



Phototoxicity Services



Leading the Field of *In Vitro* Toxicology

CeeTox offers two *in vitro* models for use as phototoxicity screens that offer alternatives to animal testing in support of the European regulations in REACH and Amendment 7 to the Cosmetics Directive.

By using the *in vitro* dermal phototoxicity testing services CeeTox offers, clients can practice the 3Rs (reduce, refine, or replace animal testing) and achieve Amendment 7 and REACH directives by reducing or eliminating the need for *in vivo* testing. These *in vitro* assays produce reliable outcomes that supplement or replace *in vivo* data at reasonable cost.

Phototoxicity tests are used to identify the phototoxic potential of a test article induced by the excited chemical after exposure to light. The tests evaluate photo-cytotoxicity by the relative reduction in viability of cells exposed to the chemical in the presence of light compared to the absence of light. Substances identified by these tests are likely to be phototoxic following systemic application and distribution to the skin, or after topical application.

Phototoxicity

Dermal Phototoxicity (photoirritation) is defined as “an acute reaction which can be caused by a single treatment with a chemical and UV or visible radiation. *In vivo*, the reaction can be evoked in all subjects provided that the concentration of chemical and dose of light are appropriate. ‘Acute’ includes both immediate and delayed (e.g. after 48 hours) reactions. The term photoirritation is used to describe phototoxic reactions in skin which are produced with topically applied substances following exposure to light.” (from ECVAM Workshop 42)

Models

It has been shown (in a joint EU/COLIPA validation project) that the phototoxic potential of chemicals can be correctly predicted by using cell culture monolayers in a specially designed cytotoxicity assay, the 3T3-Neutral Red Uptake (NRU)-Phototoxicity Test.

The NRU assay provides a quantitative estimation of the number of viable cells in a culture. It is based on the ability of viable cells to incorporate and bind the dye neutral red in the lysosomes.

For the testing of individual ingredients where solubility is acceptable, the BALB /c 3T3 NRU assay is used as the test system. While the 3T3-NRU test is often the only test required if it shows negative phototoxicity of a test article, it might be used as the first step in a tiered phototoxicity testing strategy if it shows the compound to be positive.

This is because the phototoxic potential of a chemical predicted using the 3T3 cellular system may not be relevant when topically applied to the skin at low concentrations (such as in a formulation) there is a need for adjunct tests that allow for the assessment of safe usage concentrations on a dose per area basis before testing them in humans.

Tests have revealed that substances which are known to be safe in humans, and yet have yielded positive results in the 3T3-NRU assay, exhibit their true negative phototoxic potential when topically applied to the Reconstructed Skin Model at low concentrations, such as found in a formulation. This is due to the well-developed barrier of the 3D model, which prevents the chemicals from penetrating into the deeper parts of the epidermis and subsequently causing damage to the cells.

Reconstituted skin models and epidermis models have shown the ability to predict both photoirritancy and the photoprotective action of sunscreens. In addition, skin models can handle formulations (e.g. emulsions, suspensions) which the 3T3 test cannot handle. Therefore, in a testing strategy that is based purely on *in vitro* tests, there is a need to combine the basic 3T3-NRU Phototoxicity Test with other *in vitro* tests that allow the safety or phototoxic potency of formulations to be assessed.

EpiDerm is a normal (non-transformed), human cell-derived, metabolically active, 3-dimensional organotypic *in vitro* skin model. Also known generically as reconstructed human epidermis (RhE), EpiDerm closely mimics human epidermis, both structurally and biochemically, and does so in a very reproducible manner. Proven EpiDerm dermal phototoxicity protocols, based on the well-documented MTT ET-50 Tissue Viability Assay, allow researchers to quantitatively measure the dermal phototoxicity of their experimental materials.

Types of materials that have been tested using the EpiDerm system include cosmetics and their constituents, household products, and pharmaceuticals. EpiDerm (Model EPI-200) has been used successfully as an *in vitro* alternative in a number of toxicology tests including Dermal Phototoxicity.

