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

ICH HARMONISED TRIPARTITE GUIDELINE

PHOTOSAFETY EVALUATION OF PHARMACEUTICALS

S10

Current *Step 4* version

dated 13 November 2013

This Guideline has been developed by the appropriate ICH Expert Working Group and has been subject to consultation by the regulatory parties, in accordance with the ICH Process. At Step 4 of the Process the final draft is recommended for adoption to the regulatory bodies of the European Union, Japan and USA.

S10
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PHOTOSAFETY EVALUATION OF PHARMACEUTICALS

ICH Harmonised Tripartite Guideline

Having reached *Step 4* of the ICH Process at the ICH Steering Committee meeting on 13 November 2013 this guideline is recommended for adoption to the three regulatory parties to ICH.

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PHOTOSAFETY EVALUATION OF PHARMACEUTICALS

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 authorizations for pharmaceuticals. It includes factors for initiation of and triggers for additional photosafety assessment and should be read in conjunction with ICH M3(R2), Section 14 on Photosafety Testing (Ref. 1). This guideline should reduce the likelihood that substantial differences in recommendations for photosafety assessment will exist among regions.

This guideline is divided into several sections. Section 2 discusses factors to consider in any evaluation of photosafety. Section 3 describes existing nonclinical photosafety tests, but this section does not describe specific testing strategies. Section 4 mentions clinical photosafety assessment. Section 5 provides strategies for determining how to assess photosafety for drugs given by routes intended to produce systemic exposure or by the dermal route using the considerations and tests described in Sections 2, 3 and 4.

Consideration should be given to the use of non-animal 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 the photosafety assessment relative to clinical development. It recommends that an initial assessment of phototoxicity potential be conducted, and if appropriate, an experimental evaluation be undertaken before exposure of large numbers of subjects (Phase 3). Similarly, the ICH S9 Guideline (Ref. 2) describes the timing of photosafety testing for oncology products. However, neither ICH M3(R2) nor ICH S9 provides 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), new excipients clinical formulations for dermal application (including dermal patches), and photodynamic therapy products.

Specific guidance for pharmaceuticals given *via* ocular routes is not provided because the reliability of *in vitro* approaches in predicting ocular phototoxicity is unknown and there are no standardised *in vivo* approaches for assessing phototoxicity for products administered *via* the ocular routes (see Note 1).

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 components of marketed

products unless there is a new cause for concern for either the API or an excipient (e.g., a reformulation from a tablet to a topical cream).

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. The photosafety assessment aims to determine whether risk minimization measures are warranted 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 2) 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, the term photosensitization 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 usually not present a concern for direct phototoxicity. However, increased sensitivity of skin to light can also occur through indirect mechanisms. Such mechanisms are not generally addressed by the testing outlined in this guideline (see also Section 2.4).

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 photons at any wavelength between 290 and 700 nm. A compound that does not have a Molar Extinction Coefficient (MEC) greater than 1000 L mol^{-1} cm^{-1} at any wavelength between 290 and 700 nm (Ref. 3) is not considered to be sufficiently photoreactive to result in direct phototoxicity (see Note 3 for further details).

Excitation of molecules by light can lead to generation of Reactive Oxygen Species (ROS), including superoxide anion and singlet oxygen *via* energy transfer mechanisms. Although photoreactivity can result in other molecular outcomes (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-visible light can be an indicator of phototoxicity potential.

Photostability testing (Ref. 4) 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 using high-quality scientific standards with data collection records readily available, or in compliance with Good Laboratory Practices/Good Manufacturing Practices (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. The duration of exposure depends upon clearance rates as reflected by half lives in plasma and tissue. Collectively, these parameters define the mean residence time of the photoreactive chemical in 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 half-lives in plasma, longer mean residence time in sun-exposed tissues or with higher tissue to plasma concentration ratios are more likely to produce a phototoxic reaction than compounds with shorter half-lives, 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.

