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**NOTE FOR GUIDANCE ON PHOTOSAFETY TESTING**

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## NOTE FOR GUIDANCE ON PHOTOSAFETY TESTING

### 1. INTRODUCTION

The goal of photosafety testing is to detect the adverse effects of pharmaceutical products in the presence of light. This type of testing is relevant for medicinal products that enter the skin via dermal penetration or systemic circulation. Photobiological reactions normally occur when a chemical is able to absorb UV or visible light. Four different endpoints of concern should be specifically addressed in photosafety testing:

- Phototoxicity (photoirritation), which is an acute light-induced skin response to a photoreactive chemical.
- Photoallergy, which is an immunologically mediated reaction to a chemical initiated by the formation of photoproducts (for example, the photoproducts produce an antigen).
- Photogenotoxicity, which is a genotoxic response observed after exposure to a chemical photoactivated by UV or visible light.
- Photocarcinogenicity focuses on the potential of a drug to induce skin tumours in combination with UV. This may be either an indirect enhancement of UV-induced carcinogenic effects (also termed photo co-carcinogenicity) or a carcinogenic effect of a drug photoactivated under UV irradiation (also termed photochemical carcinogenesis).

This note for guidance should be read in conjunction with the Note for Guidance on non-clinical local tolerance testing of medicinal products (Eudra/S/90/024 adopted 1990).

#### 1.1 Objectives

The objective of this guideline is to define the conditions under which photosafety evaluation of pharmaceuticals should be conducted. Additionally, this document provides guidance on approaches for evaluating the photosafety of pharmaceuticals.

#### 1.2 Scope

This guideline generally applies to new chemical entities and biotechnology-derived pharmaceuticals for human use. This guideline may be applied to marketed pharmaceuticals when appropriate (e.g. when adverse clinical events, route of administration raise concerns not previously addressed.)

### 2. NEED FOR PHOTOSAFETY TESTING

Photosafety testing is warranted for those chemicals that absorb light in the wavelength of 290 - 700 nm and are either

- topically/locally applied or
- reach skin or eyes following systemic exposure.

### 3. TEST PROCEDURES

#### 3.1 General considerations concerning experimental design

The basic principle of preclinical photosafety testing is to determine whether effects regarding phototoxicity, photoallergy, photogenotoxicity or photocarcinogenicity are produced or enhanced when animals or cell cultures are exposed to the test material plus UV radiation (i.e. sunlight simulation) compared with exposure to the test material without UV irradiation (and, where appropriate, compared with exposure to the same dose of UV radiation alone).

For some areas of photosafety testing (in particular, phototoxicity & photogenotoxicity) appropriate *in vitro* test methods are available. In compliance with *Council Directive 86/609/EEC of 24 November 1986 on protection of animals used for experimental and other scientific purposes* the use of *in vitro* tests instead of animal studies is highly encouraged if adequate. In general, alternative methods which may provide more insight into mechanisms and information on the relevance of *in vitro* or animal data relative to humans are expected to be developed in the future. When shown to be scientifically valid such tests could replace or supplement currently used tests for regulatory purposes.

#### 3.2 Light source/irradiation conditions

Normally, the irradiation spectrum should approximate the solar spectrum. Solar simulators, e.g., containing xenon arc lamps plus appropriate filters to remove the UVC and attenuate the UVB part of the emission spectrum to the levels of ambient sunlight are generally used. The characteristics of the irradiation source should be described in detail.

The irradiation dose used should have no or only slightly deleterious effects but should be high enough to ensure an efficient activation of a broad spectrum of potential photosensitizers. The rationale for the selection of doses should be given in the test report.

For *in vitro* methods additional factors affecting the irradiation spectrum and dose, like plastic lids of culture flasks, coloured pH indicators, precipitation of test compound, density of cells etc have to be carefully considered. The actual amount of UV light received by the target cells under experimental conditions should be controlled using a suitable UV-meter.

#### 3.3 Metabolic activation

The use of metabolising systems, such as rat liver S9 mix, is a general requirement for *in vitro* tests which have limited metabolic capacity. Although up to now no chemical is known for which metabolic transformation is needed for the chemical to act as a photosensitizer, the possibility of generation of photoreactive metabolites should be considered in the general toxicological evaluation (i.e. to evaluate whether main human metabolites reaching skin or eyes show structural alert with respect to photoreactivity, Note 1). Omission of S9 mix in *in vitro* phototoxicity tests may be justified due to technical reasons since it has been shown that addition of material with a high protein content such as S9 to the cell cultures can absorb or scatter light in the UV region and thus may protect the target cells from possible phototoxic and photogenotoxic effects (Gocke et al. 2000).

