厚生労働科学研究費補助金
医薬品・医療機器等レギュラトリーサイエンス総合研究事業

国際協調を指向した
薬剤性光線過敏症リスク評価方法開発の新展開

平成25年度　総括研究報告書

平成26（2014）年5月

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目　　次

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総括研究報告書

国際協調を指向した薬剤性光線過敏症リスク評価方法開発の新展開

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研究要旨

薬剤投与後に露光によって惹起される薬剤性光線過敏症は近年注目を集める副作用の一つであり，本副作用リスク回避のために効果的な予測方法の開発が国内外で急務の課題となっている．本研究では①光化学的試験方法である ROS アッセイに注目し，本試験法の多施設バリデーションスタディを行い，② ROS アッセイを応用した新規評価系構築を試みた．

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A．研究目的

薬剤性光線過敏症はその投与方法にかかわらず薬剤摂取後，露光により引き起こされる副作用である．創薬段階における本副作用リスクの回避が強く望まれており，これまでにも多くの *in vitro* ならびに *in vivo* 評価方法が開発されている．本研究の目的は薬剤性光線過敏症リスク評価のため簡便かつ信頼性の高い評価系を提示することであり，国際協調を最終的に達成することで国際的なニーズに答えようとするものである．*In vitro* 評価法として UV 吸収測定が広く実施されているが，より実質的な光化学的反応性を評価するために我々は reactive oxygen species (ROS) assay を開発した．本試験法は被験物質を光照射する際に発生する ROS 量をモニタリングすることを特徴とする光化学反応性評価法であり，種々のモデル化合物を用いた検討において薬剤性光線過敏症リスクを予測出来る可能性を示唆した．過去に ROS assay の有用性を検証するための多施設バリデーションスタディを行い，その transferability ならびに信頼性を精査することが出来た．今回，バリデーションスタディレポートを作成し，第三者評価を受けた．また，被験物質の体内動態情報と ROS アッセイデータを効率よく組み合わせることで光毒性リスクを予測する新しい試みを検討することとした．

B．研究方法

B-1）ROS assay

日本動物実験代替法検証センター(JaCVAM)主催のバリデーション運営委員会（VMT）の下，ROS assay プロトコールを確立した．VMT 主導で化合物選択を実施し，最終的に 2 種の標準物質 (quinine, sulisobenzone)，23 種の光毒性陽性化合物 (acridine, acridine HCl, amiodarone HCl, chlorpromazine HCl, doxycycline HCl,

fenofibrate, furosemide, ketoprofen, 6-methylcoumarine, 8-methoxy psoralen, nalidixic acid, nalidixic acid Na, norfloxacin, ofloxacin, piroxicam, promethazine HCl, rosigliutazone, tetracycline, anthracene, avobenzone, bithionol, hexachlorophene, rose bengal) ならびに 19 種の光毒性陰性化合物 (aspirin, benzocaine, erythromycin, phenytoin, penicillin G, bumetrizole, camphor sulfonic acid, chlorhexidine, cinnamic acid, drometrizole, histidine, methylbenzylidene camphor, octrizole, octyl methacrylate, octyl methoxycinnamate, octyl salicylate, PABA, SDS, UV-571) を選定した．バリデーションスタディには Atlas 社の Suntest CPS あるいは Seric 社の SXL-2500 を有する製薬協加盟企業が参加し，GLP の精神にのっとって各種検討を実施した．ROS assay プロトコールに従い，コード化された 42 種類の被験物質 (200 μM) を含む反応液を 96 ウェルプレートに分注して Atlas Suntest CPS series による 1 時間の擬似太陽光照射後（ca. 2.0 mW/cm^2），ROS (Singlet oxygen と Superoxide) の産生量をそれぞれ測定した．実験は 3 回繰り返し，各化合物の光毒性リスクを評価した．また，ICH topic S10 に継続して情報を発信し，ICH S10 guideline に ROS アッセイを提案した，

B-2）新規評価系構築

ROS アッセイデータと薬物動態情報を組み合わせることで創薬初期段階において有用な高効率光安全性評価系の構築を試みた．モデル薬物である 8 種の phenothiazines (PTZs; Mequitazine (MQ), promethazine HCl (PM), chlorpromazine HCl (CP), Perphenazine (PP), fluphenazine 2HCl (FP), and thiolidazine HCl (TD), Trifluoperazine 2HCl (TF), prochlorperazine dimaleate (PC)) に対し，UV や ROS アッセイ等の光化学的特性評価と cassette-dosing 法を用いた薬物動態試験を実施し，得られた結果を組み合わせて考察し各 PTZs の光毒性リスクについて順位づけした．予測した光毒性リスクと，ラットを用いた in vivo 光毒性試験の結果と比較検証した．

