



# 3T3 and RhE Phototoxicity Overview, Updates, & Considerations

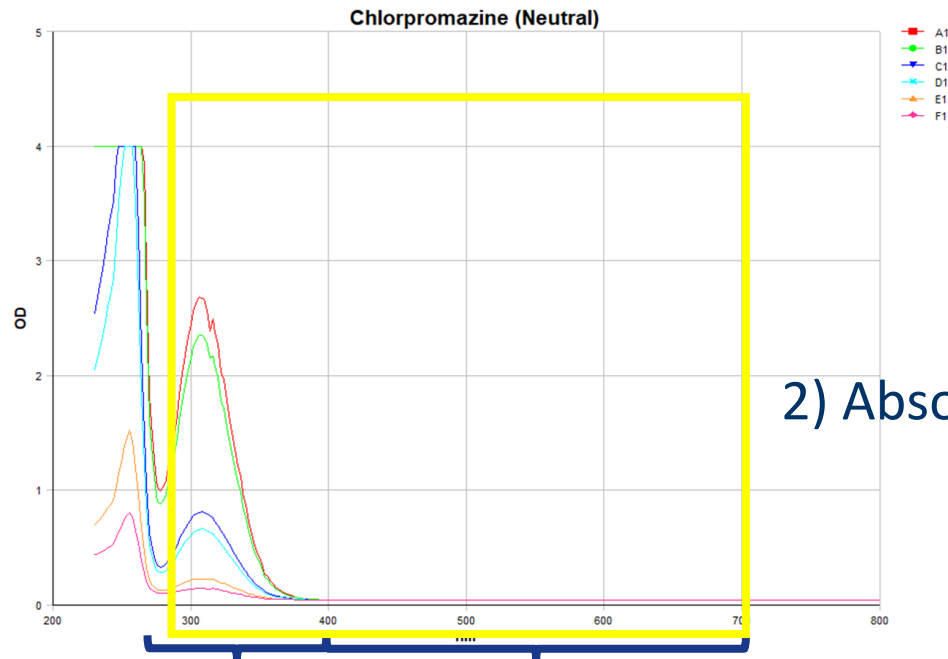
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4 October 2023



# Triggers for Photosafety Testing



## Absorption & Exposure:

- 1) Absorption: between 290-700 nm?
- 2) Absorption: significant ( $\text{MEC} > 1000 \text{ L mol}^{-1} \text{ cm}^{-1}$ )?
- 3) Exposure: skin, eye?

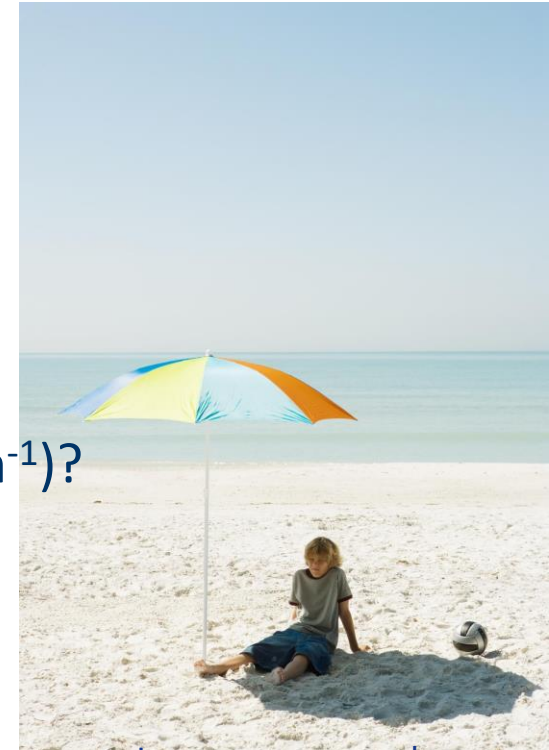
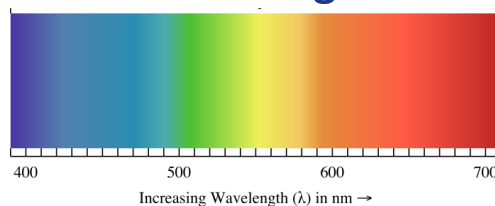


Image: [www.google.com](http://www.google.com)