#### 3.4 Phototoxicity testing

For phototoxicity testing a thoroughly validated *in vitro* method is available. The 3T3 NRU PT phototoxicity test has been officially accepted by the EU Commission and the EU member states *Annex V to Directive 67/548/EEC on the Classification, Packaging and Labelling of Dangerous Substances: Testing Method B.41 Phototoxicity – In vitro 3T3 NRU Phototoxicity Test* and is also available as an OECD guideline draft (<http://www.oecd.org/ehs/test/3t3nru.doc>).

The 3T3 NRU phototoxicity test 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 UVA/vis light. Cytotoxicity in this test is expressed as a concentration dependent reduction of the uptake of the vital dye, Neutral Red. While the use of the permanent mouse fibroblast cell line Balb/c 3T3 is recommended in the standard protocol of the EU and OECD guideline, other cells or cell lines may be used with the same test protocol, if the culture conditions are adapted to the specific needs of the cells.

For dermally applied drug products testing with the finished product formulation may be advantageous since some vehicles may affect the photosensitizing properties of the active substance. Since this may not be possible in cell cultures due to solubility problems, the conduct of *in vivo* studies in experimental animals should be taken into consideration until *in vitro* models (e.g. 3-D Skin models) for such requirements have been validated.

In most cases, data from *in vitro* tests like 3T3 NRU PT may provide sufficient information for the preclinical assessment of the phototoxic potential of a drug product and thus *in vivo* nonclinical studies are normally not warranted. For a possible clinical follow-up testing on potential risks, controlled clinical studies e.g. determination of the minimal erythema dose (MED) in volunteers are encouraged. In case of potential risks identified either *in vitro* or in phototoxicity testing in human, an appropriate clinical safety survey should be performed both before and after marketing authorisation.

### 3.5 Photoallergy testing

At present, photoallergy testing is mainly conducted using guinea pig models. Alternative test methods which may take into consideration a better objectivity in photoallergic hazard identification and animal welfare are currently under development. *In vitro* tests monitoring photochemical reactions, i.e. photoadduct formation and photooxidation of biomolecules, can be useful screening tools. *In vivo* models under development (modified LLNA-assays and MEST-assay) may become valuable in the future. However, the validation of all these tests is still limited. Photoallergy testing should be performed according to the state of the art.

### 3.6 Photogenotoxicity testing

The main purpose of photogenotoxicity testing is to make an assessment of the potential of a compound to turn into a photochemical carcinogen upon activation with UV or visible radiation.

Recommendations regarding the conduct of tests for photochemical genotoxicity have been recently elaborated by an international expert working group (Gocke et al. 2000).

Several different *in vitro* genotoxicity tests have been adapted for testing of the consequences of photoactivation of chemicals to damage the DNA. For safety testing purposes it is recommended to focus on those *in vitro* systems, which are currently used as standard tests in regulatory testing strategies. As no genotoxic photochemical is known to date which is exclusively positive for gene mutations, and the recognized photochemical reaction mechanisms are strongly clastogenic it is suggested that a test for photochemical clastogenicity (chromosomal aberration or micronucleus *in vitro* test) should have first priority.

Standard *in vivo* genotoxicity tests currently used for routine testing (bone marrow micronucleus or chromosome aberration test, rat liver UDS test) are obviously not applicable for *in vivo* photogenotoxicity testing. From a technical point of view the *in vivo* Comet assay or transgenic mutagenicity models may be appropriate for the determination of genotoxic effects (DNA strand breaks or gene mutations, respectively) in skin cells. However, experiences with these models for photogenotoxicity testing are at present very limited.

### 3.7 Photocarcinogenicity testing

The most widely used model to assess photocarcinogenesis in animals and the only model that is currently available in compliance with GLP rules is the SKH1 (hr/hr) albino hairless mouse model. However, this model has not been validated so far and the mechanistic understanding that is provided by this model is limited (Forbes 1996). Thus, the predictivity of the rodent photocarcinogenicity model for the human situation is at present unclear (Note 2).

As an alternative, *in vitro* mechanistic studies (see flow chart) including photogenotoxicity studies may be used to assess the photocarcinogenic potential of a test compound thus obviating the need for a separate mouse photocarcinogenicity study. For drugs which are positive in such *in vitro* tests warning statements with regard to photocarcinogenic potential is an adequate option and further testing in chronic rodent studies is not warranted.

