December 2012
EMA/CHMP/ICH/752211/2012
Committee for medicinal products for human use (CHMP)

ICH guideline S10
Guidance on photosafety evaluation of pharmaceuticals

Step 3

Transmission to CHMP	December 2012
Adoption by CHMP for release for consultation	December 2012
End of consultation (deadline for comments)	March 2013

Comments should be provided using this template. The completed comments form should be sent to ICH@ema.europa.eu

7 Westferry Circus ● Canary Wharf ● London E14 4HB ● United Kingdom
Telephone +44 (0)20 7418 8400 **Facsimile** +44 (0)20 7418 8416
E-mail info@ema.europa.eu **Website** www.ema.europa.eu
An agency of the European Union

S10 Photosafety evaluation of pharmaceuticals

Table of contents

1. Introduction 3
1.1. Objectives of the guideline 3
1.2. Background 3
1.3. Scope of the guideline 3
1.4. General principles 3
2. Factors to consider in the photosafety evaluation 4
2.1. Photochemical properties 4
2.2. Tissue distribution/pharmacokinetics 4
2.3. Metabolite considerations 5
2.4. Pharmacological properties 5
3. Nonclinical photosafety testing 5
3.1. General considerations 5
3.2. Photoreactivity testing using chemical assays 6
3.3. Phototoxicity testing using in vitro assays 6
3.4. Photosafety testing using in vivo assays and systemic administration 7
3.5. Photosafety testing using in vivo assays and dermal administration 8
3.6. Photosafety testing using in vivo assays and ocular administration 9
4. Clinical photosafety assessment 9
5. Assessment strategies 9
5.1. Recommendations for testing of pharmaceuticals given via systemic routes 9
5.1.1. Assessment of phototoxic potential 9
5.1.2. Experimental evaluation of phototoxicity 10
5.2. Recommendations for testing of pharmaceuticals given via dermal routes 10
5.2.1. Assessment of phototoxic potential 10
5.2.2. Experimental evaluation of phototoxicity and photoallergy 10
5.3. Recommendations for testing of pharmaceuticals given via ocular routes 11
6. Endnotes 12
7. Glossary 14
8. References 15

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 ICH M3(R2), 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.

Consideration should be given to the use of *in vitro* alternative methods or clinical data for photosafety assessment which could reduce the use of animals in accordance with the 3R (replacement/reduction/refinement) principles.

1.2. Background

The ICH M3(R2) guideline provides certain information regarding timing of photosafety assessment relative to clinical development. It recommends that an initial assessment of phototoxic potential be conducted, and if appropriate, an experimental evaluation be undertaken before exposure of large numbers of subjects (Phase III). Similarly, ICH S9 describes the timing of photosafety testing for oncology products. However, neither ICH M3(R2) nor ICH S9 provide specific information regarding testing strategies. This ICH S10 guideline outlines further details on when photosafety testing is warranted, and on possible assessment strategies.

1.3. Scope of the guideline

This guideline generally applies to new active pharmaceutical ingredients (APIs) and new excipients for systemic administration, clinical formulations for topical application, dermal patches, ocular products, and photodynamic therapy products.

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.

This guideline does not generally apply to peptides, proteins, antibody drug conjugates, or oligonucleotides. Further, this guideline does not apply to marketed products unless there is a new cause for 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 adverse events in humans.

Four different effects have been discussed in connection with photosafety testing: phototoxicity, photoallergy, photogenotoxicity and photocarcinogenicity. Testing for photogenotoxicity (Note 1) and photocarcinogenicity (Note 6 of ICH M3 (R2)) is not currently considered useful for human

pharmaceuticals. This guideline addresses only phototoxicity and photoallergy effects 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 and/or photoallergy, 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 sufficiently to light-exposed tissues (e.g., skin, eye).

If one or more of these conditions is not met, a compound will not present a photosafety concern.

