57. The test report must include the following information:

Test substance:
- identification data and CAS number, if known;
- physical nature and purity;
- physicochemical properties relevant to conduct of the study;
- UV/vis absorption spectrum;
- stability and photostability, if known.

Solvent:
- justification for choice of solvent;
- solubility of the test chemical in solvent;
- percentage of solvent present in treatment medium.

Cells:
- type and source of cells;
- absence of mycoplasma;
- cell passage number, if known;
- Radiation sensitivity of cells, determined with the irradiation equipment used in the *in vitro* 3T3 NRU phototoxicity test.

Test conditions (1); *incubation before and after treatment:*
- type and composition of culture medium;
- incubation conditions (CO_2 concentration; temperature; humidity);
- duration of incubation (pre-treatment; post-treatment).

Test conditions (2); *treatment with the chemical:*
- rationale for selection of concentrations of the test chemical used in the presence and in the absence of irradiation;
- in case of limited solubility of the test chemical and absence of cytotoxicity: rationale for the highest concentration tested;
- type and composition of treatment medium (buffered salt solution);
- duration of the chemical treatment.

Test conditions (3); *irradiation:*
- rationale for selection of the light source used;
- spectral irradiance characteristics of the light source;
- transmission and absorption characteristics of the filter(s) used;
- characteristics of the radiometer and details on its calibration;
- distance of the light source from the test system;
- UVA irradiance at this distance, expressed in $mW/cm^{2;}$
- duration of the UV/vis light exposure;
- UVA dose (irradiance x time), expressed in $J/cm^{2;}$
- temperature of cell cultures during irradiation and cell cultures concurrently kept in the dark.

Test conditions (4); *Neutral Red viability test:*
- composition of Neutral Red treatment medium;
- duration of Neutral Red incubation;
- incubation conditions (CO_2 concentration; temperature; humidity);
- Neutral Red extraction conditions (extractant; duration);
- wavelength used for spectrophotometric reading of Neutral Red optical density;
- second wavelength (reference), if used;
- content of spectrophotometer blank, if used.

Results:
- cell viability obtained at each concentration of the test chemical, expressed in percent viability of mean, concurrent solvent controls;
- concentration response curves (test chemical concentration vs. relative cell viability) obtained in concurrent +Irr and -Irr experiments;
- analysis of the concentration-response curves: if possible, computation/calculation of IC_{50} (+Irr) and IC_{50} (-Irr);
- comparison of the two concentration response curves obtained in the presence and in the absence of irradiation, either by calculation of the Photo-Inhibition-Factor (PIF), or by calculation of the Mean-Photo-Effect (MPE);
- test acceptance criteria; concurrent solvent control:
  - absolute viability (optical density of Neutral Red extract) of irradiated and non-irradiated cells;
  - historic negative and solvent control data; means and standard deviations.
- test acceptance criteria; concurrent positive control:
  - IC_{50}(+Irr) and IC_{50}(-Irr) and PIF/MPE of positive control chemical;
  - historic positive control chemical data: IC_{50}(+Irr) and IC_{50}(-Irr) and PIF/MPE; means and standard deviations.

Discussion of the results.

Conclusions.

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(19) This will be a hyperlink to the OECD web pages that contain the software.

ANNEX 1

DEFINITIONS

Irradiance: the intensity of ultraviolet (UV) or visible light incident on a surface, measured in W/m^2 or mW/cm^2.

Dose of light: the quantity (= intensity x time) of ultraviolet (UV) or visible radiation incident on a surface, expressed in Joules (= W x s) per surface area, e.g., J/m^2 or J/cm^2.

UV light wavebands: the designations recommended by the CIE (Commission Internationale de L'Eclairage) are: UVA (315-400nm) UVB (280-315nm) and UVC (100-280nm). Other designations are also used; the division between UVB and UVA is often placed at 320nm, and the UVA may be divided into UV-A1 and UV-A2 with a division made at about 340nm.

Cell viability: parameter measuring total activity of a cell population (e.g., uptake of the vital dye Neutral Red into cellular lysosomes), which, depending on the endpoint measured and the test design used, correlates with the total number and/or vitality of the cells.

Relative cell viability: cell viability expressed in relation of solvent (negative) controls which have been taken through the whole test procedure (either +Irr or -Irr) but not treated with test chemical.