It should be noted that drugs exist that may enhance UV carcinogenicity by other mechanisms than photoactivation and thus will not be detected using *in vitro* photo(geno)toxicity studies. A major class with known human relevance are immunosuppressants such as methotrexate or cyclosporin. The photo co-carcinogenic potential of immunosuppressants is believed to be directly related to the pharmacology of the drugs. Thus, potent candidate drugs of this class could be classified as potential enhancer of UV-induced skin carcinogenesis without further testing in rodent photocarcinogenicity studies.

Other mechanisms by which non-photosensitizing compounds have been shown to enhance UV-associated tumours in rodents involve changes in the optical properties of the skin or alterations in the protective layers of the epidermis induced by some topically applied vehicles and emollients. However, whether such animal findings can be extrapolated to the clinical situation and are relevant to predict human risk is questionable. Instead of testing such indirect photoeffects i.e. drug-induced changes in the UV penetration properties of the skin in albino hairless mice photocarcinogenicity studies, investigations in controlled clinical studies measuring effects in human skin (e.g. increased sensitivity to UVB) appear to be much more appropriate.

## 4. TEST STRATEGIES / PROPOSED APPROACHES TO PHOTOSAFETY TESTING

The testing scheme depicted in the flow chart is intended to give guidance on the decision-making process whether and how photosafety testing of drug products should be conducted.

As a first step of photosafety assessment of any new active substance the evaluation of the photochemical properties of the drug product is essential in making a testing decision. Only those compounds that do absorb within the 290 – 700 nm range of the electromagnetic spectrum and reach skin or eyes need to be tested. The current experiences do not allow for definition of specific levels of either the molar absorbance or the compound concentration in the skin below which photosafety testing would not be required. Findings from photostability testing and consideration of structure-activity relationship may give further valuable information concerning a possible adverse photoeffects and may help to determine whether testing is warranted. As an exception, agents used for photodynamic therapy may need to be tested even if not bioavailable in skin or eyes. If the photochemical/toxicokinetic assessment identifies a need for testing, a basic photosafety testing should include studies for the evaluation of the phototoxic, photogenotoxic and photoallergy potential. Positive findings in these studies should trigger the incorporation of phototoxicity endpoints into safety monitoring in clinical trials.

Compounds found to be clearly photogenotoxic can be considered as potential photocarcinogens and a specific testing in rodent photocarcinogenicity studies is normally not required. Accordingly, compounds devoid of photogenotoxic potential are according to present knowledge not expected to be photocarcinogenic if tested in the long-term photobioassay (with the exception of mechanisms of photo-cocarcinogenesis not depending on photoreactivity which are described in section 3.7). However, whether this assessment is acceptable without conducting a photobioassay has to be decided on a case-by-case basis taking into consideration all information such as the photochemical/photobiological data of the test compound, the clinical benefits and also information on chemically/pharmaceutically related compounds (Note 3).

## **5. REGULATORY IMPLICATIONS**

The overall risk benefit assessment of a drug product which induces adverse photoeffects will depend on considerations of the following factors:

- The quality and potency of the effects detected in the preclinical and clinical studies.
- The safety risks presented by the drug relative to its therapeutic potential.
- The availability of clinically effective alternatives with a more favourable safety profile.

If a drug with clinically relevant findings in photosafety testing is granted a marketing authorization appropriate warning statements have to be included in the SPC/PIL. These should indicate that the drug may cause adverse photoeffects (effect to be specified) and users of the drug should avoid unprotected exposure to the sun while treated with the drug.

## **6. REFERENCES**

Forbes P.D., Relevance of animal models of photocarcinogenesis to humans, *Photochem Photobiol* 63, 357-362, 1996.

Gocke E., Müller L., Guzzie P.J., Brendler-Schwaab S., Bulera S., Chignell C.F., Henderson L.M., Jacobs A., Murli H., Snyder R.D., Tanaka N., Considerations on photochemical genotoxicity: Report of the IWGTP working group, *Environ Molec Mutagen* 35, 173-184, 2000.

Stern R.S., Nichols K.T., Väkevä L.H., Malignant melanoma in patients treated for psoriasis with methoxsalen (psoralen) and ultraviolet A radiation (PUVA), *New Eng J Med* 336, 1041-1045, 1997.

## **Notes**

### **Note 1**

Molecular characteristics of many photosensitizing agents include a relatively low molecular weight and a planar, tricyclic, or polycyclic configuration that is highly conjugated.

