Summary of available in vitro topical phototoxicity data produced with RHE models

NON - PHOTOTOXIC CHEMICLAS

	Chemical	Class	In vivo human	3T3 NRU PT	EpiDerm Standard protocol and MB Research protocol (*)	EPISKIN Lelievre protocol and Portes protocol(*)	SkinEthic Bernard and Medina protocol (*)
1	SDS	surfactant	NPT	NPT	NPT	NPT, (NPT*)	NPT
2	Coumarine (not specified)	Fragrance	NPT	NPT	n.a.	NPT	n.a.
3	Penicilin G	Drug	NPT	NPT	(NPT*)	NPT	n.a.
4	L-Histidine	Essential amino acid	NPT	NPT	(NPT*)	NPT	NPT
5	Penicilin G	Drug	NPT		NPT	n.a.	NPT
6	Octyl salicylate	UV-filter	NPT	NPT	NPT	n.a.	n.a.
7	4-Methylbenzylidene camphor	UV-filter	NPT	NPT	NPT	n.a.	n.a.
8	Octyl methoxy cinnamate	UV-filter	NPT	NPT	NPT	n.a.	n.a.
9	Mexoryl SX	UV-filter	NPT		NPT	n.a	n.A
10	Benzophenone-3	UV-filter	NPT	NPT	NPT	n.a	NPT
11	benzophenone-4	UV-filter	NPT	NPT	n.a.	(NPT*)	n.a.
12	6-MC	Fragrance	NPT (allergene)	PT/NPT	NPT	(PT*)	NPT (PT*)
13	PABA	UV-filter	NPT (allergene)	PT/NPT	NPT	n.a.	NPT
14	Musk Ambrette	Fragrance	NPT (allergene)	PT	NPT	n.a.	n.a.
15	BAK	Surfactant	NPT	NPT	(NPT*)	n.a.	n.a.
16	DMSO	Solvent	NPT	NPT	(NPT*)	n.a.	n.a.
17	Ethanol	Solvent	NPT	NPT	(NPT*)	n.a	n.a.
18	Hexachlorophene	Drug	NPT	NPT	(NPT*)	n.a.	n.a.
19	BM-DBM	UV-filter	NPT	PT	NPT	n.a.	n.a.
20	Bergamot oil - purified	fragrance	NPT	PT-NPT	NPT	n.a.	n.a.
21	Eucalyptus Oil	fragrance	NPT		(NPT*)	n.a	n.a.

ECVAM's Feasibility study on Photopotency with EpiDerm model and follow-up studies

Chemical	Class	UV Absorption	3T3 NRU PT PIF	EpiDerm "IPM 30%"	First NPT dose on EpiDerm	Human photopatch data	Ref
Ethyl pale	Pharmaceutical	YES	PT	NPT	NPT in all concentration	NPT in all concentration	1
Ichthammol	Pharmaceutical	YES	PT	PT	0.316% (water)	Erythema at 5 % (aq) and 10%(aq)	1
Bergamot oil A (non purified)	Fragrance / food additive	YES	NPT/PT depending on solvent	PT	0.1% (oil) 1% (water), but 25% drop at c = 0.032%	NPT at c = 0.316% (oil) Erythema at c = 0.1 % (water)	2
Bergamot oil B (non purified)	Fragrance / food additive	YES	NPT/PT	PT	0.316% (oil) 0.1% (water)	NPT at 0.1% (n-oil) Slight E at 0.032% (water)	2
Bergamot oil C (purified)	Fragrance / food additive	Not significant in UVA/VIS	NPT (NPT - possibly PT)	NPT	NPT in all concentration	NPT in all concentration NPT in all concentration	2
Bergamot oil D (purified)	Fragrance / food additive	Not significant in UVA/VIS	NPT	NPT	NPT in all concentration	NPT in all concentration NPT in all concentration	2
Litsea Cubeba A	Fragrance / food additive	YES	PT	NPT	NPT in all concentration	NPT in all concentration NPT in all concentration	3
Litsea Cubeba B	Fragrance / food additive	YES	PT	NPT	NPT in all concentration	NPT in all concentration NPT in all concentration	3
Orange A	Fragrance / food additive		NPT/PT	PT	First NPT c = 1%	Erythema in some panelists at 1%, no effect at 0.1%	3
Orange D	Fragrance / food additive		NPT/PT	PT	First NPT c = 0.32%	No effect at 0.1% and 0.01%	3
Deterpenated Orange C	Fragrance / food additive		NPT/PT	PT	First NPT c = 0.1%	No effect at 0.1% and 0.01%	3
Lemon A	Fragrance / food additive		NPT/PT	PT	First NPT c = 3.16%	Erythema in some panelists at 1%, no effect at 0.1%	3
Lemon D	Fragrance / food additive		NPT/PT	PT	First NPT c = 0.32%	No effect at 0.1% and 0.01%	3
Deterpenated lemon	Fragrance / food additive		NPT/PT	PT	First NPT c = 0.1	- No effect at 0.1% at 24h, but delayed effects in 2/5 volunteers after 48 and 72h. No effect at 0.01%	3

Safety factor 10 should be when extrapolating from the in vitro results to situation in man

1 Jirova et al. (2005) Phototoxicity of bituminous tars—correspondence between results of 3T3 NRU PT, 3D skin model and exponmental human data. Toxicology In Vitro 19,931–934
2 Kejlova et al (2007) Phototoxicity of bergamot oil assessed by in vitro techniques in combination with human patch tests. Toxicology In Vitro 21, 1298–1303
3 Kejlova et al (2010) Phototoxicity of essential oils intended for cosmetic use. TIV

HOW TO USE THE RHE's IN A TESTING STRATEGY ?

Analytics
- Absorption and spectra determination,
- Photo-stability evaluation
- Detection of content of phototoxic impurities

Not-absorbing Photo-stable → Not phototoxic

Potential to be phototoxic

3T3 NRU PT
- Screen of biological effect,
- evaluation in different solvents important !

Negative → Not phototoxic

Positive / Equivocal results in different solvents

Skin models PT
-Evaluation of biological effect on a system with barrier properties, real use conditions
-evaluation in different vehicles important!