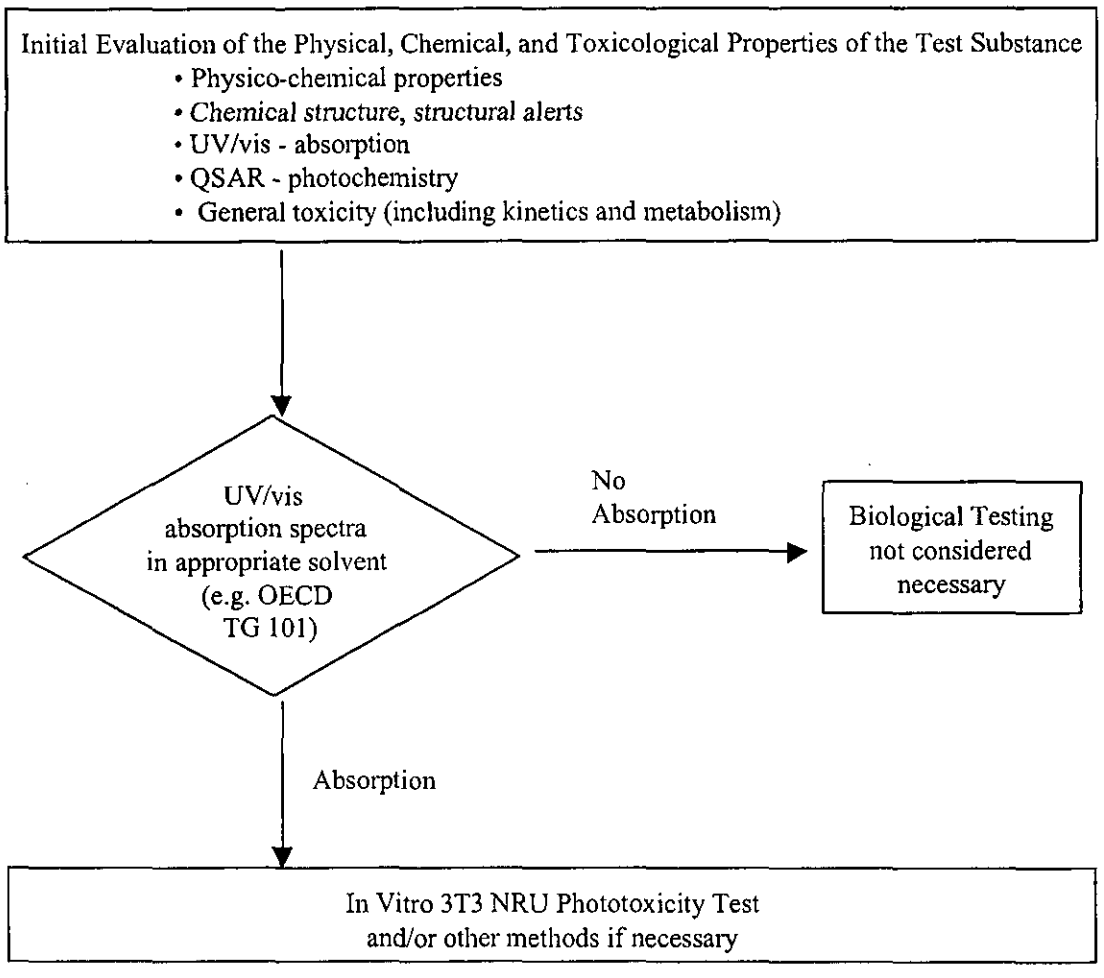
PIF (Photo-Irritation-Factor): factor generated by comparing two equally effective cytotoxic concentrations (IC_{50}) of the test chemical obtained in the absence (-Irr) and in the presence (+Irr) of a non-cytotoxic irradiation with UVA/vis light.

MPE (Mean-Photo-Effect): measurement derived from mathematical analysis of the concentration response curves obtained in the absence (-Irr) and in the presence (+Irr) of a non-cytotoxic irradiation with UVA/vis light.

Phototoxicity: acute toxic response that is elicited after the first exposure of skin to certain chemicals and subsequent exposure to light, or that is induced similarly by skin irradiation after systemic administration of a chemical.

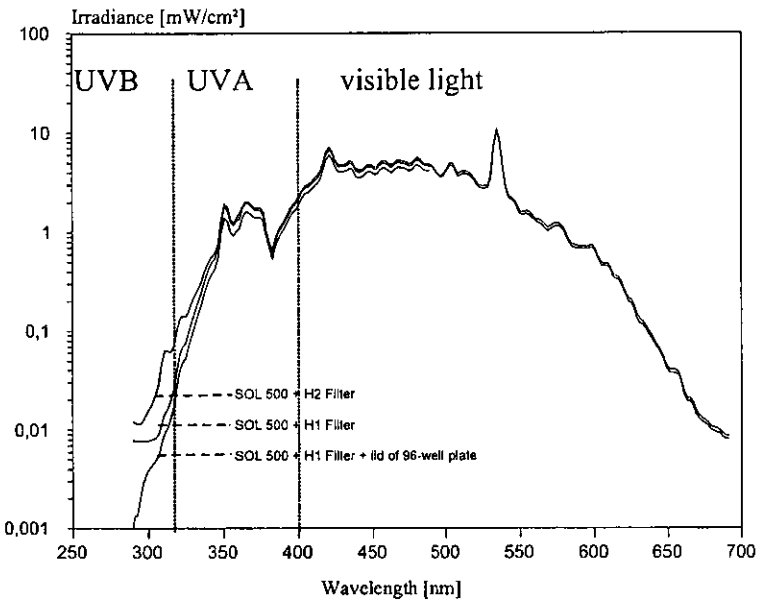
ANNEX 2

Role of the 3T3 NRU PT in a sequential approach to the phototoxicity testing of chemicals