（倫理面への配慮）

本研究において動物実験は発生しない．

C．研究結果

C-1）ROS assay

JaCVAM多施設バリデーション研究は，AtlasまたはSeric社製の擬似太陽光照射装置を用いて 7 施設により実施された．最初に，陰性及び陽性対照物質を用いて各施設における照射条件を決定した後，全 42 種類のコード化された被験物質について評価した．その結果，ROSアッセイは，施設内の日内差/日間差及び施設間差が小さく，汎用性に優れている試験法であることが示された．また，ROSアッセイによる光毒性物質の陽性検出率は，難溶性のため評価できなかった被験物質を除くと100%であった．すなわち偽陰性結果がないことから，ROS アッセイは，光毒性ポテンシャルの評価に有用であると結論された．JaCVAM の支援によってバリデーションレポートの peer review をグローバルエキスパートから受けた．Review panel から一部判断基準に関する改訂提案を受けたものの，ROS アッセイの陰性予測精度と頑健性については好意的に受け止められた．本バリデーション結果に基づき，ICH（日米EU医薬品規制調和国際会

議）の光安全性評価ガイドライン（Step 4文書，2013年11月）において，ROSアッセイは，全身及び皮膚適用薬における光毒性評価の要否判断に利用可能な試験法の一つとして採択された（添付資料①: ICH S10 guideline, step 4).

C-2）新規評価系構築

光吸収によって励起状態となった光毒性化合物は，光化学反応を起こすことで，光毒性を惹起すると考えられており，すなわち，光反応性は光毒性リスクの直接的な指標となる. PTZs のさらなる光化学的反応性について精査すべく，ROS assay を実施した．光毒性陽性化合物である quinine を陽性対照とし，光毒性陰性化合物である erythromycin を陰性対照に用いた. 以前の報告と同様に，擬似太陽光照射下においてquinine からの高い singlet oxygen および superoxide anion の産生を認めたが，一方で erythromycin からは両者の産生を認めなかった. すべての PTZs において type II 光化学反応を介した強い singlet oxygen の産生を認めた． Superoxide anion の産生は TD, FP, および PP においてわずかに認められた．特に，fluorinated PTZs において強力な ROS 産生を認め，ROS 産生能を順位づけすると以下のようになった: FP≒TF≫TD＞MQ≒PM≒CP≒PC＞PP. また，すべての PTZs における ROS データは，以前の研究において定義された ROS assay の criteria [singlet oxygen ($\Delta A_{440\ nm} \times 10^3$): 25; superoxide anion ($\Delta A_{560\ nm} \times 10^3$): 20] を上回り，PTZs は高い光化学的反応性を有することが明らかとなった．従って，ROS data および MEC 値に基づき，PTZs は光化学的反応性が高く，露光部位に分布した場合には光毒性が懸念されるであろう.

光毒性反応は露光組織である皮膚および眼に現れることから，PTZs の露光組織への分布は光安全性評価を行う上で考慮すべき重要な点であろう．本研究では創薬初期スクリーニング系に適用することを考慮し，スループット向上と動物資源の削減を可能とする cassette-dosing PK analyses を用いた．まず，8 種のPTZs をラットに経口投与 (5 mg/kg each, *p.o.*) し，血中薬物濃度推移をモニタリングし，各種 PK パラメーターを算出した．共投与されたほとんどのPTZs の血漿中濃度はゆるやかに上昇し，T_{max} は 3–4.5 h であった. 一方で，MQ の見かけの吸収は極めて遅く，T_{max} は 17 h であった. PTZs の全身曝露は薬物によって大きく異なり，C_{max} は約 10–120 ng/mL であり，C_{max} によって各 PTZs の全身曝露を以下のように順位付けした: FP＞TF＞TD＞MQ≒PP≒PC＞PM≒CP. また，PTZs は血漿中から緩やかに消失し，消失速度定数は約 0.03–0.39 h^{-1} であった. それゆえに，PTZs は長期投与によって露光組織に蓄積する可能性があり，光毒性リスクの増大につながると考える．多くの PTZs が投与後約 4.5 h において C_{max} に達したため，4.5 h における露光組織（皮膚及び目）へのPTZs の分布を調べた．ほとんどの PTZs は露光組織，特に主要な光毒性発現部位である皮膚，に分布した．FP は皮膚 (637 ng/g tissue) および眼 (548 ng/g tissue) に多く移行しており，それぞれの Kp 値は5.6 mL/g tissue, 4.8 mL/g tissue であった．この結果は FP の高い全身曝露量 (C_{max}: 120