2. Factors to consider in the photosafety evaluation

2.1. Photochemical properties

The initial consideration for assessment of photoreactive potential is whether a compound absorbs wavelengths between 290 and 700 nm. Absorption with a molar extinction coefficient (MEC) less than 1000 L mol-1 cm-1 (Ref. 2) is not considered to result in a photosafety concern (see Note 2 for further details).

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., formation of photoadducts or cytotoxic photoproducts), even in these cases, it appears that ROS are typically generated as well. Thus, ROS generation following irradiation with UV or visible light can be an indicator of phototoxic potential.

Photostability testing (see ICH Q1B, Ref. 3) can also suggest the potential for photoreactivity. However, not all photoreactive compounds are detected under these conditions, and photodegradation per se does not imply that a drug will be phototoxic. Therefore, photostability testing alone should not be used to determine whether further photosafety evaluation is warranted.

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

2.2. Tissue distribution/pharmacokinetics

The concentration of a photoreactive chemical in tissue at the time of light exposure is a very important pharmacokinetic parameter in determining whether a phototoxic reaction will occur. This concentration depends on a variety of factors, such as plasma concentration, perfusion of the tissue, partitioning from vascular to interstitial and cellular compartments, and binding, retention, and accumulation, of the chemical in the tissue.

Binding, retention, or accumulation of a compound in a tissue is not critical for a phototoxic reaction. If a molecule is sufficiently photoreactive, it might produce a phototoxic reaction at the concentration

achieved in plasma or interstitial fluid. However, compounds having longer residence times in sun-exposed tissues or with higher tissue to plasma concentration ratios are more likely to produce a phototoxic tissue reaction than compounds with shorter residence times or lower tissue to plasma ratios. Further, the longer the concentration of a compound is maintained at a level above that critical for a photochemical reaction, the longer a person is at risk for phototoxicity.

Compound binding to melanin is one mechanism by which tissue retention and/or accumulation can occur. Although melanin binding can increase tissue levels, experience with melanin binding drugs suggests such binding alone does not present a photosafety concern.

A single-dose tissue distribution study, with animals assessed at multiple time points after dosing, will generally provide an adequate assessment of tissue drug levels and the potential for accumulation.

Although a tissue concentration threshold below which the risk for phototoxic reactions would be negligible is scientifically plausible, there are currently no data to delineate such a generic threshold for all compounds. Nevertheless, on a case-by-case basis it may be possible to justify that further photosafety assessment is not warranted based upon actual or anticipated tissue drug levels, and taking into consideration the factors discussed above. One example could be a low-dose inhaled drug for which overall systemic exposure levels are very low.

For those compounds with potent *in vivo* phototoxicity (or known to be phototoxic based on their mechanism of action such as photodynamic therapy drugs), distribution to internal as well as external tissues and estimates of tissue-specific half-lives should be assessed. Compounds activated by visible light and exhibiting long elimination half-lives in internal tissues have been demonstrated to cause injury to tissues exposed to intense light during medical procedures. Drugs that only absorb ultraviolet light or have short tissue elimination half-lives are not likely to present a risk to internal tissues even if they are known to be photoreactive.

2.3. Metabolite considerations

Metabolites generally do not warrant separate photosafety evaluations as metabolism does not typically create new chromophores.

2.4. Pharmacological properties

In most cases, drug-induced phototoxicity is due to the chemical structure and not to the pharmacology. However, certain pharmacologic properties can enhance susceptibility to light-induced effects, including reactions ranging from skin irritation to carcinogenesis (e.g., immunosuppression, perturbation of heme synthesis). The testing strategies outlined in this 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)).