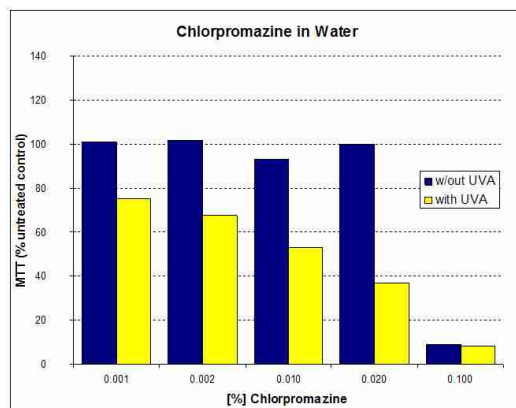
In the 3D model, viability of both irradiated and non-irradiated tissues will be determined using the recommended MTT assay. Cytokine release and histological examination can also be performed as an additional endpoint

Reporting

Reporting may take three basic forms. Our standard report includes data charts and graphs detailing results of the assays run. A detailed report is optionally available as well. This report includes:

- Executive Summary
- Objective
- Experimental Design
- Results
- Tables and Figures
- Materials and Methods
- Appendix (if necessary)

Finally, a report complying with GLP requirements is available for those studies performed according to GLP regulations.



MODEL	3T3 NRU Phototoxicity Test (<i>in vitro</i>)	Phototoxicity Test (<i>in vitro</i>) – 3D Cell Model
Purpose of Assay	The phototoxicity test is used to identify the phototoxic potential of a test substance induced by the excited chemical after exposure to light. The test evaluates photo-cytotoxicity 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 following systemic application and distribution to the skin, or after topical application.	
Cell Model	BALB/c 3T3 Mouse Fibroblasts UVA Dosage: ~5 J/cm ²	The EpiDerm™ (Model EPI-200) (MatTek Corporation) UVA Dosage: ~6 J/cm ²
Assays Performed	Solubility Microscopic Evaluation – Growth, morphology, integrity of monolayer Neutral Red Uptake - Cell Viability	MTT - Cell Viability Cytokine Release (optional) H & E stained Histology (optional)
Controls	Vehicle, positive, background	Vehicle, positive, no application
Concentrations	8	5
Number of Replicates	6 -UV, 6 +UV	2 -UV, 2 +UV
Standard Turnaround Time	2 weeks from sample receipt	2 weeks from tissue receipt
Scientific Endorsement	ECVAM	
International Regulatory Acceptance	OECD Test Guideline 432	
National Regional Regulatory Acceptance	Method B.41 of Annex to 440/2008/EC	

References:

ECVAM feasibility study: Can the pre-validated *in vitro* skin model phototoxicity assay be upgraded to quantify phototoxic potency of topical phototoxins? Kandarova, H. et. al. (presented at the 5th World Congress 2005, August 21-25).
from
<http://www.mattek.com/pages/abstracts/372>

European Community, REACH, What is REACH?
from
http://ec.europa.eu/environment/chemicals/reach/reach_intro.htm

Hayden, Patrick (2007, December 6) The Way Forward for *In Vitro* Skin Irritation Testing
from
www.alttox.org/ttrc/toxicity-tests/skin-irritation/way-forward/hayden/

MatTek Corporation, *In Vitro* Toxicology Testing
from
http://www.mattek.com/pages/in_vitro_toxicology

OECD Guidelines for the testing of chemicals/Section 4: Health effects, Test no. 432: *In vitro* 3T3 NRU phototoxicity test (2004, November 23)
from
<http://lysander.sourceoecd.org/vl=3219109/cl=37/nw=1/rpsv/ij/oecdjournals/1607310x/v1n4/s33/p1>

Spielmann, H. et. al.,(2000, Nov.-Dec.) The second ECVAM workshop on phototoxicity testing. The report and recommendations of ECVAM workshop 42. ATLA 28, 777-814.
from
<http://www.ncbi.nlm.nih.gov/pubmed/11105201>