Negative → Not acute photo-toxin

Positive

Label as phototoxic, if possible determine the non-phototoxic concentrations for "safe" use → Test for photo-allergy

Summary I

Studies with different RhE models demonstrate that Reconstructed Human Tissue models can be reliably used for identification of phototox hazard of topically applied compounds.

These tests can be used as alternative to 3T3 NTU PT, or as a second tier to identify false positive or equivocal classifications for topically applied compounds.

EpiDerm Phototoxicity test can be used also for determination of the phototoxic potency of topically applied compounds and for estimation of the first non-phototoxic dose of a phototxin. Safety factor 10 should be used when extrapolating from the in vitro results to situation in man

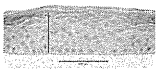
Other available & published experimental data

- Liebsch, M., Döring, B., Donelly, T.A., Logemann, P., Rheins, L.A. & Spielmann, H. (1995). Application of the human dermal model Skin² ZK 1350 to phototoxicity and skin corrosivity testing. Toxicology in Vitro 9, 557–562.
- Jones, P.A., King, A.V., Earl, L.K., Lawrence, R.S. (2003). An assessment of the phototoxic hazard of a personal product ingredient using in vitro assays Toxicology in Vitro 17, 471–480.
- Liebsch, M., Spielmann, H., Pape, W., Krul, C., Deguercy, A., Eskes, C. (2005) UV-induced effects. Alternatives to Laboratory Animals 33,131–146
- Kandarova, H. (2006) Evaluation and Validation of Reconstructed Human Skin Models as Alternatives to Animal Tests in Regulatory Toxicology – PhD Thesis. Chapter 4 – Phototoxicity. http://www.diss.fu-berlin.de/diss/receive/FUDISS_thesis_000000002248
- Pratt, L., Kirk, C., Reeder, M., DeGeorge, G. COMPARISON OF IN VITRO PHOTOTOXICITY TEST METHODS: 3T3 NRU PT VS. ENHANCED PHOTOTOXICITY SCREENING ASSAY IN RECONSTITUTED SKIN (EPARS). (2007). Society of Toxicology 46th Annual Meeting, Charlotte, NC, (2007)., Poster 441. The Toxicologist, 96, 1, 315
- Hoffmann J., Heisler E., Weimans S., Thiemann A., Schnurstein A. and Fuchs H (2008). Phototoxicity *in vitro*: Investigation of photoreactions in the skin using the reconstructed epidermis "Epidermal Skin Test 1000" (EST-1000). Toxicology Letters Volume 180, Supplement 1, 5 October 2008, Pages p.105-S106, Abstracts of the 45th Congress of the European Societies of Toxicology.

Systemic phototoxicity – overview

2006 EpiDerm protocol - for systemically applied drugs

Material	Material Class	Viability Reduction (%)	Phototoxic Range (mg%)
Acetic acid	Negative control solvents	0 - 4	Not photo-toxic
DMSO		0 - 1	
Promethazine	Histamine receptor antagonists	47	10
Chlorpromazine		33-75	1.2 - 10
Doxycycline	Tetracyclines	45 - 50	2.5 - 40
Tetracycline		51	80
5-Methoxy psoralen	Psoralens	38-49	5 - 200
8-Methoxy psoralen		42-49	10 - 100
Carprofen	NSAIDs	70	50
Ketoprofen		170	170
Ciprofloxacin	Quinolones	63	17
Fleroxacin		50	50
Nalidixic acid		60	170
Norfloxacin		34 - 62	5 - 17
Ofloxacin		35 - 45	50 - 170
Fenofibrate	Fibrates	45-73	50 - 170



Klausner, M., Neal, P., Kubilus, J. (2006). In Vitro Screen for Phototoxicity Optimised Drug Development using Highly Differentiated skin model. AAPS 2006 Biotechnology Conference, Boston, MA.

2007 EPISKIN Phototoxicity Assay – demonstration of the correct prediction of phototoxic hazard. Data selected for the systemically applied chemicals/drugs

PM: Viability or IL-1 alpha

Chemical	Known in vivo human	Known in vitro 3T3 NRU PT data	In vitro EPISKIN (viability)	In vitro EPISKIN (IL-1 alpha)
Fenofibrate	NPT/weak phototoxine	PT	PT	NPT
Ofloxacin	PT*	PT	PT	PT
Lomefloxacin	PT*	PT	PT	PT
Demeclocyclin	PT*	PT	PT	PT
Furosemide	PT*	PT	PT	PT
Angelicin	PT*	PT	PT	NPT
Ketoprofen	PT*	PT	PT	NPT

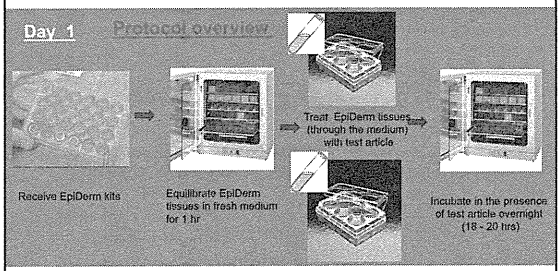
* Human systemic data available.

- Dose: 100 µL/ 100 mg
- Exposure 2 h
- Irradiation
- Post-exposure overnight
- MTT viability test
- PM Δ 25%, IL-1a

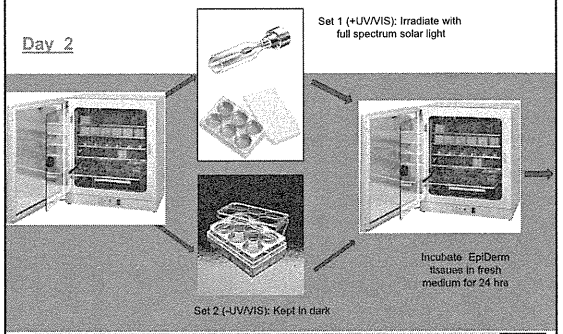
Lelievre D., Justine P., Christiaens F., Bonaventure N., Coutet J., Marrot L., Cotovio J. The episkin phototoxicity assay (EPA): Development of an in vitro tiered strategy using 17 reference chemicals to predict phototoxic potency. Toxicology in Vitro 21 (2007) 977–995