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 generic thresholds for all compounds. Nevertheless, on a case-by-case basis it can be possible to justify that further photosafety assessment is not warranted based upon actual or anticipated tissue drug levels in humans, and taking into consideration the factors discussed above. Examples could include: 1) a drug for which overall systemic exposure levels are very low, or 2) a drug with a very short plasma half-life or tissue residence.

Compound binding to tissue components (e.g., melanin, keratin) 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 timepoints after dosing, will generally provide an adequate assessment of relative tissue to plasma concentration ratios, tissue residence time and the potential for retention and accumulation. Assessment time points should be appropriately spaced in such a study to account for the drug half-life.

Compounds activated by visible light and exhibiting long elimination half-lives in internal tissues have been demonstrated to cause injury to those tissues if exposed to intense light during medical procedures. Consequently, for those compounds activated by visible light with potent *in vivo* phototoxicity or known to be phototoxic based on their mechanism of action, such as photodynamic therapy drugs, distribution to internal tissues should be measured and tissue-specific half-lives estimated. Drugs that only absorb UV 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 assessments, as metabolism does not typically result in chromophores that are substantially different from those in the parent molecule.

2.4. Pharmacological Properties

In many cases, drug-induced phototoxicity is due to the chemical structure and not to the pharmacology. However, certain pharmacologic properties (e.g., immunosuppression, perturbation of heme homeostasis) can enhance susceptibility to light-induced effects, such as skin irritation or UV-induced skin tumor formation. The testing strategies outlined in this document are not designed to detect these types of indirect mechanisms. Some of these indirect mechanisms can be identified and evaluated in other nonclinical pharmacology/toxicity testing; however, phototoxicity related to other indirect mechanisms might only become apparent with human experience.

3. NONCLINICAL PHOTOSAFETY TESTS

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 assessment strategies described in this document, it is most important that nonclinical photosafety assays show high sensitivity resulting in a low frequency of false negatives (i.e., a high negative predictive value). This is because negative assay results usually do not warrant further photosafety evaluation. 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.

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, and 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., Ref. 5) 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 of the applied spectrum. UVA doses ranging from 5 to 20 J/cm^2 are successfully used in current *in vitro* and *in vivo* phototoxicity assays. These UVA doses are comparable to those obtained during prolonged outdoor activities on summer days around noon time, in temperate zones, and at sea level. In humans, sunburn reactions caused by UVB normally limit total sunlight exposure. 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 activation by UVA for systemic drugs. However, UVB irradiation is relevant for topical formulations applied to light-exposed tissues.

The selection and monitoring of appropriate light sources (spectral distribution, irradiance, and dose) and the procedures used should be clearly described in the study methodology (e.g., Ref. 6).

3.2. Photoreactivity Tests 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 is a ROS assay (e.g., Ref. 7). Data suggest that this assay has high sensitivity for predicting direct *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, would indicate a very low probability of phototoxicity, provided a test concentration of 200 μM can be achieved, whereas a positive result (at any concentration) would only be a flag for follow-up assessment.

3.3. Phototoxicity Tests Using *in vitro* Assays

A number of *in vitro* assays have been developed for assessing the phototoxicity potential of chemicals. Some of these assays have not been qualified for use with pharmaceuticals. Some assays involve testing compounds that are dissolved in the culture medium, and such methods are often appropriate for the active ingredient or excipients in drug products, depending on their solubility. Other assays involve direct application to the surface of a tissue preparation and can be appropriate for testing entire formulations intended to be administered topically.

The most widely used *in vitro* assay for phototoxicity is the 3T3 Neutral Red Uptake Phototoxicity Test (3T3 NRU-PT) for which an Organisation for Economic Co-operation and Development (OECD) guideline (Ref. 6) is available. This is currently considered the most appropriate *in vitro* screen for soluble compounds.

