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# OECD プログラムにおいて TG と DA を開発するための AOP に関する研究

令和元年度 分担研究報告書

## 光毒性試験の AOP および IATA の開発

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**研究要旨**

外因性光線過敏症は近年注目を集める有害事情の一つであり，本毒性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 *in vitro* 光化学的試験方法である ROS アッセイを主軸とした AOP を作成するため，光毒性物質の光生物化学的ならびに光化学的特性を精査することで光毒性反応機序のさらなる解明を行った。また，得られた科学的根拠をベースに ROS assay は TG495 として OECD test guideline に採択され，OECD TG 化を達成した。

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### A. 研究目的

近年、化合物の光安全性に対する関心の高まりから光毒性リスク評価に関する数多くの研究が行われている。ICH S10 で化合物の i) 光反応性および ii) 露光部位（皮膚や眼）への分布が光毒性発現に重要な因子として明記されている。当研究室では既に光化学的評価方法として reactive oxygen species (ROS) assay を開発し、本データと皮膚内動態情報の組み合わせることで信頼性ある光安全性評価が可能となることを明らかにした。この知見を検証すべく、本研究では ROS assay による光化学的特性および Franz 型拡散セルを用いた化学物質の *in vitro* 皮膚内動態のデータを統合的に解析することで経皮適用化合物の光毒性リスクを効果的に予測できるかを検証し、その予測データを用いることで動物実験代替法の開発を指向した検討を実施した。また、検証結果を基に光毒性に関する AOP ならびに ROS assay に関する OECD TG 案を作成した。なお、TG 案については協議の結果、OECD TG 495 としてガイドライン化に成功した。

### B. 研究方法

#### B.1. ROS アッセイ

研究分担者らが既に公表している ROS assay 推奨プロトコルに基づき、quinolone derivatives (QNLs) 6 種 [enoxacin (ENX), flumequine (FLM), moxifloxacin (MFX), nalidixic acid (NLA), orbifloxacin (OFX), oxolinic acid (OXA)] について ROS assay を行った。

#### B.2. *In vitro* 皮膚内動態実験

上記 6 種の QNLs について、フランツ型拡散セルを用いてラット摘出皮膚における *in vitro* 皮膚透過性試験を実施した。ドナー側に QNLs (各 1 mg/mL) を入れ、経時的に皮膚を透過したレセプター液中の

QNLs の量を UPLC/ESI-MS にてモニタリングし、*in vitro* 皮膚透過性のデータを得た。得られたデータを基に定常状態における各 QNLs の皮膚内濃度 ($C_{ss}$) を算出した。得られた $C_{ss}$ の値と光化学的特性データを併せて考慮することで光毒性予測を実施した。

B.3. ラット *in vivo* 光毒性試験

前日に腹部を剃毛した雄性ラットに各 QNLs (10 mg/site) を塗布し、塗布後 3 h で black light にて UVA (30 J/cm$^2$) を照射した。照射終了後 24 h に色差計にて皮膚表面の色調を計測し、光毒性の指標とした。

**C. 研究結果**

C.1. 光安全性評価

ROS assay にて 6 種の ONLs (ENX, FLM, MFX, NLA, OFX および OXA) は露光時に光安全性評価における ROS assay の criteria を超える強い ROS 産生を示し、高い光反応性を有していた。特に ENX は $^1O_2$ および$O_2^-$ ならびに OFX は $^1O_2$ において他の QNLs と比し強い ROS 産生を示した。*In vitro* 皮膚透過性を基に算出した $C_{ss}$ は FLM および NLA がそれぞれ 5.0 および 8.2 μg/mL と高く、次いで MFX が 3.4 μg/mL であった。ENX, OFX および OXA の $C_{ss}$ の値はそれぞれ 1.2, 2.0 および 2.2 μg/mL と比較的低値を示した。得られたデータを基に decision matrix を用いて統合的に 6 種の QNLs の光毒性リスク予測を実施した結果は以下の通りであった。

光毒性リスク予測：

NLA＞FLM＞OFX＞ENX＞OXA＞MFX

ラットにおける *in vivo* 皮膚光毒性について MFX を除く QNLs で UVA 非照射群と比し、UVA 照射群における有意な皮膚の色の変化を確認し、5 種の QNLs は光毒性陽性と判断した。MFX においては有意な皮膚の色の変化は認めず、*in vivo* 光毒性は弱いと判断した。QNLs の *in vivo* 光毒性の強さは以下の順であった。

*In vivo* 光毒性：

FLM＞NLA＞ENX≒OFX＞OXA＞MFX

C.2. AOP および OECD TG 案の作成

ROS assay の OECD TG 化のため、ROS assay の TG 案を提出し、各国から提示されたパブリックコメントに対応し、TG 改定案を作成した。その後、細かい箇所の修正を経て 2019 年 6 月に OECD TG495 としてガイドライン化に成功した。

**D. 考察**

本研究では光化学的特性および *in vitro* 皮膚内動態に基づき被験物質の光毒性リスクが予測可能か検証した。動物実験代替法として構築した ROS assay および *in vitro* 皮膚透過性を用いた光毒性予測系を構築し、6 種の QNLs の光毒性リスク予測を実施した結果、*in vivo* 光毒性の結果と良好に対応することが分かった。本研究で構築した評価手法は良好に光毒性リスク予測が可能であろう。

**E. 結論**

動物実験代替法としての光安全性評価系を構築し、光化学的特性ならびに *in vitro* 皮膚内濃度の統合的解析により良好に被験物質の光毒性リスクを予測できた。今回構築した光安全性評価系について更なる検証試験を進めるとともに、動物試料を用いない光安全性評価系構築を試みる。本検討で得られた知見は ROS assay の OECD TG 化の実現に大きく貢献した。さらに、現在提案中の光毒性に関する IATA 構築について