2010 Development of EpiDerm In Vitro Phototoxicity Assay for Systemically Administered Pharmaceuticals to address needs of pharmaceutical industry

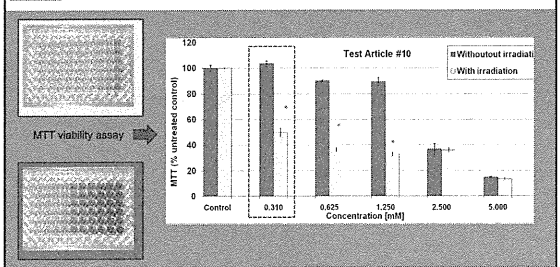
Day 1 Protocol overview



Day 2



Day 3



Prediction Model:
Decrease in viability > 30% in any of tested concentrations = Phototoxic

EpiDerm In Vitro Phototoxicity Assay for Systemically Administered Pharmaceuticals

In vivo non-phototoxic compounds

#	PT class (literature)	PT class (MatTek)	Chemical class/ type of action
1	NPT	NPT	antibiotic
2	NPT	NPT	antimicrobial agent
3	NPT	NPT	amino acid
4	NPT	NPT	NSAID
5	NPT	NPT	Benzopyrone, flavonoid
6	NPT	NPT	Surfactant
7	NPT	NPT	Organic coumpound
8	NPT	NPT	NSAID
9	NPT	NPT	antiseptic
10	NPT	NPT	food flavoring
11	NPT	NPT	loop diuretic
12	NPT	NPT	NSAID
13	NPT	NPT	chemotherapeutic antibacterial
14	NPT	NPT	antifungal
15	NPT	NPT	antibiotic
16	NPT	NPT	calcium channel blocker, antihypertensive
17	NPT	PT/NPT	calcium channel blocker, antihypertensive
18	NPT	NPT	local anesthetic
19	NPT	NPT	calcium channel blocker, antihypertensive
20	NPT	NPT	Sunscreen Agent

In vivo phototoxic compounds

#	PT class (literature)	PT class (MatTek)	Chemical class/ type of action
1	PT	PT	phenotiazine
2	PT	PT	flouroquinone antibiotic
3	PT	PT	flouroquinone antibiotic
4	PT	PT	antibiotic
5	PT	PT/NPT	anti-hyperlipidemic
6	PT	PT	diuretic
7	PT	PT	NSAID
8	PT	PT	psoralen
9	PT	PT	psoralen
10	PT	PT	phenotiazine
11	PT	PT	food flavoring
12	PT	PT	psoralen
13	PT	PT	Athelminic drug
14	PT	PT	Antiarrhythmic agent

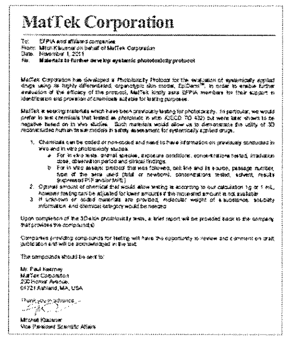
* Results and details of the study will be published by Kaluzhny et al., in 2012

EpiDerm In Vitro Phototoxicity Assay for Systemically Administered Pharmaceuticals

EpiDerm phototoxicity assay with systemic administration (n=34)	
Sensitivity	92.9 % (13/14)
Specificity	95.0 % (19/20)
Accuracy	94.1% (32/34)
Positive predictivity	92.9 %
Negative predictivity	95.0 %
False positives	5.0 %
False negatives	7.1 %

* Results and details of the study will be published by Kaluzhny et al., in 2012

EpiDerm In Vitro Phototoxicity Assay for Systemically Administered Pharmaceuticals


MatTek Corporation

To: EFPIA and affiliated companies
From: Mitch Klausner on behalf of MatTek Corporation
Date: November 1, 2011
Re: Materials to further develop systemic phototoxicity protocol

Follow-up activities:

Validation of the systemic protocol with support of EFPIA and Pharmaceutical Industry

What is needed:

- suitable chemicals with in vitro 3T3 data and in vivo human or animal data

- chemicals of special interest are those with positive in vitro but negative in vivo results

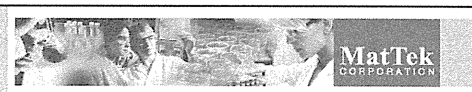
Summary II

Studies with 2 different RhE models demonstrate that Reconstructed Human tissue models can be used for screening of phototox hazard of systemically applied compounds.

These tests can be used as alternative to 3T3 NTU PT, or as a second tier to identify false positive or equivocal classifications for topically applied compounds.

EpiDerm Phototoxicity test and prediction model as developed by Kaluzhny et al., is ready for a challenge with more systemically administered substances.

Help of pharmaceutical industry in this step is crucial for development reliable and relevant in vitro phototoxicity assay.



Thank you for your attention!

Dr. Helena Kandárová

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INTERNATIONAL CONFERENCE ON HARMONISATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE

ICH HARMONISED TRIPARTITE GUIDELINE

DRAFT
Guidance on Photosafety Evaluation of Pharmaceuticals
S10

Step 1 Version

Not for Distribution

Dated 10 Jan. 2012

TABLE OF CONTENTS

1. INTRODUCTION

1.1 Objectives of the Guideline

The purpose of this document is to recommend international standards for photosafety assessment, and to harmonise such assessments supporting human clinical trials and marketing authorization for pharmaceuticals. It includes criteria for initiation of and triggers for additional photosafety testing and should be read in conjunction with ICHM3 R(2) Section 14 on Photosafety Testing (Ref. 1). This guideline for photosafety assessment should reduce the likelihood that substantial differences in testing requirements and data interpretation will exist among regions.

When appropriate, consideration should be given to the use of in vitro alternative methods for photosafety assessment which could reduce the use of animals in accordance with the 3R (reduce/refine/replace) principles.

1.2 Background

The ICH M3(R2) guideline provides certain information regarding timing of photosafety testing relative to clinical development. It recommends that an initial assessment of photoreactive potential be conducted, and if appropriate, an experimental evaluation be undertaken before exposure of large numbers of subjects (Phase III). However, ICH M3(R2) does not provide specific information regarding testing strategies. This ICH S10 guideline outlines further details on when photosafety testing is warranted, and on possible testing strategies. It represents the consensus that exists regarding assessment of photosafety to support clinical development and marketing authorization of pharmaceuticals.