ng/mL) と良好に対応している. 興味深いことに, PP の皮膚中濃度は FP の皮膚中濃度を上回り, PP の *Kp* 値は FP よりも約 4 倍高値であった. 全ての PTZs が高い全身曝露を示したにもかかわらず, TD の皮膚内濃度および眼内濃度はそれぞれ 72 ng/g tissue, 40 ng/g tissue と全 PTZs 中で最も低値を示した. 対照的に, PM は全身曝露が低いにもかかわらず, PM の皮膚内および眼内濃度はそれぞれ 336 ng/g tissue, 162 ng/g tissue と比較的高値を示した. この結果は恐らく塩基性薬物の皮膚移行が脂溶性の高さに支配されていることに起因し, PM の高い log *P* によって説明できると考える. よって, 皮膚および眼内薬物濃度の高さは以下のような順序となった: PP≫FP＞PM＞MQ≒PC≒TF＞CP≒TD. 露光部位への移行量に基づいて考えると, PP と FP は露光部位に分布し, 光吸収により反応すれば他の PTZs よりもより強く光毒性を惹起する可能性があると考える. 一方で, TD および CP は皮膚および眼への移行量が比較的少なく, 他の PTZs よりも光毒性リスクは低いであろう.

In vivo における PTZs の光安全性について精査すべく, ラットに各 PTZs と positive/negative controls (100 mg/kg) を経口投与後, UV を照射し, UV 照射前後の皮膚色調変化を指標として, 皮膚光毒性反応を評価した. マウスに 80 mg/kg の CP を投与し, UVA を照射すると顕著な光毒性反応を示したという過去の報告に基づき, PTZs における光毒性試験を計画・実行した. 陽性対照である quinine を投与し, UV を照射しなかったラットにおける皮膚表面の色調は vehicle 投与, 非照射群と比較し, 有意な差は認めなかった (data not shown). 一方で, UV 照射, quinine 投与群では主に b* の値が上昇したことにより erythromycin 投与, UV 照射群と比較し ΔE 値が 3.7±2.1 上昇した. Quinine と同様に全 PTZs において UV 照射群では非照射群と比較し顕著に高い ΔE 値を認めた (data not shown). 特に, FP 投与後の UV 照射により L* (ΔL*:

表 1　Decision matrix for photosafety evaluation

	Non-halogenated group			*Fluorinated group*		*Chlorinated group*		
	MQ	PM	TD	FP	TF	CP	PP	PC
Photochemical properties								
UV absorption $\lambda_{max}/\varepsilon$ ($M^{-1}cm^{-1}$)	**302 nm/4,350**	**299 nm/3,700**	**312 nm/4,250**	**308 nm/3,550**	**307 nm/3,350**	**306 nm/3,850**	**308 nm/4,050**	**309 nm/4,050**
	[5] [a]	[4]	[5]	[4]	[4]	[4]	[5]	[5]
ROS assay [b] $^1O_2$ ($\Delta A_{440}\times10^3$)	245	226	323	**586**	**630**	197	156	224
O_2^- ($\Delta A_{560}\times10^3$)	0	0	11	18	3	1	25	0
	[2]	[2]	[2]	[4]	[5]	[2]	[1]	[2]
Distribution to UV-exposed tissues								
Skin (ng/g tissue) [c]	211.4 (4.2) [d]	336.3 (41.5)	72.3 (1.2)	**636.5 (5.6)**	173.3 (2.5)	87.5 (8.1)	**1,109.3 (21.9)**	199.7 (4.3)
Eyes (ng/g tissue) [c]	135.1 (2.7)	162.0 (20.0)	39.8 (0.7)	**547.5 (4.8)**	114.8 (1.7)	43.1 (4.0)	**865.9 (17.1)**	152.9 (3.3)
	[1]	[2]	[1]	[4]	[1]	[1]	[5]	[2]
Total score	8	8	8	12	10	7	11	9

[a], Risk scores in parentheses. [b], ROS data for PTZs at a concentration of 200 μM. [c], tissue concentration of PTZs at 4.5 h after oral cassette-dosing. [d], K_p values (mL/g tissue) in parentheses. White cells, risk score 0–1; gray cells, 2–3; and black cells, 4–5.