UVB/UVA

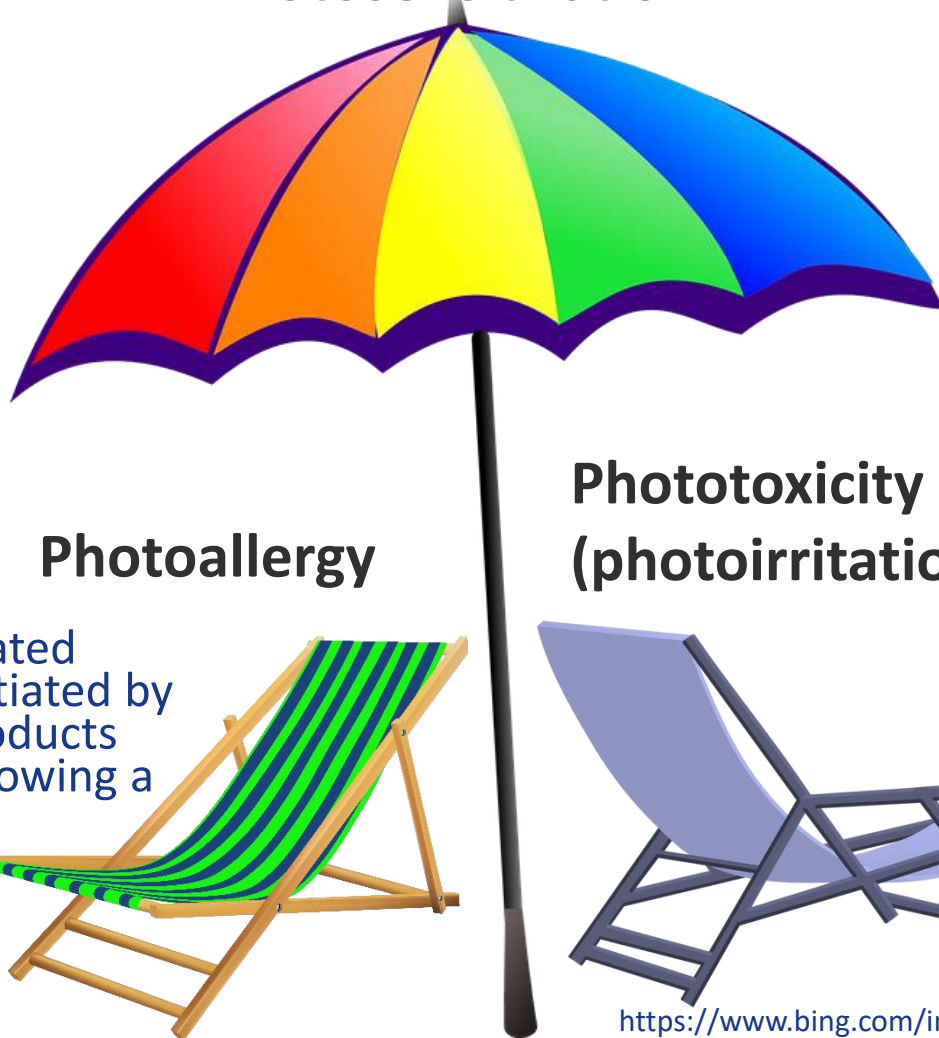
Visible Light



# Terminology\*



## Photosensitization



is a general term occasionally used to describe all light-induced tissue reactions.

## Photoallergy

An immunologically mediated reaction to a chemical, initiated by the formation of photoproducts (e.g., protein adducts) following a photochemical reaction.



