

A review of the advancements in photosafety testing with regard to ICH's new topic S10: Photosafety evaluation of pharmaceuticals

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List of abbreviations

3T3 NRU-PT	3T3 Neutral Red Uptake Phototoxicity Test
BfArM	Bundesinstitut für Arzneimittel und Medizinprodukte
c	concentration
CDER	Center for Drug Evaluation and Research
CHMP	Committee for Medicinal Products for Human Use
cm	centimeter
c_{\max}	Peak plasma concentration of a drug after administration
CPMP	Committee for Proprietary Medicinal Products
3D	Three Dimensional
DIA	Drug Information Association
EFPIA	European Federation of Pharmaceutical Industries Associations
e. g.	exempli gratia
EMA	European Medicines Agency
EMEA	European Medicines Evaluation Agency (old expression for EMA)
EPAA	European Partnership for Alternative Approaches to Animal Testing
et al.	et alia
etc.	et cetera
EU	Europe
equ	equivalent unit
EWG	Expert Working Group
FDA	United States Food and Drug Administration
g	gram
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
IC ₅₀	Mean Inhibitory Concentration
ICH	International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use

i. e.	id est
IWGT	International Workshop on Genotoxicity Testing
JPMA	Japanese Pharmaceutical Manufacturers Association
L	Liter
LOEL	Lowest Observed Effect Level
M	Multidisciplinary
MDRA	Master of Drug Regulatory Affairs
MEC	Molecular Extinction Coefficient
MHLW	Ministry of Health, Labour and Welfare
MPE	Mean Photo Effect
μ	micro
NCE	New Chemical Entity
nm	nano meter
No.	Number
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
OECD	Organization for Economic Cooperation and Development
p.	page
pos.	positive
PIF	Photoirritation Factor
Q&A	Questions & Answers
QT	Time from electrocardiogram Q wave to the end of the T wave corresponding to electrical systole
QWBA	Quantitative Whole-Body Autoradiography
R	Revision
ROS	Reactive Oxygen Species
S	Safety
SAHG	Safety Ad Hoc Group
SWP	Safety Working Party
t _{max}	time to reach c _{max} (peak time)

USA	United States of America
UV	Ultraviolet
UVA	Ultraviolet Light A (wavelengths between 320 and 400 nm)
UVB	Ultraviolet Light B (wavelengths between 290 and 320 nm)
VIS	Visible
Vol.	Volume
>	greater than
<	lesser than

Preface – General challenges in today’s drug development

From the initial idea of inventing a NCE until marketing of the corresponding product, pharmaceutical companies are in need of highly educated personnel, capital expenditures and - last but not least - time. The latter is surely accompanied by abundance of patience since drug development became more and more tedious over the last decades [1]. Amongst others, this is due to the grown complexity of global legal and regulatory obligations in the field of drug development and drug registration. Development of a new drug begins with the idea of a NCE together with the filing of the respective patent(s) and will hopefully result in a marketing authorization. Between these two milestones the development phases as displayed in table 1 have to be run through.

	Pre-Clinical Testing	Pre-Clinical Development	Clinical Phase I	Clinical Phase II	Clinical Phase III	Marketing Authorization
Years	3 - 4		1	2-5	2-6	1-2
Success Rate [compounds]	10.000 molecules under evaluation	20	13	7	2	1
Probability of Technical Success [%]		5	8	15	50	90

Table 1 [1, 2]

From the aforementioned it can be concluded that until marketing of a NCE at least 10 years will pass. This fact together with the increase in expenditure for drug development over the last decades (which is addressed in figure 1) obliges pharmaceutical companies to re-evaluate their development strategies with respect to more effective go/no-go decisions during early stages of development.

But it is not exclusively pharmaceutical industry being involved in re-evaluation of established drug development strategies. Also other key stakeholders such as regulatory bodies, academia and research organizations contribute to improvements in today’s state-of-the-art drug development strategies. In this regard, ICH shall be mentioned.

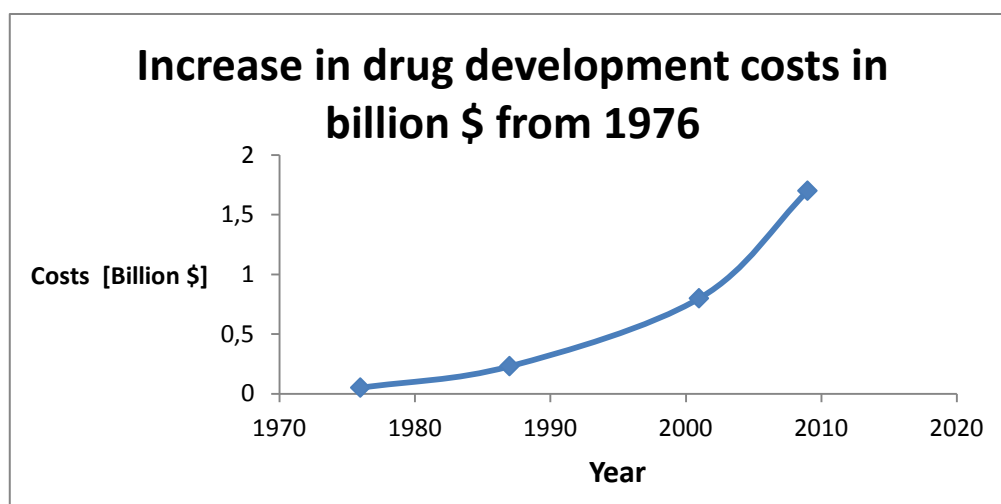


Figure 1 [1]

Rapid increase in laws, regulations and guidelines for reporting and evaluating the data on quality, safety and efficacy of new medicinal products together with the emerging detailed technical requirements resulted in duplicate work for pharmaceutical industry with respect to the conduct of many time-consuming and expensive test programs in order to market NCEs internationally. This led to the association of representatives of regulatory agencies and industry associations of Europe, Japan and the USA establishing ICH in 1990 as a response to the increasingly global face of drug development so that the benefits of international harmonized approaches for better global health can be realized worldwide [3]. In this regard, ICH’s efforts are to decrease the need for duplicate studies meaning to reduce animal testing, making research more economical and to avoid repetition of clinical studies. Besides, further objectives comprise harmonization of regulatory requirements in matters of definitions and presentation of documentation as well as bringing NCEs to the market in a lesser timeframe. By this approach, it is evident that ICH guidelines are robust and provide a greater scientific value than guidelines which only apply to certain regions (e. g. FDA guidelines for USA or EMA guidelines for Europe) and contribute to an atmosphere of mutual trust and confidence.

As a reaction of experience from both, pharmaceutical industries’ and authorities’ daily work, regulatory environment is subject to constant change. In this regard, it is no surprise that many fields of drug development have been re-evaluated in the past decades. An example is change of the requirements regarding single dose toxicity in non-clinical drug development which resulted in the withdrawal of the “Note for guidance on single dose toxicity” [4].

Other disciplines have been added completely new to today’s drug development programs. In this regard, photosafety testing within the field of toxicological testing

during the non-clinical phase can be cited an example being currently subject to re-evaluation of drug development. Furthermore, this discipline has lately been put on ICH's agenda by dedicating a new topic to photosafety testing.

Objective

This master thesis deals with a special topic attributed to non-clinical drug development, namely photosafety testing.

The upcoming chapters will briefly address general principles of safety assessment in non-clinical drug development (section 1) followed by an in-depth examination of photosafety testing. In this regard, details on the history of photosafety testing within ICH regions will be displayed as well as an overview of available regulatory guidance documents, defining the current state-of-the-art of this non-clinical discipline (section 2). In order to provide a comprehensive overview on the current points of interest related to photosafety assessment, also relevant information from recent industry associations' surveys as well as academic workshops will be displayed.

The main part of this master thesis is dedicated to the ongoing process of ICH's new topic S10: "Photosafety evaluation of pharmaceuticals". Next to a survey on the current Step 2 guideline (section 3), the related sections cover an analysis of the progress which has been made until current Step 2 (section 4) as well as an outlook and a discussion on potential outcomes of the final guideline (section 5). In this regard, upcoming changes and challenges for pharmaceutical industry within the field of photosafety testing and its implications on pre-clinical drug development will be displayed. Furthermore, also implications for regulatory authorities will be addressed.

This master thesis covers information and guidelines related to medicinal products for human use. The focus is on the regulatory situation in the ICH regions and on the development of a harmonized approach as regards ICH's new topic S10. Wherever the older abbreviation EMEA was referred to, it has been replaced by the current abbreviation EMA.

1 Safety assessment in non-clinical drug development

During the non-clinical phase of drug development usually certain types of pharmacology studies and toxicology studies support non-clinical safety assessment for marketing approval of a pharmaceutical [5]. The objective of the various non-clinical safety studies is the characterization of toxic effects in respect of target organs, dose dependence, relationship to exposure, and, if appropriate, potential reversibility in order to assess an initial safe starting dose, a dose range for clinical trials in human as well as to identify parameters which are considered relevant to clinical monitoring for potential undesirable effects [5].

1.1 Safety pharmacology studies

According to ICH S7A, pharmacology studies can be distinguished as follows: primary pharmacodynamic studies, secondary pharmacodynamic studies and safety pharmacology studies. Primary pharmacodynamic studies can be defined as examinations on the mode of action of a substance in relation to its desired therapeutic target and are thus predominantly dedicated to efficacy. In contrast, secondary pharmacodynamic studies explore the mode of action and/or effects of a substance not related to its desired therapeutic target which categorizes these studies as safety studies. Since the two former study types may in some cases contribute to the safety evaluation (i. e. the detection of potential undesirable effects in humans), they are mentioned in this regard. If appropriate, their outcomes should be considered along with the findings of safety pharmacology studies [6].

Safety pharmacology studies investigate the potential undesirable pharmacodynamic effects of a substance on physiological functions. In particular, the focus is on identification and evaluation of these undesirable effects on organs or systems acutely necessary for life, thus investigations on the following systems are mandatory since they are considered vital functions: cardiovascular system, respiratory system and central nervous system. These systems are also referred to as core battery. Certain follow-up studies for core battery need to be performed afterwards [6]. Supplemental studies on other systems may be conducted on a case-by-case basis depending on the evaluated need and, if already known, the mode of action. Regarding the timing of the named tests, current guidances request the performance of core battery testing prior to first use in humans. In case of relevant concern, also certain follow-up studies and supplemental studies must have been performed at this stage – at the latest, they must be performed

prior to approval [6]. However, additional safety pharmacology investigations may be necessary during clinical development. Safety pharmacology investigations will usually be conducted *in vitro* and *in vivo*. *In vitro*, the following models will be used by exploring a range of concentrations (with an exposure in the therapeutic range or above): isolated organs/tissues, cell cultures, cellular fragments/subcellular organelles, receptors, channels and enzymes [7]. For *in vivo* studies, typically rodents will be chosen. In this regard, the use of unanesthetized individuals is preferable and contrary to *in vitro* testing, single dose administration is state-of-the-art [7]. In general, safety pharmacology studies can be considered short-term investigations. Another aspect is that the animals tested during the *in vivo* experiments will not be killed after finalization of the studies. Thus, also information on reversibility of pharmacological effects can be obtained.

1.2 Toxicology studies

Toxicological studies comprise toxicokinetic studies and non-clinical pharmacokinetic studies, general toxicity studies, reproduction toxicity studies as well as genotoxicity studies [5]. Furthermore, for some NCEs testing of carcinogenic potential may be applicable in case there is a reasonable suspicion or the pharmaceutical is intended for a long duration of use. In addition, the following non-clinical tests may be applicable on a case-by-case basis with regard to toxicology studies: Immunotoxicity tests, studies to assess juvenile animal toxicity and abuse liability and last but not least phototoxicity studies [5].

Considering the fact that marketing authorizations will only be granted in case the benefits of a compound exceed its possible risks [8, 9], toxicology studies are of special importance during drug development since they will reveal the possible risks of a NCE. Toxicology studies concern the whole organism including all functions of organs and tissues related to hazard identification, risk assessment and risk management. The scope of toxicological studies is the identification of potential toxicities (hazard identification) as well as the extrapolation of non-clinical data to humans (risk assessment with reference to labeled and non-labeled use) and the gain of information on how to handle the obtained risk (risk management) [2].

These studies will be performed *in vitro* and *in vivo*. The former comprise experiments in bacteria, isolated organs/tissues, cells (primary cultures or cell lines), cell organelles, receptors, channels and enzymes. Within the *in vitro* approach, typical methods with respect to regulatory toxicology are the following [2]: Studies to investigate genotoxicity (e.g. Ames test), studies to investigate phototoxicity (e.g. 3T3-cells) and studies in isolated Purkinje fibers to investigate changes in the electrocardiogram (e. g. QT prolongation). As the advantages of toxicology studies (i. e. they are relatively

inexpensive and fast in processing) are accompanied by certain limitations (i. e. only selected endpoints are addressed, no information on (toxico-)kinetic effects are revealed and that it is impossible to evaluate complex mechanisms and chronic effects), *in vivo* experiments by using the most human-like animal species (based on pharmacokinetic and metabolic criteria) have to be performed, as well.

In vivo toxicological studies comprise genotoxicity studies, carcinogenicity studies and further special studies (on a case-by-case basis). They usually will be performed in two species (rodents and non-rodents) [2]. Toxicological studies have to be performed at least as long as the duration of the intended clinical use of the relevant drug or longer [2, 5]. Therefore, toxicological studies can be distinguished in matters of duration as follows: single dose studies (acute), repeat dose studies (subacute (2-4 weeks) subchronic (13 weeks) and chronic (6-12 months)) and carcinogenicity studies (2 years) [2].

The exposure in toxicology studies is higher than the anticipated maximum human dose since the detection of toxicological effects is intended. In general, all toxicological studies will comprise one control group and three different dose groups: “low dose” groups are dedicated to the definition of the NOAEL, whereas “mid dose” groups detect beginning toxicological effects and “high dose” groups will reveal the full toxicological profile, including lethality [2].

In the end of any *in vivo* toxicological study, the animals will be killed in order to examine all organs and tissues separately.

1.2.1 Photosafety testing within non-clinical safety studies

Photosafety testing belongs to the field of local tolerance testing which is a special discipline of toxicology testing of pharmaceuticals. Local tolerance testing in general aims at revealing whether NCEs are tolerated at sites of the organism which may come into contact with the medicinal product with regard to its mode of action in clinical use [10]. Hence, the testing strategy should be suitable to distinguish toxicological/pharmacodynamic effects from any mechanical effects of administration or physico-chemical actions of the formulation [10]. In addition, local tolerance testing as well as photosafety testing should also consider the physico-chemical properties of the formulation as well as the known pharmacodynamic, pharmacokinetic and toxicological data of both the active substance and the excipients [10].