もこれまでに得られた知見は大きく貢献できると期待する。

**F. 添付資料**

AOP 282: Reactive oxygen species generated from photoreactive chemicals leading to phototoxic reactions

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**H. 知的財産権の出願・登録状況**

H.1. 特許取得

なし

H.2. 実用新案登録

なし

H.3. その他

なし

# AOP ID and Title:

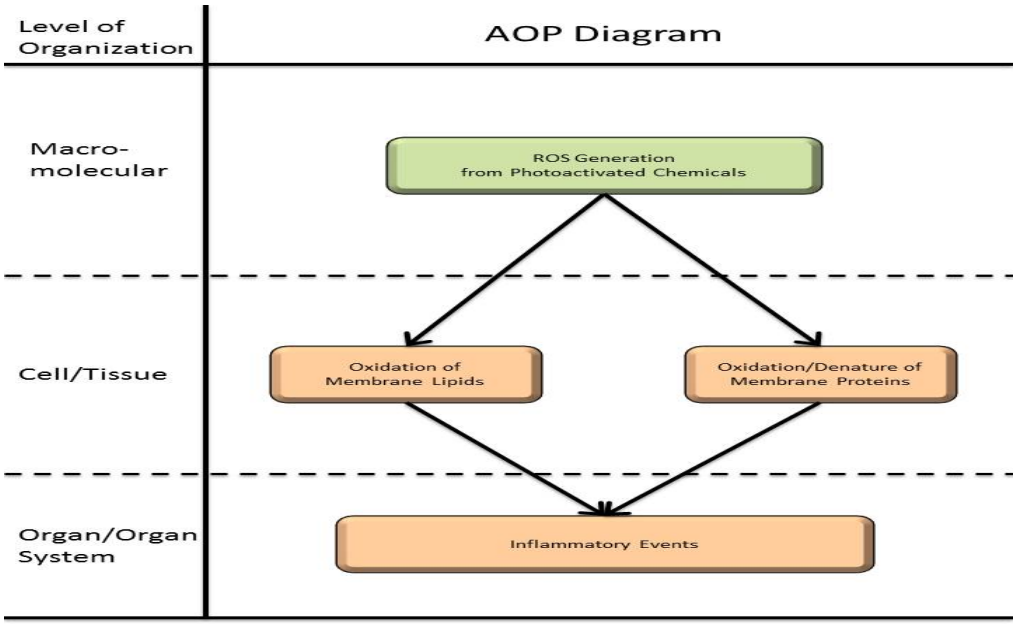
SNAPSHOT

Created at: 2020-05-26 00:39

**AOP 282: Reactive oxygen species generated from photoreactive chemicals leading to phototoxic reactions**

**Short Title: ROS-mediated chemical phototoxicity**

## Graphical Representation

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## Status

| Author status | OECD status | OECD project | SAAOP status |
|---|---|---|---|
| Under development: Not open for comment. Do not cite | EAGMST Under Review | 1.49 | Included in OECD Work Plan |

## Abstract

Phototoxicity is an adverse reaction in the light-exposed tissues triggered by normally harmless doses of sunlight (Moore, 1998; Moore, 2002; Roberts, 2001). Recently, high-intensity UV rays from the sun have reached the Earth's surface with the destruction of the ozone layer, and interest in phototoxic events has increased enormously. Notably, phototoxic reactions against exogenous agents are caused by the combined effects of environmental light and external agents, including drugs, cosmetics, and foods (Epstein, 1983; Stein and Scheinfeld, 2007).

In this AOP, the primary trigger for a compound to be considered with respect to potential to create photochemical and photobiological reactions is the absorption of photon energy from light ranging from 290 to 700 nm. The extent of absorption depends on the wavelength of light and the type of absorbing chromophores in the light-exposed tissues. A molecule is excited by absorption of photon energy, and the photoactivated molecule

induces photochemical reactions via energy transfer (type I photochemical reaction) and free radical generation (type II photochemical reaction). These photochemical reactions result in generation of radicals and reactive oxygen species, and the reactive species react with biomolecules. Generated radicals of a target chemical bind to DNA and proteins, resulting in formation of these photo-adducts, and reactive oxygen species (ROS), including singlet oxygen and superoxide, induce oxidation of biomolecules. These key events bring inflammatory events in the light-exposed tissues (Brendler-Schwaab et al. , 2004; Epstein and Wintroub, 1985; Quintero and Miranda, 2000).

This AOP describes the pathway of photochemical toxicity between attack of ROS generated from photoactivated chemicals to membranes and inflammatory events in light-exposed tissues.

# Summary of the AOP

## Events

### Molecular Initiating Events (MIE), Key Events (KE), Adverse Outcomes (AO)

| Sequence | Type | Event ID | Title | Short name |
|---|---|---|---|---|
| | MIE | 1592 | ROS generation from photoactivated chemicals (https://aopwiki.org/events/1592) | ROS generation |
| | | | | |
| | KE | 1594 | Oxidation of membrane lipids (https://aopwiki.org/events/1594) | Oxidation of membrane lipids |
| | KE | 1595 | Oxidation/denatuation of membrane proteins (https://aopwiki.org/events/1595) | Oxidation/denatuation of membrane proteins |
| | | | | |
| | AO | 1599 | Inflamatory events in light-exposed tissues (https://aopwiki.org/events/1599) | Inflammatory events |

### Key Event Relationships

| Upstream Event | Relationship Type | Downstream Event | Evidence | Quantitative Understanding |
|---|---|---|---|---|
| ROS generation from photoactivated chemicals (https://aopwiki.org/relationships/1845) | adjacent | Oxidation of membrane lipids | High | Low |
| ROS generation from photoactivated chemicals (https://aopwiki.org/relationships/1846) | adjacent | Oxidation/denatuation of membrane proteins | High | Low |
| Oxidation of membrane lipids (https://aopwiki.org/relationships/1850) | adjacent | Inflamatory events in light-exposed tissues | High | Low |
| Oxidation/denatuation of membrane proteins (https://aopwiki.org/relationships/1851) | adjacent | Inflamatory events in light-exposed tissues | High | Low |
| | | | | |

## Stressors

| Name | Evidence |
|---|---|
| Light (290-700 nm) | High |
| Photoreactive chemicals | High |
| Reactive oxygen species | High |

# Overall Assessment of the AOP

The focus of this AOP is on photochemical toxicity, especially photoactivation of target chemicals followed by generation of ROS. ROS generated from photoirradiated chemicals can react with molecules on the membranes, including lipids and proteins, and the reactions may lead to inflammatory events in the UV-exposed tissues.

