Development of ROS Assay for Photosafety Evaluation

Satomi Onoue
School of Pharmaceutical Sciences
University of Shizuoka, Japan

Phototoxicity webinar

Oct. 4th, 2023

Scope of talks

1. Drug-induced phototoxicity
2. Reactive oxygen species (ROS) assay for photosafety testing
3. International harmonization on ROS assay
   - Multi-lab validation study
   - ICH S10 guideline (2014) and OECD TG495 (2019)
4. Photosafety testing
   - Combination use of ROS and PK data
5. Summary

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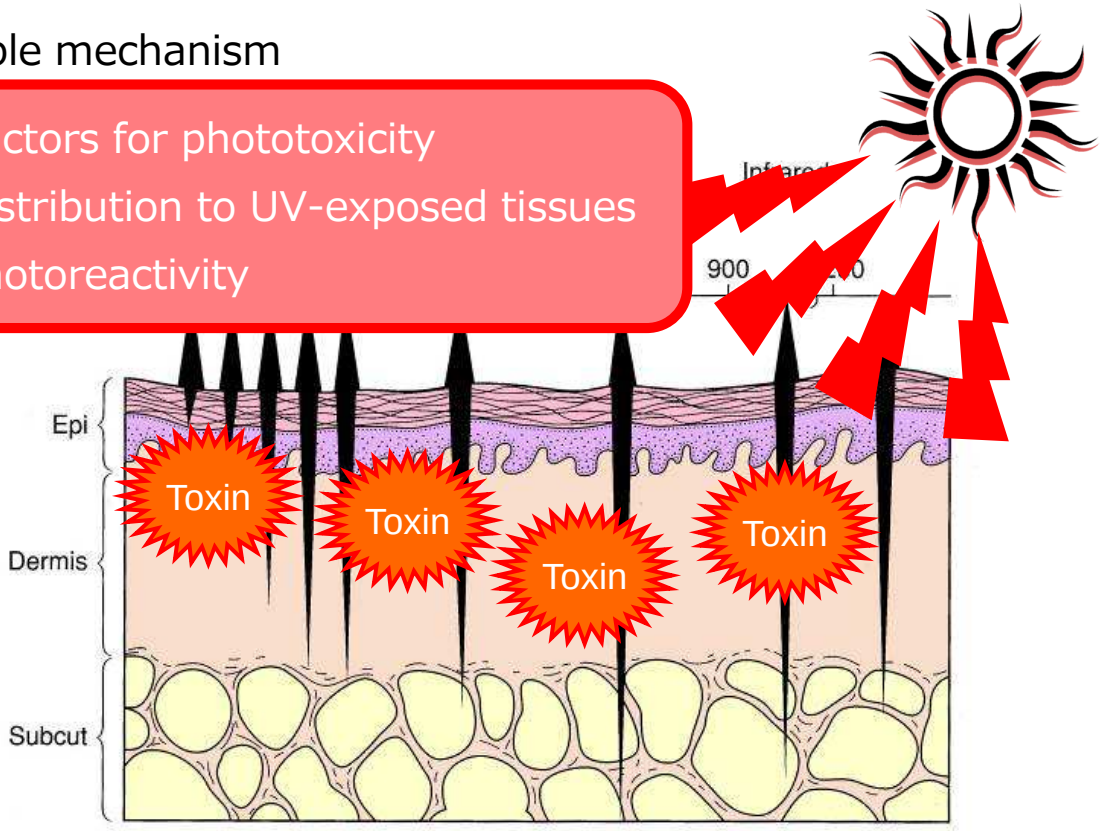
Chemical phototoxicity

➢ Possible mechanism

Risk factors for phototoxicity
1) Distribution to UV-exposed tissues
2) Photoreactivity



Onoue *et al.*, *Curr Drug Saf*, **4:** 123 (2009); Onoue *et al.*, *High-Throughput Screening Methods in Toxicity Testing* (John Wiley&Sons) 177 (2013)

Laws of photochemistry

- First law of photochemistry (Grotthus-Draper law)

  ✓ Only light that is absorbed can be active in photochemical processes.