11.4±0.7) 値および b* (Δb*: 3.4±0.7) 値の上昇と a* (Δa*: -8.2±0.9) 値の減少による皮膚表面の色調の顕著な変化が惹起され，FP による著しく強い光毒性反応を示唆した．TF および PC においても ΔE 値の顕著な変化を認め，quinine と比較して有意に強い皮膚光毒性反応が明らかとなった ($P < 0.05$)．対照的に non-halogenated PTZs を投与し，UV を照射したラットにおける ΔE 値は他の PTZs を投与して UV を照射した場合と比較して低い値となり，すなわち non-halogenated PTZs は PTZs の中でも比較的光毒性が弱いことを示唆した．

D．考察

D-1）ROS assay

Atlas Suntest series ならびに Seric SXL-2500V2 を用いた ROS assay の多施設バリデーションスタディの結果，ROS assay の高い transferability と predictive capacity を確認できた．同評価系を広く運用していくためには，推奨プロトコールの積極的な公表と講習会による訓練などが必要になるものと考える．

D-2）新規評価系構築

光化学的反応性および皮膚，眼への移行量を合わせて decision matrix を構築し，PTZs の光毒性リスクを推測した (表 1)．decision matrix は多数の実験結果から系統的に分析し，判断するために用いられる定性的指標および定量的数値のデータを集約した模式的なデータ表である．Matrix decision による PTZs の光毒性リスク判定においては，光化学的反応性および PK の両データにおいて高いスコアとなった化合物は，その光毒性リスクが高いことを表し，どちらか一方，あるいは両方のスコアが低い値となった化合物は中程度または低い光毒性リスクを有することを表す．光化学的特性評価に基づくスコアリングにおいては，UV 吸収スペクトルおよび ROS データを用いた．UV 吸収スペクトルからは，化合物の光励起性についての情報が得られ，ROS データからは化合物の光化学的反応性がわかる．化合物の光励起およびそれに続く光化学反応は，光毒性メカニズム初期において起こりうる現象であり，光励起性および光反応性はそれぞれ，光毒性リスクの指標となる．従って，UV 吸収スペクトルと ROS データの両者を光化学的特性評価における指標として用いた．UV 吸収スペクトルにおいては極大吸収波長における MEC 値 1,000 $M^{-1}cm^{-1}$ 以上の化合物は光毒性を有する可能性が高いことが報告されており，本研究においても MEC 値に基づきスコアリングを行った．PK データにおいては皮膚および眼への移行量を decision matrix におけるスコアリングに用いた．ICH S10 draft guideline にも記載されているように，光反応性を有する薬物で，なおかつ露光部位である皮膚や眼に多く曝露される，または皮膚などに滞留しやすく長時間に渡って曝露される薬物は，曝露量が少なく，曝露時間も短いものと比較し，光毒性を引き起こしやすい．一方で，薬物に光反応性がなければ，たとえそれが露光部位に長時間，多量に曝露されたとしても光毒性を起こす可能性は低くなる．従って，光励起性/光反応性と同様に皮膚/眼移行性は，光毒性の発現を左右する重要な要因であり，両者のデータを組み合わせて光安全性評価に用いることで，信頼性のある光安