## Phototoxicity (photoirritation)



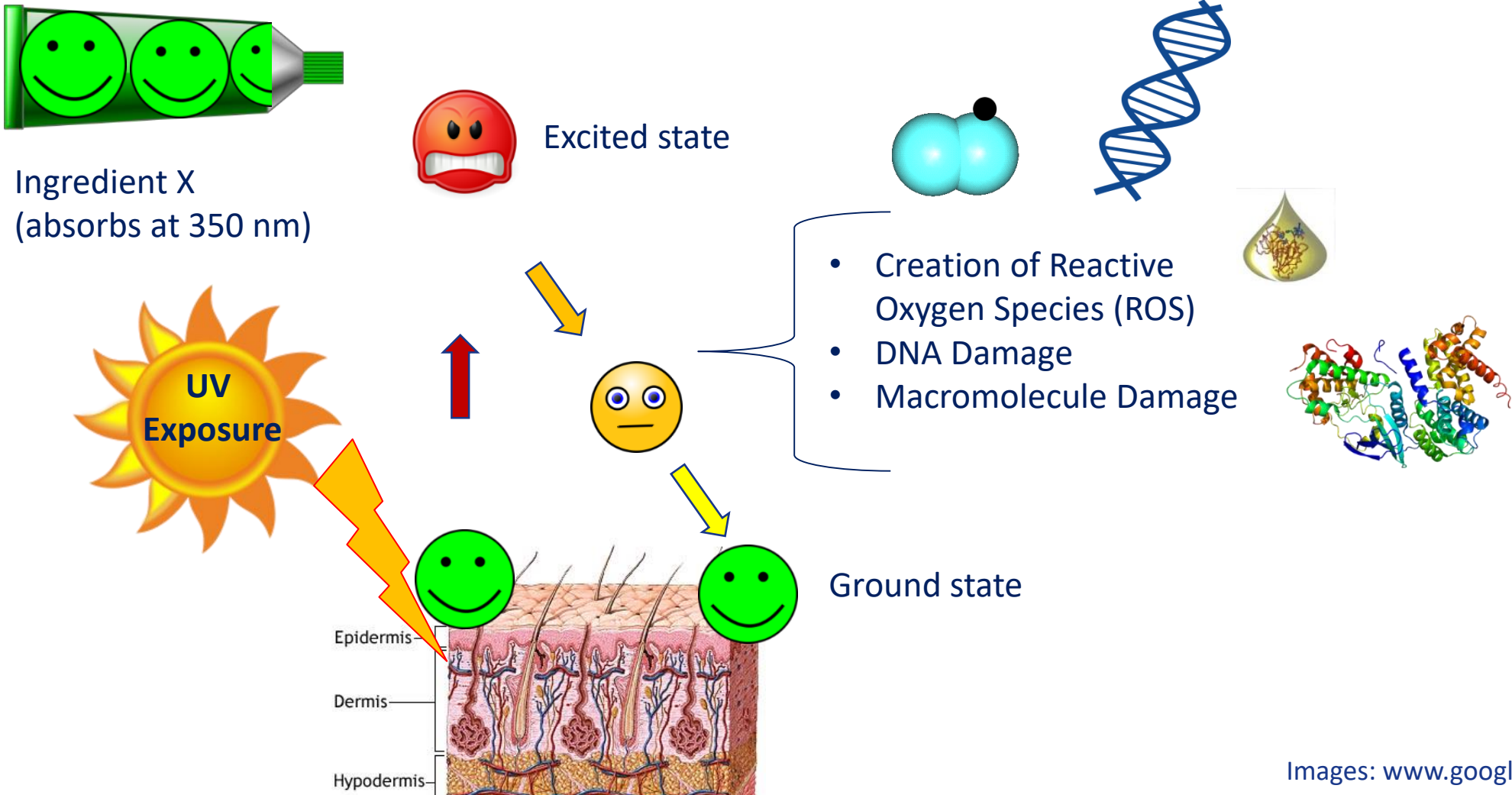
[www.istockphotos.com](http://www.istockphotos.com)

An acute light-induced tissue response to a photoreactive chemical.

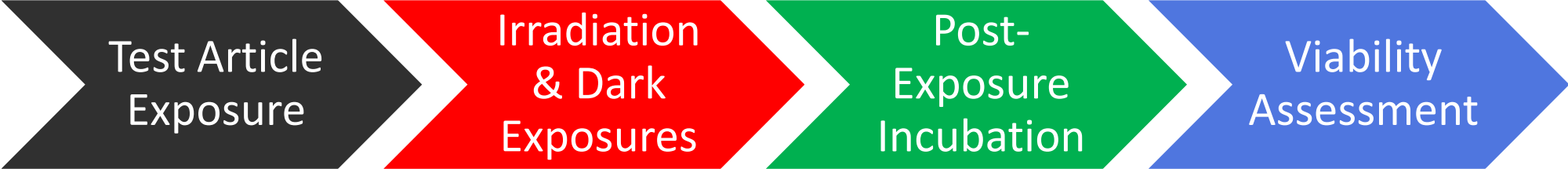
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# General Mechanism of Phototoxicity