  ✓ Photobiological processes can be included here.

- Second law of photochemistry (Einstein-Stark law)

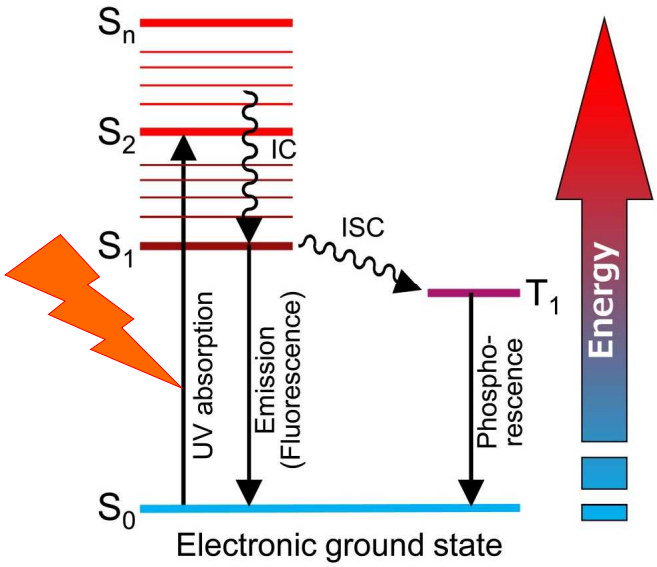
  ✓ A single photon can excite only one electron.

ROS in phototoxic events

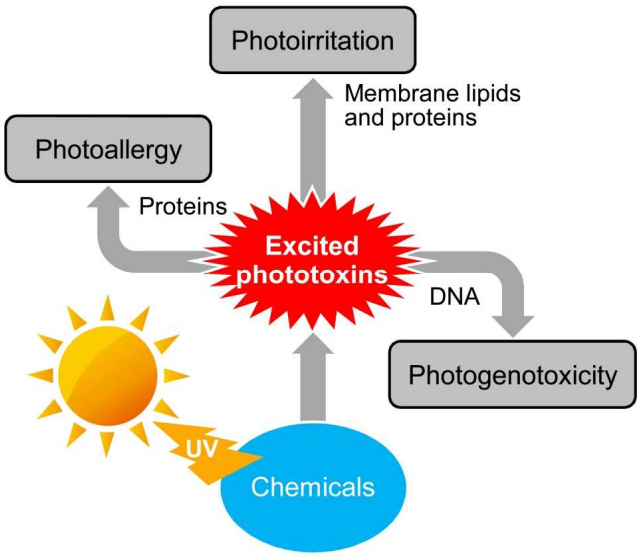
- Working hypothesis;

  "ROS may induce photochemical/toxic reactions"

Jablonski diagram



Several phototoxic responses



Onoue *et al.*, *J. Dermatol. Sci.*, 85: 4–11 (2017)

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Determination of ROS

- Superoxide (O_2^-) as indicator of type I photochemical reaction

  Reduction of Nitroblue tetrazolium (NBT)

O_2^- + Nitroblue tetrazolium → O_2 + Nitroblue diformazan ($\Delta A_{560\ nm}\uparrow$)

- Singlet oxygen ($^1O_2$) as indicator of type II photochemical reaction

  Bleaching of p-nitrosodimethylaniline (RNO)

$$^1O_2 + A \rightarrow [AO_2] \rightarrow AO_2$$

$$[AO_2] + RNO \rightarrow -RNO + Products \ (\Delta A_{440\ nm}\downarrow)$$

(A, $^1O_2$ acceptor, imidazole; RNO: nitroso compounds)