Although the formal European Centre for the Validation of Alternative Methods (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. 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 Note 4). These proposed changes are appropriate for the testing of pharmaceuticals. The sensitivity of the 3T3 NRU-PT is high 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 initially recommended irradiation conditions (Ref. 6) involve the use of filters to attenuate wavelengths below 320 nm. However, depending on the light source and filters used, the ratio of UVB to UVA can be adjusted such that it is possible to assess UVB-induced phototoxicity in this test. UVB-induced phototoxicity is rarely a problem for pharmaceuticals with systemic exposure since UVB minimally penetrates beyond the epidermis. However, UVB-induced phototoxicity is more relevant for topical products. For components of topically applied products that absorb predominately in the UVB range, and where *in vitro* assessment is desired, the use of the 3T3 NRU-PT with modified irradiation conditions (see above) can be considered. Alternatively, *in vitro* skin models, which better tolerate UVB, could be considered.

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 assays developed with reconstructed human skin to date measure cell viability with and without irradiation. These assays appear to be capable of detecting known human acute dermal phototoxicants. However, the sensitivity of some assays can be less than that of human skin *in vivo*, wherein the lowest concentration eliciting a positive response can be higher than in human skin *in vivo*. Consequently, it is important to understand the sensitivity of any assay selected and, if appropriate and feasible, 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, regardless of the route of administration. While negative results in the 3T3 NRU-PT or a reconstructed human skin assay might suggest a low risk, the predictive value of these assays for ocular phototoxicity is unknown.

3.4. Photosafety Tests Using *in vivo* Assays and Systemic Administration

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 factors might be considered as best practices.

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, pigmented skin should be considered for APIs that bind significantly to melanin (see Section 2.2) if appropriate exposures in target tissues cannot be ensured otherwise.

If an *in vivo* phototoxicity study is conducted, it is desirable to have some information about the pharmacokinetic profile of the compound before designing the study. This is to ensure that irradiation of the animals is conducted at the approximate T_{max} and to assist in the selection of an appropriate study duration in relation to the intended clinical exposure. Relevant pharmacokinetic data, if not already available, should be collected as part of the *in vivo* phototoxicity study.

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 after repeated administration might lead to an increased phototoxic response. Similarly, repeated irradiation after each dose might also lead to an increased phototoxic response due to the accumulation of damage. Generally, studies of a single day or up to a few days' duration of dosing are appropriate, using the clinical route of administration, if feasible. Single or repeated daily irradiations after dosing (around T_{max}) can be used.

Dose selection for *in vivo* nonclinical phototoxicity 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, typically considering C_{max} comparisons. Vehicle and non-irradiated controls can help identify compound-related phototoxicity and distinguish irradiation-induced from 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.

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 No Observed Adverse Effect Level (NOAEL) should be established. The hazard identification might be further supported by additional endpoints (e.g., early inflammatory markers in skin or lymph node reactions indicative of acute irritation).

If a phototoxicity study is conducted in animals for a systemic drug that absorbs light above 400 nm, phototoxicity of the retina should be assessed using a detailed histopathological evaluation. For compounds that only absorb light below 400 nm, retinal assessment is usually not warranted because such wavelengths do not reach the retina of the adult human eye due to limited penetration of the cornea, lens and vitreous body.

Adequate performance of *in vivo* phototoxicity assays, which are not formally validated, should be demonstrated using suitable reference compounds, including pharmaceuticals. Compounds that are phototoxic in humans and that represent different chemical classes and mechanisms of phototoxicity should be included to establish adequacy of the assays. For retinal phototoxicity, 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* assay 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. Photoallergy reactions in humans following systemic administration are rare and there are no established nonclinical photoallergy assays for systemically administered compounds.

3.5. Photosafety Tests 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 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 (see Section 3.4). 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, contact photoallergy has often been assessed in a nonclinical study along with acute phototoxicity (photoirritation). However, no formal validation of such assays has been performed. While the acute photoirritation observed in these studies is considered relevant to humans, the predictivity of these studies for human photoallergy is unknown. For regulatory purposes, such nonclinical photoallergy testing is generally not recommended.