Phototoxicity is an adverse reaction triggered by normally harmless doses of sunlight. There are two types of photosensitive disorders, endogenous and exogenous phototoxicity, and the observable changes to the sunlight-exposed tissues are essentially detrimental, and include the following appearance; (i) immediate faint erythema during exposure, (ii) delayed erythemal responses, (iii) abnormal keratinisation and vacuolated cells, (iv) formation of desquamating layer, and (v) desquamation (peeling) (Moore, 1998; Moore, 2002; Roberts, 2001). Recently, high-intensity UV rays from the sun have reached the Earth's surface with the destruction of the ozone layer, and interest in phototoxic events has increased enormously. Notably, phototoxic reactions against exogenous agents are caused by the combined effects of UV irradiation and external agents, including drugs, cosmetics and foods (Stein and Scheinfeld, 2007). Phototoxic skin responses after administration of photosensitive drugs, so-called drug-induced phototoxicity, have been recognized as undesirable side effects, and several classes of drugs, even when not toxic by themselves, may become reactive under exposure to environmental light, inducing undesired phototoxic responses (Epstein, 1983).

The primary trigger for a compound to be considered with respect to potential to create photochemical and photobiological reaction is the absorption of UV and visible light ranging from 290 to 700 nm. The extent of absorption depends on the wavelength of light and the type of absorbing chromophores in the UV-exposed tissues. UV radiation is usually divided into several ranges based on its physiologic effects: (1) UVA (near UV): 320–400 nm (UVA I: 340–400 nm and UVA II: 320–340 nm), (2) UVB (middle UV): 290–320 nm, and (3) UVC (far UV): 180–290 nm (Svensson et al., 2001; Vassileva et al., 1998). The sun emits ultraviolet radiation in the UVA, UVB, and UVC bands, but because of absorption by the atmosphere's ozone layer, the main ultraviolet radiation that reaches the Earth's surface is UVA (Dubakiene and Kupriene, 2006). Absorption of light through the skin and eyes, primarily in the 290–700 nm range, varies with wavelength, such that light in the red region of the spectrum reaches well into the subcutis layer; whereas at 300 nm or shorter wavelength, only an estimated 10% passes through the epidermis (Epstein, 1989). Thus, penetration and absorption of light in the UV-exposed tissues is important factor in drug-induced phototoxicity as Grotthus-Draper law of photobiology states; only light that is absorbed can be active in photochemical and photobiological processes.

When a drug molecule absorbs a photon energy, electrons can be prompted from occupied orbitals (the ground state) to an unoccupied orbital (S1, S2) depending upon bond type and associated energy level. Furthermore, unpaired singlet state electrons (opposite spin) may be converted to triplet state (parallel spin) by inversion of the spin via intersystem crossing of the absorbed energy. To return to the ground state from S1, S2/T1, T2, energy must be dissipated by internal conversion, fluorescence (from singlet state), phosphorescence (from triplet state) or via chemical reaction, giving rise to photoproducts and/or potential external reactions with biomolecules.

In addition, molecular oxygen, a triplet radical in its ground state, appears to be the predominant acceptor of excitation energy as its lowest excited level (singlet state) has a comparatively low value. An energy transfer from excited triplet photosensitizer to the oxygen (type II photochemical reaction) could produce excited singlet oxygen which might, in turn, participate in a lipid- and protein-membrane oxidation or induce DNA damage. An electron or hydrogen transfer could lead to the formation of free radical species (type I photochemical reaction), producing a direct attack on the biomolecules or in the presence of oxygen, to evolve towards secondary free radicals such as peroxyl radicals or the very reactive hydroxyl radical, a known intermediate in the oxidative damage of biomolecules. This toxic pathway corresponds to successive reactions which involve the appearance of superoxide anion radical, its dismutation to from hydrogen peroxide followed with the hydrogen peroxide reduction to form hydroxyl radical. Herein, excitation of the drug by light may give rise to ROS such as singlet oxygen and superoxide, which may be one of causative molecules for the drug-induced phototoxicity (Brendler-Schwaab et al., 2004; Epstein and Wintroub, 1985).

## Domain of Applicability

**Life Stage Applicability**

| Life Stage | Evidence |
|---|---|
| All life stages | High |

**Taxonomic Applicability**

| Term | Scientific Term | Evidence | Links |
|---|---|---|---|
| human | Homo sapiens | High | NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606) |

**Sex Applicability**

| Sex | Evidence |
|---|---|
| Mixed | High |

**Chemicals:** This AOP applies to a wide range of chemicals. Phototoxic chemicals are recognized to have following characteristics: (i) absorption of light within the range of natural sunlight (290-700 nm); (ii) generation of a reactive species following absorption of UV-visible light; (iii) distribution to light-exposed tissues (e.g., skin and eye) (ICH S10).

**Sex:** This AOP applies to both males and females.

**Life stages:** The relevant life stages for this AOP are all life stages after born.

**Taxonomic:** This AOP mainly applies to human.

## Essentiality of the Key Events

The essentiality of KEs for this AOP was rated high on the basis of experimental evidence in the investigations related to each of KEs and published guidelines. For details see the table on “Support for Essentiality of KEs”.

