretation of Results

- 1. PIF of <2 or an MPE <0.1 indicates 'no phototoxicity'
- 2. PIF of >2 and <5, or an MPE of >0.1 and <0.15 indicates 'probable phototoxicity,'
- 3. PIF of >5 or an MPE of >0.15 indicates 'phototoxicity.' Where, PIF = IC50 (-Irr)/ IC50 (+Irr)

Results and Discussion

The average IC50 value, with SSL and without SLL were 0.516 µg/mL and 33.703 µg/mL, respectively for the Chlorpromazine Technical. Thus, the mean value of PIF and MPE factor of Chlorpromazine Technical was 66.994 and 0.432, respectively.

Based upon the observed results and under the test-conditions chosen, Chlorpromazine Hydrochloride was phototoxic as the PIF was > 5 and MPE was > 0.15.

As Chlorpromazine showed phototoxic potential as suggested by the test guideline OECD432, study will be further validated with proficiency chemicals recommended by the test guideline.

References:

- 1. OECD guideline for testing of chemicals (Guideline No. 432) on conduct of 'In Vitro 3T3 NRU Phototoxicity Test' adopted by the council on 18 June 2019.
- 2. INVITTOX Protocol 78. 3T3 Neutral Red Uptake (NRU) Phototoxicity Assay. ECVAM DB-ALM; 2008. http://ecvam-dbalm.jrc.ec.europa.eu/

Figure 1: 96 well plate for the 3T3 phototoxicity assay (without SSL and with SSL)