The aim of photosafety testing is to provide information on a certain medicinal product’s adverse effects in the presence of light [11]. Photosafety testing is warranted for chemicals that absorb light in the spectrophotometric range between 290 and 700 nm (UV/VIS) and if they are either locally applied or are dedicated to reach/significantly partition to the skin or the eyes following systemic exposure [2, 11, 12, 13].

The following four different effects should be taken into account when photosafety testing is referred to [2, 11]:

1. **Phototoxicity (photoirritation):** This can be regarded as an acute light-induced skin response to a photoreactive chemical. The 3T3 NRU-PT serves as an established *in vitro* assay since it is currently the only method which is validated. Its principle is to compare the cytotoxicity of a chemical in a permanent mouse fibroblast cell line (Balb/c 3T3) both, in the presence and in the absence of a non-cytotoxic dose of UVA/visible light. *In vivo* tests can be conducted in hairless mice or guinea pigs: Here, the animal is treated with the chemical in combination with UV, followed by an examination of skin reactions (erythema, pain and swelling) and a histopathological investigation of the skin.
2. **Photoallergy:** Is defined as an immunologically mediated reaction to a chemical initiated by the formation of photoproducts (e. g. the latter produce antigens). For the assessment of photoallergy, next to the modified local lymph node assay (which is not fully validated), guinea pigs are mostly used.
3. **Photogenotoxicity:** Is a genotoxic response observed after exposure to a chemical photoactivated by UV or visible light and is being evaluated by the conduct of photoclastogenicity tests. The latter is an *in vitro* chromosomal aberration or micronucleus test.
4. **Photocarcinogenicity:** This reaction is related to the potential of a pharmaceutical to induce skin tumors in combination with UV. Photocarcinogenicity is no longer recommended. However, a one year long-term study in albino hairless mice (SKH1) was conducted during which the animals were treated systemic or topical in combination with UV.

At present, the named effects or endpoints of photosafety testing are to some extent subject to different approaches regarding their individual conduct and the question of whether they should all be evaluated at all, or in parallel or if even a tiered approach in testing might be applicable. This is currently different in ICH regions which gave rise to development of an independent guidance document related to photosafety testing within ICH in order to achieve harmonization in this non-clinical discipline. Further details will be addressed in the following chapters.

2 Regulatory history of photosafety testing

First efforts in establishing a regulatory framework in matters of photosafety testing were made during the early 1990s [12]. It was OECD's initiative in 1995 which basically started the process of discussing the need for a defined regulatory environment for photosafety testing: OECD made a draft proposal for a new guideline "Acute dermal photoirritation screening test" (TGP951) [12]. This draft addressed general principles for the use of the rabbit or the guinea pig for the first time [12]. However, after the comment period, the process until finalization of the guideline did not proceed.

In Europe, the "Note for guidance on non-clinical local tolerance testing of medicinal products" (first adopted in 1990) [10] provided brief information on photosafety testing until EMA's release of the "Note for guidance on photosafety testing" [11] in 2002. With the latter, a comprehensive guideline on photosafety testing was available, for the first time. Almost at the same time, FDA's CDER published its "Guidance for industry on photosafety testing" in 2003 [13]. Hence, a regulatory environment for the conduct of photosafety testing in the course of drug development was also available in the USA.

In the following years, pharmaceutical industry, regulatory authorities as well as academia generated data and increased their knowledge regarding the then in force approaches as regards photosafety testing. Since regulatory environment is subject to constant change and since the aforementioned experiences revealed certain shortcomings in the recommended photosafety approaches [12], further efforts and discussions on the available approaches of photosafety testing can be regarded a logical consequence. Hence, industry associations (EFPIA and JPMA) commissioned surveys on photosafety testing and also workshops related to photosafety testing (e. g. 2007 DIA workshop on photosafety testing and 2009 IWGT) took place triggering the advancement of the same.

EMA's adoption of the "Concept paper on the need for revision of the note for guidance on photosafety testing" [14] in beginning of 2008 can be regarded a first specific step towards a new, harmonized approach in the regulatory field of photosafety testing since this efforts finally resulted in ICH's consensus to start with its work on an independent guidance for photosafety testing.

2.1 Regulatory milestones in photosafety testing

This section provides further information on the aforementioned guidance documents in more detail. Additionally, other regulatory documents referring to photosafety evaluation are covered in this section since they contribute to the overall context and

understanding of this non-clinical discipline. The following guidances are addressed chronologically with regard to their individual release.

2.1.1 EMA “Note for guidance on non-clinical local tolerance testing”

The named guidance document addresses principles of local tolerance testing in the course of non-clinical drug development. Phototoxicity testing is not addressed as independent discipline. However, the note for guidance states that an evaluation of the phototoxicity and photosensitization potential should be done for products intended for administration to the skin. As general principle, it is referred to the fact that evaluation of local tolerance should be performed in laboratory experiments prior to first in man exposure of the product [10]. Furthermore, it is stated that the purpose of these studies is to ascertain whether medicinal products (both active ingredients and excipients) are tolerated at sites in the body which may come into contact with the product as a result of its administration in clinical use [10]. In addition, reference is made to the general testing strategy which should take into consideration that any mechanical effects of administration or purely physico-chemical actions of the product should be distinguishable from toxicological or pharmacodynamic effects [10].

Next to these general considerations, the “Note for guidance on non-clinical tolerance testing” addresses both, tolerance testing at the site of administration and systemic toxicity testing. In addition, also advice for the conduct of local tolerance tests is given (e. g. the choice of species, frequency and duration of administration, choice of dose, etc.) as well as for testing procedures for the following routes of administration: oral, dermal, parenteral, rectal, and vaginal. Last but not least, it is referred to two different test systems which address local sensitizing potential for chemicals applied to the skin by dermal, rectal or vaginal administration. These tests are the guinea pig assay and the lymph node assay.

This note for guidance was first released in 1990 [15]. From this version to the latest version which was adopted by CPMP in 2001, the following approach was newly included: Studies on animals can be substituted by validated *in vitro* tests provided that the test results are of comparable quality and usefulness for the purpose of safety evaluation [16, 17]. In this regard, it is emphasized that consideration should be given to developments in alternative testing methods.

In 2011, CPMP started efforts to improve the note for guidance on non-clinical local tolerance testing. This became necessary since from the release of the named note for guidance in 2001, the focus on local tolerance testing has broadened with regard to different routes of administration such as transdermal therapeutic systems [18]. Furthermore, newer methods of drug delivery have been established such as the fact that a shift has been observed towards the regulatory acceptance of scientifically valid *in*

vitro methods as well as formally validated *in vitro* methods as part of an integrated testing strategy. In addition, the possibility of reducing or refining animal studies is addressed with regard to the question of whether animal studies could be substituted by validated *in vitro* tests [19].

The revision should aim at harmonization of local tolerance requirements as outlined in the ICH guideline M3(R2). The aspired timeline until end of consultation was end of 2011 – until finalization of this master thesis, no new note for guidance on non-clinical local tolerance testing was released.

2.1.2 EMA “Note for guidance on photosafety testing”

The “Note for guidance on photosafety testing” refers to the aim of photosafety testing as being the detection of adverse effects of a medicinal product in the presence of ultraviolet or visible light. Next to NCEs, also biotechnology-derived medicinal products for human use are considered in this note for guidance. Reference is made to the four endpoints of photosafety testing, namely phototoxicity (photoirritation), photoallergy, photogenotoxicity and photocarcinogenicity. Instructions on the definitions of the conditions under which photosafety testing should be conducted and on the approaches for evaluation of photosafety tests of medicinal products are provided. This guidance requires photosafety testing for chemicals that absorb light in the wavelength of 290 – 700 nm and that are applied locally/topically or reach the skin or the eyes following systemic exposure [11].

Next to the aforementioned general considerations, the note for guidance provides information on test procedures with regard to photosafety testing. These comprise the conduct of phototoxicity testing, photoallergy testing, photogenotoxicity testing and photocarcinogenicity testing. In addition, also information on the experimental design in general as well as information on light sources/irradiation conditions and on metabolic activation is included. In general, the use of validated *in vitro* methods on all photoreactive compounds bioavailable to skin or eyes is encouraged regardless of level of exposure [11]. *In vivo* non-clinical studies are not warranted. A possible clinical follow-up may be conducted using the minimal effective dose in volunteers [20].

The use of *in vitro* assays, especially the 3T3 NRU-PT assay, is emphasized and the guinea pig model is recommended for photoallergy testing (the modified local lymph node assay as well as the mouse ear swelling test are considered not yet validated) [11, 12]. Regarding photogenotoxicity assays, the note for guidance considers the *in vitro* model (especially the photoclastogenicity assays) useful for evaluation of photocarcinogenic potential whilst the *in vivo* assay is considered subject to limited experience [11, 12]. With regard to photocarcinogenicity testing, it is emphasized that the established mouse model (SKH1) is neither validated nor has mechanistic

understanding been achieved, so far [21]. It is suggested that *in vitro* mechanistic studies, including photogenotoxicity, may be useful [12]. Compounds which revealed a positive phototoxic or photogenotoxic potential *in vitro* are recommended to be subject to warning statements and no further testing is required. Pharmaceuticals like immunosuppressants can be presumed enhancer of UV-induced skin carcinogenesis (which is due to their pharmacology) without testing using photocarcinogenicity models [12].

The note for guidance summarizes the aforementioned principles in one single flow chart, referring to a parallel approach in testing, which is provided in attachment 1.

2.1.3 FDA “Guidance for industry on photosafety testing”

The FDA guidance addresses similar principles as the European note for guidance on photosafety testing – however, the two documents differ in certain points as described in the following.

Its scope is to reduce unnecessary testing while ensuring an appropriate and science-based assessment of photosafety with regard to medicinal products for human use (biologicals are not addressed) [13]. The FDA guidance provides many background information on photoirritation, photoallergy and photocarcinogenesis which is significantly more than provided in the European note for guidance [12]. Furthermore, a rationale for photosafety evaluation and many scientific references are included as well as an overview on the historic approaches of photosafety testing.

The guidance refers to general tests which are available for evaluation of photoirritation (phototoxicity), photochemical carcinogenicity potential, or the potential to enhance UV-associated skin carcinogenesis [13]. In contrast to the EMA note for guidance on photosafety testing, this guidance does not recommend non-clinical test models for testing photoallergy since these tests are considered not predictive of clinical effects [13]. Another difference is that the FDA does not recommend the conduct of specific tests but refers to some available test methods.

Mentioned animal models are mice or guinea pigs but also rabbits or swine; as *in vitro* test for photoirritation, the 3T3 NRU-PT assay is named [13]. The latter is considered useful for materials which absorb UV/VIS radiation but is not recommended for products which are water-insoluble or for the evaluation of complete formulations [13]. Thus, in general the use of traditional animal tests is encouraged which is another distinction from the EMA “Note for guidance on photosafety testing”. Furthermore, the fact that follow-up measures (e. g. clinical studies) are recommended on photoreactive compounds which are bioavailable in skin or eyes at levels sufficient to cause photoirritation (and are known to exhibit clinical evidence or class effects), emphasizes

the consideration of a compound's level of exposure which is currently not present in the mentioned EMA guidance.

This guidance document provides different decision tree-based approaches for the conduct of photosafety testing. In particular, these comprise one decision tree each for

- Phototoxicity testing (photoirritation) (provided in attachment 2),
- The need for phototoxicity testing in the case of reformulation of an approved topical medicinal product (provided in attachment 3),
- Evaluation of photocarcinogenesis of phototoxic medicinal products (provided in attachment 4),
- Evaluation of photocarcinogenesis of materials that could indirectly enhance UV-induced skin carcinogenesis (e. g. immunosuppressants) (provided in attachment 5).

FDA's "Guidance for industry on photosafety testing" emphasizes at different text passages the impact of excipients of topically administered formulations which also results in the provision of a single topic within this guideline, namely the impact of reformulation of approved topical products (please also refer to attachment 4).

2.1.4 OECD test guideline 432 on the *in vitro* 3T3 NRU phototoxicity test

This OECD guidance of April 2004 provides a definition of phototoxicity which is in line with the aforementioned guidances of EMA and FDA. The guidance centers on the *in vitro* 3T3 NRU-PT assay and defines its scope as being the identification of the phototoxic potential of a test substance induced by the excited chemical after exposure to light through evaluation of photo-cytotoxicity being achieved via relative reduction in viability of cells exposed to the chemical in the presence *versus* the absence of light [22].

A MEC of $> 10 \text{ L mol}^{-1} \text{ cm}^{-1}$ is recommended as a compound's trigger for further photosafety testing which is based on a further OECD guidance dealing with UV measurement technique [23]. However, there is no reference made to any specific relationship with photosafety issues *per se*. Since the stated MEC value is very low, in practice it represents only a slight increase in absorption over standard spectrophotometer baseline measurements leading to most test compounds exceeding this value [24]. Thus, a MEC of $\leq 10 \text{ L mol}^{-1} \text{ cm}^{-1}$ represents the practical limit of detection for most NCEs in pre-clinical pharmaceutical development.

The main part of OECD test guidance 432 is designated to the principles of the 3T3 NRU-PT assay as well as to its conduct providing detailed information on preparation of cells, media and culture conditions, preparation of cultures and test substances, irradiation conditions and conditions of the final test. Furthermore, also information on

quality and quantity of data, on the evaluation and interpretation of results as well as on the mandatory content to be included in the test report is summarized. In addition, also a flow chart referring to the role of the 3T3 NRU-PT in a sequential approach to the phototoxicity testing of chemicals is provided in the guideline (please refer to attachment 6).

2.1.5 EMA “Questions and answers on the note for guidance on photosafety testing”

Prior to the adoption of this guidance document, EMA planned to revise the “Note for guidance on photosafety testing” in order to refine the criteria relevant for deciding whether photosafety testing is warranted, since the current attributes are rather non-specific and result in testing of too many new pharmaceuticals. A respective concept paper was released where the following shortcomings were outlined [25]:

- In the current note for guidance a parallel approach including tests covering the endpoints phototoxicity, photoallergy, and photogenotoxicity is recommended which does not seem suitable and effective anymore. Instead, a tiered approach shall be established, where photoallergy and photogenotoxicity testing would usually not be required in case the compound in question is clearly negative in an initial *in vitro* phototoxicity study.
- Problems regarding oversensitivity with regard to the 3T3 NRU-PT and the occurrence of “pseudo-effects” (the mammalian cell test for photogenotoxicity) cannot be justified for regulatory purposes any longer. Hence, they need to be replaced by more appropriate approaches.
- Timing of photosafety testing during drug development was not addressed, so far. This needs to be updated in order to avoid uncertainty and to obtain more significance.