## Weight of Evidence Summary

Support for biological plausibility of KERs

| | | |
|---|---|---|
| MIE => KE 1 | Generated ROS from photoactivated chemicals can react with membrane lipids, and oxidation of membrane lipids could be occurred. | Biological Plausibility of the MIE => KE 1 is high.<br><br>The relationship between MIE and KE 1 is consistent with chemical and biological knowledge (Girotti, 1990; Girotti, 2001; Onoue and Tsuda, 2006). |
| MIE => KE 2 | Generated ROS from photoactivated chemicals can react with membrane proteins, and oxidation/denaturation of membrane proteins could be occurred. | Biological Plausibility of the MIE => KE 2 is high.<br><br>The relationship between MIE and KE 2 is consistent with biological knowledge (Dalle Carbonare and Pathak, 1992; Valenzeno, 1987). |
| KE 1 => AO | Oxidation of membrane lipids relates with damage produced in the cellular membrane, leading to inflammatory events. | Biological Plausibility of the KE 1 => AO is high.<br><br>The relationship between KE 1 and AO is consistent with biological knowledge (Castell et al., 1994). |
| KE 2 => AO | Oxidation/denaturation of protein provides the necrosis of the living tissues as an inflammatory event. | Biological Plausibility of the KE 2 => AO is high.<br><br>The relationship between KE 2 and AO is consistent with biological knowledge (Dalle Carbonare and Pathak, 1992; Opie, 1962). |

Support for Essentiality of KEs

| | | |
|---|---|---|
| MIE | ROS generation from photoactivated chemicals | High; well-accepted generation of reactive oxygen species from photo-activated chemicals associated with phototoxic reactions with 200 of chemicals evaluated in qualitative endpoints (Onoue et al., 2014; Onoue et al., 2013a; Onoue et al., 2008a; Onoue and Tsuda, 2006; Seto et al., 2013b). The event has described in ICH S10 guideline as a crucial factor of phototoxic reactions (ICH, 2014). |
| KE 1 | Oxidation of membrane lipids | High; Oxidative stress to lipids associated with the phototoxic reactions (Girotti, 1990; Girotti, 2001; Onoue and Tsuda, 2006). |
| KE 2 | Oxidation/denaturation of membrane proteins | High; accepted oxidation/denaturation of proteins associated with the phototoxic reactions (Dalle Carbonare and Pathak, 1992; Valenzeno, 1987). |
| Adverse outcome | Inflammatory events in sunlight-exposed tissues | Photoreactive agents indicated inflammatory events, including edema, dyskeratosis, and necrosis, in light-exposed tissues after sunlight exposure (Moore, 1998; Moore 2002; Roberts, 2001). |

Empirical Support for KERs

| | |
|---|---|
| MIE => KE 1: ROS generation leads to Oxidation of membrane lipids | Empirical support of the MIE => KE 1 is strong.<br>Rationale:<br>Lipid peroxidation was occurred by ROS-generating chemicals under exposure to simulated sunlight (Onoue et al., 2011, Onoue and Tsuda, 2006).<br>A photoreactive chemical indicated dose-dependent increases in ROS generation and lipid peroxidation after exposure to a fixed dose of simulated sunlight (Seto et al., 2013a). |
| MIE => KE 2: ROS generation leads to Oxidation/denaturation of membrane proteins | Empirical support of the MIE => KE 2 is moderate.<br>Rationale:<br>ROS generated from photosensitizing agents led to oxidation and denaturation of proteins (Dalle Carbonare and Pathak, 1992). |
| KE 1 => AO: Oxidation of membrane lipids leads to Inflammatory events | Empirical support of the KE 1=> AO is strong.<br>Rationale:<br>Increases in lipid peroxidation and inflammatory-related cytokines were observed in the murine skin, and naringenin, an anti-oxidant, attenuated these increases in a dose-dependent manner (Martinez et al., 2015).<br>Benzoyl peroxide, a ROS generator, led to lipid peroxidation and GSH depletion, and the changes caused the gene expression of pro-inflammatory cytokines (Valacchi et al., 2001). |

| | |
|---|---|
| KE 2 => AO: Oxidation/denaturation of membrane proteins leads to Inflammatory events | Empirical support of the KE 2=> AO is moderate.<br>Rationale:<br>Denaturation of proteins induced necrosis and inflammatory in the skin (Opie, 1962). |

## Quantitative Consideration

Although there is empirical information on KERs as described above sections, the overall quantitative understanding of the AOP is insufficient to directly link a measure of KEs to a quantitative prediction of KERs.

As a pre-MIE, light absorption of chemicals is an important event for phototoxic reactions induced by photoreactive chemicals. Quantitative endpoint on absorption of light (290–700 nm) was recognized in the previous report (Henry et al., 2009), and, for photoreactive chemicals, the criterion on molar extinction coefficient (MEC) was determined to be 1,000 $M^{-1}\cdot cm^{-1}$. Most of chemicals with MEC values of over 1,000 $M^{-1}\cdot cm^{-1}$ generated significant ROS, including singlet oxygen and/or superoxide (Onoue et al., 2013b; Onoue and Tsuda, 2006), and the qualitative criteria on ROS generation was determined to evaluate chemical phototoxicity (Onoue et al., 2014; Onoue et al., 2013a; Onoue et al., 2008b).

# Considerations for Potential Applications of the AOP (optional)

The MIE and KEs in this AOP could contribute to assays development for photosafety evaluation and an AOP-based IATA construction. AOP-based IATA can be applied for various aims including screening of chemicals, prioritization of chemicals for further testing, and risk assessment.

The regulatory applicability of the AOP would be to use experimental results from assays based on MIE and KEs as indictors for the risk of phototoxic reactions.