Potential for a compound to become more toxic in the presence of light



# General Overview of Assay Steps for 3T3 PT (TG 432) & RhE PT (TG 498)



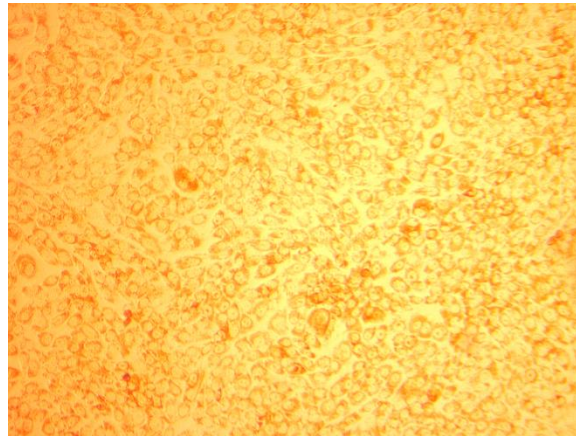
# Data Analyses:

Compare viability of cultures or tissues in the presence and absence of UVA/visible light

## 3T3 Phototoxicity



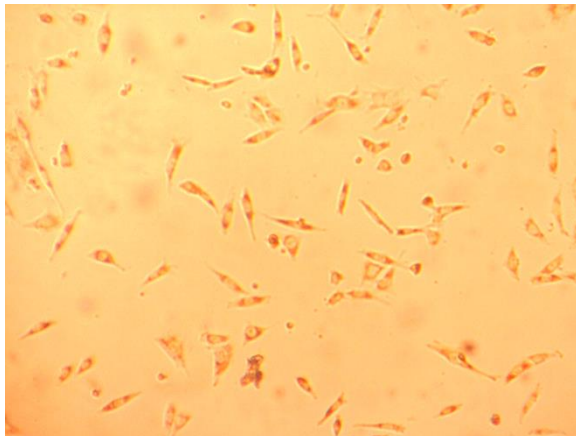
-UVA (dark)



## RhE Phototoxicity



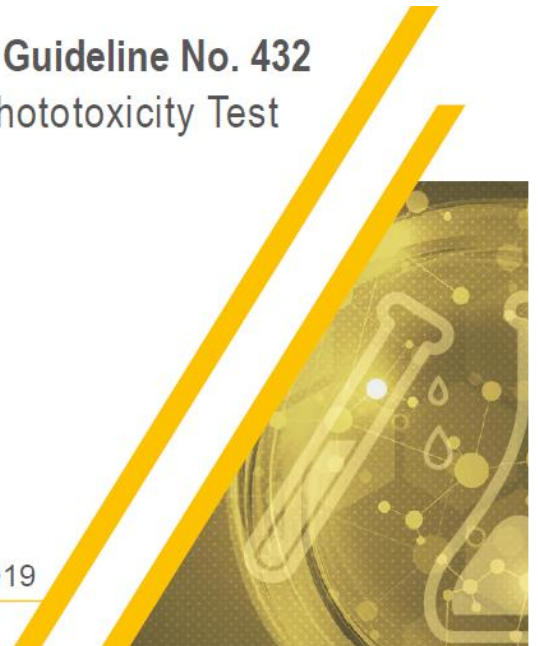
+UVA (light)



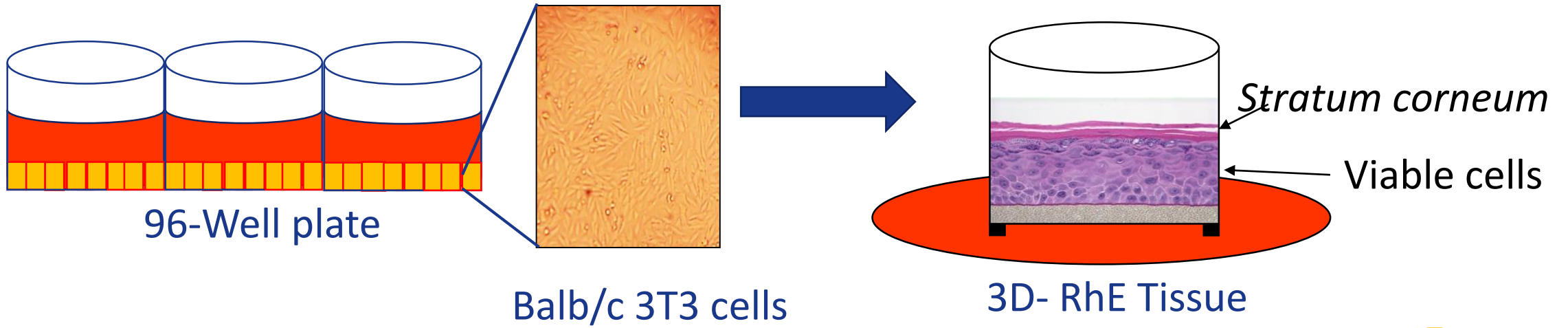
# Updates to 3T3 NRU Phototoxicity Assay (2019)