Since this approach was not followed in the end, and since ICH already initiated its work on the new topic S10 on photosafety testing, it was concluded to release a “questions and answers” document in order to provide an interim solution until the final adoption of the ICH guideline. The following six issues are addressed in the named document [14]:

1. The first question concerns the refinement of criteria for the need of photosafety testing (absorption in the range 290-700 nm). It is discussed whether certain levels of the MEC could serve as a threshold under which photosafety testing could be regarded not warranted since Henry et al. published in 2009 that compounds with a MEC $< 1000 \text{ L mol}^{-1} \text{ cm}^{-1}$ are of sufficiently low concern of photosafety issues [26]. This applies also to the condition listed in the OECD 432 guidance for triggering testing with the 3T3 NRU-PT assay [12, 22]. Hence,

this approach of negligible testing for compounds with a MEC $< 1000 \text{ L mol}^{-1} \text{ cm}^{-1}$ is confirmed.

In addition, it is referred to whether an acceptable concentration threshold for a compound's exposure in target tissues can be defined below which photo-adverse reactions are unlikely and therefore no testing is required. Since there are no data available, delineating such a threshold for new compounds, an assessment of exposure in target tissues like skin or eyes has to be made on a case-by-case basis.

2. The next point deals with the question of establishing a tiered approach instead of testing all endpoints (phototoxicity, photoallergy, and photogenotoxicity) in parallel. It is stated that in case a compound is negative in one or more relevant phototoxicity assays, further testing is not necessary. Hence, a tiered approach is acceptable. In addition, phototoxic compounds being administered cutaneously should be tested for photoallergenicity.
3. Question number three centers around current recommendations for photogenotoxicity testing since the mammalian cell photogenotoxicity test will not be accepted any longer for regulatory purposes. This is due to the examination of Lynch et al. who proved that the established photoclastogenicity assays (e. g. mammalian cell test for photogenotoxicity) are oversensitive and even subject to pseudo-photoclastogenicity [27]. In brief, it is concluded that photogenotoxicity testing should be excluded as routine part of the standard photosafety testing program.
4. This question is about the concerns regarding the established use of the 3T3 NRU-PT assay and its perceived high incidence of positive responses as well as its perceived poor predictivity of phototoxicity *in vivo* which was discovered by Lynch and Wilcox [12, 28]. The question was raised whether this assay could be replaced by an appropriate *in vivo* animal study or clinical trial. The SWP comments this saying that despite the recognized shortcomings of the 3T3 assay, replacement cannot be recommended since the 3T3 assay does not result in false negative results. Moreover, negative results serve as satisfactory evidence that the compound is not phototoxic. In addition, it is emphasized that next to the 3T3 test which has undergone formal validation and is being supported by an OECD guideline [22], no validated *in vitro* assays exist which prohibits the conduct of an animal study for the same endpoints [19]. However, the initial performance of a well-designed study evaluating phototoxicity in humans would be an acceptable alternative to the conduct of the 3T3 assay. In general, a negative *in vivo* response of an appropriately conducted phototoxicity test will always transcend a positive *in vitro* response. Furthermore, a human negative response will always transcend a positive non-clinical response [12].

5. Next, timing of evaluation of photosafety testing within drug development is addressed. Here, reference is made to ICH's guideline M3(R2) where is referred to the conduct of photosafety evaluation prior to phase III (before exposure of large numbers of people) [5]. In addition, ICH's guidance S9 is cited, saying that for patients with advanced cancer, testing should be conducted prior to marketing [29].
6. The last point deals with the need for conducting photosafety testing of peptides and proteins since these molecules absorb with a peak at 280 nm and shoulder at 290 nm. Being a class effect (due to the aromatic amino acids), this is not related to any photosafety concern. Thus, no general testing for photosafety is warranted.

2.2 Further regulatory sources for photosafety testing

Apart from the regulatory key guidance documents dealing with the conduct and evaluation of photosafety testing, this topic is also shortly addressed as additional point to consider in guidances dedicated to superior topics in non-clinical drug development. These are ICH's topics M3(R2) and S9 which will be addressed in the following [5, 29]. Furthermore, also industry associations' surveys have been conducted on the current approaches of photosafety testing and shall be mentioned as well to provide a comprehensive overview on the latest points of interest in the field of photosafety assessment.

2.2.1 ICH topic M3(R2): "Guideline on non-clinical safety studies for the conduct of human clinical trials and marketing authorization for pharmaceuticals"

This guideline deals with recommendations on the conduct and evaluation of non-clinical safety studies to support clinical trials in humans as well as marketing authorization for medicinal products, applying for all ICH regions and thus aiming at achieving international standards.

The chapter on photosafety testing addresses the appropriateness together with the timing of photosafety testing in relation to human exposure. In this regard, the molecule's photochemical properties, available information on the phototoxic potential of chemically related molecules, as well as tissue distribution and clinical or non-clinical findings indicating phototoxic potential are being named the general criteria for the conduct of phototoxicity testing [5]. An initial testing on evaluation of a compound's phototoxicity is recommended which should result in protective measures during clinical trials for humans in case the compound has been proven to exhibit a human phototoxicity risk. Furthermore, the non-clinical examination of drug

distribution to the skin and eyes should take place to further define potential risks [12]. In case further testing is deemed necessary, appropriate experimental evaluation (non-clinical, *in vitro* or *in vivo*, or clinical) is required before exposure to large numbers of patients (phase III) [5]. The aforementioned describes a stepwise approach on testing phototoxicity. Alternatively, a direct assessment of phototoxic potential in a clinical or non-clinical study is possible. In this case, no further testing with regard to skin or eye distribution is required if the study is negative [5]. Finally, the guideline refers to the case when phototoxicity testing revealed a potential risk for photocarcinogenicity. It is stated that this risk can be managed by the inclusion of warning statements in the informed consent (clinical trials), or product information (marketing authorization), respectively. In this regard, the use of the currently available rodent model for evaluation of photocarcinogenicity (i. e. hairless rodent) is addressed. It is recognized that this assay can neither be considered useful in support of pharmaceutical development nor can be recommended.

2.2.2 ICH topic S9: “Non-clinical evaluation for anticancer pharmaceuticals”

This guidance provides particular information for the class of anticancer pharmaceuticals for the ICH regions. It is recommended to conduct initial assessment of phototoxic potential prior to phase I, taking into consideration photochemical properties and information of the class of the molecule [29]. In case a potential risk to humans will be identified, appropriate measures to control the anticipated risk have to be taken during outpatient trials [29]. If the risk cannot be adequately qualified based on the collected data, an evaluation consistent with the recommendations of the ICH M3(R2) should be provided prior to marketing [12].

2.2.3 Industry associations’ surveys

Since the adoption of both, the EMA “Note for guidance on photosafety testing” in 2002 and the FDA “Guidance for industry on photosafety testing” in 2003, growing concern within pharmaceutical industry accumulated with regard to the performance of the *in vitro* photosafety tests and their predictivity to *in vivo* (in animals and in humans) [28]. This was due to the fact that positive results in the 3T3 NRU-PT assay trigger a great deal of follow-up studies to assess whether there is any risk to humans, particularly, in the absence of any formal regulatory guidance as to which follow-up *in vivo* assays are suitable to verify and quantify phototoxic risk [28]. In addition, follow-up testing in humans is subject to high expenditure and also time consuming and may result in significant delay in the conduct and finalization of clinical trials. Furthermore, positive *in vitro* photosafety testing has consequences on patient information documents (e. g. labeling).

In consequence of the aforementioned and due to the inconsistencies regarding the regulatory agreed approaches for triggering photosafety testing of NCEs (i. e. absorption of light in the range 290-700 nm of locally/topically applied drugs which reach (EMA [11, 14]), or significantly partition to (FDA [13]) the skin or eyes), pharmaceutical industry felt that there was need for action. Finally, it was GlaxoSmithKline, who petitioned the SAHG of the EFPIA in 2007 in order to commission a survey of member companies with the scope to better understand the triggers for photosafety testing and how as well as to what extent hazard characterization *in vitro* is related to *in vivo* risk. In particular, the aims of the survey were the investigation of the triggers for photosafety testing, the assessment of the frequency of positive results in photosafety studies *in vitro* and the conditions under which these arise and last but not least to correlate *in vitro* results with *in vivo* data and if available with respect to tissue distribution [28]. The latter should also take into consideration NOELs and LOELs to assess whether there is sufficient evidence for the establishment of thresholds based on tissue exposure.

The survey collected data on 361 development compounds which were subject to *in vitro* phototoxicity testing in the standard 3T3 NRU-PT assay from ten EFPIA members by submission of a questionnaire to the EFPIA member companies. Where available, also significant *in vitro* and *in vivo* study data were requested together with further information of importance with regard to the problem (e. g. PIF and MEC, etc.). In summary, the survey revealed the following results: 85 % of the *in vitro* 3T3-NRU PT assay positives (which were 44% of the entire compounds) were negative when tested *in vivo*. Regarding photogenotoxicity, 74% of the tested compounds were positive for photoclastogenicity in mammalian cells *in vitro* which was surprising since more than 75% of compounds in this category were negative in the 3T3 NRU-PT assay [28]. The authors emphasize that although bias cannot be excluded which is due to the comparable small number of compounds evaluated and due to some company's strategies to submit data being a balanced representative subset of their in house data, significant inconsistencies between the two endpoints phototoxicity and photogenotoxicity appeared. The latter was also regarded remarkable, since the underlying mechanism responsible for both endpoints, namely the chemical photoactivation leading to the generation of free radicals and/or active oxygen species is considered the same [28].

From the results obtained by the EFPIA survey, it can be concluded that the currently used *in vitro* photosafety assays are substantially over-predicting animal photosafety hazard *in vivo* and also human photosafety risk in the clinic [28]. The obtained data raise concern with regard to the use of the currently recommended *in vitro* photosafety assays, such as the 3T3 NRU-PT assay, for regulatory purposes. Furthermore, the data revealed significant inconsistencies with regard to the correlation of the 3T3 NRU-PT

assay and mammalian *in vitro* photoclastogenicity assays. It is recommended to at least review the available regulatory guidances on photosafety assessment and to refine the requirements which are currently recognized triggers of photosafety testing [28].

Next to the EFPIA survey on photosafety testing, also JPMA commissioned a survey for photosafety evaluation in 2009 which drew a similar conclusion to the EFPIA survey: Both the EFPIA and the JPMA surveys clearly question the specificity and usefulness of the *in vitro* photosafety assays, raising concern over interpretation of positive *in vitro* photosafety tests for the assessment of NCE's photosafety for regulatory purposes [24].

2.3 Workshops with impact on photosafety testing

Apart from the above mentioned regulatory sources related to photosafety testing which have in common being available in written form, also two workshops of the recent past were of importance for the advancement of photosafety testing. These are the DIA workshop of 2007 and the IWGT of 2009 which will be addressed in the following.

2.3.1 2007 DIA “Workshop on photosafety evaluation of drugs”

It was in November 2007, when DIA organized a “Workshop on photosafety evaluation of drugs”. During this workshop a comprehensive revision of basic photochemistry and physics related to non-clinical and clinical evaluation of phototoxicology, as well as regulatory photosafety assessment and risk assessment was carried out [12].

Amongst other shortcomings, the fact of only having available one validated assay for phototoxicity, namely the 3T3 NRU-PT assay, next to the “quasi validated” lymph node assay, was criticized [12]. Since the presence of these two assays was considered insufficient, DIA's consensus in 2007 was that there was a lack of validated assays *in vitro* as well as of further *in vitro* and *in vivo* tests. Furthermore, the lack of established standards on how to perform those assays, the usage of varying endpoints and varying ways of interpreting the generated data together with missing consistency in the performance of phototoxicity test assays revealed a general lack of clarity with regard to photosafety testing [12]. This was ascribed to the fact of the existence of both EMA's and FDA's guidelines at the same time [12]: Although they were applicable for different continents, the fact that many pharmaceutical companies follow a world wide development approach, resulted in a inconsistency with regard to the conduct of photosafety testing (e. g. the utility of the photocarcinogenesis assay). Thus, consensus was reached with regard to the need of certain changes in the regulatory environment of photosafety testing.

2.3.2 2009 “International workshop on genotoxicity testing”

In August 2009, the 5th IWGT took place in Basel, Switzerland during which international representatives from industry, academia and regulatory bodies came together in order to re-evaluate the current recommendations for photogenotoxicity testing and the outcomes of the latest IWGT of 1999. In particular, the progress in development of photo(geno)toxicity testing over the past decade during which regulatory key guidances (as displayed above) were introduced was critically reviewed with regard to pharmaceutical industries’ worries referring to the performance of *in vitro* photosafety tests and their predictivity to *in vivo* systems. Especially, the performance of both, old and new photogenotoxicity assays was debated with special regard to the occurrence of pseudoclastogenicity.

The expert panel which was chaired by Peter Kaspar, BfArM, discussed the parallel approach of photosafety testing which was established by current EU guidances which consider photogenotoxicity testing as a screen for photocarcinogenic potential and recommend a test for photochemical photoclastogenicity, especially the photo-chromosome aberration assay, as the preferred model for this purpose [14, 24]. It was argued whether other tests, e. g. photo-Ames test or *in vivo* tests for photogenotoxicity might serve as alternative for the photo-chromosome aberration assay. In summary, the following problems were of special interest to the expert panel [24]:

1. The existence of a threshold level for the MEC below which testing could be neglected.
2. The existence of a threshold level regarding drug exposure in the skin or the eyes which entails a photogenotoxic response.
3. Whether available information on photoreactivity or photostability, respectively could predict potential phototoxicity (which would require follow-up testing on photogenotoxicity).
4. Whether there was a need for photogenotoxicity testing of established non-phototoxic compounds.
5. Which test models can be considered appropriate for photogenotoxicity testing.
6. The positioning of photogenotoxicity within a photosafety strategy: should it be case-by-case or pivotal part?

The expert group’s final conclusion regarding the six key questions is summarized hereafter [24]:

1. Based on the latest publications (e. g. Henry et al. [26]) and since the harmonization of MEC determination is in progress, there should be no requirement for regulatory photosafety testing for NCEs with a MEC < 1000 L mol⁻¹ cm⁻¹. However, further data post-meeting are considered necessary for confirmation.