Combined use of photobiochemical properties and tissue exposure data would be of help for photosafety evaluation of chemicals. Risk assessment would be possible when exposure data in light-exposed tissues combined with assay data based on AOP.

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# Appendix 1

## List of MIEs in this AOP

### Event: 1592: ROS generation from photoactivated chemicals (https://aopwiki.org/events/1592)

Short Name: ROS generation

#### AOPs Including This Key Event

| AOP ID and Name | Event Type |
|---|---|
| Aop:282 - Reactive oxygen species generated from photoreactive chemicals leading to phototoxic reactions (https://aopwiki.org/aops/282) | MolecularInitiatingEvent |

#### Stressors

| Name |
|---|
| Light (290-700 nm) |
| Photoreactive chemicals |

#### Biological Context

| Level of Biological Organization |
|---|
| Molecular |

## Evidence for Perturbation by Stressor

## Overview for Molecular Initiating Event

Several classes of chemicals cause ROS generation under light exposure, and the ROS generation can be monitored by ROS assay (Onoue et al., 2014, Onoue et al., 2013, Onoue et al. , 2008, Seto et al. , 2013). The criteria of ROS assay for photosafety assessment of chemicals were defined (Onoue et al., 2014, Onoue et al., 2013).

## Domain of Applicability

**Taxonomic Applicability**

| Term | Scientific Term | Evidence | Links |
|---|---|---|---|
| human | Homo sapiens | High | NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606) |

**Life Stage Applicability**

| Life Stage | Evidence |
|---|---|
| All life stages | High |

**Sex Applicability**

| Sex | Evidence |
|---|---|
| Mixed | High |

**Chemicals:** This MIE applies to a wide range of chemicals. The chemicals absorb photon energy from light within the range of natural sunlight (290-700 nm) (ICH, 2014, Onoue and Tsuda, 2006).

**Sex:** This MIE applies to both males and females.

**Life stages:** The relevant life stages for this MIE are all life stages after born.

**Taxonomic:** This MIE mainly applies to human.

## Key Event Description

In the primary event, photoreactive chemicals are excited by the absorption of photon energy. The energy of the photoactivated chemicals transfer to oxygen and then generates the reactive oxygen species (ROS), including superoxide ($O_2^-$) via type I reaction and singlet oxygen ($^1O_2$) via type II reaction, as principal intermediate species in phototoxic reaction (Foote, 1991, Onoue et al. , 2009).

## How it is Measured or Detected

On the basis of the pathogenesis of drug-induced phototoxicity, a reactive oxygen species (ROS) assay was proposed to evaluate the phototoxic risk of chemicals. The ROS assay can monitor generation of ROS, such as singlet oxygen and superoxide, from photoirradiated chemicals, and the ROS data can be used to evaluate the photoreactivity of chemicals (Onoue et al. , 2014, Onoue et al. , 2013, Onoue and Tsuda, 2006). The ROS assay is a recommended approach by guidelines to evaluate the phototoxic risk of chemicals (ICH, 2014, PCPC, 2014).

## References

Foote CS. Definition of type I and type II photosensitized oxidation. Photochem Photobiol. 1991;54:659.

ICH. ICH Guideline S10 Guidance on Photosafety Evaluation of Pharmaceuticals.: International Council on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use; 2014.

Onoue S, Hosoi K, Toda T, Takagi H, Osaki N, Matsumoto Y, et al. Intra-/inter-laboratory validation study on reactive oxygen species assay for chemical photosafety evaluation using two different solar simulators. Toxicology in vitro : an international journal published in association with BIBRA. 2014;28:515-23.

Onoue S, Hosoi K, Wakuri S, Iwase Y, Yamamoto T, Matsuoka N, et al. Establishment and intra-/inter-laboratory validation of a standard protocol of reactive oxygen species assay for chemical photosafety evaluation. Journal of applied toxicology : JAT. 2013;33:1241-50.

Onoue S, Kawamura K, Igarashi N, Zhou Y, Fujikawa M, Yamada H, et al. Reactive oxygen species assay-based risk assessment of drug-induced phototoxicity: classification criteria and application to drug candidates. J Pharm Biomed Anal. 2008;47:967-72.

Onoue S, Seto Y, Gandy G, Yamada S. Drug-induced phototoxicity; an early *in vitro* identification of phototoxic potential of new drug entities in drug discovery and development. Current drug safety. 2009;4:123-36.

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## List of Key Events in the AOP

### Event: 1594: Oxidation of membrane lipids (https://aopwiki.org/events/1594)

Short Name: Oxidation of membrane lipids

### AOPs Including This Key Event

| AOP ID and Name | Event Type |
|---|---|
| Aop:282 - Reactive oxygen species generated from photoreactive chemicals leading to phototoxic reactions (https://aopwiki.org/aops/282) | KeyEvent |

### Stressors

| Name |
|---|
| Photoactivated chemicals |
| Reactive oxygen species |

### Biological Context

| Level of Biological Organization |
|---|
| Cellular |

### Domain of Applicability

**Taxonomic Applicability**

| Term | Scientific Term | Evidence | Links |
|---|---|---|---|
| human | Homo sapiens | High | NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606) |

**Life Stage Applicability**

| Life Stage | Evidence |
|---|---|
| All life stages | High |

**Sex Applicability**

| Sex | Evidence |
|---|---|
| Mixed | High |

**Chemicals:** This KE applies to a wide range of chemicals. The chemicals generate a reactive species, such as reactive oxygen species, following absorption of photon energy from light within the range of natural sunlight (290-700 nm) (ICH, 2014, Onoue and Tsuda, 2006).