1.3 Scope of the Guideline

This guideline generally applies to new low molecular weight active pharmaceutical ingredients (APIs) for systemic administration, clinical formulations for topical application (including dermal patches), intraocular injections, and photodynamic therapy products. The testing strategies described focus on the identification of APIs showing direct photochemical reactivity under clinically relevant conditions. The guideline does not apply to previously marketed products unless there is a new cause for concern.

Photodynamic therapy drugs are developed with photochemical reactivity as an inherent aspect of their intended pharmacology and additional assessment of their phototoxicity is not usually warranted. However, an evaluation of the toxicokinetics and tissue distribution of photodynamic therapy drugs is warranted to enable appropriate risk management in patients.

Photosafety testing is not appropriate for peptides and proteins. Such products can show absorption in the UVB region due to the absorption profile of certain

aromatic amino acids. However, this absorption is also seen with endogenous proteins and is of no concern.

1.4 General Principles

The photosafety assessment of a pharmaceutical is an integrated process that can involve an evaluation of photochemical characteristics, data from nonclinical studies and human safety information. This information is used to determine adequate risk minimization measures to prevent light induced adverse events in clinical trials and for marketed products.

Four different endpoints have been discussed in connection with photosafety testing; phototoxicity, photoallergy, photogenotoxicity and photocarcinogenicity. Photogenotoxicity and photocarcinogenicity testing (ICH M3 (R2), Note 1) are no longer considered appropriate for human pharmaceuticals. This guideline focuses only on phototoxicity and photoallergy endpoints as defined below:

- Phototoxicity (photoirritation): An acute light-induced tissue response to a photoreactive chemical.

- Photoallergy: An immunologically mediated reaction to a chemical initiated by the formation of photoproducts (e.g., protein adducts) following a photochemical reaction.

- Photosensitization is a general term occasionally used to describe all light-induced tissue reactions. However, in order to clearly distinguish between photoallergy and phototoxicity, this term is not used in this guideline.

For a chemical to demonstrate phototoxicity, the following characteristics are critical:

- absorbs light within the range of natural sunlight (290-700 nm);
- generates a reactive species following absorption of UV/Visible light;
- distributes to light-exposed tissues (e.g., skin, eye).

If one or more of these conditions is not met, a compound will not elicit phototoxicity.

2. FACTORS TO CONSIDER IN THE PHOTOSAFETY EVALUATION

2.1 Photo-chemical Properties

The initial consideration for assessment of photoreactive potential is whether a compound absorbs UVB, UVA, or visible radiation (290-700 nm). Further, absorption with a molar extinction coefficient less than 1000 L mol^{-1} cm^{-1} is not considered to result in a photosafety concern (See Note 2).

Photoinstability can also suggest potential for photoreactivity. However, photostability testing results (See ICH Q1B, Ref. 2) should not be used solely to determine whether further photosafety evaluation is warranted, since not all photoreactive compounds are detected under these conditions.

Excitation of molecules by light can lead to generation of reactive oxygen species (ROS) including superoxide and singlet oxygen via energy transfer mechanisms.

Although other mechanisms for phototoxicity are known (e.g., via formation of radicals) which might be more relevant in vivo for particular cases, it appears that ROS are typically generated as well. Therefore, chemical surrogate assays detecting ROS generation may represent a sensitive and sufficient endpoint. Compounds that are negative in these assays are not recommended for further photosafety evaluation.

Assessments of photo-chemical properties should be conducted under high-quality scientific standards with data collection records readily available, or in compliance with GLP regulations.

2.2 Tissue Distribution/Pharmacokinetics

The concentration of the photoactive chemical in the light-exposed tissue at the time of light exposure is the critical pharmacokinetic parameter in determining whether a photochemical and subsequent phototoxic reaction will occur. This concentration depends on a variety of factors such as serum drug concentration and perfusion of the tissue, accumulation of the drug in the tissue, and binding of the drug to tissue components (e.g., melanin).

Binding or accumulation of a drug in a tissue is not required for a phototoxic reaction. If a molecule is sufficiently photochemically active, it might produce a phototoxic reaction at the concentration achieved in blood or interstitial fluid. However, drugs that have a higher ratio of tissue to plasma concentration can be more likely to produce a phototoxic reaction in the tissue than drugs with a lower tissue to plasma ratio.

While drug binding in a tissue is not critical for phototoxicity, such binding can slow the elimination of the drug from the tissue. This can lead to an accumulation of a drug in these tissues or maintenance of higher tissue concentrations for a longer

period of time than would occur in the absence of such binding. The longer the concentration is maintained at a level above that critical for a photochemical reaction, then the longer a person is at risk for a phototoxic reaction.

Drug binding to melanin is one mechanism by which tissue binding can occur. Although melanin binding can increase tissue levels and this might increase the risk for a phototoxic reaction from a photoreactive drug, melanin binding alone does not present an additional photosafety concern. Information from studies on melanin-binding or tissue distribution studies in pigmented animals can be useful in understanding the potential impact melanin binding might have on tissue levels of drug.

When the apparent half-life of the test compound (and/or metabolites) in skin or the eye significantly exceeds the apparent half-life of the elimination phase in plasma, it is considered that there is retention of the drug in the skin or eye. For most compounds, it is expected that single dose tissue distribution studies with sufficient sensitivity and specificity will provide an adequate assessment of tissue distribution and the potential for accumulation (see also ICH S3B 1994).

Internal tissues can be exposed to light during routine or emergency medical procedures. Compounds with phototoxicity that accumulate in and exhibit long elimination half-lives in internal tissues have been demonstrated to possess the potential to cause harm in these tissues should they be exposed to light. For those drugs with potent in vivo photoreactivity (or known to be phototoxic based on their mechanism of action such as photodynamic therapy drugs), tissue distribution studies and estimates of tissue-specific half-lives should consider internal tissues as well as external tissues normally exposed to light. Identification of such a risk allows appropriate patient precautions to be instituted. Drugs that only absorb ultraviolet light or have short tissue elimination half-lives are not likely to present a risk to internal tissues during medical procedures and additional precautions are not likely to be warranted.