3. Nonclinical photosafety testing

3.1. General considerations

Carefully selected conditions that consider both the model system and exposure to a relevant radiation spectrum are critical for nonclinical photosafety testing. Ideally, a nonclinical assay should exhibit both high sensitivity and specificity (i.e., low false negative and low false positive rates). However, to support the integrated assessment strategy described in this document, it is most important that nonclinical photosafety assays show high sensitivity (i.e., produce a low frequency of false negatives). This is because negative assay results usually do not warrant further photosafety evaluation. It is not

essential that positive assay results always predict a clinically relevant phototoxic response. The available nonclinical assays, both *in vitro* and *in vivo*, are focused primarily on detecting potential phototoxicity, which might or might not translate into clinically relevant phototoxicity. Therefore, the false positive rate for an assay should still be considered when deciding whether or not to use an assay.

Selection of irradiation conditions is critical for both *in vitro* and *in vivo* assays. Natural sunlight represents the broadest range of light exposure that humans might be exposed to regularly. However, sunlight per se is not well defined and depends on many factors (such as latitude, altitude, season, time of day, weather). In addition, sensitivity of human skin to natural sunlight depends on a number of individual factors (e.g., skin type, anatomical site and tanning status). Standardized sunlight exposure conditions have been defined by various organizations. Such standards (e.g., CIE-85-1989, Ref. 4) should be considered in order to assess suitability of a sunlight 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/cm2 have successfully been used to establish *in vitro* and *in vivo* phototoxicity assays. 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 sunburn reactions caused by the UVB part of sunlight. In nonclinical phototoxicity assays, however, the amount of UVB should not limit the overall irradiation and might be attenuated (partially filtered) so that relevant UVA doses can be tested without reducing assay sensitivity. Penetration of UVB light into human skin is mainly 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. However, UVB irradiation is relevant for topical formulations.

3.2. Photoreactivity testing using chemical assays

If a drug developer chooses to assess photoreactivity, the assay should be qualified using pharmaceutical agents under appropriate conditions to demonstrate assay sensitivity. One such assay that is subject of a validation exercise is a ROS assay (e.g., Ref. 5). Preliminary data suggest that this assay has high sensitivity for predicting *in vivo* phototoxicants. However, it has a low specificity, generating a high percentage of false positive results. A negative result in this assay, conducted under the appropriate conditions for the particular assay, would indicate a very low probability of phototoxicity, whereas a positive result would only be a flag for follow-up assessment.

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 the active ingredient or excipients in systemic drug products, depending on the solubility. Other models involve direct application to the surface of a tissue preparation and can be appropriate for entire topical formulations.

The most widely used *in vitro* assay for phototoxicity is the "*in vitro* 3T3 Neutral Red Uptake Phototoxicity Test" (3T3 NRU-PT) for which a guideline (Ref. 6) is available. This is currently considered the most appropriate *in vitro* screen for soluble compounds that are not exclusively UVB absorbers.

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 (see Note 3). The original OECD protocol was not validated for pharmaceuticals specifically. Thus, some modifications to the original OECD protocol have been proposed to address the low specificity observed with drug substances (see 3T3 Workshop Report, Ref. 7, and Note 4). The sensitivity of the 3T3 NRU-PT 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 should not be regarded as indicative of a likely clinical phototoxic risk, but rather a flag for follow-up assessment.

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 minimally penetrate beyond the epidermis and hence UVB absorbers in systemic circulation are unlikely to be photoactivated. However, this is not true for topical products that absorb in the UVB range or for systemically administered compounds that distribute to the epidermis. For topical products that absorb predominately in the UVB range, and where *in vitro* assessment is desired, alternative models (e.g., reconstructed human skin models) which better tolerate UVB might be used.

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, to adjust the assay conditions accordingly (e.g., testing higher strength formulations, increasing exposure time).

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 nonclinical *in vivo* phototoxicity or photoallergy assay 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, if a decision is made by the drug developer to conduct *in vivo* studies in animals.

For species selection, irradiation sensitivity (i.e.., minimal erythema dose), heat tolerance, and performance of reference substances should be considered. Models with both pigmented and non-pigmented animals are available. Although non-pigmented skin tends to be more sensitive than pigmented skin for detecting phototoxicity, the influence of melanin-binding (see section 2.2) should be considered when selecting a species/strain 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. Accumulation of compound in relevant light-exposed tissues might lead to an increased sensitivity after repeated administration. Similarly, repeated irradiation after each dose might also lead to an increased sensitivity due to the accumulation of damage. 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. Whenever feasible, the clinical route of administration should be used. Single or repeated daily irradiations after dosing (around Tmax) can be used.