**Sex:** This KE applies to both males and females.

**Life stages:** The relevant life stages for this KE are all life stages after born.

**Taxonomic:** This KE mainly applies to human.

### Key Event Description

Lipid peroxidation of membrane lipids has been considered to be one of the major mechanisms in phototoxic skin responses induced by photoreactive chemicals (Castell et al., 1994, Girotti, 1990, 2001, Onoue et al., 2011, Onoue and Tsuda, 2006, Seto et al., 2013).

### How it is Measured or Detected

An *in vitro* system using thiobarbituric acid can monitor lipid peroxidation by photoactivated chemicals (Onoue et al., 2011, Onoue and Tsuda, 2006).

### References

Castell JV, Gomez-Lechon MJ, Grassa C, Martinez LA, Miranda MA, Tarrega P. Photodynamic lipid peroxidation by the photosensitizing nonsteroidal antiinflammatory drugs suprofen and tiaprofenic acid. Photochem Photobiol. 1994;59:35-9.

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Seto Y, Inoue R, Kato M, Yamada S, Onoue S. Photosafety assessments on pirfenidone: photochemical, photobiological, and pharmacokinetic characterization. J Photochem Photobiol B. 2013;120:44-51.

## Event: 1595: Oxidation/denatuation of membrane proteins (https://aopwiki.org/events/1595)

Short Name: Oxidation/denatuation of membrane proteins

### AOPs Including This Key Event

| AOP ID and Name | Event Type |
|---|---|
| Aop:282 - Reactive oxygen species generated from photoreactive chemicals leading to phototoxic reactions (https://aopwiki.org/aops/282) | KeyEvent |

### Stressors

| Name |
|---|
| Photoactivated chemicals |
| Reactive oxygen species |

### Biological Context

| Level of Biological Organization |
|---|
| Cellular |

### Domain of Applicability

**Taxonomic Applicability**

| Term | Scientific Term | Evidence | Links |
|---|---|---|---|
| human | Homo sapiens | High | NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606) |

**Life Stage Applicability**

| Life Stage | Evidence |
|---|---|
| All life stages | High |

**Sex Applicability**

| Sex | Evidence |
|---|---|
| Mixed | High |

**Chemicals:** This KE applies to a wide range of chemicals. The chemicals generate a reactive species, such as reactive oxygen species, following absorption of photon energy from light within the range of natural sunlight (290-700 nm) (ICH, 2014, Onoue and Tsuda, 2006).

**Sex:** This KE applies to both males and females.

**Life stages:** The relevant life stages for this KE are all life stages after born.

**Taxonomic:** This KE mainly applies to human.

### Key Event Description

Reactive oxygen species yielded from photoactivated chemicals can cause cross-linking of proteins and oxidation of sulfydryl groups resulting in disulfide cross-links (Dalle Carbonare and Pathak, 1992).

### How it is Measured or Detected

As for in vitro systems, sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) and other gel electrophoresis methodologies can detect denaturation of proteins (Dalle Carbonare and Pathak, 1992).

### References

Dalle Carbonare M, Pathak MA. Skin photosensitizing agents and the role of reactive oxygen species in photoaging. J Photochem Photobiol B. 1992;14:105-24.

ICH. ICH Guideline S10 Guidance on Photosafety Evaluation of Pharmaceuticals.: International Council on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use; 2014.

Onoue S, Tsuda Y. Analytical studies on the prediction of photosensitive/phototoxic potential of pharmaceutical substances. Pharmaceutical research. 2006;23:156-64.

## List of Adverse Outcomes in this AOP

Event: 1599: Inflamatory events in light-exposed tissues (https://aopwiki.org/events/1599)

Short Name: Inflammatory events

### AOPs Including This Key Event

| AOP ID and Name | Event Type |
|---|---|
| Aop:282 - Reactive oxygen species generated from photoreactive chemicals leading to phototoxic reactions (https://aopwiki.org/aops/282) | AdverseOutcome |

### Stressors

| Name |
|---|
| Light (290-700 nm) |
| Photoreactive chemicals |
| Reactive oxygen species |

### Biological Context

| Level of Biological Organization |
|---|
| Organ |

## Domain of Applicability

**Taxonomic Applicability**

| Term | Scientific Term | Evidence | Links |
|---|---|---|---|
| human | Homo sapiens | High | NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606) |

**Life Stage Applicability**

| Life Stage | Evidence |
|---|---|
| All life stages | High |

**Sex Applicability**

| Sex | Evidence |
|---|---|
| Mixed | High |

**Chemicals:** This AO applies to a wide range of chemicals.  Phototoxic chemicals are recognized to have following characteristics: (i) absorption of light within the range of natural sunlight (290-700 nm); (ii) generation of a reactive species following absorption of UV-visible light; (iii) distribution to light-exposed tissues (e.g., skin and eye) in ICH S10 guideline for photosafety assessment (ICH, 2014).

**Sex:** This AO applies to both males and females.

**Life stages:** The relevant life stages for this AO are all life stages after born.

**Taxonomic:** This AO mainly applies to human.

## Key Event Description

Photoirritation is frequently characterized as exaggerated sunburn sometimes mediated by oxidative stress in the cell membrane, and hyperpigmentation and desquamation may occur as a residual effect of a phototoxic reaction. Theoretically, if a high concentration of a phototoxic drug accumulates in the skin and the appropriate wavelength of light is present, any individual will develop a phototoxic reaction. In particular, peroxidation of membrane lipid could be induced by some photosensitizers under photo-irradiation, and this photochemical reaction certainly correlates with damage produced in the cell membrane, leading to the skin photoirritation (Castell et al. , 1994, Onoue et al. , 2009).

## How it is Measured or Detected

Inflammatory events induced by photoreactive chemicals can be detected *in vivo* phototoxicity testing and photopatch test in clinical (Epstein, 1964, ICH, 2014, Onoue et al., 2009).