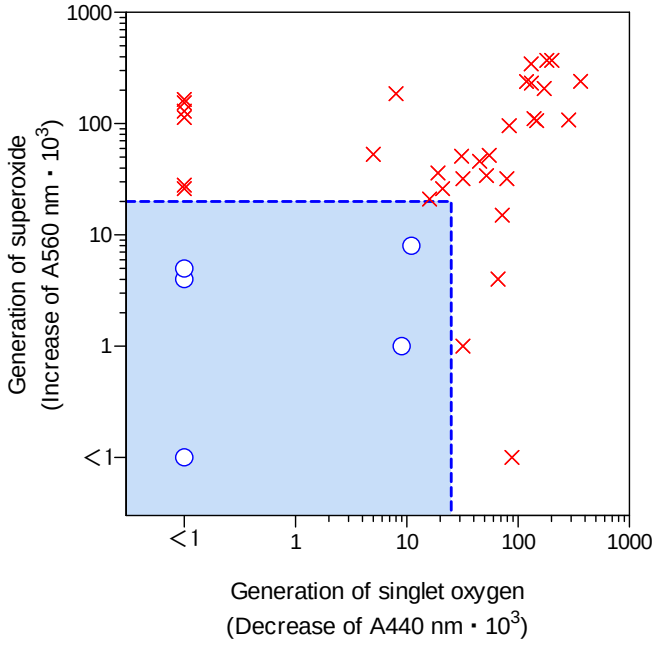
*Recommended protocol is now available in JaCVAM web site.

http://www.jacvam.jp/files/news/ROS_protocol_v3.1_130920_clean.pdf

Onoue *et al.*, *Pharm. Res.*, **23**: 156 (2006); Onoue *et al.*, *Pharm. Res.*, **25**: 861 (2008)

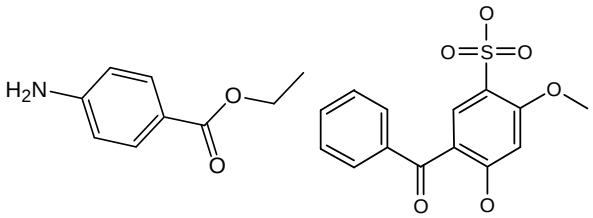
ROS assay on marketed drugs

- ROS generation from light-exposed phototoxins



○, non-phototoxic drugs (6 compounds)
×, phototoxic drugs (33 compounds)