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 trial. The precise strategy is determined on a case-by-case basis.

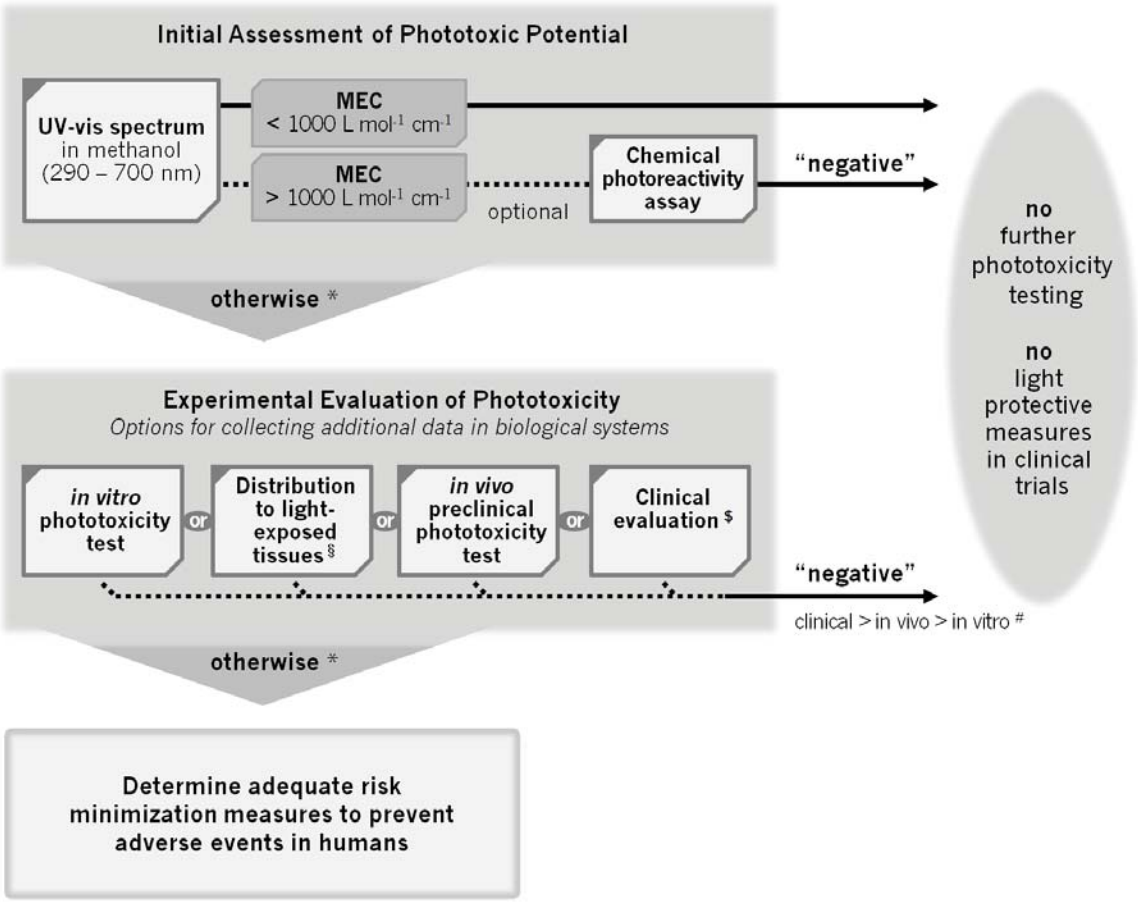
5. ASSESSMENT STRATEGIES

The choice of the photosafety assessment strategy is up to the drug developer. ICH M3(R2) suggests that an initial assessment of the phototoxicity potential based on

photochemical properties and pharmacological/chemical class be undertaken before outpatient studies. Characterization of the UV-visible absorption spectrum is recommended as the initial assessment because it can obviate any further photosafety evaluation. In addition, the distribution to skin and eye can be evaluated to inform further on the human risk and the recommendations for further testing. Then, if appropriate, an experimental evaluation of phototoxicity potential (*in vitro* or *in vivo*, or clinical) should be undertaken before exposure of large numbers of subjects (Phase 3).