## Regulatory Significance of the AO

Inflammatory events in light-exposed tissues are considered to be the endpoint of ROS-mediated chemical phototoxicity, especially photoirritant reactions (ICH, 2014, Onoue et al., 2009).

## References

Castell JV, Gomez-Lechon MJ, Grassa C, Martinez LA, Miranda MA, Tarrega P. Photodynamic lipid peroxidation by the photosensitizing nonsteroidal antiinflammatory drugs suprofen and tiaprofenic acid. Photochem Photobiol. 1994;59:35-9.

Epstein S. The Photopatch Test; Its Technique, Manifestations, and Significance. Ann Allergy. 1964;22:1-11.

ICH. ICH Guideline S10 Guidance on Photosafety Evaluation of Pharmaceuticals.: International Council on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use; 2014.

Onoue S, Seto Y, Gandy G, Yamada S. Drug-induced phototoxicity; an early *in vitro* identification of phototoxic potential of new drug entities in drug discovery and development. Current drug safety. 2009;4:123-36.

# Appendix 2

# List of Key Event Relationships in the AOP

## List of Adjacent Key Event Relationships

Relationship: 1845: ROS generation leads to Oxidation of membrane lipids (https://aopwiki.org/relationships/1845)

AOPs Referencing Relationship

| AOP Name | Adjacency | Weight of Evidence | Quantitative Understanding |
|---|---|---|---|
| **Reactive oxygen species generated from photoreactive chemicals leading to phototoxic reactions (https://aopwiki.org/aops/282)** | **adjacent** | **High** | **Low** |

Evidence Supporting Applicability of this Relationship

**Taxonomic Applicability**

| Term | Scientific Term | Evidence | Links |
|---|---|---|---|
| human | Homo sapiens | High | NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606) |

**Life Stage Applicability**

| Life Stage | Evidence |
|---|---|
| All life stages | High |

**Sex Applicability**

| Sex | Evidence |
|---|---|
| Mixed | High |

**Chemicals:** This KER applies to a wide range of chemicals. The chemicals absorb photon energy from light within the range of light (290-700 nm) (ICH, 2014, Onoue and Tsuda, 2006).

**Sex:** This KER applies to both males and females.

**Life stages:** The relevant life stages for this KER are all life stages after born.

**Taxonomic:** This KER mainly applies to human.

### Key Event Relationship Description

Some photoactivated chemicals can generate reactive oxygen species (ROS) after photoactivation of chemicals by irradiation of light (290–700 nm). ROS generated from photoactivated chemicals can react with membrane lipids and lead to oxidation of membrane lipids.

### Evidence Supporting this KER

**Biological Plausibility**

Photoactivated chemicals generate ROS, and the ROS-generating chemicals cause lipid peroxidation under exposure to light (290–700 nm) in chemical and biological systems (Girotti, 1990, 2001, Onoue and Tsuda, 2006).

**Empirical Evidence**

Lipid peroxidation was occurred by ROS-generating chemicals under exposure to simulated sunlight (Onoue et al. , 2011, Onoue and Tsuda, 2006).

A photoreactive chemical indicated dose-dependent increases in ROS generation and lipid peroxidation after exposure to a fixed dose of simulated sunlight (Seto et al. , 2013).

### References

Girotti AW. Photodynamic lipid peroxidation in biological systems. Photochem Photobiol. 1990;51:497-509.

Girotti AW. Photosensitized oxidation of membrane lipids: reaction pathways, cytotoxic effects, and cytoprotective mechanisms. J Photochem Photobiol B. 2001;63:103-13.

ICH. ICH Guideline S10 Guidance on Photosafety Evaluation of Pharmaceuticals.: International Council on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use; 2014.

Onoue S, Seto Y, Ochi M, Inoue R, Ito H, Hatano T, et al. In vitro photochemical and phototoxicological characterization of major constituents in St. John's Wort (Hypericum perforatum) extracts. Phytochemistry. 2011;72:1814-20.

Onoue S, Tsuda Y. Analytical studies on the prediction of photosensitive/phototoxic potential of pharmaceutical substances. Pharmaceutical research. 2006;23:156-64.

Seto Y, Inoue R, Kato M, Yamada S, Onoue S. Photosafety assessments on pirfenidone: photochemical, photobiological, and pharmacokinetic characterization. J Photochem Photobiol B. 2013;120:44-51.

## Relationship: 1846: ROS generation leads to Oxidation/denatuation of membrane proteins (https://aopwiki.org/relationships/1846)

### AOPs Referencing Relationship

| AOP Name | Adjacency | Weight of Evidence | Quantitative Understanding |
|---|---|---|---|
| **Reactive oxygen species generated from photoreactive chemicals leading to phototoxic reactions (https://aopwiki.org/aops/282)** | **adjacent** | **High** | **Low** |

### Evidence Supporting Applicability of this Relationship

**Taxonomic Applicability**

| Term | Scientific Term | Evidence | Links |
|---|---|---|---|
| human | Homo sapiens | High | NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606) |

**Life Stage Applicability**

| Life Stage | Evidence |
|---|---|
| All life stages | High |

**Sex Applicability**

| Sex | Evidence |
|---|---|
| Mixed | High |

**Chemicals:** This KER applies to a wide range of chemicals. The chemicals absorb photon energy from light within the range of light (290-700 nm) (ICH, 2014, Onoue and Tsuda, 2006).

**Sex:** This KER applies to both males and females.

**Life stages:** The relevant life stages for this KER are all life stages after born.

**Taxonomic:** This KER mainly applies to human.

### Key Event Relationship Description

Some photoactivated chemicals can generate reactive oxygen species (ROS) after photoactivation of chemicals by irradiation of light (290–700 nm). ROS generated from photoactivated chemicals can react with membrane proteins and lead to oxidation/denaturation of membrane proteins.