ROS data on UV absorbers

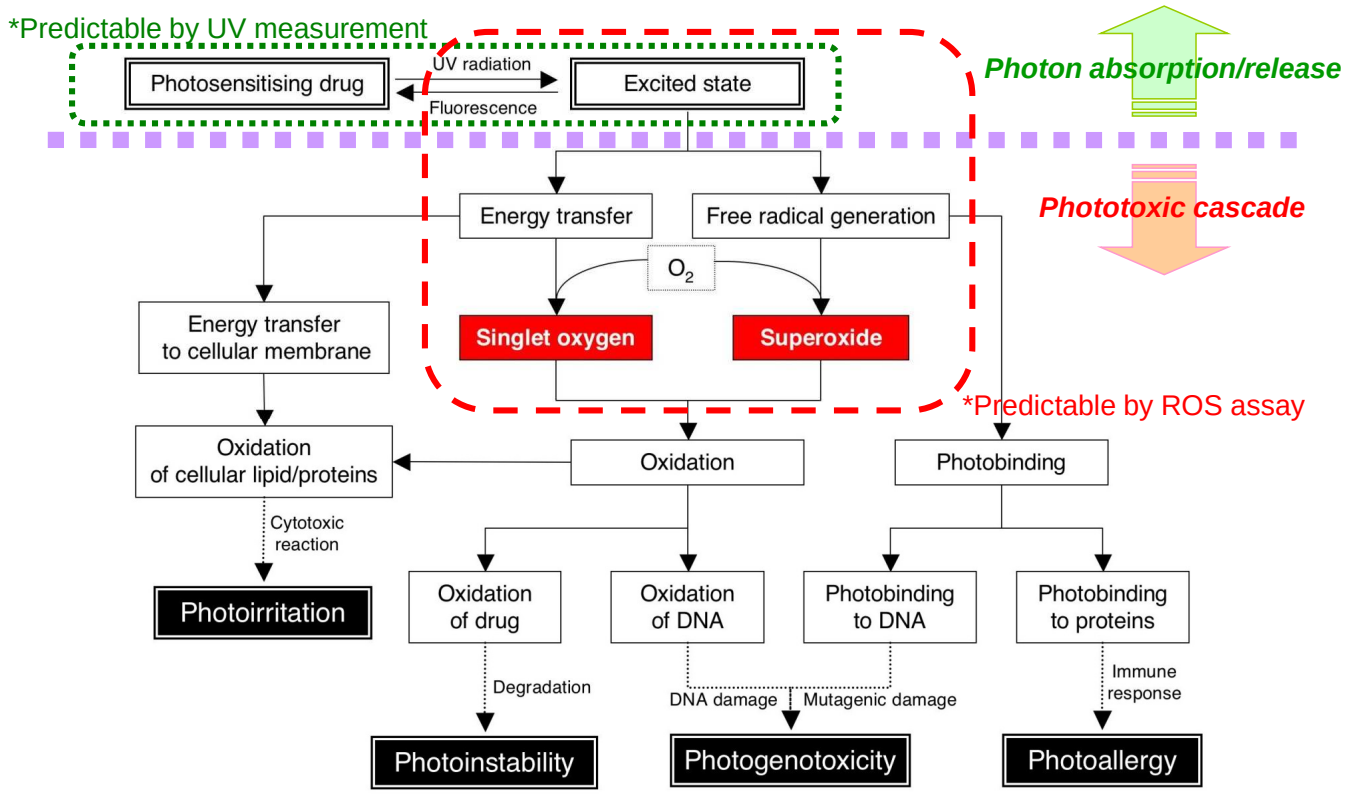


Benzocaine		Sulisobenzone	
MEC:	17,025 $M^{-1}cm^{-1}$ (290 nm)	MEC:	6,525 $M^{-1}cm^{-1}$ (319 nm)
ROS: $^1O_2$	3±1	ROS: $^1O_2$	1±2
O_2^-	N.D.	O_2^-	N.D.

Proposed threshold
25 for singlet oxygen ($\Delta A_{440\ nm}$・10^3)
20 for superoxide ($\Delta A_{560\ nm}$・10^3)

Onoue *et al.*, *J Pharm Biomed Anal* **46** (2008) 187–193.

UV and ROS assay for photosafety assessment



Onoue *et al.*, *Pharm. Res.*, **23:** 156–64 (2006)