2. Since appropriate data are missing, no threshold level for skin exposure below which testing could be omitted was recommended. In particular, the problem of the lack of quantitative data was recognized since the sensitivity of modern analytical methodology for measurement of drug concentrations in skin and/or eyes (e. g. lowest LOQ) is often below 1 µg equ/g using QWBA.
3. The recommendation from the 1999 IWGT was that photostability could not serve as a sufficient argument to omit testing. This approach should be reconsidered based on data presented by GlaxoSmithKline which indicate that there is a correlation between photochemical reactivity assays and phototoxic liability *in vitro* and may serve as more appropriate trigger for photosafety testing than absorption of compounds between 290 and 700 nm [28]. Regarding photogenotoxicity, the correlation with photochemical reactivity was lower than phototoxicity. However, a good predictability for known photogenotoxicants in the SKH-1 mouse model such as psoralens and fluoroquinolones was shown. The expert panel finally agreed that further data need to be collected, before this approach might be included into regulatory guidelines.
4. It was concluded that established non-phototoxic compounds do not require photogenotoxicity testing since the underlying mechanisms for phototoxicity and photogenotoxicity are considered identical.
5. Based on the available data from Dufour et al. and from a 4-lab ring-trial organized by BfArM [30], clear evidence for pseudo-photoclastogenicity of the standard *in vitro* photoclastogenicity assays was accepted. Consequently, these tests could no longer be recommended for regulatory phototoxicity purposes. Furthermore, data on alternative tests to the standard *in vitro* assays were discussed. Regarding the photo-Ames test, there was concern with respect to sensitivity and the endpoint gene mutation so that a final conclusion could not be drawn. Also the lack of recommendations for *in vivo* photogenotoxicity assays (animal testing) was addressed with regard to the additional benefit of the gained data which would be more applicable to human risk assessment than solely *in vitro* data which mainly identify hazard. In this regard, the photocomet assay, the photomicronucleus assay and the human 3-D skin model were addressed (the latter was used in the cosmetic industry, so far [24]). The mentioned tests were considered promising, however they are all still subject to limited data. Thus, the expert panel could not recommend a preferred test for routine photogenotoxicity testing.
6. Taking into account the high incidence of positive 3T3 NRU-PT assay results being false positives, the expert group concluded that a follow-up study for phototoxicity (either non-clinical by using human skin models/rodents or clinical) should have priority rather than another *in vitro* study with a mechanistically related endpoint such as photogenotoxicity. Consensus was

reached that the impact of photogenotoxicity data on overall assessment can be regarded negligible. Thus, no specific test model for photogenotoxicity to predict photocarcinogenicity should be included routinely in regulatory photosafety testing.

3 ICH S10: “Photosafety evaluation of pharmaceuticals”

3.1 Why a new topic in ICH’s framework was deemed necessary

The years 2007 until 2009 can be regarded as being of special importance with regard to advancement of photosafety testing. As mentioned earlier, certain workshops related to photosafety testing took place in 2007 and 2009 (DIA’s workshop on photosafety testing and 5th IWGT) which revealed many shortcomings in the current approaches of photosafety testing. Furthermore, industry associations’ surveys (from EFPIA and JPMA) were issued which mirrored the conclusions from the aforementioned workshops.

Almost at the same time, in January 2008 EMA adopted its concept paper on the need for revision of the “Note for guidance on photosafety testing”. The problem statement makes reference to the fact that since the release of the named guidance in 2002, accumulating data and experiences as well as new developments in the field of photosafety testing became available which revealed certain deficiencies in the current approach towards this topic (details are addressed in section 2.1.4). The initial timetable aimed at finalizing consultation by end of 2008 and to release a new guideline which shall replace the current “note for guidance on photosafety testing”. Instead, the “Questions and answers on the note for guidance on photosafety testing” was adopted in March 2011, saying that the plans for revising the EMA guideline as indicated by the concept paper will no longer be pursued since ICH started a new process in order to implement photosafety testing as a new topic in the ICH framework [14]. The mentioned question and answer document provides an interim solution until the final ICH guideline on evaluation of photosafety testing will be publicly available.

3.2 First steps: adoption of ICH’s final concept paper

Based on the recognized shortcomings of the available guidance documents dealing with photosafety testing as outlined before, ICH began its work with key stakeholders in this field. The initial effort was the adoption of the “Final concept paper for the new topic S10: photosafety evaluation of pharmaceuticals” on 8th April 2010 which was endorsed by the ICH Steering Committee on 9th April 2010. In this document, ICH officially identifies the need for a harmonized guideline on the evaluation of photosafety

testing which aims at being a valuable adjunct to the released ICH M3(R2) guideline [31].

3.2.1 Recognized deficiencies in today's photosafety testing

In the statement of the perceived problem, ICH outlines that currently no definite threshold criteria for the conduct of photosafety others than the absorbance of ultraviolet and visible lights within the range of 290 to 700 nm testing have been established, yet. Rather, every test compound is being subject to phototoxicity testing, especially in Europe and Japan since the MEC value suggested by the OECD is $< 10 \text{ L mol}^{-1} \text{ cm}^{-1}$ which in fact can be regarded so low as to be meaningless (i. e. the chemical is unlikely to be photoreactive) [31]. In this regard, a MEC value $< 1000 \text{ L mol}^{-1} \text{ cm}^{-1}$ based on the absorptivity range of known human phototoxic compounds as appropriate threshold is mentioned as a possible alternative approach in the latest publication of Henry et al. [26, 31]. Furthermore, the lack of clarity concerning phototoxicity testing in Europe and the USA is addressed since EMA requires validated *in vitro* assays, namely the 3T3 NRU-PT assay, while FDA recommends short-term testing for photoirritation in animals or humans. Together with the outcomes of both the EFPIA and the JPMA survey, concluding that *in vitro* testing in general can be regarded too sensitive, this issue should be subject to harmonization [31]. Also the fact that photoclastogenicity testing often classifies compounds genotoxic which even do not absorb light between 290 and 700 nm is discussed, putting the general value of genotoxicity testing in the field of photosafety testing into question. The following issues were consequently identified and shall be resolved by the preparation of one single ICH tripartite guideline on photosafety testing [31]:

- Criteria of light absorbance and skin exposure to initiate phototoxicity testing should be defined;
- Criteria of tissue levels achieved and/or retained in the skin and eyes should be defined;
- A consensus on the triggers for photosafety testing should be developed;
- The need for the photosafety testing of drug metabolites should be assessed;
- The values of several *in vitro* and *in vivo* phototoxicity and photoclastogenicity tests should be described after examining their correlation with clinical data;
- The value or lack of value of photogenotoxicity testing for non-phototoxic agents should be clarified;
- A consensus on the need for photogenotoxicity testing should be developed

The above mentioned points are in-line with the recognized shortcomings of EMA's "Questions and answers on the note for guidance on photosafety testing".

3.3 Current status of the ICH process

According to ICH's final concept paper on photosafety evaluation of pharmaceuticals, the aspired timeline for publishing the Step 2 document initially was June 2012. It was further assumed that collecting and incorporating public comments of Step 3 consultation would take one year so that the final Step 4 document could be released in June 2013 - in case the EWG decided not to conduct a data survey setting the criteria to initiate the phototoxicity testing and to examine the correlation between non-clinical and clinical data, (or Step 4 might be reached even six months earlier).

In the end, the process was not as fast as defined in the concept paper. However, in November 2012, ICH topic S10 reached Step 2 of the process which was closely followed by Step 3 - the open consultation period - as of December 2012. After achievement of Step 2 of the ICH process, the draft guidance was released in all three ICH regions which particular details regarding timelines are as follows [32]:

- In Europe, the draft guideline was transmitted to CHMP in December 2012 and issued as EMA/CHMP/ICH/752211/2012; deadline for comments was March 2013.
- Japan's MHLW released the draft guideline on 28th December 2012 for consultation under reference PFSB/ELD and defined 28th February 2013 as deadline for comments.
- In the USA, the draft guideline was published by the FDA in the Federal Register on 4th February 2013 with reference to Vol. 78, No. 23, p. 7786-7. Deadline for comments was 21st March 2013.

Until finalization of this work, neither deviant information on the estimated timelines for achievement of Step 4 were published nor was the final Step 4 document made publically available. But since consultation was just finished in March 2013, the final Step 4 guideline on evaluation of photosafety testing may not be expected to be adopted by ICH's Steering Committee before end of 2013 [33]. Thus, in the following the current Step 2 guideline which was subject to public consultation will be analyzed since it represents the current status of ICH's progress in the topic S10.

3.4 The current Step 2 guideline on photosafety evaluation

The current Step 2 document is fragmented into a general part and a scientific section. The former refers to the scope and general considerations with regard to photosafety testing. It is mentioned that harmonization in the regions with regard to photosafety assessment is aimed at and that it shall provide substantial adjunct to the ICH guidances M3(R2) and S9 in matters of specific testing strategies. In addition, it is referred to "the principle of 3R (replacement/reduction/refinement)" which means that consideration should be given to *in vitro* alternative methods or clinical data for photosafety

assessment in order to reduce the use of animals [34]. The scope of the Step 2 guidance states that it applies to NCEs but not to peptides, proteins, antibody drug conjugates, oligonucleotides or marketed products (unless there is a cause for concern). The NCEs comprise pharmaceuticals for systemic administration, clinical formulations for topical application, as well as dermal patches, ocular products and products for photodynamic therapy.

The scientific section addresses the following issues:

- Factors to consider in the photosafety evaluation
- Non-clinical photosafety testing
- Clinical photosafety testing
- Assessment strategies

In the following, these major points of the Step 2 document of ICH topic S10 will be displayed in detail.

3.4.1 Factors to consider in the photosafety evaluation

This section addresses photochemical properties and points out conditions which make a NCE subject to photosafety evaluation. In particular, these are [34]:

- The absorption of UV or visible light between 290 and 700 nm together with a MEC above $1000 \text{ L mol}^{-1} \text{ cm}^{-1}$.
- The formation of ROS (e.g. singlet oxygen and superoxide) following irradiation with light of the UV/VIS spectrum since this is an indicator of phototoxic potential.

Additionally, it is emphasized that photostability testing alone does not serve as a validated source for deciding whether full photosafety evaluation is necessary since photodegradation in itself does not necessarily define a NCE as being phototoxic [34]. Furthermore the Step 2 guidance states that all tests related to assessment of photochemical properties should be subject to GLP/GMP regulations.

Next to photochemical properties, reference is made to tissue distribution since the concentration of a photoreactive NCE in tissue during light exposure will determine whether a phototoxic reaction can be expected. Being a pharmacokinetic issue, tissue distribution especially depends on plasma concentration, perfusion of the tissue, partitioning from vascular to interstitial and cellular compartments and residence times in sunexposed skin. Binding, retention, and accumulation of the NCE in the tissue yet are considered negligible – in this regard the example of melanin binding of compounds is given which does not present a photosafety concern *per se* although tissue retention or accumulation is likely to occur. It is rather recommended to conduct single-dose tissue distribution studies with animals assessed at multiple time points after dosing in order to

receive adequate information regarding tissue drug levels and the potential for accumulation [34].

According to the authors, the definition of tissue drug levels which might serve as threshold below which further photosafety assessment could be regarded not warranted is currently not delineated by significant data. However, depending on tissue levels of the compound and the general pharmacokinetic properties of the drug, discussions may be held on a case-by-case basis (e. g. for pharmaceuticals that will be administered by inhalation at low doses and are subject to low systemic exposure due to extensive biotransformation *in vivo*) [34].

The Step 2 guideline also refers to compounds with potential *in vivo* toxicity due to their mode of action (e. g. photodynamic therapy drugs) concluding that for these compounds other pharmacokinetic properties should be assessed or taken into consideration, namely distribution to internal and external tissues as well as tissue-specific half-lives. Furthermore, metabolites must not intrinsically be assessed with regard to photosafety because the common phase I and phase II biotransformation reactions do not trigger the formation of new chromophores. In addition, it is emphasized that the future S10 guideline and the outlined testing strategies are not intended to detect so-called indirect phototoxicity. The latter can be regarded as caused by certain pharmacological properties which might enhance susceptibility to certain light-induced effects such as immunosuppression and carcinogenesis and can be characterized well by non-clinical pharmacology tests or non-clinical toxicity tests [34].

3.4.2 Non-clinical photosafety testing

The Step 2 document emphasizes that all non-clinical photosafety tests should be subject to both high sensitivity (e. g. low frequency of false negatives will be obtained) and specificity (e. g. low amount of false negatives and false positives). Since the described *in vitro* and *in vivo* assays primarily focus on detection of potential phototoxicity, which might or might not translate into clinically relevant phototoxicity, the specificity of the chosen assay should always be considered [34].

Also the current lack of clarity with regard to applicable irradiation conditions, being a critical factor in photosafety testing, is addressed: Reference is made to standardized sunlight exposure conditions such as CIE-85-1989 [35]. The predominance of tests conducted with respect to irradiation conditions based on the UVA part (320 to 400 nm) of the assessed spectrum is pointed out because UVA is known to reach capillary blood whilst penetration of UVB light into human skin is mainly limited by the epidermis [34]. The latter makes reference to the fact that non-clinical photosafety testing for topical formulations should also comprise UVB light.

In case photoreactivity testing is part of the non-clinical photosafety evaluation, qualified assays with known sensitivity should be first choice. In this regard, the ROS assay is discussed although it is known for its low specificity with respect to false positives. However, 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 [34].

Regarding non-clinical *in vitro* phototoxicity testing, the Step 2 guidance recommends the 3T3 NRU-PT assay being the most appropriate *in vitro* screen for soluble compounds that are not exclusively UVB absorbers [34]. However, also the results of the above mentioned EFPIA survey are mentioned which indicate low specificity of this test with regard to false positives [28]. It is hence concluded that a positive result in the 3T3 NRU-PT should not serve as indicative of a likely clinical phototoxic risk, but rather as a hint for follow-up assessment [34].

Usage of the BALB/c 3T3 cell line is considered problematic for topical products that absorb in the UVB range or for systemically administered compounds that distribute to the epidermis as the mentioned test is sensitive to UVB and the recommended irradiation conditions involve the use of filters to attenuate wavelengths below 320 nm [34]. Thus, for topical products which absorb in the UVB range, other *in vitro* systems which feature higher tolerance to UVB should be considered [34]. The latter might be reconstructed human skin models that detect cell viability in the tissue preparation with compared to without irradiation. However, the current test models are not fully understood with respect to sensitivity, hence a case-by-case adjustment of certain assay conditions (e. g. testing higher strength formulations, increasing exposure time) should be done [34].

Regarding ocular phototoxicity tests, the ICH Step 2 guideline admits that there are no specific *in vitro* models available and that the predictive values of tests like the 3T3 NRU-PT assay or reconstructed human skin models for ocular phototoxicity is unknown.

If *in vivo* studies for non-clinical photosafety testing for systemically administered compounds are considered necessary, tests in the guinea pig, rat and mouse have been established so far. However, none of these tests is validated up to now [12, 34]. In this regard, the following criteria should be considered:

- Species selection: Here, irradiation sensitivity (e. g. minimal erythema dose), heat tolerance and performance of reference substances are of interest. The question whether pigmented or non-pigmented animals will be selected should not only be asked in connection with sensitivity (higher sensitivity in non-

pigmented skin) but also regarding a possible influence of melanin-binding in order to assure an appropriate exposure in target tissues [34].

- Duration: Problems like the possible accumulation of the test compound in relevant light-exposed tissues might lead to an increased sensitivity following repeated administration [34]. The same applies to repeated irradiation after each dose and should thus be considered. It is recommended to use single or repeated daily irradiations after dosing (around t_{max}) [34].
- Dose selection: A meaningful human risk assessment in line with the recommendations of ICH M3(R2) with regard to a maximum dose level should be done. Negative tests at a maximum dose do not require testing of lower doses. In case a positive result is anticipated, additional dose groups can support a NOAEL-based risk assessment [34].