### Evidence Supporting this KER

**Biological Plausibility**
Photoactivated chemicals by UVA generate ROS including singlet oxygen, superoxide, and hydroxyl radicals, and the generated ROS cause cross-linking of proteins and denaturation of proteins as oxidative damages by photoactivated chemicals (Dalle Carbonare and Pathak, 1992).

**Empirical Evidence**
ROS generated from photosensitizing agents led to oxidation and denaturation of proteins, resulting in cross-linking of proteins and oxidation of sulfydryl groups (Dalle Carbonare and Pathak, 1992).

### References

Dalle Carbonare M, Pathak MA. Skin photosensitizing agents and the role of reactive oxygen species in photoaging. J Photochem Photobiol B. 1992;14:105-24.

ICH. ICH Guideline S10 Guidance on Photosafety Evaluation of Pharmaceuticals.: International Council on Harmonisation of Technical

Requirements for Registration of Pharmaceuticals for Human Use; 2014.

Onoue S, Tsuda Y. Analytical studies on the prediction of photosensitive/phototoxic potential of pharmaceutical substances. Pharmaceutical research. 2006;23:156-64.

## Relationship: 1850: Oxidation of membrane lipids leads to Inflammatory events (https://aopwiki.org/relationships/1850)

### AOPs Referencing Relationship

| AOP Name | Adjacency | Weight of Evidence | Quantitative Understanding |
|---|---|---|---|
| **Reactive oxygen species generated from photoreactive chemicals leading to phototoxic reactions (https://aopwiki.org/aops/282)** | **adjacent** | **High** | **Low** |

### Evidence Supporting Applicability of this Relationship

**Taxonomic Applicability**

| Term | Scientific Term | Evidence | Links |
|---|---|---|---|
| human | Homo sapiens | High | NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606) |

**Life Stage Applicability**

| Life Stage | Evidence |
|---|---|
| All life stages | High |

**Sex Applicability**

| Sex | Evidence |
|---|---|
| Mixed | High |

**Chemicals:** This KER applies to a wide range of chemicals. The chemicals generate ROS under exposure to light (290-700 nm) (ICH, 2014, Onoue and Tsuda, 2006).

**Sex:** This KER applies to both males and females.

**Life stages:** The relevant life stages for this KER are all life stages after born.

**Taxonomic:** This KER mainly applies to human.

### Key Event Relationship Description

Oxidation of membrane lipids can lead to inflammatory events via oxidative damage of cellular membranes and increases in expression of pro-inflammatory cytokines.

### Evidence Supporting this KER

**Biological Plausibility**

Lipid peroxidation in cellar membrane is an oxidative damage of cellular membrane, and viability of cells decreases by lipid peroxidation of photoreactive chemicals (Castell et al., 1994).

**Empirical Evidence**

Increases in lipid peroxidation and inflammatory-related cytokines were observed in the murine skin, and naringenin, an anti-oxidant, attenuated these increases in a dose-dependent manner (Martinez et al., 2015).

Benzoyl peroxide, a ROS generator, led to lipid peroxidation and GSH depletion, and the changes caused the gene expression of pro-inflammatory cytokines in human keratinocytes (Valacchi et al., 2001).

### References

Castell JV, Gomez-Lechon MJ, Grassa C, Martinez LA, Miranda MA, Tarrega P. Photodynamic lipid peroxidation by the photosensitizing nonsteroidal antiinflammatory drugs suprofen and tiaprofenic acid. Photochem Photobiol. 1994;59:35-9.

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Onoue S, Tsuda Y. Analytical studies on the prediction of photosensitive/phototoxic potential of pharmaceutical substances. Pharmaceutical research. 2006;23:156-64.

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## Relationship: 1851: Oxidation/denatuation of membrane proteins leads to Inflammatory events (https://aopwiki.org/relationships/1851)

### AOPs Referencing Relationship

| AOP Name | Adjacency | Weight of Evidence | Quantitative Understanding |
|---|---|---|---|
| **Reactive oxygen species generated from photoreactive chemicals leading to phototoxic reactions (https://aopwiki.org/aops/282)** | **adjacent** | **High** | **Low** |

### Evidence Supporting Applicability of this Relationship

**Taxonomic Applicability**

| Term | Scientific Term | Evidence | Links |
|---|---|---|---|
| human | Homo sapiens | High | NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606) |

**Life Stage Applicability**

| Life Stage | Evidence |
|---|---|
| All life stages | High |

**Sex Applicability**

| Sex | Evidence |
|---|---|
| Mixed | High |

**Chemicals:** This KER applies to a wide range of chemicals. The chemicals generate ROS under exposure to light (290-700 nm) (ICH, 2014, Onoue and Tsuda, 2006).

**Sex:** This KER applies to both males and females.

**Life stages:** The relevant life stages for this KER are all life stages after born.

**Taxonomic:** This KER mainly applies to human.

### Key Event Relationship Description

Oxidation/denaturation of membrane proteins can lead to inflammatory events via oxidative damage of cellular membranes and increases in expression of pro-inflammatory cytokines.

Necrosis in living tissues can be occurred as an inflammatory events via oxidative damages of membrane proteins.

### Evidence Supporting this KER

**Biological Plausibility**
Oxidative damage of membrane proteins by photoactivated chemicals can cause oxidation and denaturation of proteins, and damaged proteins induces inflammatory events (necrosis) (Dalle Carbonare and Pathak, 1992, Opie, 1962).

**Empirical Evidence**
Denaturation of proteins induced necrosis and inflammatory in the skin (Opie, 1962).

### References

Dalle Carbonare M, Pathak MA. Skin photosensitizing agents and the role of reactive oxygen species in photoaging. J Photochem Photobiol B. 1992;14:105-24.

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Opie EL. On the relation of necrosis and inflammation to denaturation of proteins. The Journal of experimental medicine. 1962;115:597-608.