Figure 1 provides an outline of possible phototoxicity assessment strategies. The figure is based on the strategies outlined in this section of this document. The strategies are flexible. Depending on the particular situation, some portions of the assessment are optional and might not be conducted.

Figure 1. Outline of possible phototoxicity assessment strategies for pharmaceuticals given *via* systemic and dermal routes



***** "otherwise": data do not support a low potential for phototoxicity or have not been generated (assay/test/evaluation not conducted)

A "negative" result in an appropriately conducted *in vivo* phototoxicity study supersedes a positive *in vitro* result. A robust clinical phototoxicity assessment indicating no concern supersedes any positive nonclinical results. A positive result in an *in vitro* phototoxicity test could also, on a case-by-case basis, be negated by tissue distribution data (see text). In the United States, for products applied dermally, a dedicated clinical trial for phototoxicity on the to-be-marketed formulation can be warranted in support of product approval.

$ Clinical evaluation could range from standard reporting of adverse events in clinical studies to a dedicated clinical photosafety trial.

§ Tissue distribution is not a consideration for the phototoxicity of dermal products.

5.1. Recommendations for Pharmaceuticals Given *via* Systemic Routes

5.1.1 Assessment of Phototoxicity Potential

If the substance does not have a MEC greater than 1000 L mol^{-1} cm^{-1} (between 290 and 700 nm), no photosafety testing is recommended and no direct phototoxicity is anticipated in humans. However, it should be noted that phototoxicity by indirect mechanisms (e.g., pseudoporphyria or porphyria), although rare, could still occur. For compounds with MEC values of 1000 L mol^{-1} cm^{-1} or higher, if the drug developer chooses to conduct a test for photoreactivity a negative result could support a decision that no further photosafety assessment is warranted (see Section 3.2). Otherwise, nonclinical and/or clinical photosafety assessment of the substance should be conducted. Available data on the phototoxicity of chemical class-related compounds should be evaluated as this could inform on the approach to be taken.

5.1.2 Experimental Evaluation of Phototoxicity

In order to reduce the use of animals in accordance with the 3R principles, a validated *in vitro* method should generally be considered before conducting animal testing (e.g., see Directive 2010/63/EU). If the drug developer chooses an *in vitro* approach, the 3T3 NRU-PT is currently the most widely used assay and in many cases could be considered as an initial test for phototoxicity. 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 direct 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. Alternatively, if drug distribution data are available, they could, on a case-by-case basis, support a decision that no further photosafety assessment is warranted (see Section 2.2).

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 a response *in vivo*. Alternatively, drug distribution data could, on a case-by-case basis, support a position that the risk of phototoxicity *in vivo* is very low and that no further photosafety assessment is warranted (see Section 2.2). As another option, the photosafety risk could be assessed in the clinical setting, or managed by the use of light-protective measures. A negative result in an appropriately conducted phototoxicity study either in animals or humans supersedes a positive *in vitro* result. In such cases no further testing is recommended and no direct phototoxicity is anticipated in humans.

A positive result in an *in vivo* animal study can, in certain circumstances, be mitigated using a NOAEL-based risk assessment, typically considering C_{max} comparisons. Otherwise, a clinical assessment is warranted. In all cases a robust clinical phototoxicity assessment indicating no concern supersedes any positive nonclinical results.

A positive result in an *in vitro* phototoxicity test would not be negated by a negative result in a subsequently conducted chemical photoreactivity assay (e.g., a ROS assay).