Phototoxicity assessment in the retina is also shortly addressed. However, no specific recommendations regarding test models or irradiation conditions are given.

Wherever applicable, phototoxicity assessment of any compound should at least comprise the evaluation of time and dose dependency as well as establishment of the NOAEL [34]. In general, the performance of a chosen *in vivo* phototoxicity model should be demonstrated using suitable reference compounds. Irradiation during any *in vivo* study should be conducted at t_{max} which requires knowledge of the pharmacokinetic profile of the drug before designing any phototoxicity study. Photoallergy testing is generally not warranted for systemically administered compounds [34].

The above mentioned principles, relating to systemic drugs such as species selection, study duration, and irradiation conditions may also be recommended for dermal administration taking into consideration that the dermal formulation should be tested [34].

3.4.3 Clinical photosafety testing

Here, brief information is provided, saying that clinical photosafety testing should be subject to a case-by-case evaluation and that no general strategies could be recommended since there were various options for collecting human data.

3.4.4 Assessment strategies

In general, it is pointed out that there cannot be defined one global approach how photosafety assessment needs to be conducted – this is up to the drug developer and hence has to be estimated on a case-by-case basis. The Step 2 guidance also refers to the established stepwise approach to photosafety assessment, as mentioned in ICH M3(R2) [5, 34]: Prior to start of outpatient studies, an initial assessment of phototoxic potential

based on photochemical properties and pharmacological/chemical class is recommended. In addition, the distribution to skin and eyes can be evaluated in order to gain further information on the human risk as well as the need for additional testing. Finally, if appropriate, an experimental evaluation of phototoxic potential (nonclinical, *in vitro* or *in vivo*, or clinical) should be conducted before exposure of large numbers of subjects (phase III).

Since the testing recommendations are separated with regard to their individual route of administration, this sectioning will also be displayed in the following.

3.4.4.1 Testing of pharmaceuticals via systemic route

With regard to assessment of phototoxic potential, no testing is warranted if the compound exhibits a MEC below $1000 \text{ L mol}^{-1} \text{ cm}^{-1}$ between 290 and 700 nm, since no phototoxicity will be anticipated in humans [34]. However, it is mentioned that class-effects should be taken into account by evaluation of available phototoxicity data of class-related compounds. In case tests for photoreactivity or assessment of drug distribution to light-exposed tissues will be conducted, the outcomes may support an approach not to undertake further photosafety assessment. Else, non-clinical and/or clinical photosafety assessment of the compound is warranted [34].

The recommendations for experimental evaluation of phototoxicity center on the 3T3 NRU-PT in case an *in vitro* assay is chosen: This test should be the initial test for phototoxicity testing since its good sensitivity with regard to negative results will support the approach not to conduct further testing in case the outcome will be negative [34]. In case the 3T3 NRU-PT assay's result is positive, further testing *in vivo* (in animals or in humans) could be conducted in order to assess whether the potential phototoxicity identified *in vitro* correlates with an *in vivo* response [34]. It is emphasized that negatives in either animal testing, testing in humans or in the clinical setting supersede any positive results from *in vitro* tests.

In addition, it is pointed out that in the EU, animal testing should only be considered after having consulted an alternative validated *in vitro* test – no information is given for any of the other ICH regions. However, for compounds being insoluble, or other respective scenarios which make appropriate *in vitro* testing impossible, a phototoxicity assessment *in vivo* is considered possible.

3.4.4.2 Testing of pharmaceuticals via dermal route

The aforementioned relating to assessment of phototoxic potential (no testing is warranted for compounds with a MEC below $1000 \text{ L mol}^{-1} \text{ cm}^{-1}$ between 290 and 700 nm) also applies for pharmaceuticals via dermal route with the addition that this must be the case for both active substance and new excipients. As for systemic drugs, available data on the phototoxicity of class-related compounds shall also be consulted [34]. For

compounds with higher MEC values than $1000 \text{ L mol}^{-1} \text{ cm}^{-1}$, the EU and Japan accept negative results in photoreactivity tests such as the ROS assay, as justification for omitting further photosafety assessment. In the USA, negative results in photoreactivity assays do not generally preclude further clinical photosafety assessment of the to-be-marketed formulation [34]. In addition, it is demonstrated that tissue distribution must not be considered for these products.

Recommended *in vitro* tests for assessment of the phototoxicity potential comprise the 3T3 NRU-PT (for the API and excipients) and reconstructed 3D skin models (for the clinical formulation). It is emphasized that appropriate test conditions in order to guarantee sensitivity must be achieved. Differences between ICH regions are pointed out regarding negative results in reconstructed 3D skin models: Whereas in the EU and Japan, no further phototoxicity testing is recommended, the USA do not generally preclude further clinical photosafety tests in humans [34]. The same is stated with regard to negative outcomes of *in vivo* animal tests [34].

In addition to phototoxicity testing, photoallergy testing is recommended for formulations containing APIs or excipients exhibiting MECs greater than $1000 \text{ L mol}^{-1} \text{ cm}^{-1}$ which should be conducted with the formulation intended for marketing.

3.4.4.3 Testing of pharmaceuticals via ocular route

In general, also ocular products should be assessed regarding photosafety. As well as for systemic and dermal products, ocular drugs do not require phototoxicity testing if the compound's MEC is below $1000 \text{ L mol}^{-1} \text{ cm}^{-1}$ between 290 and 700 nm for the aforementioned reasons [34]. In case absorption is known for wavelengths over 400 nm only, and if the administration is as intraocular injection behind the lens there is low concern with regard to phototoxicity, since only light of wavelengths greater than 400 nm reaches the back of the adult eye.

Despite this general information, the Step 2 guidance does not recommend specific tests as regards *in vitro* or *in vivo* assessment of photosafety. However, it is mentioned that in the EU experimental assessment is recommended by means of *in vitro* approaches or *in vivo* studies using other routes of administration when the available data are considered insufficient for hazard identification [34]. In contrast, for the USA and Japan no recommendations for ocular products are available.

3.4.5 Further points for consideration

Genotoxicity testing is no longer recommended as a general approach in photosafety evaluation since its significance for clinically relevant enhancement of UV-mediated skin cancer has not been proven up to now.

Furthermore, it is referred to the importance of standardization of conditions for MEC determination with regard to the chosen solvent. In most cases, methanol might serve as adequate solvent; however, it is emphasized that also other UV/VIS spectra obtained under aqueous (pH adjusted) conditions may provide valuable information regarding differences in the shape of the absorption spectrum and in the MEC. If significant differences are present between measurements obtained in methanol *versus* pH-adjusted aqueous conditions, the MEC threshold of $1000 \text{ L mol}^{-1} \text{ cm}^{-1}$ cannot be used to support a definitive assessment [34].

Also the reduction of the maximum test concentration from 1000 to 100 $\mu\text{g/mL}$ is addressed since compounds without any significant cytotoxicity (under irradiation) up to this limit can be considered as being devoid of relevant phototoxicity [34]. Moreover, the allocation of compounds with PIF values between 2 and 5 and MPE values between 0.10 and 0.15 as being "probable phototoxic" (as per OECD) is questioned with regard to 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 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 [34].

Furthermore, it is referred to the above mentioned EFPIA survey and the concerns regarding the high rates of false positive results obtained in the 3T3 NRU-PT.

Last but no least, consideration should be given to the scenario if a systemically administered drug does not have higher tissue to plasma concentration ratios or does not accumulate in the skin. In this case, further assessment of the phototoxicity potential is generally not warranted in the USA, whereas this phenomenon is considered important in the EU and in Japan [34]. However, the presence of compound in skin is considered to be the critical factor in determining whether further testing is warranted and possibilities for omitting photosafety testing should be individually addressed at the relevant regulatory authorities [34].

4 Analysis of the current Step 2 guideline

The following sections individually analyze the single topics as listed in the current Step 2 guidance together with a qualitative assessment of the distinct subjects which have been included until now in ICH's new topic S10.

4.1 Compound's characteristics requiring photosafety testing

According to the current Step 2 guideline, the mentioned criteria making a compound subject to photosafety concerns are light absorption of the substance between 290 and 700 nm together with a MEC $> 1000 \text{ L mol}^{-1} \text{ cm}^{-1}$, generation of ROS following absorption of UV/VIS light and the sufficient distribution to light-exposed tissues. If one of these criteria is not met, there is no evidence for any photosafety concern of the particular compound.

The approach of the incorporation of the MEC value greater than $1000 \text{ L mol}^{-1} \text{ cm}^{-1}$ in the considerations of whether compounds exhibit a phototoxic potential is not completely new since it is based on the data from Henry et al. of 2009 which already found entry in the scientific discussion and in the EMA's Q&A [14, 26]. However, the inclusion of the mentioned MEC value as a relevant criterion for light absorbance to initiate photosafety testing seems very reasonable and will lead to a reduction of phototoxicity testing of compounds, especially in the EU and Japan [31]. In addition, the fact that there is provided information on the critical point of MEC determination with regard to standardized test conditions (e. g. choice of solvent) can be regarded an advancement triggering comparability and significance of MEC values.

In contrast, the incorporation of photoreactivity testing (formation of ROS) in an official guideline as an indicator for phototoxic potential is new. Although the named ROS assay to some extent lacks specificity, negative results will indeed provide further evidence that a compound is unlikely to be phototoxic.

Another point which was not present in the older European guidelines on photosafety testing is the importance of pharmacokinetic parameters with special regard to tissue distribution and the formation of metabolites. Up to now, the bioavailable amount of a compound in the skin or the eyes at levels sufficient to cause photoirritation was only mentioned in the FDA guideline of 2003 which considers the compound's level of exposure as a critical parameter in matters of photosafety testing [13]. Probably, this led to the fact that tissue distribution is referred to quite in detail in the current Step 2 guidance: It is concluded that a compound's level in tissue is a critical parameter with regard to photochemical reactions. In case of long residence times, the risk for

phototoxic reactions will increase. It is recommended and regarded sufficient to conduct a single-dose tissue distribution study with multiple time-points. In addition, the phenomenon of melanin binding alone is pointed out as not being a photosafety concern by itself. Regarding the formation of metabolites, brief information is given saying that metabolites in general do not create new chromophores and hence do not require separate photosafety evaluation. The provided information on pharmacokinetic problems provides more insight and clarity with regard to harmonization of initiation of photosafety testing.

All in all, the addressed points contribute to harmonization of photosafety evaluation for the ICH regions in terms of compound's characteristics serving as triggers for initiation of photosafety testing.

4.2 Non-clinical photosafety testing

General information on non-clinical assays is provided as regards the requirements for sensitivity and specificity. This information of the Step 2 document can be read in conjunction with the recent findings and publications of pharmaceutical industry which are related to the comparable high frequency of false positive results of the 3T3 NRU-PT assay [28]. Thus, the latest concerns of pharmaceutical industry regarding the occurrence of false positive *in vitro* results (which might trigger follow-up studies *in vivo*), have been recognized. However, as can be seen in the following section referring to *in vitro* assays, no specific recommendations regarding applicable alternatives for the 3T3 NRU-PT are made.

It is generally emphasized that negative results do not warrant any further photosafety evaluation. In addition, the importance of standardized irradiation conditions is pointed out which is an advancement towards harmonization of test conditions in non-clinical photosafety evaluation.

4.2.1 *In vitro* assays

The Step 2 guideline still refers to the 3T3 NRU-PT assay as the most appropriate *in vitro* test for soluble compounds being not exclusively UVB absorbers. Although the recent concerns of pharmaceutical industry as regards the sensitivity of this assay are recognized, the 3T3-NRU PT's sensitivity remains unquestioned since negative results are related to a very low probability of the tested compound being phototoxic. This might be supported by the fact that this assay is still the only validated *in vitro* test for phototoxicity. However, the Step 2 guidance admits that the original OECD protocol of the 3T3 NRU-PT was not validated specifically for pharmaceuticals which again might elucidate its lower specificity for pharmaceuticals. Hence, it is recommended to take positive results as a flag for follow-up evaluation. So far, the aforementioned is neither

advancement (since no validated alternatives can be recommended despite the recognized shortcomings of this test) nor a step backwards (since the 3T3 NRU-PT assay is still the only validated phototoxicity test). In this regard, the naming of the conducted retrospective analysis of the 3T3 NR-PT for drugs can at least be regarded a step in the right direction [12, 34]: It is recommended to reduce the maximum test concentration from 1000 to 100 µg/mL and to remove the probable phototoxicity PIF and MPE criteria. Furthermore, the retrospective analysis led to scrutinization of the relevance of OECD's term for compounds as being "probable phototoxic" under the conditions as mentioned before.

The naming of reconstructed human skin models (with the presence of stratum corneum) for testing of dermal formulations can be regarded a progress in the development of photosafety evaluation. This is a reasonable approach since the 3T3 NRU-PT is not appropriate for topically-applied formulations since the chemical must dissolve in an acceptable medium (i. e. methanol) at a relevant concentration which is hardly achievable for most of dermal formulations [12]. Furthermore, reconstructed human skin models are more sensitive to UVB which should be a criterion for testing of topical formulations according to the current Step 2 guideline. Although these models are considered useful tests since they may be applicable for the testing of various types of topically administered materials, specific test conditions or types of tests are not mentioned. It is solely referred to the fact that some of the models developed to date may lack sensitivity compared to the situation *in vivo*.

Ocular tests are mentioned in the current Step 2 guideline which can be regarded progress with regard to provision of information in matters of phototoxicity tests for ocular products. However, it is admitted that at present no specific *in vitro* models are available and that negative results in the 3T3 NRU-PT assay or reconstructed skin models might suggest a low risk but that the predictive value for ocular phototoxicity is still unknown. Hence, the practical value of this information is rather questionable.

4.2.2 *In vivo* assays

With regard to *in vivo* phototoxicity assays, it is summarized that to date, there are no formally validated *in vivo* tests for evaluation of phototoxicity. Thus, no standard study design can be recommended. Nevertheless, general reference points for consideration in order to have available best practice information are displayed (as described under 3.4.2). The provided general information contributes to harmonization of testing conditions and endpoints to consider as regards relevant *in vivo* assays. Yet, further data should be collected and assessed in order to provide standard recommendations for (validated) *in vivo* phototoxicity assays.

In matters of photoallergy testing, it is stated that testing is only applicable for cutaneously administered products but that no non-clinical tests are recommended for regulatory purposes since the predictivity of non-clinical models is unknown (no formal validation available). No tests must be conducted for systemic drugs. This distinction can be regarded progress in the development of photosafety evaluation.

For ocular products, the situation as presented in the current Step 2 guideline is similar to that of *in vitro* assays: No standardized non-clinical *in vivo* tests for the assessment of phototoxicity of ocular products are available. Thus, the collection of new data which might serve as a basis for further recommendations is desirable.