Generally, the elimination of APIs from the ocular surface is rapid when applied topically to the eye, and a very small amount of UV radiation reaches the retina since most is absorbed by the lens. These factors should be considered when assessing phototoxic potential of APIs in formulations for ocular administration.

2.3 Pharmacological Properties

In most cases, drug-induced phototoxicity is due to the chemical structure and not the pharmacology. However, certain pharmacologic properties can enhance susceptibility to light-induced effects, including reactions ranging from photoirritation to skin carcinogenesis (e.g., immunosuppression, perturbation of heme synthesis). The testing strategies outlined in this guidance document are not designed to detect these types of indirect phototoxicity. Many of these mechanisms can be identified and evaluated in nonclinical pharmacology/toxicity testing (see ICH M3(R2)).

2.4 Metabolite Considerations

Metabolites generally do not warrant separate photosafety evaluations. This is supported by the fact that in vitro assays (e.g. 3T3 NRU assay, ROS assay) which do not incorporate a metabolic activation system, appear to detect the vast majority of known human phototoxicants.

3. NON-CLINICAL PHOTOSAFETY TESTING

3.1 General Considerations

Carefully selected conditions considering both the model system and exposure to a relevant light spectrum are critical for non-clinical testing intended to support photosafety evaluation of pharmaceuticals. Ideally, a nonclinical assay should exhibit both high sensitivity and specificity (i.e., low false negative and low false positive rates). To support the tiered testing approach described in this guidance, it is most important that non-clinical photosafety assays show high sensitivity (i.e., produce a low frequency of false negatives) since definitively negative assay results usually do not warrant further photosafety evaluation. Therefore, it is not important that positive results always predict a clinically relevant phototoxic or photoallergic response. The available non-clinical assays, both in vitro and in vivo, are focused primarily on detecting a photoreactivity hazard, which might or might not translate into a clinically relevant photosafety risk.

Selection of irradiation conditions is critical for both in vitro and in vivo assays. Natural sun light represents the broadest range of light exposure that humans might be exposed to regularly. However, sun light per se is not well defined and depends on many factors (such as latitude, altitude, season, time of day, weather, etc.). In addition, sensitivity of human skin to natural sun light depends on a number of individual factors (e.g., skin type, anatomical site and tanning status). Standardized sun light exposure conditions have been defined by various organizations. Such standards (e.g., CIE-85-1989, Ref. 3) should be considered in order to assess suitability of a sun light simulator light source, and irradiance and irradiation dose should be normalized based on the UVA part (320 to 400 nm) of the applied spectrum. UVA doses ranging from 5 to 20 J/cm^2 have successfully been used to establish in vitro and in vivo photosafety models. These UVA doses are comparable to those obtained during longer outdoor activities on summer days at noon time, in temperate zones, and at sea level. In humans, total sunlight exposure is normally limited by sun burn reactions caused by the UVB part of sun light. In non-clinical photosafety assays, however, the amount of UVB should not limit the overall irradiation and might be attenuated (partially filtered) in order that relevant UVA doses can be tested without reducing assay sensitivity. Penetration of UVB radiation into human skin is limited to the epidermis, while UVA can reach capillary blood. Therefore, clinical relevance of photochemical activation by UVB is considered less important than UVA for systemic drugs.

3.2 Photosafety Testing Using Chemical Assays

The initial event in any photoreactive process is the absorption of photons of the appropriate wavelength, which allows a chromophore to reach an excited state. The excitation energy can be transferred to oxygen molecules, resulting in generation of superoxide anion and singlet oxygen (ROS).

From the standpoint of hazard identification, monitoring ROS generation from pharmaceutical substances irradiated with UV and visible light can be an indicator of photoreactive potential. In an ROS assay, chemicals in solution are irradiated using simulated sunlight and generation of ROS is detected by various methods. An appropriate ROS assay should be qualified using pharmaceutical agents and a minimum concentration that demonstrates assay sensitivity (See Ref. 4).

3.3 Phototoxicity Testing Using *In Vitro* Assays

A number of in vitro models have been developed for assessing the phototoxic potential of chemicals. Some of these models have not been qualified for use with pharmaceuticals. Some models involve testing compounds that are dissolved in the culture medium, and such methods are often appropriate for systemic drugs. Other models involve direct application to the surface of a tissue preparation and can be appropriate for topical formulations.

The most widely used in vitro assay for phototoxicity testing is the "in vitro 3T3 Neutral Red Uptake Phototoxicity Test" (3T3-NRU-PT) which is available as an OECD guideline (OECD TG 432, adopted 13 April 2004, Ref. 5). This is considered the most appropriate in vitro screen for soluble compounds that are not exclusively UVB absorbers. The 3T3-NRU-PT is based on a comparison of the cytotoxicity of a chemical when tested in the presence and in the absence of exposure to a non-cytotoxic dose of simulated sun light. Cytotoxicity in this test is expressed as a concentration dependent reduction of the uptake of the vital dye, Neutral Red. The irradiation-dependent shift of the observed cytotoxicity is defined as the Photo-Irritation-Factor (PIF). An alternative analysis method, the Mean-Photo-Effect (MPE), can be useful for compounds showing no cytotoxicity in the absence of irradiation up to the solubility limit. While the use of the mouse fibroblast cell line Balb/c 3T3 is described in the standard OECD protocol, other cells or cell lines can be used with the same test protocol, if the culture conditions are adapted to the specific needs of the cells.

Since the original OECD protocol was not validated for pharmaceuticals specifically, modifications to the original OECD protocol have been proposed (See Ref. 6). Following a retrospective review of data for pharmaceuticals, a reduction of the maximum test concentration from 1000 to 100 ug/mL appears justified. Compounds without any significant cytotoxicity (under irradiation) up to this limit can be considered as being devoid of relevant phototoxicity. In addition, PIF values between 2 and 5 (the category named "probable phototoxicity" per OECD)

are of questionable toxicological relevance for systemic drugs and do not warrant further experimental photosafety evaluations.