Dose selection for *in vivo* nonclinical phototoxicity testing of systemic drugs, if conducted, 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. A vehicle group as well as non-irradiated controls can support adequate analyses and can distinguish between irradiation-induced and non-irradiation-induced adverse reactions. If the maximum systemic exposure achieved in animals is lower than clinical exposure, the reliability of a negative result in predicting human risk is questionable.

If an *in vivo* phototoxicity study is conducted, it is desirable to know the pharmacokinetic profile of the compound before designing the study, to ensure that irradiation of the animals is conducted at the approximate Tmax. Relevant systemic exposure data (e.g., Cmax), if not already available, should be collected as part of the *in vivo* phototoxicity study.

The most sensitive early signs of compound-induced phototoxicity are usually erythema followed by edema at a normally sub-erythemogenic irradiation dose. The type of response might vary with the compound. Any identified phototoxicity reaction should be evaluated regarding dose and time dependency and, if possible, the NOAEL should be established. The hazard assessment might be further supported by additional endpoints (e.g., early inflammatory markers in skin or lymph node reactions indicative of acute irritation).

In some cases, phototoxicity in the retina should be assessed (usually only warranted for substances absorbing light above 400 nm considering the optical properties of the human eye, see Ref. 8). 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 might not be relevant to humans. 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 eyelids.

Adequate performance of *in vivo* phototoxicity models, which are not formally validated, should be demonstrated using suitable reference compounds. Compounds that are phototoxic in humans and that represent 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 (i.e., above 400 nm) is recommended. The concurrent use of a positive control compound 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 that 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 to dermal administration, including those for species selection, study duration, and irradiation conditions. For dermal drug products in general, the clinical formulation should be tested. The intended clinical conditions of administration (e.g., occluded, non-occluded, intradermal) should be used to the extent possible. 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 nonclinical skin sensitization testing. However, no formal validation of such models has been performed and their predictivity for human photoallergy is unknown. For regulatory purposes, such nonclinical photoallergy testing is generally not recommended.

3.6. Photosafety testing using in vivo assays and ocular administration

Currently, there are no standardised nonclinical *in vivo* approaches for assessing phototoxicity following ocular administration.

4. Clinical photosafety assessment

There are various options for collecting human data, if warranted, ranging from standard reporting of adverse events in clinical studies to a dedicated clinical photosafety study. The precise strategy is determined on a case-by-case basis.

5. Assessment strategies

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

ICH M3(R2) suggests a stepwise approach to photosafety assessment. An initial assessment of phototoxic potential based on photochemical properties and pharmacological/chemical class should be undertaken before outpatient studies. In addition, the distribution to skin and eye can be evaluated to inform further on the human risk and the need for further testing. Then, if appropriate, an experimental evaluation of phototoxic potential (nonclinical, *in vitro* or *in vivo*, or clinical) should be undertaken before exposure of large numbers of subjects (Phase III).

5.1. Recommendations for testing of pharmaceuticals given via systemic routes

5.1.1. Assessment of phototoxic potential

If the substance has an MEC less than 1000 L mol-1 cm-1 (between 290 and 700 nm), no further photosafety testing is recommended and no phototoxicity is anticipated in humans. Any available data on the phototoxicity of class-related compounds should also be assessed, as this could inform on the decision taken for further assessment. If the drug developer chooses to conduct a test for photoreactivity (see Section 3.2) the resulting data can support a decision that no further photosafety assessment is warranted. Similarly, if a drug developer chooses to assess drug distribution to light-exposed tissues (see Section 2.2), the resulting data can support a decision that no further photosafety assessment is warranted (see Note 5). Otherwise, non-clinical and/or clinical photosafety assessment of the substance should be conducted.