4.3 Clinical photosafety testing

For this discipline of photosafety assessment, no specific information is provided by the current Step 2 document. It is only stated that there exist various options for the collection of human data and that any precise strategy must be developed on a case-by-case basis [34]. This information is neither definite nor does it give specific hints for drug developers on how to conduct clinical tests for photosafety assessment in order to obtain significant and comparable data which will serve as reasonable benefit for regulatory purposes as regards registration of pharmaceuticals.

4.4 Assessment strategies

In case the conditions regarding phototoxic potential as outlined before (light absorption, photoreactivity and significant presence in light exposed tissue), will apply to a compound, the Step 2 guideline arrives at the conclusion that photosafety testing is warranted. However, no predefined assessment strategies for photosafety testing are recommended. Instead, it is solely referred to the availability of both non-clinical and clinical tests. It is further mentioned that in case any of these will reveal negative results in phototoxicity testing, no further phototoxicity testing is required whereas negative *in vivo* tests supersede positive *in vitro* results. The emphasis of the latter can be regarded as advancement compared to the former guidances (EMA, FDA, etc.) which do not clearly state this aspect. Hence, more clarity is provided as regards the valuation of *in vivo* versus *in vitro* results.

Class effects, based on available data, should be considered in general, which seems to be a reasonable approach.

Also the mention of timing of photosafety testing relevant to clinical development is appreciated since it eliminates uncertainties with regard to the planning and conduct of photosafety tests.

4.4.1 Pharmaceuticals for systemic use

It is stated that for assessment of the phototoxic potential the light absorbance criteria as mentioned before have to apply. In addition, the consultation of data gained from photoreactivity tests or tissue distribution studies may support the decisions of whether further photosafety testing is warranted, or not. Hence, this can be regarded an unambiguous statement. Contrarily, the situation regarding recommendations of tissue distribution data as trigger for further testing is quite unclear since there are still differences between the single ICH regions: Whereas the presence of the compound itself in the skin is classified as being critical in the EU and Japan, the USA require no further assessment if the tissue to plasma concentration ratio is low or if the compound will not accumulate in the skin. Hence, for now this issue cannot be considered as harmonized.

Furthermore, it is not harmonized which test represents the current state-of-the-art in phototoxicity evaluation. The decision on which test for phototoxicity evaluation should be conducted, is in general left up to the drug developer – with one exemption: In the EU, the 3T3 NRU-PT assay is recommended since in general a validated *in vitro* test should be used prior to taking animal testing into consideration.

Indeed, it can be appraised as positive that negative results of tests for phototoxicity in general do not require further testing. Opponent to this is the mentioning of various options for follow-up assessment in case an *in vitro* test for phototoxicity reveals positive results. Here, it is up to the drug developer whether to conduct additional phototoxicity studies in animals or to address the photosafety risk in a clinical study. With regard to significance and reproducibility of photosafety data, one homogeneous approach on follow-up testing would have been desirable.

4.4.2 Pharmaceuticals for dermal use

For dermal products, the characteristics regarding phototoxic potential for either the active substance or any of the new excipients are sufficient to make a compound a candidate to photosafety evaluation. Furthermore, it is consensus that the 3T3 NRU-PT assay should serve as *in vitro* test for the active substance and/or the new excipients individually, whereas reconstituted 3D skin models are designated for the *in vitro* evaluation of clinical formulations. If appropriate *in vitro* models are not available, the collection of *in vivo* data in animals or humans is considered a possible approach.

Certainly, there are some discrepancies in recommendations concerning both, negative results in 3D skin models and *in vivo* animal studies. While negative results in the named tests are in principle considered an indicator for a low phototoxic potential of the formulation, different follow-up approaches are referred to for the single ICH regions: Whereas in the EU and Japan the mentioned negative results would serve as sufficient

evidence that no further testing for phototoxicity is necessary, in the USA further clinical photosafety assessment using the formulation intended for marketing cannot be precluded. Furthermore, regional differences are also mentioned as regards negative results of photoreactivity tests (e. g. the ROS assay) for substances with a MEC > 1000 L mol⁻¹ cm⁻¹. Here the USA again do not preclude further testing, whereas the EU and Japan follow the approach that non-clinical *in vitro* or *in vivo* data could support the decision that no further testing might be warranted. Hence, further harmonization between the ICH regions regarding assessment strategies for dermal products is definitely worthwhile.

It is welcome that photoallergy testing is mentioned in matters of pharmaceuticals for dermal use as being warranted in general (in case the criteria for designating the ingredients as subject for photosafety testing are met) in addition to phototoxicity testing. Furthermore, clarity is provided by stating that the formulation intended for marketing shall be used for clinical photoallergy evaluation and that there is the possibility of conducting this study during phase III of the development program.

4.4.3 Pharmaceuticals for ocular use

With respect to ocular products, the provided information in the current Step 2 guidance is on the one hand welcome since they recommend testing for relevant substances, in general. On the other hand, the given information is rather non-specific. For example, no *in vitro* test for the assessment of phototoxicity can be recommended since their reliability for ocular products is unknown. Moreover, it is admitted that no standardized *in vivo* approaches can be recommended. In fact, it is emphasized that basic principles of phototoxicity assessment apply which are considered to be self-evident.

Yet there are discrepancies between ICH regions' approaches, also with regard to ocular products: In the USA and Japan, no specific recommendations in matters of experimental phototoxicity assessment are available. Contrarily, in the EU experimental assessment is generally recommended, using *in vitro* or *in vivo* approaches with other routes of administration in case that the available data are insufficient for hazard identification.

Thus, a harmonized approach in photosafety evaluation of ocular medicinal products needs to be developed.

5 Conclusion and outlook

In order to recall its scope, the identified issues of the underlying concept paper, which should be subject to improvement by incorporation in the final guideline, are shortly repeated in the following [31]:

- Definition of both, criteria of light absorbance and skin exposure to initiate phototoxicity testing;
- Definition of criteria of tissue levels achieved/retained in the skin and eyes;
- Reach a consensus on the triggers for photosafety testing;
- Assessment of the need for the photosafety testing of drug metabolites;
- Description of the values of several *in vitro* and *in vivo* phototoxicity and photoclastogenicity tests;
- Clarification of the value of photogenotoxicity testing for non-phototoxic agents;
- Provision of a consensus on the need for photogenotoxicity testing.

These issues will be assessed in the upcoming sections. Furthermore, consequences for the daily practice of both, drug developers in pharmaceutical industry and assessors in regulatory authorities will be highlighted.

5.1 Achievements of the ICH Step 2 “Guidance on photosafety evaluation”

As summarized before, the Step 2 guidance names the following criteria which require the initiation of phototoxicity testing: Light absorption of the substance between 290 and 700 nm together with a MEC $> 1000 \text{ L mol}^{-1} \text{ cm}^{-1}$, generation of ROS following absorption of UV/VIS light and the sufficient distribution to light-exposed tissues. Thus, the ambition of defining criteria of light absorbance to initiate phototoxicity testing can be regarded accomplished.

The definition of skin exposure to initiate phototoxicity testing cannot be considered fully resolved. This is due to the fact that it is indeed mentioned that sufficient distribution of the compound to light-exposed skin is deemed necessary to initiate phototoxicity testing but no specific criteria are defined. As an example, it is recommended to conduct a single-dose tissue distribution study as outlined above, but no definite endpoints are referred to.

The aforementioned issue is closely associated to the aim of defining criteria for tissue levels achieved/retained in the skin and eyes. The Step 2 guideline concludes that no generic threshold for all compounds can be recommended because of the lack of

delineating data. Thus, the collection of significant data which might support a certain threshold is essential for achievement of the desired harmonization as regards a generic threshold for tissue levels in skin and eyes.

What has definitely been achieved is the provision of one standard definition of triggers for photosafety testing in ICH regions which is closely connected to the criteria requiring phototoxicity testing as outlined before (if one of those is not met, the compound in question will not present a photosafety concern).

Furthermore, the need for photosafety assessment of drug metabolites has been addressed explicitly in the Step 2 guideline, concluding that there is no need for testing of drug metabolites.

The description of the values of available *in vitro* and *in vivo* phototoxicity and photoclastogenicity tests in connection with their individual correlation with clinical data can be regarded partly achieved:

- The 3T3 NRU-PT, which was and still is subject to controversial discussions within pharmaceutical industry as regards its low sensitivity, has been classified as first choice for evaluation of *in vitro* phototoxicity for soluble pharmaceuticals. The fact that it is still the only validated *in vitro* assay for phototoxicity evaluation supersedes its alleged deficiencies in matters of false positive results since a negative result in this assay is still regarded significant evidence for the absence of photosafety concerns.
- Reconstructed human skin models were included in recommendations for *in vitro* testing of topically-applied formulations. However, further data are needed to give more precise guidance for the conduct of these tests and for the evaluation of the collected data in order to obtain more significance.
- Photoreactivity tests were also included in the framework of *in vitro* photosafety assessment since they represent one of the criteria of the newly defined triggers for phototoxicity testing. This is surely also due the publication by Lynch et al. which indicates that there is a correlation between photochemical reactivity assays and phototoxic liability *in vitro* and that those assays may serve as appropriate trigger for photosafety testing [28].
- Although no specific *in vivo* tests for phototoxicity are recommended, there are however named several important points for consideration when planning and conducting these tests.
- Photoclastogenicity tests are not referred to in the Step 2 guideline at all because photogenotoxicity testing is generally not considered useful in the framework of photosafety evaluation.

The last named matter picks up the problem regarding the need for consensus of photogenotoxicity testing as addressed in the concept paper. Since the Step 2 guideline

considers photogenotoxicity testing, and as a consequence also photocarcinogenicity testing, insignificant in the context of photosafety assessment, no further information except the recommendation of photogenotoxicity and photocarcinogenicity testing being negligible, is provided.

Next to the aforementioned, the Step 2 guideline supports a tiered approach in photosafety evaluation which is welcome since it facilitates photosafety testing within pharmaceutical industry and eliminates present uncertainties due to the fact that the guidelines in the EU and USA are currently not consistent in matters of whether all endpoints should be tested in parallel (according to EMA's "Note for guidance on photosafety testing" [11]) or if a tiered approach will be sufficient (as recommended in FDA's "Guidance on photosafety testing" [13] and EMA's "Q&A on the note for guidance on photosafety testing" [14]). Although, this is not explicitly outlined in the Step 2 document, the following statements underline this fact: That all of the above mentioned criteria must be met in order to initiate phototoxicity testing; that photoallergy testing is only warranted for dermal products, meeting the defined criteria; that *in vivo* results in general supersede *in vitro* tests together with the general information that photogenotoxicity testing is considered insignificant.

Last but not least, the fact that timing of photosafety testing relative to clinical development is addressed in the Step 2 guideline is also an achieved harmonization since so far information on the appropriate timing of photosafety testing has only been available in ICH M3(R2) and ICH S9 (for anticancer pharmaceuticals).

5.2 Photosafety issues requiring further advancement

In general, the summarized situation in terms of achievements of the ongoing ICH process regarding the new topic S10, provides valuable adjunct to the available guidances M3(R2) and S9 in the context of photosafety evaluation of pharmaceuticals. However, there are some issues which although having been addressed in the Step 2 guideline should be still subject to further refinement or improvement, respectively. These points of interest are displayed in the following.

Further harmonization is desired regarding achievement of consistent standards in all three ICH regions. This is currently not reached since the Step 2 guidance mentions divergent approaches being applicable for the independent regions at several stages: For example, it is stated in the introduction that animal testing should only be considered in case no validated *in vitro* model is available but later (section 5.1.2 Experimental evaluation of phototoxicity), this approach is explicitly mentioned for the EU, only. Taking into account the aforementioned, this is quite contradictory and does not contribute to reduce animal testing at all or even to support the establishment of validated *in vitro* tests.

Moreover, the recommendations in the context of non-clinical *in vitro* and/or *in vivo* data for dermal products mirror the above mentioned disharmony between approaches in ICH regions. Here, both the EU and Japan accept negative results as sufficient evidence for lack of photosafety concerns whereas the USA require further clinical assessment with the formulation intended for marketing. The same applies to the provided information on tissue distribution data as trigger for follow-up testing since the EU and Japan consider the presence of the compound itself in the skin as critical, whereas the USA require no further assessment if the tissue to plasma concentration ratio is low or if the compound will not accumulate in the skin. The named inconsistencies are again mirrored by the provided information regarding ocular products since there are no specific recommendations made for experimental assessment for the USA and Japan. At least, it is referred to the European approach of consulting appropriately conducted *in vitro* or *in vivo* studies for hazard identification, if deemed necessary. Considering the aforementioned differences between ICH regions, it can be concluded that the current Step 2 guideline to some extent fails to provide international standards and to harmonize the photosafety assessment for pharmaceuticals in order to reduce the likelihood that substantial differences in testing requirements and data interpretation will exist among regions [34]. Hence, further harmonization is indeed eligible.

Next to the need for a more generalized approach in photosafety testing which means that it should be aimed at implementation of one single approach for each category of photosafety evaluation (e. g. non-clinical testing for dermal products, etc.) being applicable in all ICH regions, also the provision of meaningful flow charts representing the outlined assessment approaches might be useful. Therefore, the presence of such flow charts in the final guideline is eligible as well. A general concept for a possible flow chart underlining the recommendations of the Step 2 guideline is provided in attachment 7.

Another issue which is discussed controversially within academia and pharmaceutical industry is the value of the 3T3 NRU-PT assay and its predictivity regarding obtained *in vitro* data and their clinical relevance for *in vivo* (in animals or in humans). The Step 2 guidelines admits that false positive results are known to be sustained for this tests, however the sensitivity of the assay remains unquestioned. Since this is also (next to the latest data obtained as per revised OECD protocol) supported by the fact that this assay is still the only validated *in vitro* test, efforts should be made to generate significant data to validate further *in vitro* tests for phototoxicity. Preferably, this would result in an appropriate alternative to the 3T3 NRU-PT being accepted in all ICH regions so that a termination of the ongoing discussions could be achieved. Even if this will not be reached, further data on alternative *in vitro* tests are necessary anyhow in order to gain

more insight and to develop and advance new approaches in photosafety evaluation of pharmaceuticals.

5.3 Implications for daily practice in photosafety evaluation

Although the current Step 2 guideline on photosafety evaluation of pharmaceuticals does not yet represent the final ICH guideline, its up-to-date status, as analyzed above, has certain implications for daily practice in photosafety evaluation. This will be addressed for both, affairs in pharmaceutical industry and regulatory bodies.

5.3.1 Pharmaceutical industry

Due to the grown complexity of global legal and regulatory obligations in the field of drug development and drug registration, pharmaceutical industry is obliged to re-evaluate current development strategies with respect to more effective go/no-go decisions during drug development as outlined in the beginning of this thesis. Since duplicate work and the lack of clarity as regards single topics during drug development result in loss of time and hence money, harmonization processes triggered by stakeholders such as ICH are always appreciated. Thus, the up-to-date Step 2 guideline on the new topic S10 in general provides valuable assistance regarding the establishment of refined conditions for the design and conduct of relevant photosafety tests.