Although the formal ECVAM validation exercise conducted on this assay indicated a sensitivity of 93% and a specificity of 84%, experience within the pharmaceutical industry suggests a much lower specificity. A survey of EFPIA member companies indicated that the 3T3-NRU-PT assay, as described in the OECD guideline, generates a high percentage of positive results (industry mean of approximately 50%), the majority of which do not correlate with in vivo responses in animals or humans (Lynch and Wilcox, 2011). The sensitivity of the 3T3 NRU assay remains unquestioned, and if a compound is negative in this assay, it would have a very low probability of being phototoxic in humans. However, a positive result in the 3T3-NRU-PT assay should not be regarded as indicative of a likely clinical phototoxic risk, but rather a flag for follow-up assessment which might or might not include in vivo testing. Compounds that are positive in the 3T3-NRU-PT only at high in vitro concentrations that are many times higher than drug concentrations likely to be achieved in light-exposed tissues in humans, might in certain circumstances be considered to be 'low risk' without follow-up in vivo testing.

The Balb/c 3T3 cell line is sensitive to UVB and the recommended irradiation conditions involve the use of filters to attenuate wavelengths below 320 nm. UVB attenuation should not present a problem for systemic pharmaceuticals since these wavelengths do not penetrate beyond the epidermis and hence UVB absorbers in systemic circulation are unlikely to be activated. However, this argument cannot be made for topical products that absorb in the UVB range. In such cases, and where in vitro assessment is desired, alternative models (e.g., reconstructed human skin models) which better tolerate UVB might be employed.

Reconstructed human skin models, with the presence of a stratum corneum, permit testing of various types of topically applied materials ranging from neat chemicals to final clinical formulations. The models developed to date measure cell viability in the tissue preparation with and without irradiation. While such models appear to be capable of detecting known human dermal phototoxicants, the sensitivity of some models with respect to the dose eliciting a positive response can be lower than in the *in vivo* human situation. Consequently, it is important to understand the sensitivity of any model selected and, if appropriate, adjust the assay conditions accordingly, e.g., testing higher strength formulations, increasing exposure time, etc.

There are no in vitro models that specifically assess ocular phototoxicity. While negative results in the 3T3 NRU-PT or a reconstructed skin model might suggest a low risk, in the absence of data, the predictive value of these assays for ocular phototoxicity is unknown.

3.4 Photosafety Testing Using In Vivo Assays and Systemic Administration

To date, no in vivo photosafety model has been formally validated. Phototoxicity testing for systemically administered compounds has been conducted in a variety of species, including guinea pig, mouse and rat. No standardized study design has been established and thus the following criteria might be considered as best practices.

For species selection, irradiation sensitivity, heat tolerance, and performance of reference substances should be considered. Strains (or skin areas such as ears) without fur might be used to avoid shaving procedures. In general, there is no preference regarding use of pigmented or non-pigmented strains. However, melanin-binding (see section 2.2) should be considered when selecting a model to ensure appropriate exposures in target tissues.

Although phototoxicity is typically an acute reaction, the duration of an in vivo assay should be carefully considered. Both accumulation of compound (towards a steady state) in relevant light-exposed tissues as well as accumulation of damage might lead to an increased sensitivity after repeated application followed by subsequent irradiation. Generally, studies of a few days duration of dosing are appropriate, but pharmacokinetic properties as well as the intended clinical treatment regimen should be taken into consideration. Single or repeated daily irradiations after dosing (around T_{max}) is appropriate; however, repeated daily irradiations can increase the sensitivity of the assay. Wherever feasible the clinical route of administration should be used.

Dose selection for in vivo nonclinical photosafety testing of systemic drugs should support a meaningful human risk assessment. For such studies a maximum dose level that complies with the recommendations for general toxicity studies in ICH M3(R2) section 1.5 is considered appropriate. If a negative result is obtained at the maximum dose, testing of lower doses is usually not warranted. However, if a positive result is anticipated, additional dose groups can support a NOAEL-based risk assessment. If the systemic exposure achieved in animals is lower than clinical exposure, the reliability of a negative result in predicting human risk is questionable. Vehicle groups as well as non-irradiated dose groups should be included in order to support adequate analyses.

If an in vivo phototoxicity study is conducted, it is desirable to know the pharmacokinetic profile of the compound prior to designing the study to ensure that irradiation of the animals is carried out at the approximate Tmax. Toxicokinetic data (Cmax) at the selected doses are also valuable for risk assessment, and if this is not already available, it should be collected as part of the in vivo phototoxicity study.

The most sensitive early signs of phototoxicity are usually erythema followed by edema at sub-erythemogenic irradiation doses. However, objective assessment of minimally reddened skin areas relies upon specifically trained laboratory personnel. In addition, the type of onset might vary with the respective substance (mechanism of phototoxicity). Any identified

phototoxicity reaction should be evaluated regarding dose and time dependency and, if possible, the NOAEL and LOEL should be established. Human risk assessment might be further supported by additional endpoints (e.g., early inflammatory markers in skin and lymph node reactions indicative of acute irritation).

In some cases, phototoxicity of the retina should be assessed (usually only warranted for substances absorbing light above 400 nm considering the optical properties of the human eye). However, wavelength-dependent penetration of light through the eye of typical animal species might vary significantly (related to species, age, and gender) and occurs in some cases even in the UVA range. In such cases it is possible that findings observed in the animal model may not be relevant to man. If warranted, phototoxicity of the retina should be assessed in established animal models using a careful histopathological analysis. No preference is made whether to restrain the animals during irradiation or whether to enforce open lids.

Adequate performance of in vivo photosafety models, which are not formally validated, should be demonstrated using suitable reference compounds. Clinically relevant phototoxic compounds covering different chemical classes and mechanisms of phototoxicity should be evaluated to establish adequacy. For retinal toxicity a reference compound with a light absorption profile within the visible light range is recommended (above 400 nm, e.g., Sparfloxacin). The concurrent use of a positive control compound during regulatory tests might not be warranted, if an in vivo model has been formally validated or has reached general acceptance and is established in the testing facility.

Testing for photoallergy is not recommended for compounds which are administered systemically.