ANNEX 3

Figure 1: Spectral power distribution of a filtered solar simulator



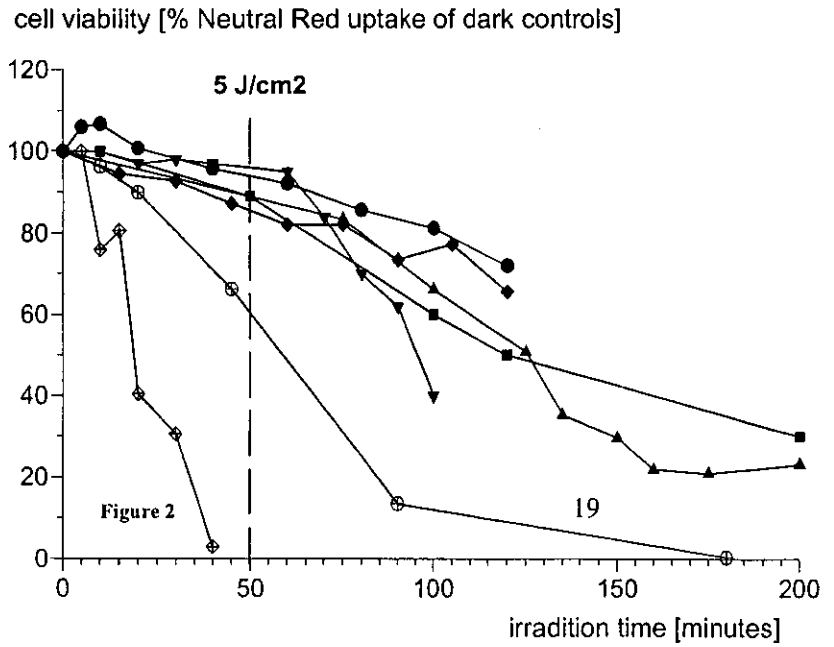
(see paragraph 22)

Figure 1 gives an example of an acceptable spectral power distribution of a filtered solar simulator. It is from the doped metal halide source used in the validation trial of the 3T3 NRU PT (1)(3)(12). The effect of two different filters and the additional filtering effect of the lid of a 96-well cell culture plate are shown. The H2 filter was only used with test systems that can tolerate a higher amount of UVB (skin model test and red blood cell photo-hemolysis test). In the 3T3 NRU-PT the H1 filter was used. The figure shows that additional filtering effect of the plate lid is mainly observed in the UVB range, still leaving enough UVB in the irradiation spectrum.

Figure 2

Irradiation sensitivity of Balb/c 3T3 cells (as measured in the UVA fa

Figure 2: Irradiation sensitivity of (as measured in the UV



(see paragraphs 24, 28, 29)

Sensitivity of Balb/c 3T3 cells to irradiation with the solar simulator used in the validation trial of the 3T3NRU-

Phototoxicity Test, as measured in the UVA range. Figure shows the results obtained in 7 different laboratories in the pre-validation study (1). While the two curves with open symbols were obtained with aged cells (high number of passages), that had to be replaced by new cell stocks the curves with bold symbols show cells with acceptable irradiation tolerance.
From these data the highest non-cytotoxic irradiation dose of 5 J/cm² was derived (vertical dashed line). The horizontal dashed line shows in addition the maximum acceptable irradiation effect given in paragraph 29.

This Draft Guideline is open for public comment until 12th April 2002

Comments should be submitted as follows:

1. If you are a citizen of an OECD Member country or a non-member country participating in the work on Test Guidelines (see list) and:

- working in the public sector (local/ federal government, academia) please submit your comments to your National Co-ordinator (see list);

- working in the private sector (industry, contract laboratory) please submit to your comments either to your National Co-ordinator (see list) to the Business and Industry Advisory Council (BIAC) to OECD;

- commenting on behalf of a public interest group, please submit your comments to your National Co-ordinator (see list) or to the EEB (for environmental interest groups) or ICAPO (for animal welfare interest groups).

2. If you are not a citizen of an OECD Member country or a non-member country participating in the work on Test Guidelines (see list) please submit your comments directly to the Secretariat.

For inquiries related to the work on Test Guidelines, please contact the Head of the Test Guidelines Programme

研究成果の刊行に関する一覧表

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
田中憲穂 若栗忍	光細胞毒性試験における照射条件と細胞株の基礎的検討	秦野研究所年報	24	32-38	2001
N,TANAKA Y,NAKAGAWA Y,TANIGAWA	The rapid screening of photogenotoxic compounds using	Environmental Mutagen Research	23	107-118	2001
田中憲穂	安全性試験としての遺伝毒性（変異原性）試験の意義と試験概要	JHOSPA(Journal of Japan Hygeinic Olefin and Styrene Plastics Association)	12	2-16	2001
田中憲穂 若栗忍	動物モデルによる新しい評価法67、In Vitor 光細胞毒性試験について	アニテックス	14	44-48	2002

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