- MEC trigger increased from  $>10 \text{ L mol}^{-1} \text{ cm}^{-1}$  to  $>1000 \text{ L mol}^{-1} \text{ cm}^{-1}$   
(based on work from Bauer, *et al.*, Henry *et al.*, & ICH S10)
- Additional guidance on solubility & solvents
- Harmonization with other TG, regulatory documents for photosafety
  - Max conc., evaluation of PM
- Procedural clarifications

Test Guideline No. 432  
*In Vitro* 3T3 NRU Phototoxicity Test



# Moving from Monolayer to Tissue Model



**Test Guideline No. 432**  
*In Vitro* 3T3 NRU Phototoxicity Test

18 June 2019

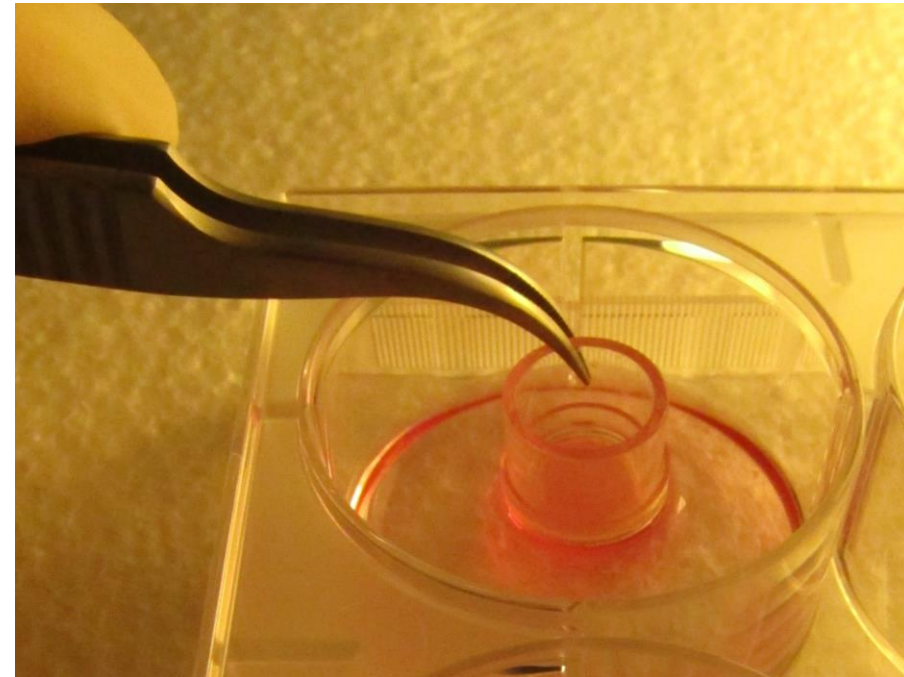
**Test Guideline No. 498**  
*In vitro* Phototoxicity: Reconstructed  
Human Epidermis Phototoxicity test  
method