3.5 Photosafety Testing Using in vivo Assays and Dermal Administration

The main recommendations provided for investigating the systemic route of administration also apply for dermal administration, including those for species selection, study duration, and irradiation conditions. For dermal drugs, in general, the clinical formulation should be tested as well as lower strength formulations, and a placebo. The intended clinical conditions of administration should be used to the extent possible (e.g., occluded, non-occluded, intradermal). Irradiation of the exposed area should take place at a specified time after application, and the interval between application and irradiation should be justified based on the specific properties of the formulation to be tested. Signs of phototoxicity should be assessed based on relevant endpoints. The sensitivity of the assay should be demonstrated using appropriate reference compounds. Assessment of systemic drug levels is generally not warranted in dermal phototoxicity studies.

For dermal drug products, acute phototoxicity (photoirritation) and contact photoallergy have often been investigated in conjunction with skin sensitization testing. Although no formal validation has been performed and the predictivity for human photoallergy is unknown, these contact photoallergy models (e.g., in guinea

pig or mouse) are well-described and follow the validated protocols for skin sensitization. If this approach is chosen, it is important that the general recommendations for in vivo phototoxicity testing of dermal formulations (see above) are considered for the design of the induction and challenge phases in skin sensitization protocols. Photoallergy can also be assessed clinically following appropriate standards for human contact allergy testing.

3.6 Photosafety Testing Using In Vivo Assays and Ocular Administration

To date, no standardized in vivo photosafety models for testing APIs or formulations via ocular administration have been described. Until appropriate models for assessment of phototoxicity following ocular administration are available, the use of existing in vivo phototoxicity models, using alternative routes of administration (e.g., systemic, dermal) could be considered for photosafety assessment. However, the reliability of using non-ocular routes of administration for predicting ocular phototoxicity is unknown.

4. PHOTOSAFETY ASSESSMENT IN CLINICAL EVALUATIONS

Prior to exposure of large numbers of subjects (see ICH M3(R2)), there are various options for assessing photosafety in a clinical setting. When appropriate, photosafety can be assessed in a dedicated clinical study. Alternatively, clinical studies with embedded questionnaires or the inclusion of special photosafety endpoints in existing clinical development studies, can also contribute to photosafety assessment. In situations where the risk of a clinical response is judged to be low, no specific photosafety monitoring other than standard clinical monitoring of adverse events may be appropriate. The precise strategy is determined on a case-by-case basis in consultation with regulatory authorities.

5. TESTING STRATEGIES

For a compound that has characteristics consistent with photoreactivity, nonclinical in vitro and in vivo tests and clinical alternatives are available for photosafety testing. The choice of the photosafety testing strategy is up to the sponsor. If any one of these tests, having been conducted in an appropriate way, is negative, a compound is unlikely to elicit phototoxicity and further testing is not recommended.

The photosafety testing strategy of a compound with structural similarity to chemicals with known photosafety concern should take into account existing knowledge of the chemical class.

5.1 Recommendations For Testing Of Pharmaceuticals Given Via Systemic Route

Tier 1

An initial assessment of phototoxic potential should be based on the photochemical properties of the active substance, also taking into consideration the pharmacological/chemical class and the pharmacokinetic properties, including tissue distribution.

If the active substance does not have an MEC above 1000 L mol^{-1} cm^{-1}(between 290 and 700 nm), or does not generate ROS, or is not present in light-exposed tissues, no further photosafety testing is recommended and no phototoxicity is anticipated in humans. If the criteria above are not met (or have not been assessed), non-clinical and/or clinical assessment of the active substance is warranted. If the overall human risk assessment regarding photosafety is solely based on the MEC, appropriate documentation of the methods used in determining the MEC should be provided (see Note 2).

Tier 2

The in vitro 3T3 NRU-PT is generally recommended as the initial test for phototoxicity. The high sensitivity of the 3T3 NRU PT results in a good negative predictivity, and negative results are generally accepted as sufficient evidence that a substance is not phototoxic. In such cases no further testing is recommended and no phototoxicity is anticipated in humans.

In some situations (e.g., poorly soluble compounds) an initial assessment of phototoxicity in animals or humans could be an alternative to conducting a 3T3 NRU-PT. Provided the study design is shown to be appropriate and sufficiently sensitive, a negative result would generally be accepted to indicate no phototoxic risk.

If the 3T3 NRU-PT assay gives a positive result, an in vivo animal phototoxicity study could be conducted to assess whether the potential phototoxicity identified in vitro correlates with an in vivo response. Alternatively, the photosafety risk should be addressed/managed in the clinical setting. This could include a recommendation for protective measures in Phase I and II trials in lieu of testing, or until photosafety risk has been adequately evaluated. The photosafety assessment should be completed prior to exposure of large numbers of subjects (Phase III, see ICH M3(R2)). A negative result in an appropriately conducted in vivo phototoxicity study (either in animals or humans) supersedes a positive 3T3 NRU-PT result. In such cases no further testing is recommended and no phototoxicity is anticipated in humans. In addition, a negative result in an appropriately conducted clinical phototoxicity evaluation supersedes any positive nonclinical result(s).

In cases where an in vivo animal phototoxicity study or clinical phototoxicity study had already been conducted, there is no reason to subsequently conduct a 3T3 NRU-PT.

5.2 Recommendations For Testing Of Pharmaceuticals Given Via Dermal Route

Tier 1

The Tier I assessment, which applies to the API and novel excipients of the dermal product, is as described for systemic drugs except for the consideration as to whether the drug is present in light exposed tissues. Dermal products are administered directly to the skin and hence, unless they are applied to areas not exposed to light (e.g., vaginal creams), are present in light-exposed tissues.

Tier 2

The in vitro 3T3 NRU-PT can be used to assess individually the photoreactive potential of the API and any novel excipient(s) provided that appropriate testing conditions can be achieved (e.g., test concentrations not limited by poor solubility, relevant UVB dose can be applied). In cases where no photoreactive ingredient has been identified in vitro the overall phototoxicity potential of the clinical formulation can be regarded as low.