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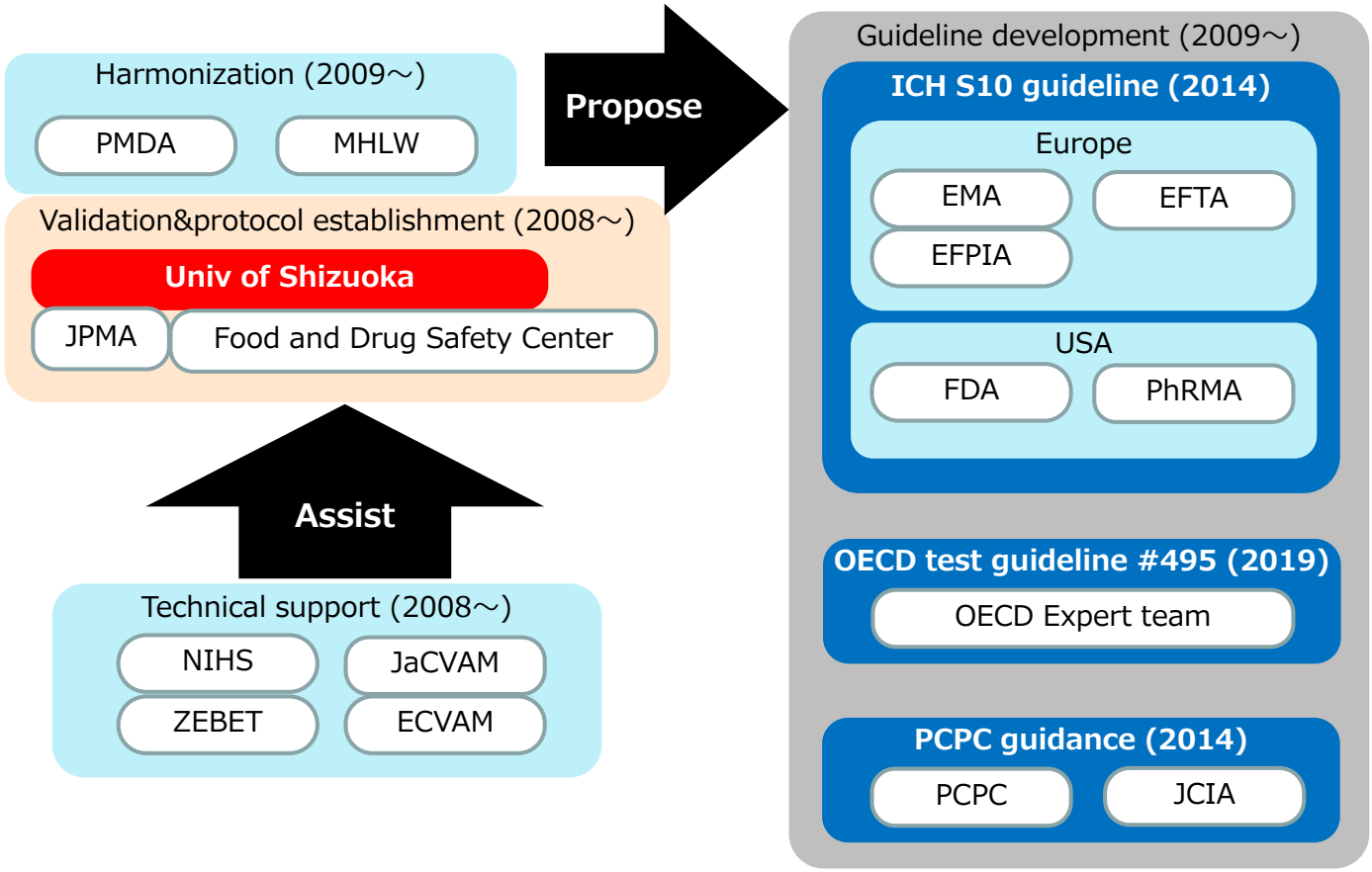
International harmonization on ROS assay



Validation study (supervised by JaCVAM)