4 July 2023

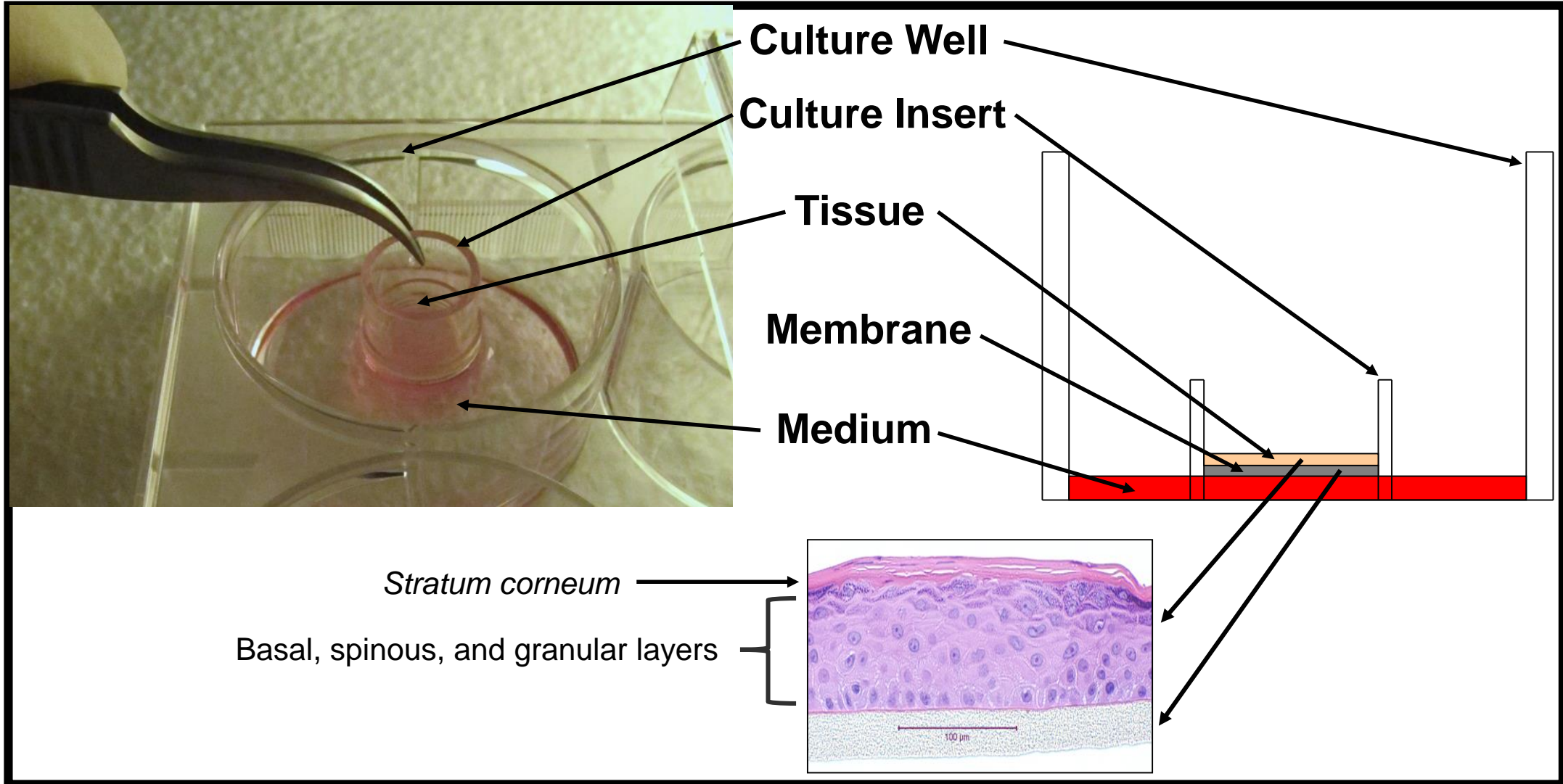


# Advantages over Monolayer Model

- Overcome solubility limitations
- Flexibility in Exposure conditions
  - Incorporation of UVB (as needed)
  - Topical & systemic application
  - Exposure time
- Model end use application
- Address hazard AND risk

