PHOTOTOXICITY

Phototoxicity (or photoirritation) is a toxic response to a topically or systemically administered substance that occurs after exposure to light and does not involve the immune system. It can cause symptoms ranging from first-degree burns (e.g. redness, itching, or pain) to full-thickness third-degree burns. Compounds that absorb light in the UV and visible range of 290–700 nm and could come into contact with the skin or eyes may require testing for potential phototoxicity if their molar extinction coefficient (MEC) is greater than 1,000 L mol-1 cm-1.

The below OECD test guidelines can be combined with other physico-chemical assessments and *in vitro* and *in silico* approaches, as outlined in the OECD's Guidance Document on Integrated Approaches to Testing and Assessment (IATA) for Phototoxicity Testing, without animal testing to assess a substance's phototoxic potential.[1] For pharmaceuticals, ICH S10 (2013) gives initial guidance on these methods.[2]

	METHOD	TEST PRINCIPLE	APPLICABILITY DOMAIN	DATA INTERPRETATION
IN CHEMICO	OECD TG 495: ROS (Reactive Oxygen Species) Assay for Photoreactivity[3] Adopted by OECD in 2019	1. The test substance is added to the reaction mixtures. 2. Before and after 1 hour of light exposure, absorbance at 440 and 560 nm is measured to detect superoxide anion (SA) or singlet oxygen (SO)—the principal intermediate species in phototoxic reactions. 3. SO generation is detected by *p*-nitrosodimethylaniline bleaching (decrease of A_{440}), and SA generation is detected by observing the reduction of nitroblue tetrazolium (increase of A_{560}).	This test is applicable to substances that are soluble in dimethyl sulfoxide (DMSO) or sodium phosphate buffer. Solubility enhancers may be used but further characterisation and standardisation of procedures should be performed. For substances that directly interfere with the reaction mixture, OECD TG 432 or 498 (see below) should be used. The test does not address metabolic activation, but there is no evidence that any phototoxic compound would be missed in the absence of metabolic activation.[4]	**Substance tests negative:** Likely to test negative in humans. **Substance tests positive:** May require further testing using *in vitro* methods.
IN VITRO	OECD TG 432: 3T3 Neutral Red Uptake Phototoxicity Test[5] Adopted by OECD in 2004	1. Increasing concentrations of the test substance are applied to BALB/c 3T3 cells for 1 hour. 2. Half of the samples are exposed to a non-cytotoxic dose of simulated sunlight while the other half are kept in the dark. 3. 18-24 hours later, cytotoxicity is measured as a concentration-dependent reduction of neutral red (vital dye) uptake.	This test is applicable to substances that are soluble in buffered salt solutions. Test substances with limited solubility in water can be dissolved in an appropriate solvent, such as DMSO or ethanol. Solvent controls must be included, and precipitation or cloudy solutions should be avoided. To reduce false positives, test only relevant substances (MEC>1,000 $L\ mol^{-1}cm^{-1}$), limit the maximum concentration under irradiation to 100 mg mL^{-1}, and consider a higher maximum concentration, without irradiation, to establish IC_{50} values for photo irritation factor calculations.	**Substance tests negative**: Likely to test negative in humans. **Substance tests positive**: May require further testing using OECD TG 492 or clinical photosafety assessment in volunteers for medical drugs.
	OECD TG 498: Reconstructed Human Epidermis Phototoxicity Test (RhE PT)[6] Adopted by OECD in 2021	1. Three to five concentrations of the test substance are applied topically to RhE models for 18-24 hours. 2. Half of the tissues are exposed to a non-cytotoxic dose of simulated sunlight while the other half are kept in the dark. 3. 18-24 hours later, relative viability is determined using the MTT assay.	This test is applicable to a wide selection of substances and final formulations—even in complex mixtures, dermatological patches, or substances with an extreme pH. Test results of substances that may interfere with the MTT assay directly should be interpreted with caution, and appropriate controls should be included. The absorption and penetration of the test chemical during topical exposure could provide more relevant results than tests performed using simpler systems.	**Substance tests negative**: Likely to test negative in humans. **Substance tests positive**: May require further clinical photosafety assessment in volunteers for medical drugs.

References:

1. OECD. Guidance Document on Integrated Approaches to Testing and Assessment (IATA) for Phototoxicity Testing. OECD Series on Testing and Assessment No. 397. 2024. https://doi.org/10.1787/8a979653-en
2. ICH. Photosafety Evaluation for Pharmaceuticals – S10. Safety Guidelines. 2013. https://www.ich.org/page/safety-guidelines
3. OECD. Test No. 495: Ros (Reactive Oxygen Species) Assay for Photoreactivity. OECD Guidelines for the Testing of Chemicals. 2019. https://doi.org/10.1787/915e00ac-en
4. Ceridono M, Tellner P, Bauer D, et al. The 3T3 neutral red uptake phototoxicity test: practical experience and implications for phototoxicity testing—the report of an ECVAM-EFPIA workshop. *Regul Toxicol Pharmacol.* 2012;63(3):480-488. https://doi.org/10.1016/j.yrtph.2012.06.001
5. OECD. Test No. 432: In Vitro 3T3 NRU Phototoxicity Test. OECD Guidelines for the Testing of Chemicals. 2019. https://doi.org/10.1787/9789264071162-en
6. OECD. Test No. 498: In Vitro Phototoxicity - Reconstructed Human Epidermis Phototoxicity test method. 2023. . https://doi.org/10.1787/7b2f9ea0-en

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