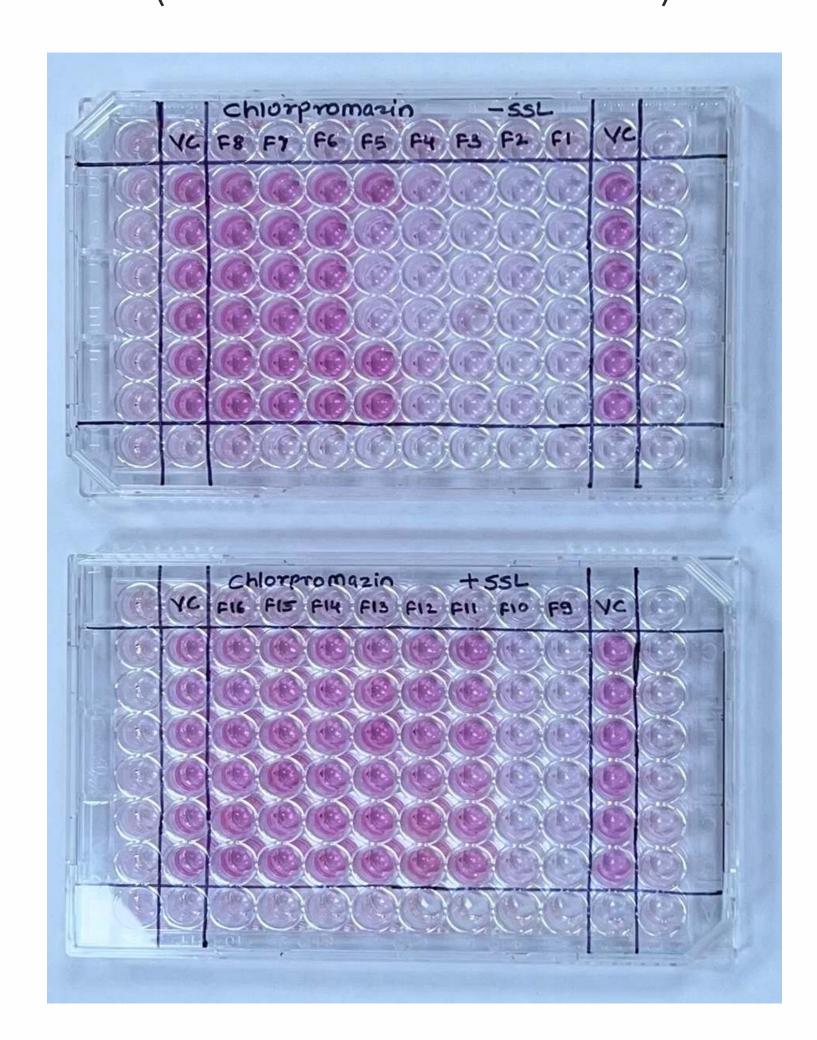
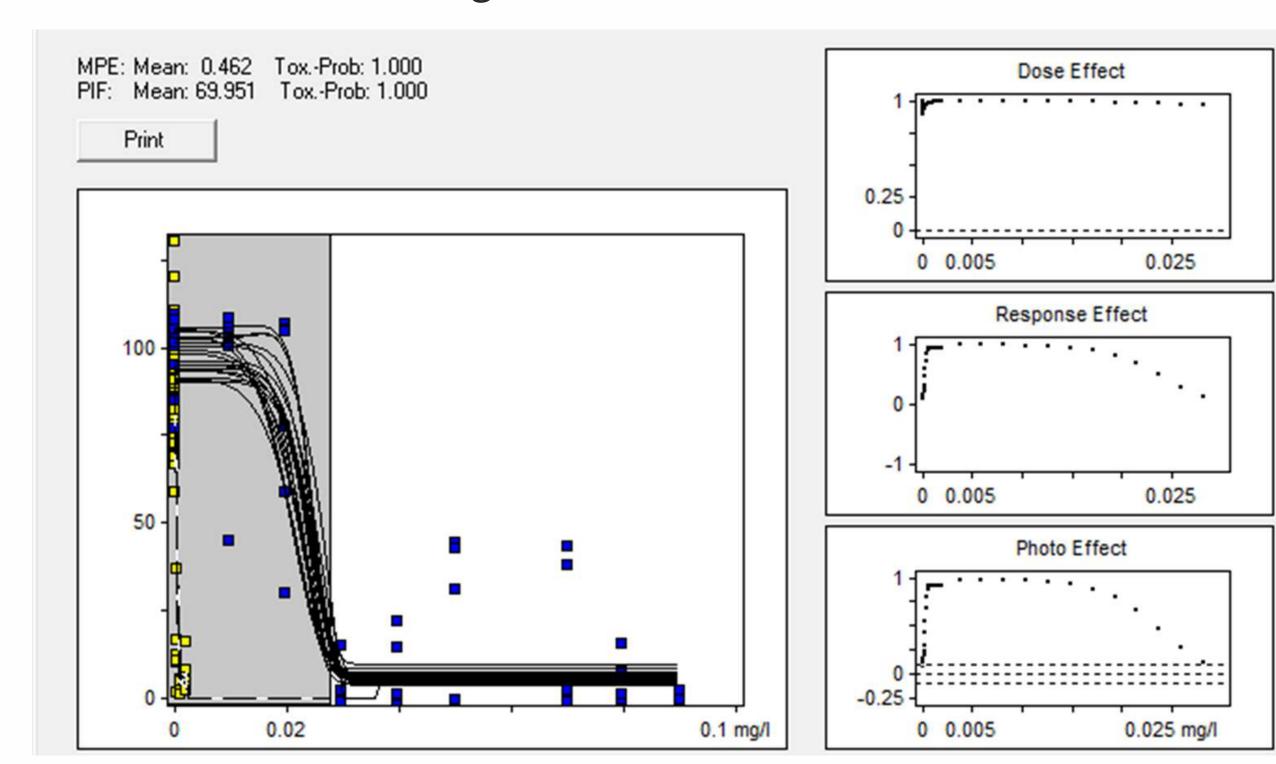


Figure 2: Pair Evaluation using Phototox 2.0 software



Keywords:

Phototoxicity, Neutral Red Uptake, Solar simulator, Photo irradiation factor, Mean photo irradiation factor, BALB/c, clone 31