In cases where an animal or clinical phototoxicity study has already been conducted, there is no reason to subsequently conduct either a chemical photoreactivity or an *in vitro* phototoxicity assay.

5.2. Recommendations for Pharmaceuticals Given *via* Dermal Routes

5.2.1 Assessment of Phototoxicity Potential

If the active substance and excipients do not have MEC values greater 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. For compounds with MEC values of 1000 L mol^{-1} cm^{-1} or higher, negative photoreactivity test results (e.g., a ROS assay) can support a decision that no further photosafety assessment is warranted (see Note 5 for exception). If further assessment is warranted, available data on the phototoxicity of chemical class-related compounds should be evaluated, as this could inform on the approach to be taken.

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

5.2.2 Experimental Evaluation of Phototoxicity and Photoallergy

The 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 that 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.

Reconstructed human skin models can be used to assess the phototoxicity potential of clinical formulations. Under adequate test conditions (see Section 3.3), a negative result in a reconstructed human skin assay indicates that the direct phototoxicity potential of the formulation can be regarded as low. In this case, generally no further phototoxicity testing is recommended (see Note 5 for exception).

If an appropriate *in vitro* assay is not available, the initial test could be an *in vivo* phototoxicity test on the clinical formulation. A negative result in an appropriately conducted *in vivo* animal phototoxicity study would be sufficient evidence that the formulation is not directly phototoxic and no further phototoxicity testing is recommended (see Note 5 for exception). Alternatively, the phototoxicity potential can be assessed in the clinical setting.

For dermal products where the API or any new excipient has a MEC value greater than 1000 L mol^{-1} cm^{-1} at any wavelength between 290 and 700 nm, a photoallergy assessment is generally warranted in addition to phototoxicity testing. As the predictivity of nonclinical photoallergy tests is unknown, this would typically be a clinical assessment using the to-be-marketed formulation and conducted during Phase 3.

Photosafety evaluation of the clinical formulation delivered *via* dermal patches can follow the above described principles for clinical dermal formulations. For transdermal patches, the principles for both dermal and systemic drugs should be applied. In addition, the intended clinical use (e.g., skin area recommended for use, duration of application) and the properties of the patch matrix (e.g., being opaque to UV and visible light) should be considered for the overall risk assessment.

6. ENDNOTES

Note 1 For compounds that absorb at relevant wavelengths, have a MEC value greater than 1000 L mol^{-1} cm^{-1}, and are given *via* ocular routes (e.g., eye drops, intraocular injections), an evaluation of the phototoxicity potential should be undertaken in accordance with the general principles of phototoxicity assessment. Biodistribution of drug in the eye, and optical properties of the eye should also be considered. Any available information on the compound or chemical class-related compounds should be considered in the overall assessment.

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 for retinal phototoxicity, as only light of wavelengths greater than 400 nm reaches the back of the adult eye. However, the lens in children of less than approximately 10 years of age is not completely protective against wavelengths below 400 nm.

Note 2 Testing for photogenotoxicity is not recommended as a part of the standard photosafety testing program. In the past, some regional guidelines (e.g., CPMP/SWP/398/01) have recommended that photogenotoxicity testing 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 guideline was issued has indicated that these tests are substantially oversensitive and even incidences of pseudo-photoclastogenicity have been reported (Ref. 8). Furthermore, the interpretation of photogenotoxicity data regarding its meaning for clinically relevant enhancement of UV-mediated skin cancer is unclear.

Note 3 Standardized conditions for determination of the MECs are critical. Selection of an adequate solvent is driven by both analytical requirements (e.g., dissolving power, UV-visible light transparency) and physiological relevance (e.g., pH 7.4-buffered aqueous conditions). Methanol is recommended as a preferred solvent and was used to support the MEC threshold of 1000 L mol^{-1} cm^{-1} (Ref. 3). When measuring UV-visible light spectra, potential limitations (e.g., artifacts due to high concentrations or low solubility, including 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 MECs. 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 obviate further photosafety assessment.