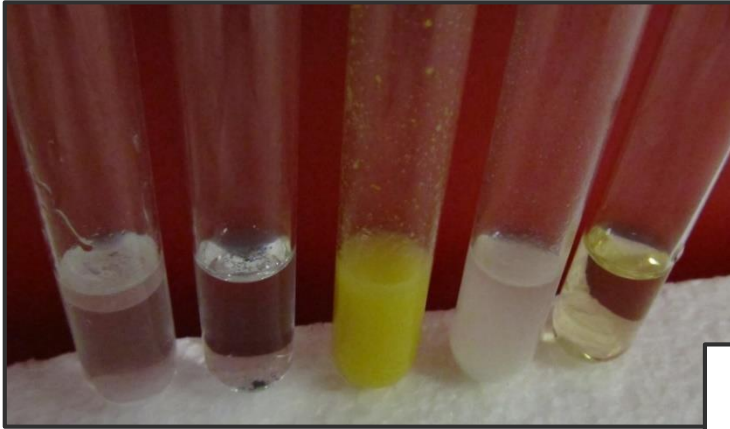


# Reconstructed Human EpiDermis (RhE) Model cultured at Air/Liquid Interface (ALI)



Pictured: EpiDerm™ (EPI-200), MatTek Corporation

# Preliminary Assessments



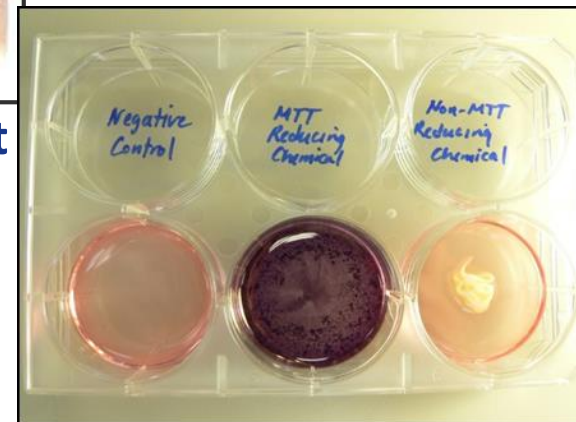
**Solubility Evaluation**

- Max Concentration = 10%
- Suggested Solvents (vehicles): DPBS, HBSS, sesame seed oil, mineral oil, ethanol, acetone:olive oil mix, & others w/ consideration



**Colorant Control Test**

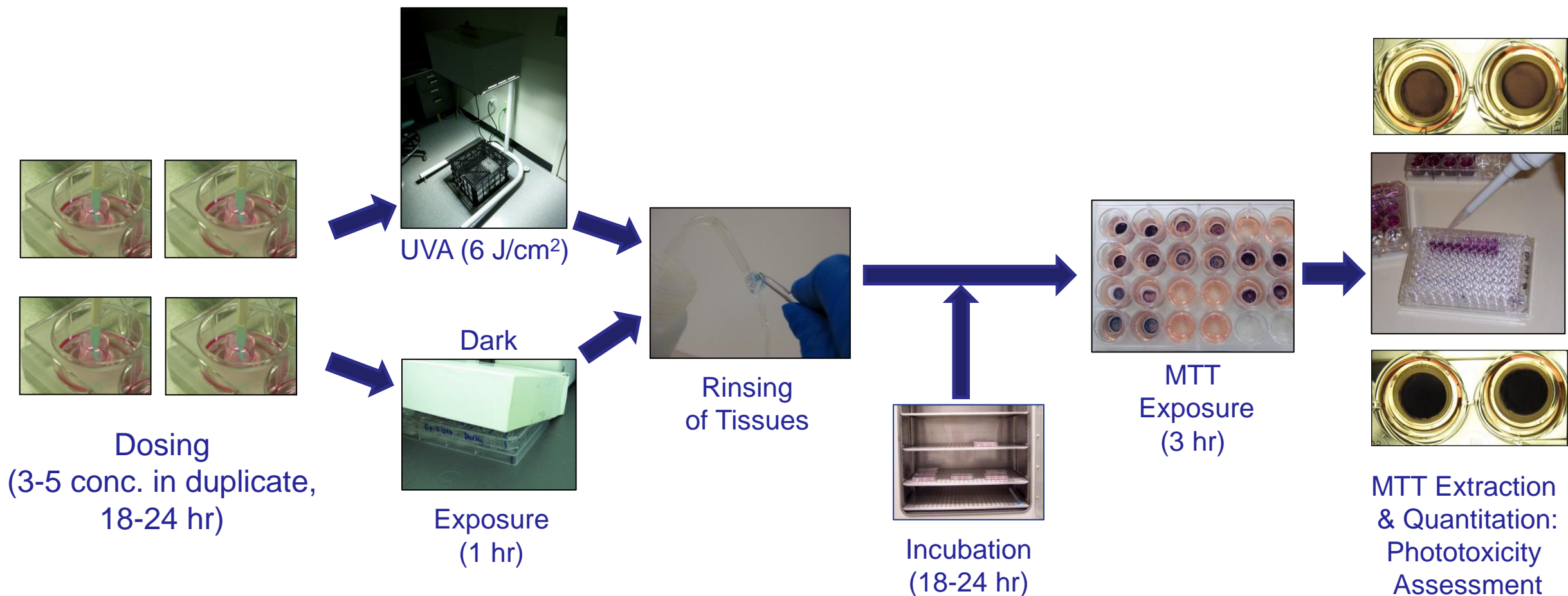
- Can test article cause interference with OD reading?
- If yes, include colorant controls (viable tissues w/o MTT)  
+Irr & -Irr
  - Also consider HPLC/UPLC