全性評価を実施できる．そこで MEC 値，ROS データ，および皮膚/眼移行量のそれぞれにおいて，光毒性リスクを 5 段階でスコアリングし，そのトータルスコアを算出して光毒性リスク評価に用いた．UV/ROS データおよび皮膚/眼組織濃度の全てにおいてリスクスコアが 4 と高い値を示した FP は最も高い光毒性リスクを有すると推測できる．また，高い光反応性を示した TF (total score: 10) および皮膚/眼への極めて高い曝露を示した PP (total score: 11) もまた，高い光毒性リスクを有すると推察できる．Total score が 8–9 となった PC, MQ, PM, および TD は光反応性および皮膚/眼への曝露量が中程度から低いレベルとなり，光毒性リスクがそれほど高くないと推察できる．CP は光反応性が高いものの (リスクスコア: 6) 極めて皮膚/眼への曝露量が低く (リスクスコア: 1)，その光毒性リスクは比較的低いと推測できる (total score: 7)．これらのスコアリングに基づき PTZs の光毒性リスクの高さを順位づけした: FP＞PP＞TF＞PC＞MQ≒PM≒TD＞CP．ラット *in vivo* 光毒性試験の結果から，PTZs のラットに対する光毒性の強さにはその化学構造と関連性があることが明らかとなった: fluorinated PTZs＞chlorinated PTZs＞non-halogenated PTZs．本知見は，decision matrix から推測した光毒性リスクと比較的良好に対応し，本研究における光安全性評価系の良好な予測能量を示唆した．一方で，CP において，予測した光毒性リスクと光毒性試験の結果との大きな乖離を認め，すなわち，本評価系では CP の光毒性リスク予測できないことを示唆した．CP の強力な光毒性については実験的および臨床的に明らかにされており，実際に本研究における *in vivo* 光毒性試験においても強力に光毒性を惹起している．経口的に摂取された CP は比較的速やかに，cytochrome P450 (CYP) 1A2 および CYP 3A4 による *N*-demethylation や CYP 2D6 による 7-hydroxylation を受け，desmethylchlorpromazine などに代謝されることが明らかとなっている．Desmethylchlorpromazine および didesmethylchlorpromazine は CP よりも光毒性が強いことが分かっている．従って，これらの報告から，生体内における光毒性代謝物の産生が CP の光毒性リスク予測において過誤を生じた一因であると推察する．しかしながら，皮膚における CP とこれら代謝物の比率は明らかではなく，さらなる精査を必要とする点であるだろう．また，他の可能性として，CP の臨床における投与量が他の薬物と比較して多いことから，高用量の CP においてのみ光毒性が発現する可能性や，生体内における CP の光化学反応によって，強い光毒性を有する生成物が生じた可能性もあるだろう．本評価系が親薬物のみに着目した評価系であるが故に，予測精度の低下を招いたものと考え，信頼性の高い光安全性評価を実施するために主要代謝物の光化学的および薬物動態学的特性を考慮した評価系を構築していく必要があると考える．

E．結論

光安全性の高い医薬品を生み出すために，医薬品開発における光安全性評価への関心は高まっていくだろう．本研究においては ROS assay を中心とした光化学的特性評価

ツールを提示し，さらに本手法とcassette-dosing 法を用いた皮膚，眼への移行性評価の組み合わせにより PTZs の in vivo 光安全性を，一部の例外はあるものの，効率的かつ良好に予測できた．近年では規制当局から基礎研究などに対し，3Rs (refinement, reduction, replacement) への貢献を求める動きが強まっており，本研究において新規に構築した光安全性評価系は，reduction と replacement の面からこの要求に応えることが可能であろう．本評価系の他の化合物群に対する適用可能性の検証など，課題はあるものの，本研究において提案した decision matrix approach は信頼性のある光安全性評価，ならびに新薬の創出における補助となるものと期待する．

F．健康危険情報

特になし

G．研究発表

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H. 知的財産権の出願・登録状況

1. 特許取得

なし

2. 実用新案登録

なし

3.その他

なし

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

ICH HARMONISED TRIPARTITE GUIDELINE

PHOTOSAFETY EVALUATION OF PHARMACEUTICALS

S10

Current *Step 4* version
dated 13 November 2013

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

S10
Document History

Code	History	Date
S10	Approval by the Steering Committee under *Step 2* and release for public consultation.	15 November 2012

Current *Step 4* version

S10	Approval by the Steering Committee under *Step 4* and recommendation for adoption to the three ICH regulatory bodies.	13 November 2013

PHOTOSAFETY EVALUATION OF PHARMACEUTICALS

ICH Harmonised Tripartite Guideline

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

TABLE OF CONTENTS

PHOTOSAFETY EVALUATION OF PHARMACEUTICALS

1. INTRODUCTION

1.1. Objectives of the Guideline

The purpose of this document is to recommend international standards for photosafety assessment, and to harmonise such assessments supporting human clinical trials and marketing authorizations for pharmaceuticals. It includes factors for initiation of and triggers for additional photosafety assessment and should be read in conjunction with ICH M3(R2), Section 14 on Photosafety Testing (Ref. 1). This guideline should reduce the likelihood that substantial differences in recommendations for photosafety assessment will exist among regions.