Some properties of the clinical formulation which could influence the potential phototoxic response (e.g., penetration into skin, intracellular uptake) cannot be evaluated using the 3T3 NRU-PT alone. Therefore, confirmation of the overall negative result in an evaluation using the clinical formulation and/or dedicated monitoring during clinical trials can still be warranted.

Reconstituted 3D skin models can be used to assess the phototoxicity potential of clinical formulations. It is important to understand the sensitivity of the particular 3D model selected and, if appropriate, adjust the assay conditions accordingly, e.g., testing higher strength formulations, increasing exposure time, etc. However, if these conditions can be met, a negative result in a reconstituted skin model could be sufficient evidence that the formulation is not phototoxic. In this case, no further phototoxicity testing is recommended and no phototoxicity is anticipated in humans.

If an appropriate in vitro model is not available, the initial test could be an in vivo animal phototoxicity test on the clinical formulation. Alternatively, the phototoxic potential in humans can be assessed prior to exposure of large numbers of subjects (ICH M3(R2)). A negative result in an appropriately conducted in vivo animal or human phototoxicity study would be sufficient evidence that the formulation is not phototoxic. In such cases no further phototoxicity testing is recommended and no phototoxicity is anticipated in humans.

A negative result in an appropriately conducted in vivo phototoxicity study (either in animals or humans) supersedes a positive in vitro result. In such cases no further phototoxicity testing is recommended and no phototoxicity is anticipated in

humans. In addition, a negative result in an appropriately conducted clinical phototoxicity study supersedes any positive in vivo nonclinical result(s).

For dermal products where the Tier I assessment suggests the potential for photoreactivity, in vivo photoallergy assessment in animals or humans is also warranted in addition to phototoxicity testing. Some regulatory authorities (EU and Japan) generally consider that negative results in a well-conducted photoallergy study in animals provide sufficient evidence that a compound is not likely to be photoallergenic. Other regions (US) think that animal models of photoallergy have low predictivity to humans and thus would generally recommend a clinical photoallergy assessment for dermal products.

5.3 Recommendations For Testing Of Pharmaceuticals Given Via Ocular Route

Tier 1

The Tier I assessment, which applies to API and novel excipient(s), is as described for systemic drugs. Ocular products are administered directly to the eye and hence are present in light-exposed tissues.

Tier 2

The in vitro 3T3 NRU-PT can be used to assess individually the photoreactive potential of the API and any novel excipient(s) provided that appropriate testing conditions can be achieved (e.g. test concentrations not limited by poor solubility, relevant UVB dose can be applied). In cases where no photoreactive ingredient has been identified in vitro the overall phototoxicity potential of the clinical formulation may be regarded as low.

While testing the complete clinical formulation in an in vivo nonclinical study is desirable, currently there are no standardized nonclinical in vivo photosafety testing procedures via ocular routes (topical or intraocular injection) and the predictive value of either positive or negative results is unknown. Therefore, no specific in vivo nonclinical photosafety evaluation is recommended for ocular pharmaceuticals until models have been developed and their predictivity for ocular phototoxicity in humans established.

Clinical testing of APIs/formulations having demonstrated photoreactivity should be avoided unless well justified.

6. ENDNOTES

Note 1: Testing of photogenotoxicity is not recommended as a part of the standard photosafety testing programme. In the past some regional guidances (e.g., CPMP/SWP/398/01) have recommended that photogenotoxicity testing should be conducted, preferentially using a photoclastogenicity assay (chromosomal aberration or micronucleus test) in mammalian cells in vitro. However, experience with these models since this guideline was issued has indicated that these tests are substantially oversensitive and even incidences of pseudo-photoclastogenicity have been reported (Ref. 7). Furthermore, the interpretation of photogenotoxicity data regarding its meaning for clinically relevant enhancement of UV-mediated skin cancer is unclear in most cases. In the majority of cases the mechanism by which compounds induce photogenotoxic effects are identical to those that produce phototoxicity, i.e. via the generation of active oxygen species, and separate testing of both endpoints is not warranted.

Note 2: UV-visible light absorption with a molar extinction coefficient (MEC) of less than 1000 L mol-1 cm-1 in the range of 290 to 700 nm is not considered to result in a photosafety concern. However, standardized measurement conditions are critical in order to support this conclusion and are described below.

Selection of an adequate solvent is driven by both analytical requirements (e.g., dissolving power, UV-vis transparency) and physiological relevance (e.g. pH-buffered aqueous conditions). Methanol has been selected as a good compromise solvent and was used to support the initially defined MEC threshold of 1000 L mol-1 cm-1 by a larger data base. It is expected that for the majority of new drug substances meaningful UV-vis spectra can be obtained, typically at concentrations around 100 µM. Nevertheless, potential limitations (e.g., linear range of the absorption measurement, artifacts due to high concentrations or slow precipitation) should be carefully considered. If the chromophore structure of the molecule appears to be pH-sensitive (e.g., phenolic structure, aromatic amines/carboxylic acids, etc.) a spectrum obtained under aqueous, pH = 7.4-buffered conditions could be of particular interest and can add valuable information (regarding differences in shape of the absorption spectrum and molar extinction) for a definitive assessment.

When the overall human risk assessment regarding photosafety is solely based on the obtained MEC appropriate documentation of the recorded UV-vis light absorption spectrum is critical. The analyzed sample should be representative for the drug substance as intended for clinical use (e.g., obtained from batches released under GLP, GMP, or equivalent). The analytical method should allow for reproducible quantitative recordings (e.g., dedicated UV-vis spectrophotometers instead of HPLC-UV-detectors). The analytical report should include all relevant settings and conditions used for the described measurements. In some cases, this information might already be available within the Quality section of submission

dossiers (part of the physiochemical properties documentation of the drug substance) and can be referenced directly.

Note 3: A positive Tier 1 assessment/outcome (e.g., MEC above 1000 L mol^{-1} cm^{-1}(between 290 and 700 nm), generates ROS (if tested), and is present in light-exposed tissues) for a systemically administered compound should initiate analysis of whether implementation of Tier 2 testing is appropriate. Although a threshold for 'presence' in light sensitive tissue cannot be precisely defined, a case could be presented wherein compounds with negligible systemic exposure (e.g., very low dose inhaled drugs) present a negligible photosafety risk and further testing is not warranted.