In particular, the achievements as displayed in section 5.1 will support the aim of reducing development times since certain tests (i. e. tests for photogenotoxicity and photocarcinogenicity) are no longer recommended as per the current Step 2 guideline. Since this makes clear that a tiered approach is recommended for photosafety testing, pharmaceutical industry will be content because this was already the favored approach when EMA adopted its Q&A [14].

Also the provision of defined criteria making a compound subject to a photosafety concern, contribute to improve certainty of whether NCEs should be tested or not. In this regard, also the provision of explicit information on appropriate timing of photosafety testing enhances clarity. Therefore, improvements of significance and increased comparability of photosafety data in the ICH regions will surely be a result of adopting the final ICH guidance on photosafety evaluation into pharmaceutical industry's daily practice. Furthermore, the forecited will contribute to an important gain of knowledge in terms of daily practice in photosafety evaluation and to a noteworthy reduction of duplicate work.

Next to the above-named positive aspects of the new topic S10 for pharmaceutical industry, some of the issues which have not yet been harmonized (e. g. the differences

in approaches related to the collection of non-clinical *in vitro* or *in vivo* data for dermal products, the divergent recommendations for testing of ocular products, the differences in the value of avoiding animal tests, etc.) will surely challenge non-clinical development of companies which follow global development approaches.

Taking the new ICH guidance on photosafety evaluation into consideration, a general challenge with respect to daily practice in non-clinical drug developments will be the harmonization of results which have been achieved until now with the data which will be collected in the future. Although, there will probably be defined transition periods until exclusively data according to ICH's new approach in photosafety testing will be accepted for registration purposes of NCEs, this aspect will surely bring about some extra work within pharmaceutical industry. However, there is always the general possibility – adequate justification given – to propose divergent approaches apart from those named in up-to-date guidelines.

Based on the latest publication from Lynch et al. [24, 28], it is evident that pharmaceutical industry hoped for a clear statement in the final ICH guideline regarding a recommendation of an alternative test for the 3T3 NRU-PT assay as per its above described shortcomings (e. g. the lack in sensitivity with respect to false positive results). But since the current Step 2 guidance states that there are insufficient data available of possible alternative *in vitro* or *in vivo* tests and that the 3T3 NRU-PT is still the only validated assay for *in vitro* phototoxicity testing, the usage of the same must be continued. However, this should be a motivation for pharmaceutical industry to collect representative and significant data for other test models (e.g. reconstructed human skin models, etc.) in order to contribute to respective validation so that alternative tests can find entry in standard recommendations for photosafety evaluation of pharmaceuticals in the future.

5.3.2 Regulatory bodies

The assessment of the non-clinical part of submitted dossiers for marketing authorizations in regulatory bodies will be facilitated as soon as the final ICH guideline on photosafety evaluation of pharmaceuticals will be available. This is due to the fact that the achievements of the current Step 2 guideline, as displayed above, will contribute to certainty and clarity regarding which conditions must be met for photosafety testing and regarding the requirement to apply certain tests. Hence, in case companies intend to omit specific tests for photosafety evaluation although they are recommended in the final guideline, this must be based on an adequate and science-based justification. Otherwise, regulatory bodies are in the safe position of pointing to the generally acknowledged rules of science (i. e. the final ICH guideline on photosafety evaluation).

Furthermore, regulatory bodies will benefit from the receipt of all the collected data from pharmaceutical industry being based on the same conditions because this will result in a pool of significant and comparable data as regards non-clinical photosafety evaluation. On the one hand, this supports the gain of knowledge within regulatory environment which will lead to the reduction of duplicate assessment of data and could also contribute to the release of further guidances being related to photosafety testing. On the other hand, the data to be obtained according to the harmonized ICH approach might be useful to conduct a survey on specific new findings in order to review the new recommendations and hence to achieve further improvements within the field of non-clinical photosafety evaluation of pharmaceuticals. The latter of course implies that agreements from the relevant companies have been obtained in order to make their individual data publicly available.

An issue which is not only desired by pharmaceutical industry but also by regulatory bodies dealing with worldwide applications for marketing authorization is the achievement of further harmonization of current divergent approaches in the single ICH regions as mentioned in the current Step 2 guideline (e. g. the differences in approaches of non-clinical *in vitro* or *in vivo* data for dermal products, the divergent recommendations for testing of ocular products, the differences in the value of avoiding animal test, etc.). This is eligible since different recommendations for different ICH regions might give the impression that – especially with regard to regulatory requirements and the related assessment of the data by authorities – specific recommendations would not be of the same value. This should be avoided.

5.4 Outlook

The latest publications [14, 24, 26, 28], workshops and industry surveys indicating the above mentioned shortcomings regarding the currently available guidance documents in the EU and USA underline the desire for one harmonized approach in photosafety testing. The final Step 4 guideline is expected by end of 2013 or beginning of 2014 and will ideally succeed in eliminating the yet existing different approaches related to specific issues (as analyzed above) between the EU, Japan and the USA which are to some extent still present in the Step 2 guideline.

All in all, the ongoing process within ICH, i. e. implementation of a tripartite guideline for harmonization of photosafety evaluation is highly appreciated by both, pharmaceutical industry and regulatory bodies. Whether the final guideline will fulfill the expectations of providing substantial benefit for “real life” in drug development remains to be seen. However, the identified achievements up until the current Step 2 guideline are promising and there is no doubt that more significance of global data on photosafety evaluation will be obtained.

In this regard, the continuous collection of data in order to improve the currently available test models for both, *in vitro* and *in vivo* photosafety testing is eligible. Furthermore, this also contributes to the desired development of further validated *in vitro* tests in addition to the 3T3 NRU-PT, as well as to the advancement of significant *in vivo* models with defined endpoints.

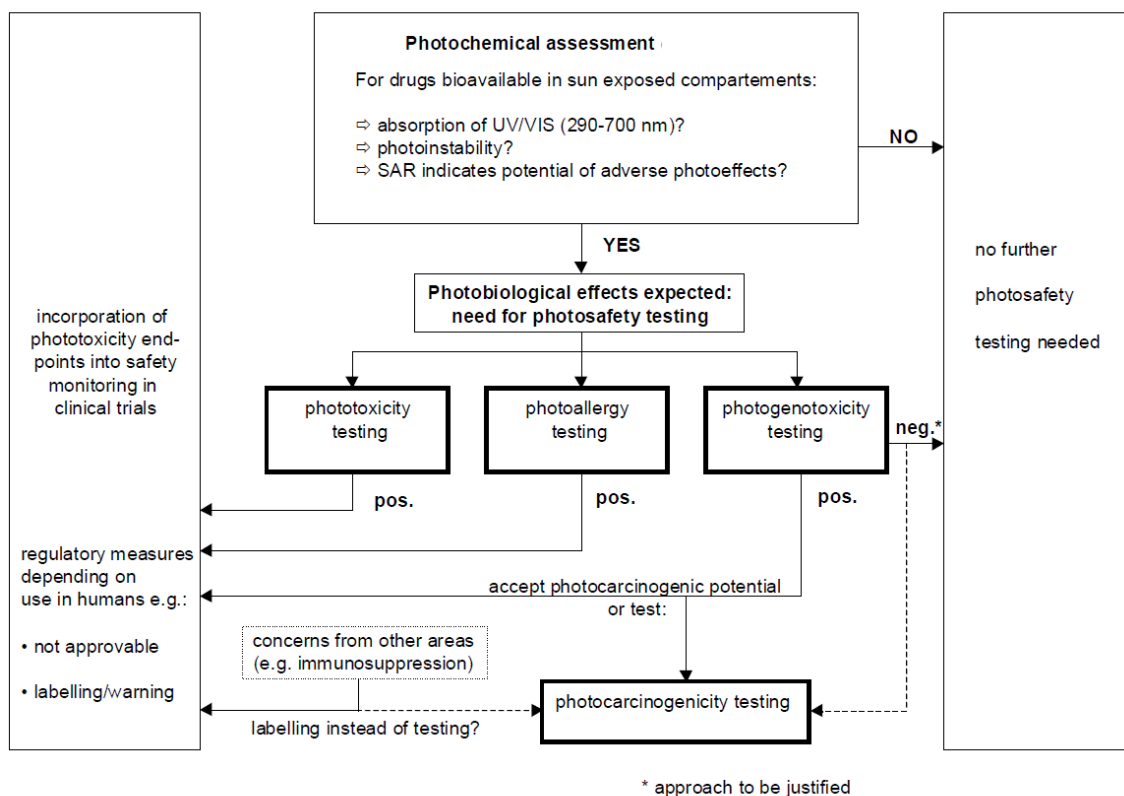
Abstract

Photosafety evaluation of pharmaceuticals is an integral part of non-clinical toxicity testing during drug development. However, first steps towards a defined regulatory environment for photosafety testing were made during the 1990s when EMA adopted a guideline on local tolerance testing [10] which provided brief information on photosafety evaluation until the release of EMA's "Note for guidance on photosafety testing" [11] in 2002. Almost at the same time, FDA's CDER published its "Guidance for industry on photosafety testing" in 2003 [13].

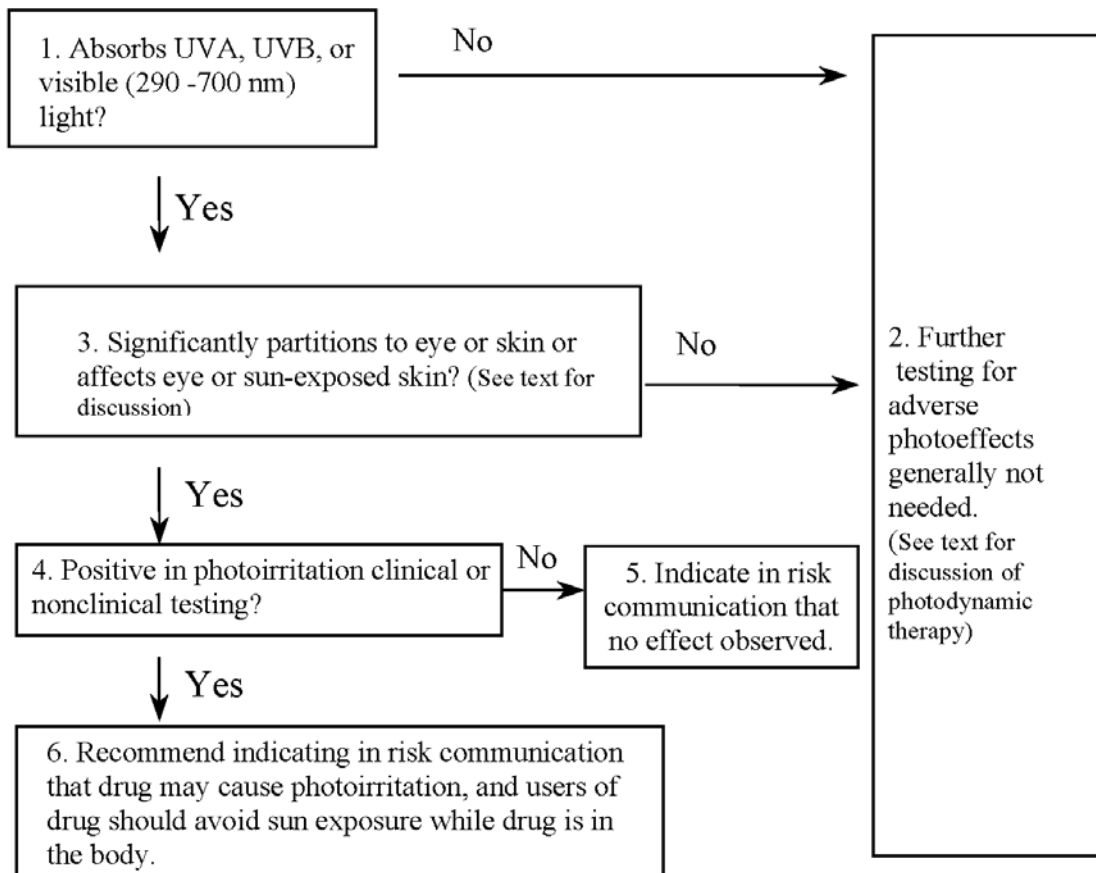
Since the release of the named guidances, pharmaceutical industry, regulatory authorities as well as academia generated data and increased their knowledge in the field of photosafety testing. This resulted in the revealing of certain shortcomings in the up-to-date approaches which comprise the lack of clarity in certain scientific questions related to photosafety evaluation (i. e. the conduct of a parallel testing approach *versus* a tiered approach, the lack of specificity of the 3T3 NRU-PT, the general lack of validated *in vitro* alternatives, the need for defined standards of *in vivo* tests as well as the question of whether tests for photogenotoxicity (and photocarcinogenicity) should be a pivotal part of any photosafety evaluation program). Furthermore, also the missing harmonization between ICH regions as regards testing strategies was identified as a subject to advancement of photosafety evaluation since global development programs of pharmaceutical companies require standard approaches in order to avoid duplicate work and to shorten development times.

A specific step towards a new, harmonized approach in the regulatory field of photosafety testing was ICH's consensus in 2010 to start with its work on an independent guidance for photosafety testing, namely ICH S10: "Photosafety evaluation of pharmaceuticals". Until finalization of this master thesis, the ICH process reached Step 2. The related draft guideline is comprehensively displayed and analyzed with regard to the above mentioned shortcomings for both, pharmaceutical industry and regulatory authorities.