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

Consideration should be given to the use of non-animal methods or clinical data for photosafety assessment which could reduce the use of animals in accordance with the 3R (Replacement/Reduction/Refinement) principles.

1.2. Background

The ICH M3(R2) Guideline provides certain information regarding timing of the photosafety assessment relative to clinical development. It recommends that an initial assessment of phototoxicity potential be conducted, and if appropriate, an experimental evaluation be undertaken before exposure of large numbers of subjects (Phase 3). Similarly, the ICH S9 Guideline (Ref. 2) describes the timing of photosafety testing for oncology products. However, neither ICH M3(R2) nor ICH S9 provides specific information regarding testing strategies. This ICH S10 Guideline outlines further details on when photosafety testing is warranted, and on possible assessment strategies.

1.3. Scope of the Guideline

This guideline generally applies to new Active Pharmaceutical Ingredients (APIs), new excipients clinical formulations for dermal application (including dermal patches), and photodynamic therapy products.

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

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

This guideline does not generally apply to peptides, proteins, antibody drug conjugates, or oligonucleotides. Further, this guideline does not apply to components of marketed

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

1.4. General Principles

The photosafety assessment of a pharmaceutical is an integrated process that can involve an evaluation of photochemical characteristics, data from nonclinical studies and human safety information. The photosafety assessment aims to determine whether risk minimization measures are warranted to prevent adverse events in humans.

Four different effects have been discussed in connection with photosafety testing: phototoxicity, photoallergy, photogenotoxicity and photocarcinogenicity. Testing for photogenotoxicity (Note 2) and photocarcinogenicity (Note 6 of ICH M3 (R2)) is not currently considered useful for human pharmaceuticals. This guideline addresses only phototoxicity and photoallergy effects as defined below:

- Phototoxicity (photoirritation): An acute light-induced tissue response to a photoreactive chemical.
- Photoallergy: An immunologically mediated reaction to a chemical, initiated by the formation of photoproducts (e.g., protein adducts) following a photochemical reaction.

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

For a chemical to demonstrate phototoxicity and/or photoallergy, the following characteristics are critical:

- Absorbs light within the range of natural sunlight (290-700 nm);
- Generates a reactive species following absorption of UV-visible light;
- Distributes sufficiently to light-exposed tissues (e.g., skin, eye).

If one or more of these conditions is not met, a compound will usually not present a concern for direct phototoxicity. However, increased sensitivity of skin to light can also occur through indirect mechanisms. Such mechanisms are not generally addressed by the testing outlined in this guideline (see also Section 2.4).

2. FACTORS TO CONSIDER IN THE PHOTOSAFETY EVALUATION

2.1. Photochemical Properties

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

Excitation of molecules by light can lead to generation of Reactive Oxygen Species (ROS), including superoxide anion and singlet oxygen *via* energy transfer mechanisms. Although photoreactivity can result in other molecular outcomes (e.g., formation of photoadducts or cytotoxic photoproducts), even in these cases, it appears that ROS are typically generated as well. Thus, ROS generation following irradiation with UV-visible light can be an indicator of phototoxicity potential.

Photostability testing (Ref. 4) can also suggest the potential for photoreactivity. However, not all photoreactive compounds are detected under these conditions, and

photodegradation *per se* does not imply that a drug will be phototoxic. Therefore, photostability testing alone should not be used to determine whether further photosafety evaluation is warranted.

Assessments of photochemical properties should be conducted using high-quality scientific standards with data collection records readily available, or in compliance with Good Laboratory Practices/Good Manufacturing Practices (GLP/GMP) regulations.

2.2. Tissue Distribution/Pharmacokinetics

The concentration of a photoreactive chemical in tissue at the time of light exposure is a very important pharmacokinetic parameter in determining whether a phototoxic reaction will occur. This concentration depends on a variety of factors, such as plasma concentration, perfusion of the tissue, partitioning from vascular to interstitial and cellular compartments, and binding, retention, and accumulation of the chemical in the tissue. The duration of exposure depends upon clearance rates as reflected by half lives in plasma and tissue. Collectively, these parameters define the mean residence time of the photoreactive chemical in tissue.