5.1.2. Experimental evaluation of phototoxicity

If the drug developer chooses an *in vitro* approach, the 3T3 NRU-PT is currently the most widely used assay and in most cases could be considered as an initial test for phototoxicity. In the EU, a validated *in vitro* alternative method should generally be used before considering animal testing. The high sensitivity of the 3T3 NRU-PT results in 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 an *in vitro* assay might not be appropriate. In this case, an assessment in animals or in humans could be considered.

If an *in vitro* phototoxicity assay gives a positive result, a phototoxicity study in animals could be conducted to assess whether the potential phototoxicity identified *in vitro* correlates with an *in vivo* response. Alternatively, the photosafety risk could be addressed/managed in the clinical setting. This could include a recommendation for protective measures in clinical trials in lieu of photosafety testing, or until the risk has been assessed (see ICH M3(R2)). 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 testing is recommended and no phototoxicity is anticipated in humans. In addition, a robust clinical phototoxicity assessment indicating no concern supersedes any positive nonclinical results.

In cases where an *in vivo* animal phototoxicity study or clinical phototoxicity study had already been conducted, there is no reason to subsequently conduct an *in vitro* phototoxicity assay.

5.2. Recommendations for testing of pharmaceuticals given via dermal routes

5.2.1. Assessment of phototoxic potential

If the active substance and excipients have MEC values less than 1000 L mol-1 cm-1 (between 290 and 700 nm), no further photosafety testing is recommended and no phototoxicity is anticipated in humans. Any available data on the phototoxicity of chemical class-related compounds should also be assessed as this could inform on the approach taken for further assessment. For compounds with MEC values of 1000 L mol-1 cm-1 or higher, in the EU and Japan, negative photoreactivity test results (e.g., a ROS assay) can support a decision that no further photosafety assessment is warranted. In the U. S., negative test results in photoreactivity assays do not generally preclude further clinical photosafety assessment using the to-be-marketed formulation.

Tissue distribution is not a consideration for dermal products. Dermal products are administered directly to the skin and hence, unless they are applied to areas not exposed to light, are assumed to be present in light-exposed tissues.

5.2.2. Experimental evaluation of phototoxicity and photoallergy

The *in vitro* 3T3 NRU-PT can be used to assess individually the phototoxicity potential of the API and any new 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 phototoxic component 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 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 skin model selected and, if appropriate, adjust the assay conditions accordingly (e.g., testing higher strength formulations, increasing exposure time). However, under adequate test conditions, a negative result in a 3D skin model indicates that the phototoxicity potential of the formulation can be regarded as low. In this case, in the EU and Japan generally no further phototoxicity testing is recommended. In the U. S., negative test results do not generally preclude further clinical photosafety assessment using the to-be-marketed formulation.

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)). In the EU and Japan, a negative result in an appropriately conducted *in vivo* animal phototoxicity study would be sufficient evidence that the formulation is not phototoxic and no further phototoxicity testing is recommended. In the U. S., negative test results do not generally preclude further clinical photosafety assessment using the to-be-marketed formulation.

For dermal products where the API or any new excipient has a MEC value of 1000 L mol-1 cm-1 or higher, a photoallergy assessment is generally warranted in addition to phototoxicity testing. A clinical photoallergy assessment is generally recommended using the to-be-marketed formulation, and a study can be conducted during Phase III, if warranted.

5.3. Recommendations for testing of pharmaceuticals given via ocular routes

For compounds that have an MEC value less than 1000 L mol-1 cm-1 (between 290 and 700 nm) no phototoxicity is anticipated in humans. Compounds that only absorb light at wavelengths below 400 nm and are to be administered as intraocular injections behind the lens (e.g., in the vitreous) are of low concern, as only light of wavelengths greater than 400 nm reaches the back of the adult eye. However, the lens in children is not completely protective against wavelengths below 400 nm.