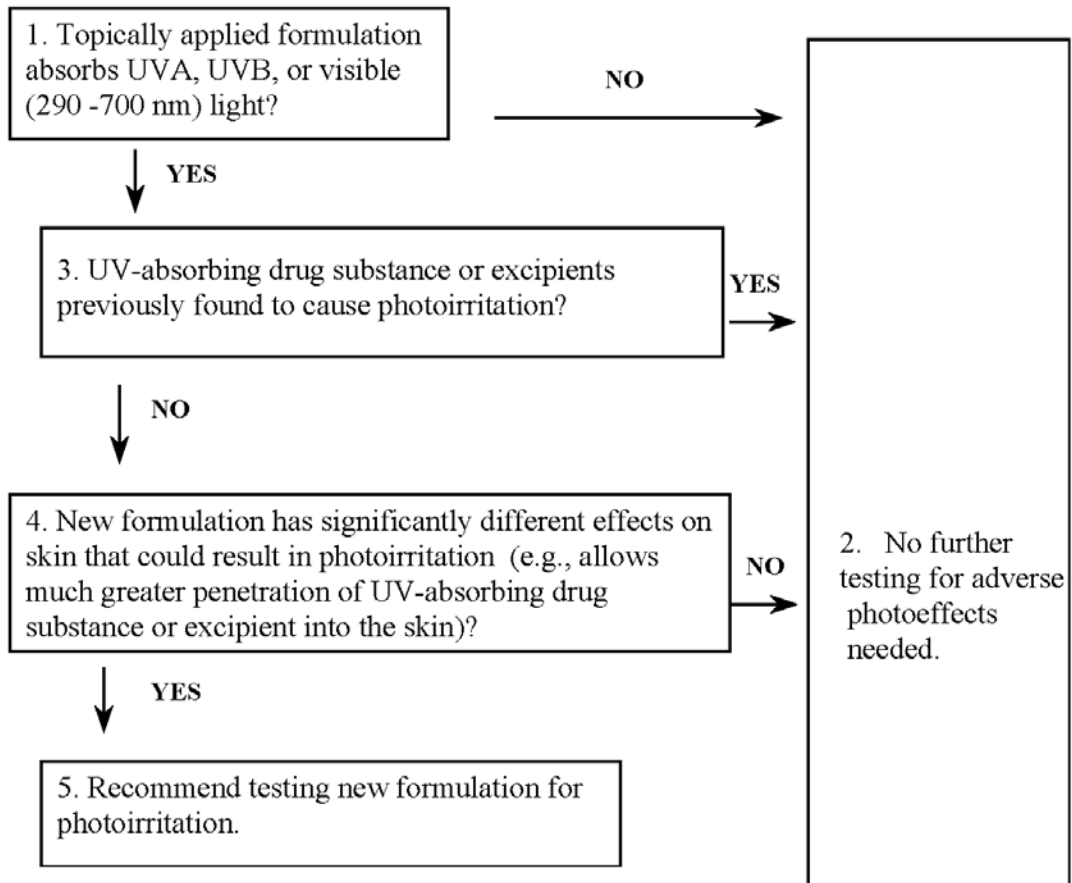
Attachment 1: Flow chart on the parallel approach in photosafety testing, according to CPMP/SWP/398/01



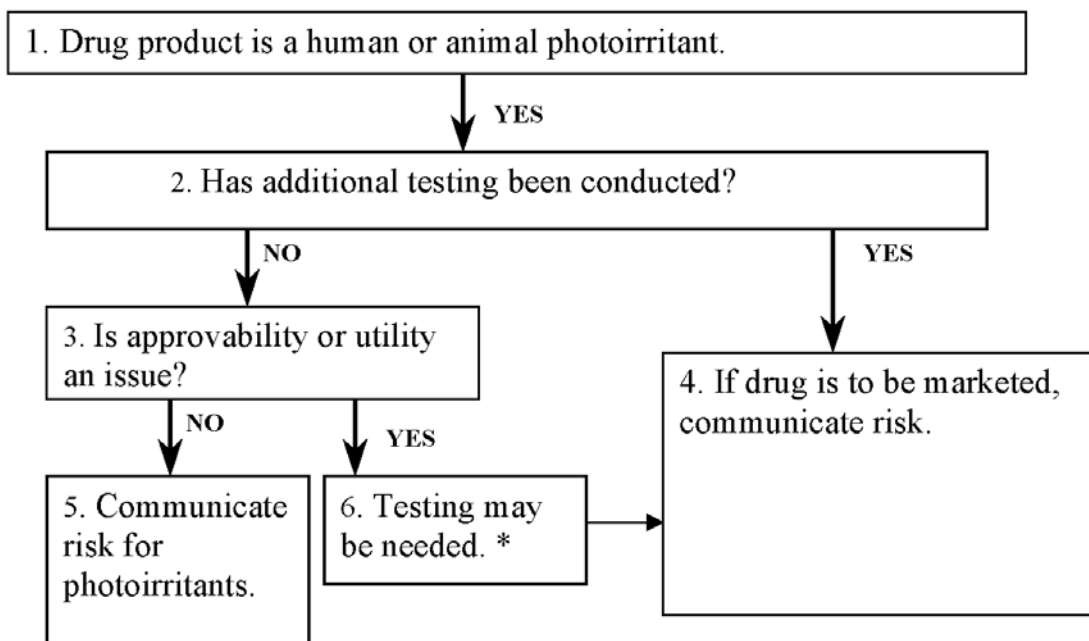
Attachment 2: Flow chart on the conduct of short-term photochemical irritation, according to FDA's guidance for industry on photosafety testing



Attachment 3: Flow chart on the conduct of short-term photochemical irritation after reformulation of a topical formulation, according to FDA's guidance for industry on photosafety testing

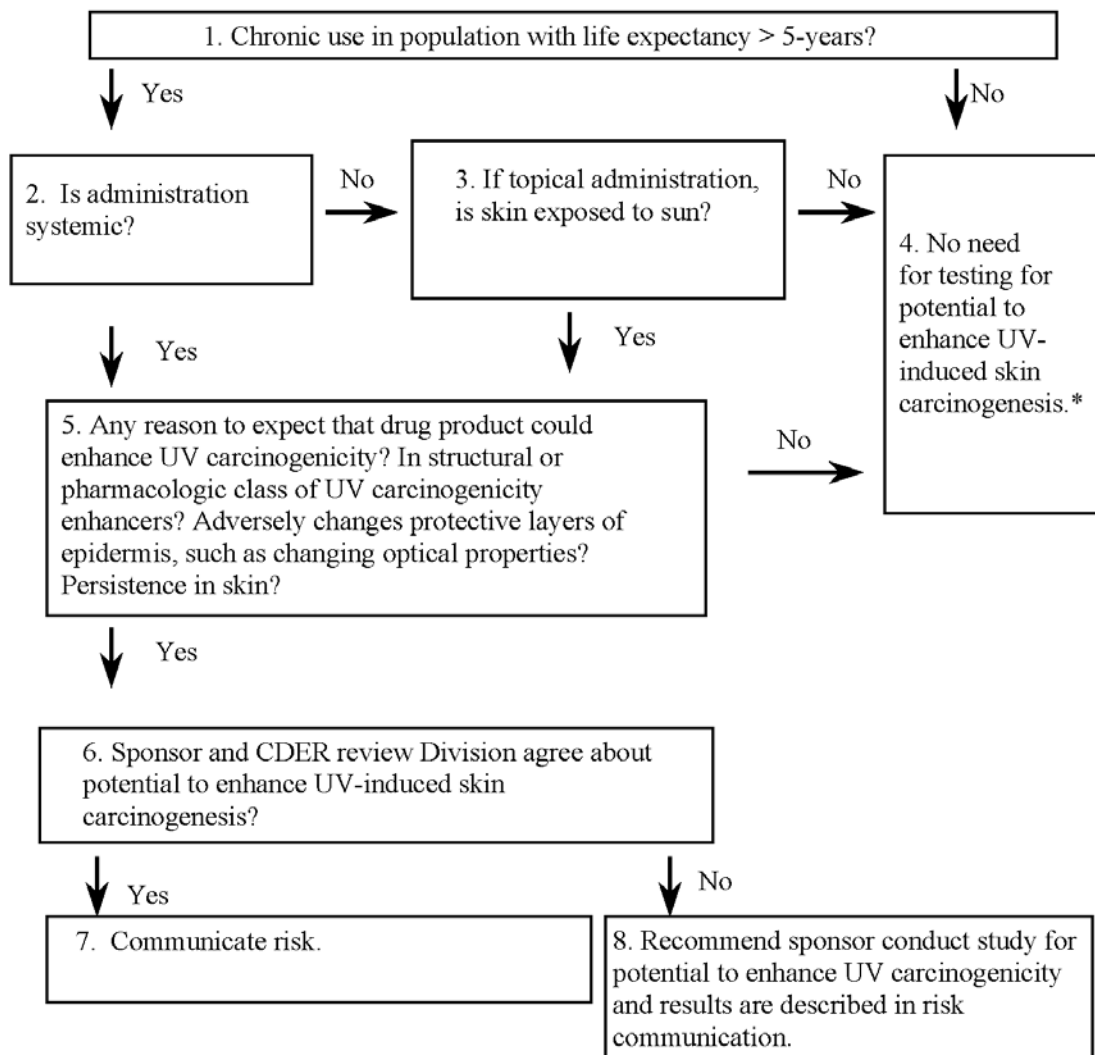


Attachment 4: Flow chart on the testing for photochemical carcinogenicity potential of photoreactive irritating drug products and labeling outcomes, according to FDA's guidance for industry on photosafety testing



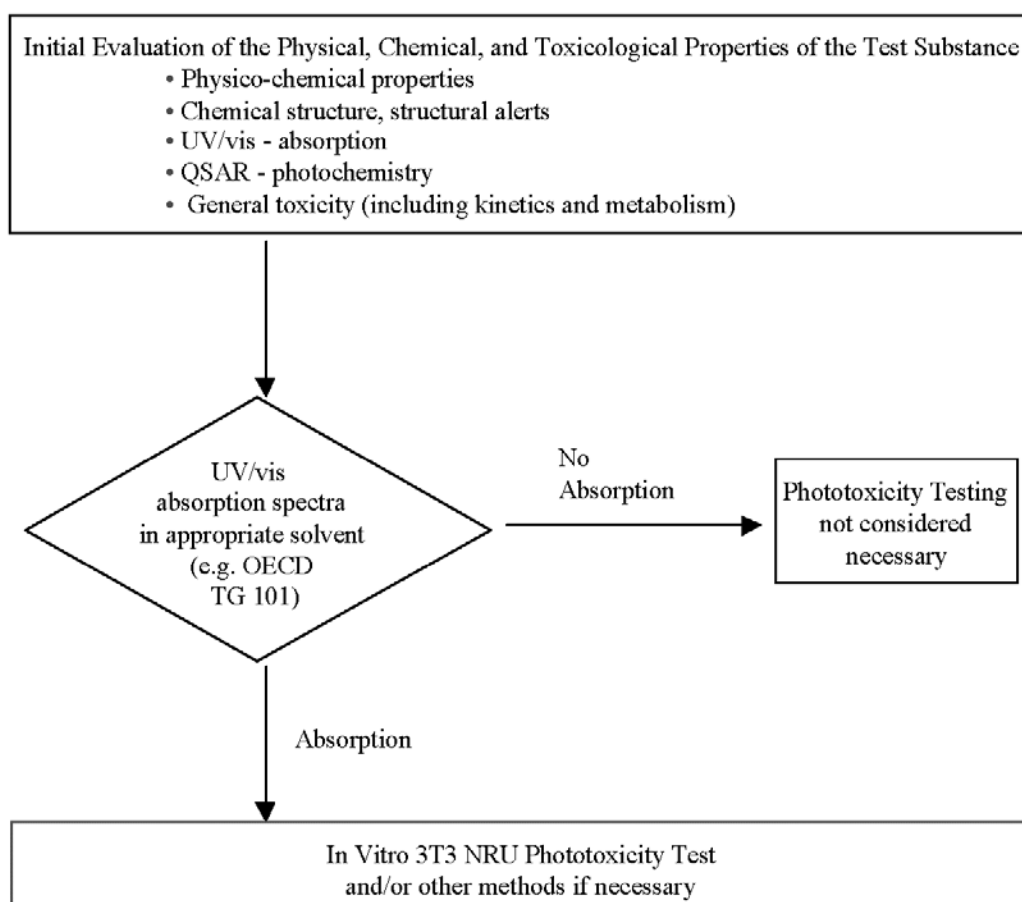
* Testing should be in an appropriate model. Assays of appropriate in vivo biomarkers can be used; consultation with CDER staff is recommended.

Attachment 5: Flow chart on the testing of non-photoreactive drug products for potential to enhance UV-induced skin carcinogenesis, according to FDA's guidance for industry on photosafety testing

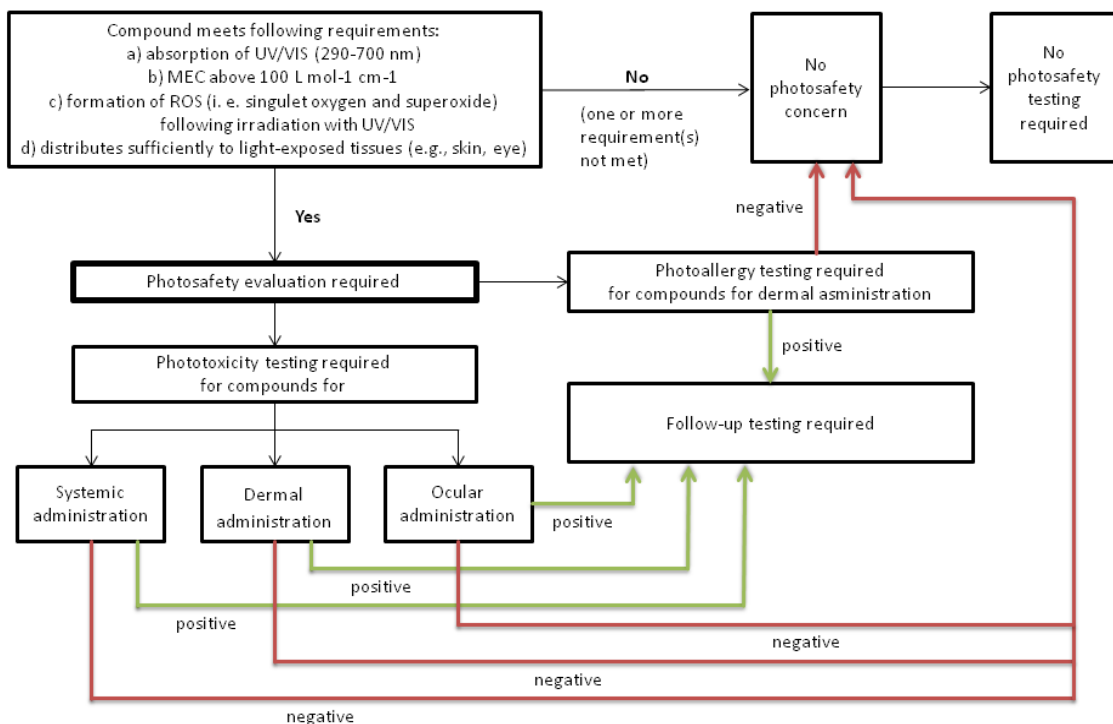


*Products specifically intended for use in sunlight should be tested for potential to enhance UV carcinogenicity. Testing should be in an appropriate model. Assays of appropriate in vivo biomarkers of increased UV exposure may be used; consultation with CDER is recommended.

Attachment 6: Flow chart on the role of the 3T3 NRU-PT in a sequential approach to the phototoxicity testing of chemicals, according to OECD TG 432



Attachment 7: Proposal for a flow chart on photosafety evaluation, according to ICH draft consensus guideline, photosafety evaluation of pharmaceuticals, S10, current Step 2



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Eidesstattliche Versicherung

Hiermit versichere ich, Annette Kienapfel, an Eides statt, dass ich die vorliegende Masterarbeit mit dem Titel „A review of the advancements in photosafety testing with regard to ICH’s new topic S10: Photosafety evaluation of pharmaceuticals“ selbständig und ohne fremde Hilfe verfasst und keine anderen als die angegebenen Hilfsmittel benutzt habe.

Ort, Datum

Unterschrift