Binding, retention, or accumulation of a compound in a tissue is not critical for a phototoxic reaction. If a molecule is sufficiently photoreactive, it might produce a phototoxic reaction at the concentration achieved in plasma or interstitial fluid. However, compounds having longer half-lives in plasma, longer mean residence time in sun-exposed tissues or with higher tissue to plasma concentration ratios are more likely to produce a phototoxic reaction than compounds with shorter half-lives, residence times or lower tissue to plasma ratios. Further, the longer the concentration of a compound is maintained at a level above that critical for a photochemical reaction, the longer a person is at risk for phototoxicity.

Although a tissue concentration threshold below which the risk for phototoxic reactions would be negligible is scientifically plausible, there are currently no data to delineate such generic thresholds for all compounds. Nevertheless, on a case-by-case basis it can be possible to justify that further photosafety assessment is not warranted based upon actual or anticipated tissue drug levels in humans, and taking into consideration the factors discussed above. Examples could include: 1) a drug for which overall systemic exposure levels are very low, or 2) a drug with a very short plasma half-life or tissue residence.

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

A single-dose tissue distribution study, with animals assessed at multiple timepoints after dosing, will generally provide an adequate assessment of relative tissue to plasma concentration ratios, tissue residence time and the potential for retention and accumulation. Assessment time points should be appropriately spaced in such a study to account for the drug half-life.

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

2.3. Metabolite Considerations

Metabolites generally do not warrant separate photosafety assessments, as metabolism does not typically result in chromophores that are substantially different from those in the parent molecule.

2.4. Pharmacological Properties

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

3. NONCLINICAL PHOTOSAFETY TESTS

3.1. General Considerations

Carefully selected conditions that consider both the model system and exposure to a relevant radiation spectrum are critical for nonclinical photosafety testing. Ideally, a nonclinical assay should exhibit both high sensitivity and specificity (i.e., low false negative and low false positive rates). However, to support the assessment strategies described in this document, it is most important that nonclinical photosafety assays show high sensitivity resulting in a low frequency of false negatives (i.e., a high negative predictive value). This is because negative assay results usually do not warrant further photosafety evaluation. The available nonclinical assays, both *in vitro* and *in vivo*, are focused primarily on detecting potential phototoxicity, which might or might not translate into clinically relevant phototoxicity.

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

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

3.2. Photoreactivity Tests Using Chemical Assays

If a drug developer chooses to assess photoreactivity, the assay should be qualified using pharmaceutical agents under appropriate conditions to demonstrate assay sensitivity. One such assay is a ROS assay (e.g., Ref. 7). Data suggest that this assay has high sensitivity for predicting direct *in vivo* phototoxicants. However, it has a low specificity, generating a high percentage of false positive results. A negative result in this assay, conducted under the appropriate conditions, would indicate a very low probability of phototoxicity, provided a test concentration of 200 μM can be achieved, whereas a positive result (at any concentration) would only be a flag for follow-up assessment.

3.3. Phototoxicity Tests Using *in vitro* Assays

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

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

Although the formal European Centre for the Validation of Alternative Methods (ECVAM) validation exercise conducted on this assay indicated a sensitivity of 93% and a specificity of 84%, experience within the pharmaceutical industry suggests a much lower specificity. The original OECD protocol was not validated for pharmaceuticals specifically. Thus, some modifications to the original OECD protocol have been proposed to address the low specificity observed with drug substances (see Note 4). These proposed changes are appropriate for the testing of pharmaceuticals. The sensitivity of the 3T3 NRU-PT is high and if a compound is negative in this assay it would have a very low probability of being phototoxic in humans. However, a positive result in the 3T3 NRU-PT should not be regarded as indicative of a likely clinical phototoxic risk, but rather a flag for follow-up assessment.

The BALB/c 3T3 cell line is sensitive to UVB and the initially recommended irradiation conditions (Ref. 6) involve the use of filters to attenuate wavelengths below 320 nm. However, depending on the light source and filters used, the ratio of UVB to UVA can be adjusted such that it is possible to assess UVB-induced phototoxicity in this test. UVB-induced phototoxicity is rarely a problem for pharmaceuticals with systemic exposure since UVB minimally penetrates beyond the epidermis. However, UVB-induced phototoxicity is more relevant for topical products. For components of topically applied products that absorb predominately in the UVB range, and where *in vitro* assessment is desired, the use of the 3T3 NRU-PT with modified irradiation conditions (see above) can be considered. Alternatively, *in vitro* skin models, which better tolerate UVB, could be considered.