Note 4 A survey of pharmaceutical companies indicated that the 3T3 NRU-PT, as described in Organisation for Economic Co-operation and Development, Test Guideline (OECD TG) 432, generates a high percentage of positive results (approximately 50%), the majority of which do not correlate with phototoxicity responses in animals or humans (Ref. 9). Following a retrospective review of data for pharmaceuticals, a reduction of the maximum test concentration from 1000 to 100 µg/mL appears justified (Ref. 10). 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 TG 432 (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 in this category generally do not warrant further photosafety evaluations. For compounds with a PIF value between 2 and 5, and for which it is not possible to determine an IC_{50} 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 phototoxicity in humans , without follow-up *in vivo* testing.

Note 5 In the United States, for products applied dermally, a dedicated clinical trial for phototoxicity (photoirritation) on the to-be-marketed formulation (API plus all excipients) can be warranted in support of product approval.

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.

Dermal Drugs:

Products applied topically to the skin.

Direct Phototoxicity:

Phototoxicity induced by absorption of light by the drug or excipient.

Indirect Phototoxicity:

Phototoxicity due to cellular, biochemical or physiological alterations caused by the drug or excipient, but not related to photochemical reactivity of the drug or excipient (e.g., perturbation of heme homeostasis).

Irradiance:

The intensity of UV or visible light incident on a surface, measured in W/m^2 or mW/cm^2.

Irradiation:

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

MEC:

Molar Extinction Coefficient (also called molar absorptivity) reflects the efficiency with which a molecule can absorb a photon at a particular wavelength (typically expressed as $L\ mol^{-1}\ cm^{-1}$) and is influenced by several factors, such as solvent.

MPE:

The Mean Photo Effect is calculated for results of the 3T3 NRU-PT. 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.

Outpatient Study:

A clinical study in which patients are not restricted to a clinical site.

Photoproducts:

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

Photoreactivity:

The property of chemicals to react with another molecule as a consequence of absorption of photons.

PIF:

Photo Irritation Factor is calculated for results of the 3T3 NRU-PT by comparing the IC_{50} values obtained with and without irradiation.

ROS:

Reactive Oxygen Species, including superoxide anion and singlet oxygen.

Systemic drugs:

Products administered by a route that is intended to produce systemic exposure.

UVA:

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

UVB:

Ultraviolet light B (wavelengths between 280 and 320 nm; as a part of sunlight wavelengths between 290 and 320 nm).

8. REFERENCES

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

2. ICH S9 Guideline: Nonclinical Evaluation for Anticancer Pharmaceuticals; October 2009.

3. Bauer D, Averett LA, De Smedt A, Kleinman MH, Muster W, Pettersen BA, et al. Standardized UV-vis spectra as the foundation for a threshold-based, integrated photosafety evaluation. Regul Toxicol Pharmacol 2014;68(1):70-5.

4. ICH Q1B Guideline: Stability Testing: Photostability Testing of New Drug Substances and Products; November 1996.

5. Solar spectral irradiance. CIE 1989 Jan;85.

6. Test No. 432: In vitro 3T3 NRU phototoxicity test. OECD Guidelines for the Testing of Chemicals Section 4 2004 Nov.

7. Onoue S, Igarashi N, Yamada S, Tsuda Y. High-throughput reactive oxygen species (ROS) assay: An enabling technology for screening the phototoxic potential of pharmaceutical substances. J Pharm Biomed Anal 2008;46(1):187-93.

8. Lynch AM, Robinson SA, Wilcox P, Smith MD, Kleinman M, Jiang K, et al. 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.

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

10. Ceridono M, Tellner P, Bauer D, Barroso J, Alépée N, Corvi R, 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. Regul Toxicol Pharmacol 2012;63(3):480-8.