For compounds that absorb at relevant wavelengths and are given via ocular routes (e.g., ocular eye drops, intraocular injections), an assessment of photosafety is generally recommended. The reliability of *in vitro* approaches in predicting phototoxicity following ocular administration is unknown and there are no standardised *in vivo* approaches for assessing phototoxicity for products administered via the ocular route. Nevertheless, the basic principles of phototoxicity assessment still apply and any available data on the phototoxicity of the compound in question or of chemical class-related compounds should be considered in the overall assessment. In the U.S. and Japan there are no specific recommendations to experimentally assess the phototoxic potential of ocular products. In the EU, an experimental assessment would be recommended using *in vitro* approaches or *in vivo* studies using other routes of administration when the available data are considered insufficient for hazard identification.

6. Endnotes

Note 1: Testing of photogenotoxicity is not recommended as a part of the standard photosafety testing programme. In the past, some regional guidance (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 the CPMP/SWP guidance was issued has indicated that these tests are substantially oversensitive and even incidences of pseudo-photoclastogenicity have been reported (Ref. 9). Furthermore, the interpretation of photogenotoxicity data regarding its meaning for clinically relevant enhancement of UV-mediated skin cancer is unclear in most cases. In most cases, the mechanism by which compounds induce photogenotoxic effects is identical to those that produce phototoxicity, and thus separate testing of both endpoints is not warranted.

Note 2: Standardized conditions for determination of MEC are critical. Selection of an adequate solvent is driven by both analytical requirements (e.g., dissolving power, UV-vis transparency) and physiological relevance (e.g., pH 7.4-buffered aqueous conditions). Methanol has been selected as a preferred solvent and was used to support the MEC threshold of 1000 L mol-1 cm-1 (data to be published). For most compounds, useful UV-vis spectra can be obtained, at concentrations around 100 µM. Nevertheless, potential limitations (e.g., artifacts due to high concentrations or slow precipitation) should be considered. If the chromophore of the molecule appears to be pH-sensitive (e.g., phenolic structure, aromatic amines, carboxylic acids, etc.) an additional spectrum obtained under aqueous, pH 7.4-buffered conditions, could add valuable information regarding differences in the shape of the absorption spectrum and in the MEC. If significant differences are seen between measurements obtained in methanol versus pH-adjusted conditions, the MEC threshold of 1000 L mol-1 cm-1 cannot be used to support a definitive assessment.

Note 3: A survey of EFPIA member companies indicated that the 3T3 NRU-PT, as described in the OECD guideline, generates a high percentage of positive results (approximately 50%), the majority of which do not correlate with phototoxicity responses in animals or humans (Ref. 10)

Note 4: Following a retrospective review of data for pharmaceuticals, a reduction of the maximum test concentration from 1000 to 100 µg/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, the category named “probable phototoxicity” per OECD (i.e., photo irritation factor (PIF) values between 2 and 5 or mean photo effect (MPE) values between 0.10 and 0.15) is of questionable toxicological relevance for systemic drugs. Compounds falling into this category generally do not warrant further photosafety evaluations. For compounds that give a PIF value between 2 and 5, and for which it is not possible to determine an IC50 in the absence of irradiation, it is important to check that the compound is not classified as positive using the MPE calculation, i.e., that the MPE is less than 0.15.

Systemic drugs that are positive in the 3T3 NRU-PT only at *in vitro* concentrations that are many times higher than drug concentrations likely to be achieved in light-exposed tissues in humans, can, on a case-by-case basis, and in consultation with regulatory authorities, be considered to be ‘low risk’ for human phototoxicity, without follow-up *in vivo* testing.

Note 5: If a systemically administered drug does not have higher tissue to plasma concentration ratios or does not accumulate in skin, in the U.S. further assessment of the phototoxicity potential is generally not warranted. In the EU and Japan higher tissue to plasma concentration ratios and/or tissue accumulation are also considered to be important. However, the presence of compound in skin is considered to be the critical factor in determining whether further testing is warranted. If a drug

developer believes there is a rationale for not testing based on very low tissue levels, this can be discussed with the regulatory authority on a case-by-case basis.