Reconstructed human skin models, with the presence of a stratum corneum, permit testing of various types of topically applied materials ranging from neat chemicals to final

clinical formulations. The assays developed with reconstructed human skin to date measure cell viability with and without irradiation. These assays appear to be capable of detecting known human acute dermal phototoxicants. However, the sensitivity of some assays can be less than that of human skin *in vivo*, wherein the lowest concentration eliciting a positive response can be higher than in human skin *in vivo*. Consequently, it is important to understand the sensitivity of any assay selected and, if appropriate and feasible, to adjust the assay conditions accordingly (e.g., testing higher strength formulations, increasing exposure time).

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

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

Phototoxicity testing for systemically administered compounds has been conducted in a variety of species, including guinea pig, mouse, and rat. No standardized study design has been established and thus the following factors might be considered as best practices.

For species selection, irradiation sensitivity (i.e., minimal erythema dose), heat tolerance, and performance of reference substances should be considered. Models with both pigmented and non-pigmented animals are available. Although non-pigmented skin tends to be more sensitive than pigmented skin for detecting phototoxicity, pigmented skin should be considered for APIs that bind significantly to melanin (see Section 2.2) if appropriate exposures in target tissues cannot be ensured otherwise.

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

Although phototoxicity is typically an acute reaction, the duration of an *in vivo* assay should be carefully considered. Accumulation of compound in relevant light-exposed tissues after repeated administration might lead to an increased phototoxic response. Similarly, repeated irradiation after each dose might also lead to an increased phototoxic response due to the accumulation of damage. Generally, studies of a single day or up to a few days' duration of dosing are appropriate, using the clinical route of administration, if feasible. Single or repeated daily irradiations after dosing (around T_{max}) can be used.

Dose selection for *in vivo* nonclinical phototoxicity testing of systemic drugs should support a meaningful human risk assessment. For such studies a maximum dose level that complies with the recommendations for general toxicity studies in ICH M3(R2) Section 1.5 is considered appropriate. If a negative result is obtained at the maximum dose, testing of lower doses is usually not warranted. However, if a positive result is anticipated, additional dose groups can support a NOAEL-based risk assessment, typically considering C_{max} comparisons. Vehicle and non-irradiated controls can help identify compound-related phototoxicity and distinguish irradiation-induced from non-irradiation-induced adverse reactions. If the maximum systemic exposure achieved in animals is lower than clinical exposure, the reliability of a negative result in predicting human risk is questionable.

The most sensitive early signs of compound-induced phototoxicity are usually erythema followed by edema at a normally sub-erythemogenic irradiation dose. The type of response might vary with the compound. Any identified phototoxicity reaction should be

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

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

Adequate performance of *in vivo* phototoxicity assays, which are not formally validated, should be demonstrated using suitable reference compounds, including pharmaceuticals. Compounds that are phototoxic in humans and that represent different chemical classes and mechanisms of phototoxicity should be included to establish adequacy of the assays. For retinal phototoxicity, a reference compound with a light absorption profile within the visible light range (i.e., above 400 nm) is recommended. The concurrent use of a positive control compound might not be warranted if an *in vivo* assay has been formally validated or has reached general acceptance and is established in the testing facility.

Testing for photoallergy is not recommended for compounds that are administered systemically. Photoallergy reactions in humans following systemic administration are rare and there are no established nonclinical photoallergy assays for systemically administered compounds.

3.5. Photosafety Tests Using *in vivo* Assays and Dermal Administration

The main recommendations provided for investigating the systemic route of administration also apply to dermal administration, including those for species selection, study duration, and irradiation conditions. For dermal drug products in general, the clinical formulation should be tested. The intended clinical conditions of administration should be used to the extent possible. Irradiation of the exposed area should take place at a specified time after application, and the interval between application and irradiation should be justified based on the specific properties of the formulation to be tested. Signs of phototoxicity should be assessed based on relevant endpoints (see Section 3.4). The sensitivity of the assay should be demonstrated using appropriate reference compounds. Assessment of systemic drug levels is generally not warranted in dermal phototoxicity studies.

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

4. CLINICAL PHOTOSAFETY ASSESSMENT

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

5. ASSESSMENT STRATEGIES

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