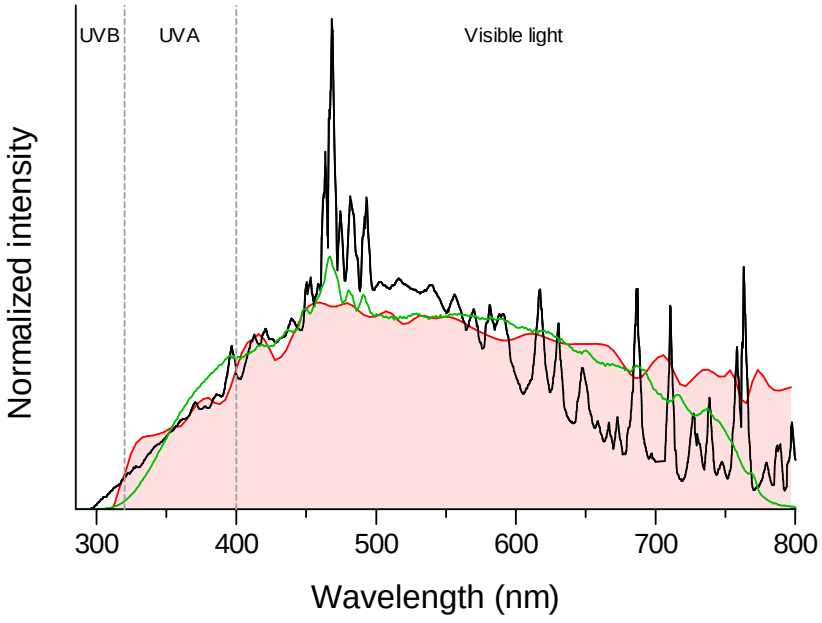
- Study design

No. of labs	7 laboratories	
Test chemicals	Coded 42 compounds 200 μM (final concentration) When precipitation is found microscopically, assay at lower concentrations, 20 μM or 2 μM, was conducted.	
Controls	Qunine HCl (positive control) Sulisobenzone (negative control)	
Criteria	Calculation	$^1O_2$: Decrease of $A_{440\ nm} \times 1000$ O_2^-: Increase of $A_{560\ nm} \times 1000$
	Positive	$^1O_2$:≧25 and/or O_2^-≧20 at 2–200 μM
	Negative	$^1O_2$:＜25 and O_2^-＜20 at 200 μM

Recommended protocol in JaCVAM web site.
http://www.jacvam.jp/files/news/ROS_protocol_v3.1_130920_clean.pdf

Solar simulators

- Standard and simulated daylights



Lab#1–3:
CPS/CPS+ (Atlas)

Lab#4–7:
SXL-2500V2 (Seric)

Standard daylight (CIE85/1989)
Atlas Suntest CPS/CPS+ (ca. 2.0 mW/cm^2): Lab#1–3
Seric SXL-2500V2 (3.0–5.0 mW/cm^2): Lab#4–7

Predictive capacity (Suntest and SXL-2500V2)

- Applicability and predictive capacity of ROS assay

	Suntest CPS series (Atlas)			SXL-2500V2 (Seric)			
	Lab#1	Lab#2	Lab#3	Lab#4	Lab#5	Lab#6	Lab#7
Evaluable chemicals (%)	78.6	73.8	81	81	81	71.4	76.2
Sensitivity (%)	100	100	100	100	100	100	100
Specificity (%)	81.8	60	41.7	53.8	46.2	60	63.6
Positive predictivity (%)	91.7	84	75.9	77.8	75	83.3	84
Negative predictivity (%)	100	100	100	100	100	100	100

S. Onoue *et al.*, *Toxicol In Vitro*, **28**: 515 (2013)

ICH S10 guideline &

- **ROS assay**

"A negative result in this
conditions, would indicate
provided a test concentrati

International conferenc

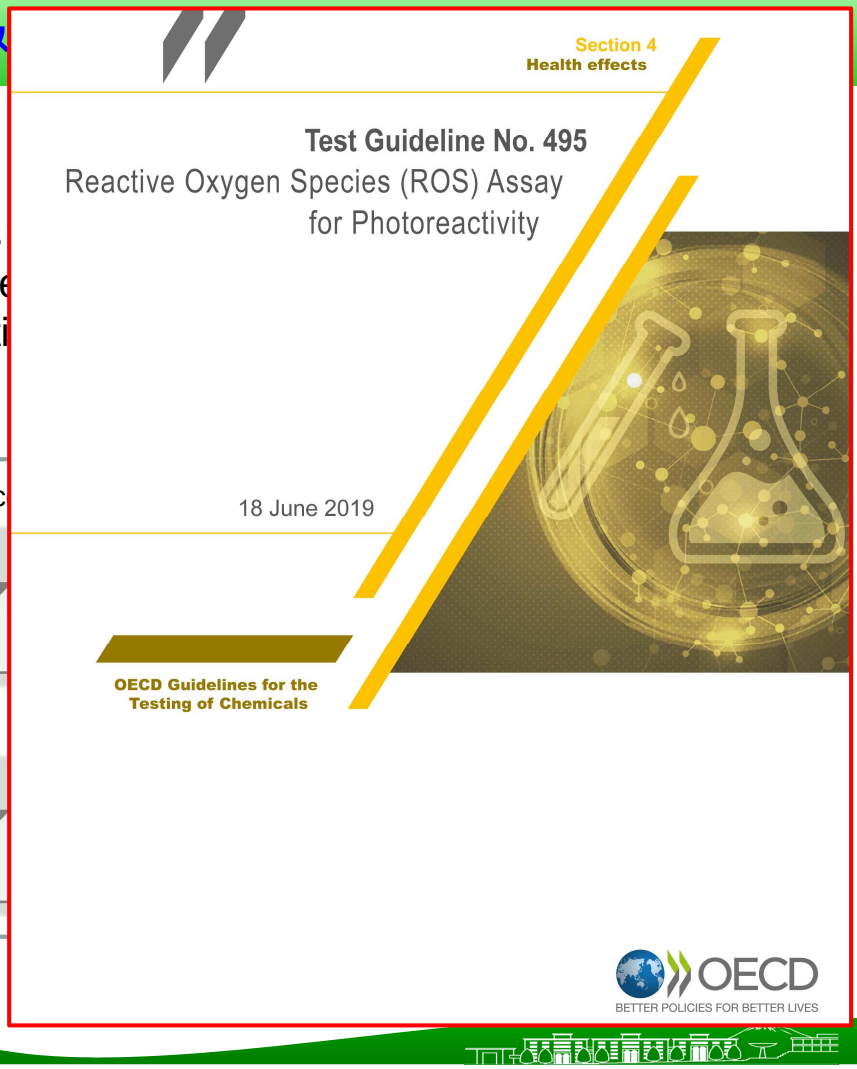
INTERNATIONAL CONFERENCE ON HARMONISATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE

DRAFT CONSENSUS GUIDELINE

PHOTOSAFETY EVALUATION OF PHARMACEUTICALS

S10

Current *Step 2* version
dated 15 November 2012



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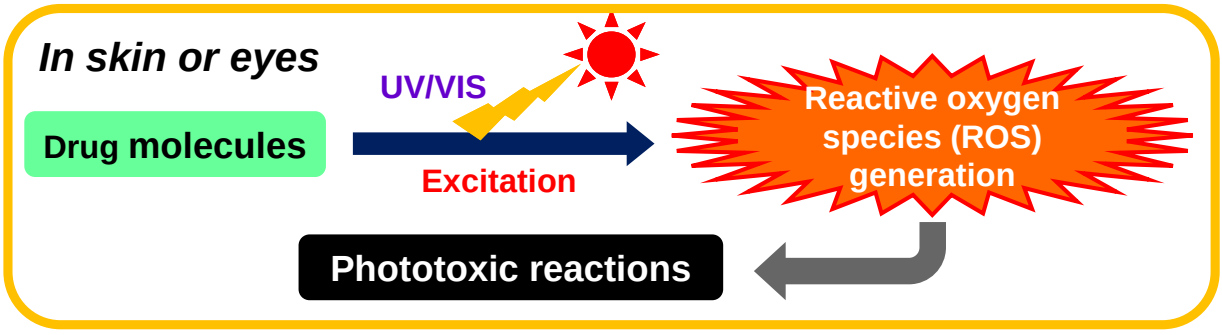
Drug-induced phototoxicity

✓ **Possible mechanism of phototoxicity**



✓ **Strategic photosafety screening**

ROS assay — Photoreactivity

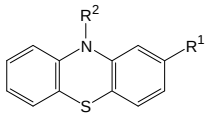
\+

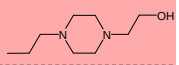
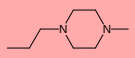
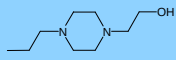
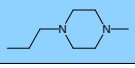
Pharmacokinetic data — Skin exposure

→ **Phototoxic risk prediction?**

Combination use of ROS and PK data (1)

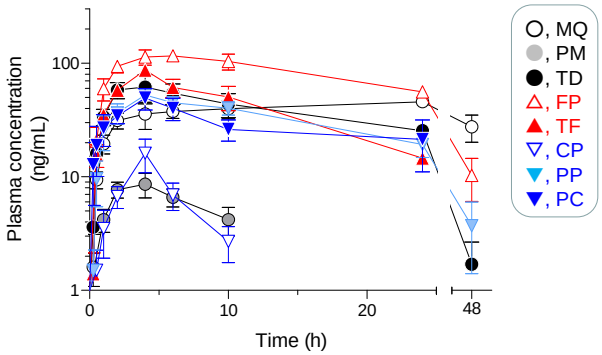
Phenothiazines (PTZs)