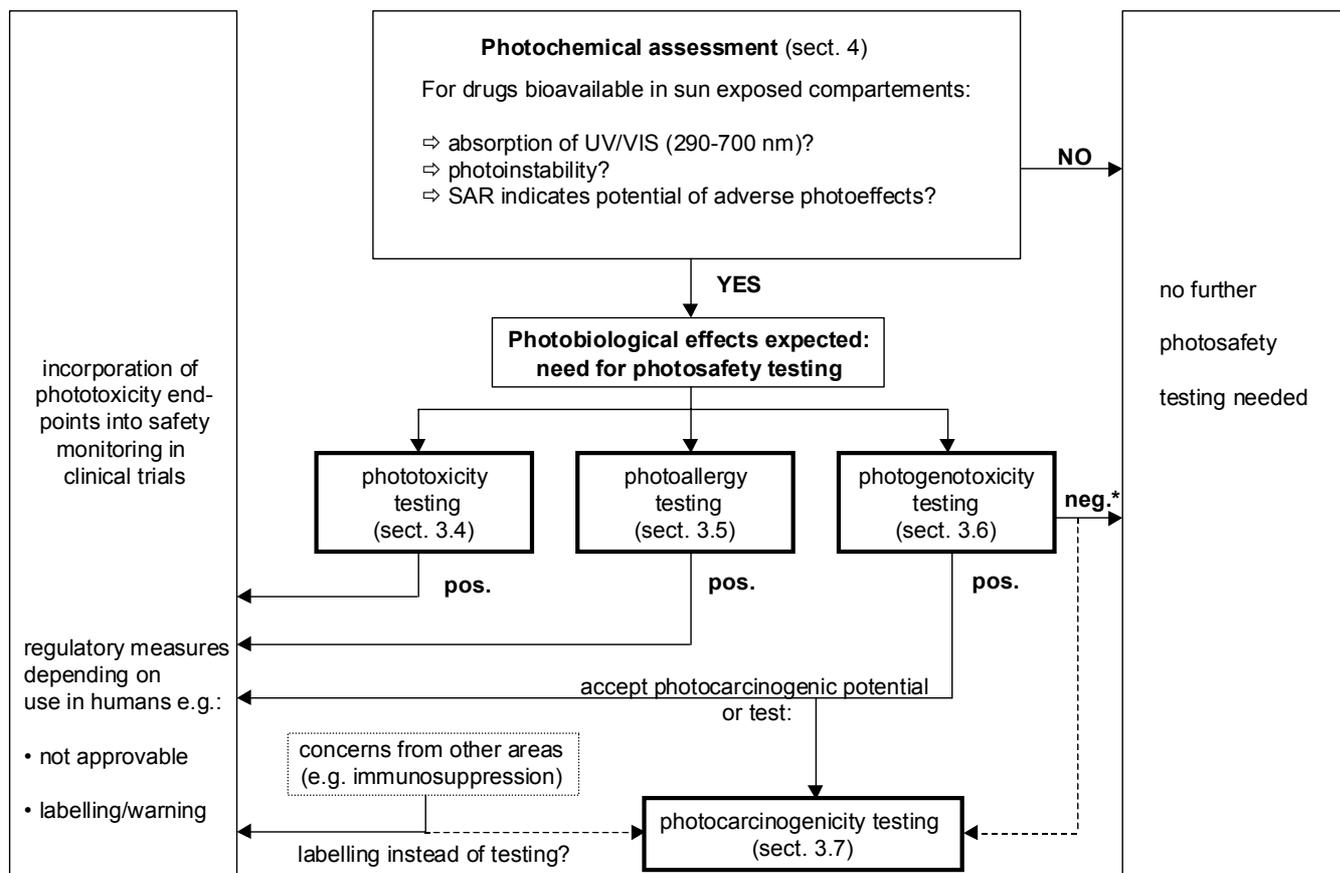
### **Note 2**

The main problem for evaluating the predictivity of the chronic rodent photocarcinogenicity study is the lack of established human photocarcinogens. Presently, the only proven positive human example of a photoactivated carcinogen is the psoriasis treatment 8-methoxypsoralen (8-MOP) plus UV radiation, i.e. PUVA (Stern et al. 1994). So far, only for PUVA the rodent proved to be a sensitive predictor of the human response. To further substantiate findings from rodent photocarcinogenicity studies for humans, mechanistic and supportive data are essential.

### **Note 3**

E.g., experimental data for the class of the fluoroquinolones show a good qualitative and quantitative correlation between the effects found in photogenotoxicity studies and the potential as photocarcinogenes in the hairless mouse model. Thus, a new fluoroquinolone found to be negative when tested in comparison with known photogenotoxic/photocarcinogenic fluoroquinolones may be evaluated as non-photocarcinogenic without conducting a photobioassay.

## Flow Chart: Assessment of photosafety of new active substances



\* approach to be justified (sect. 4)