7. Glossary

3T3 NRU-PT: *In vitro* 3T3 neutral red uptake phototoxicity test.

Assessment: In the context of this document, an assessment is an evaluation of all available information and does not always mean an additional test is conducted.

Chromophore: The substructure of a molecule that absorbs visible or ultraviolet light.

Irradiance: The intensity of UV or visible light incident on a surface, measured in W/m2 or mW/cm2.

Irradiation: The process by which an object/subject is exposed to UV or visible radiation.

MEC: Molar extinction coefficient (also called molar absorptivity) is a constant for any given molecule under a specific set of conditions (e.g., solvent, temperature, wavelength) and reflects the efficiency with which a molecule can absorb a photon (typically expressed as L mol-1 cm-1).

MPE: The mean photo effect is calculated for results of the 3T3 NRU-PT when two equally effective concentrations (IC50), both with and without irradiation, cannot be determined. The MPE is based on comparison of the complete concentration response curves (see OECD TG 432).

NOAEL: No observed adverse effect level.

OECD TG: Organisation for Economic Co-operation and Development Test Guideline.

Photoproducts: New compounds/structures formed as a result of a photochemical reaction.

Photoreactivity: The property of chemicals that react with another molecule as a consequence of absorption of photons.OLE_LINK5

PIF: Photo irritation factor is calculated for results of the 3T3 NRU-PT by comparing the IC50 with and without irradiation.

ROS: Reactive oxygen species, including superoxide anion radicals and singlet oxygen.

UVA: Ultraviolet light A (wavelengths between 320 and 400 nm).

UVB: Ultraviolet light B (wavelengths between 290 and 320 nm)

8. References

ICH M3(R2) Guideline: Guidance on Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals; June 2009.

Henry B, Foti C, Alsante K. Can light absorption and photostability data be used to assess the photosafety risks in patients for a new drug molecule? J Photochem Photobiol B Biol 2009: 96: 57-62

ICHQ1B Guideline: Stability Testing: Photostability Testing of New Drug Substances and Products; Nov. 1996.

CIE-85-1989: Solar Spectral Irradiance; Jan. 1989.

Onoue S, Hosoi K, Wakuri S, Iwase Y, Yamamoto T, Matsuoka N, Nakamura K, Toda T, Takagi H, Osaki N, Matsumoto Y, Kawakami S, Seto Y, Kato M, Yamada S, Ohno Y, Kojima H. Establishment and intra-/inter-laboratory validation of a standard protocol of reactive oxygen species assay for chemical photosafety evaluation. J Appl Toxicol, Article first published online, 13 June, 2012 (DOI: 10.1002/jat.2776).

OECD (2004), Test No. 432: *In vitro* 3T3 NRU Phototoxicity Test, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing.

Ceridono M, et al. Workshop Report: The 3T3 neutral red uptake phototoxicity test: Practical experience and implications for phototoxicity testing – The report of an ECVAM–EFPIA workshop. Reg Tox Pharm 2012 Aug: 63(3): 480–488.

Wielgus, AR, Rogerts, JE. Retinal Photodamage by Endogenous and Xenobiotic Agents. Photochemistry and Photobiology 2012 Jul: 88 (6): 1320–1345.

Lynch AM, Robinson SA, Wilcox P, Smith MD, Kleinman M, Jiang K, Rees RW. Cycloheximide and disulfoton are positive in the photoclastogenicity assay but do not absorb UV irradiation: another example of pseudophotoclastogenicity? Mutagenesis 2008 Mar;23(2):111-8.

Lynch, AM, Wilcox, P. Review of the performance of the 3T3 NRU *in vitro* phototoxicity assay in the pharmaceutical industry. Exp Toxicol Pathol 2011Mar: 63(3): 209-14.