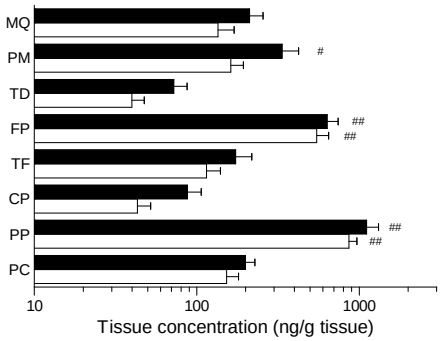
PTZs	CAS No.	Clog *P* [a]	R^1	R^2
Non-halogenated group				
Mequitazine (MQ)	88598-74-7	4.91	−H	
Promethazine HCl (PM)	58-33-3	4.60	−H	
Thioridazine HCl (TD)	130-61-0	6.20	$-SCH_3$	
Fluorinated group				
Fluphenazine 2HCl (FP)	146-56-5	4.32	$-CF_3$	
Trifluoperazine 2HCl (TF)	440-17-5	4.89	$-CF_3$	
Chlorinated group				
Chlorpromazine HCl (CP)	69-09-0	5.50	−Cl	
Perphenazine (PP)	58-39-9	4.01	−Cl	
Prochlorperazine dimaleate (PC)	84-02-6	4.58	−Cl	

Onoue *et al.*, *Toxicol Sci*, **137**: 469 (2014)

Cassette-dosing PK study



Plasma concentrations of PTZs in rats after oral cassette-dosing of 8 PTZs (5 mg/kg, each). Mean±SE (n=4).



Deposition in skin (filled bars) and eyes (open bars).
#, $P<0.05$ and ##, $P<0.01$ with respect to TD.

Combination use of ROS and PK data (2)

Decision matrix

	Obtained data/prediction
Predicted	
Phototoxicity	**FP** > PP > **TF** > PC > MQ ≒ PM ≒ TD > CP
Chemical series	**Fluorinated PTZs** > Chlorinated > Non-halogenated
Observed	
In vivo phototoxicity	**FP** ≒ **TF** > PC > PP ≒ CP ≫ MQ ≒ PM ≒ TD

Eyes (ng/g tissue)	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

Kp: tissue to plasma concentration ratio.

Onoue *et al.*, *Toxicol Sci*, **137**: 469 (2014)

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Summary

1. Reactive oxygen species (ROS) assay was newly developed as *in chemico* photosafety testing tool.
2. The validation study indicates satisfactory outcomes in terms of transferability, intra- and inter-laboratory variability, and predictive capacity.
3. ROS assay was successfully adopted as ICH S10 guideline (2014) and OECD test guideline 495 (2019).
4. Combined use of ROS and PK data might enable evaluation of phototoxic risk with high clinical relevance.

Acknowledgement

- Collaborators in Pfizer

  Dr. Yoshiko Tsuda (Pfizer, Japan)
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  Dr. Karen Alsante (Pfizer, US)
  Dr. Chris Foti (Pfizer, US)
  Dr. Brian Henry (Pfizer, UK)
  Dr. Graham Gandy (Pfizer, UK)

- Validation study

  JaCVAM/NIHS
  Validation management team
  Peer-review panel
  Dr. Manfred Liebsch (ZEBET)
  ICCVAM
  ECVAM
  Dr. Dai Nakae
  Dr. Hiroshi Onodera (PMDA)
  Dr. Horst Spielmann (Freie Universität Berlin)

- University of Shizuoka

  Dr. Yoshiki Seto
  Mr. Masashi Kato
  Mr. Gen Suzuki
  Mr. Masanori Ochi
  Mr. Hiroto Ohtake
  Mr. Yosuke Iyama