**Direct MTT Test**

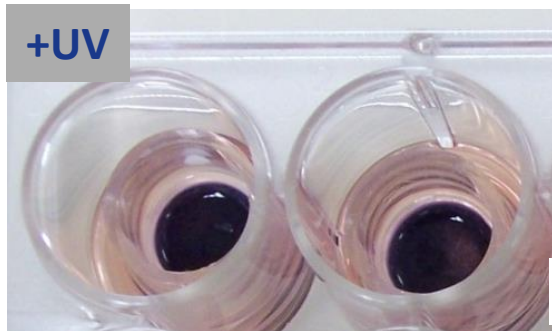
- Can test article directly reduce MTT?
- If yes, addition of killed control tissues (non-viable tissue)  
+Irr & -Irr
  - Also consider HPLC/UPLC

# RhE Phototoxicity Assay Procedures

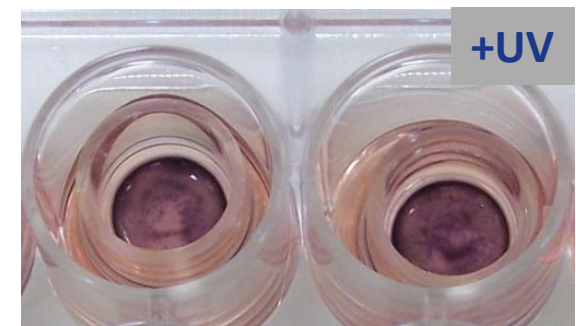
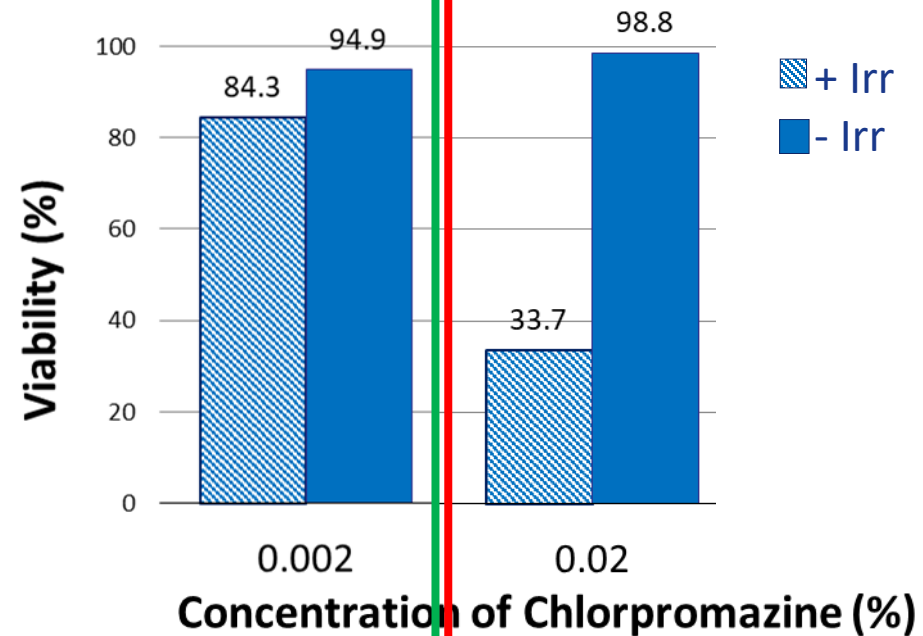


# RhE Data

$$\% \text{ Relative Viability} = \frac{\text{Final Corrected OD}_{570} \text{ of Test Article or Positive Control}}{\text{Corrected OD}_{570} \text{ of Negative/Solvent Control}} \times 100$$



No Phototoxicity Potential

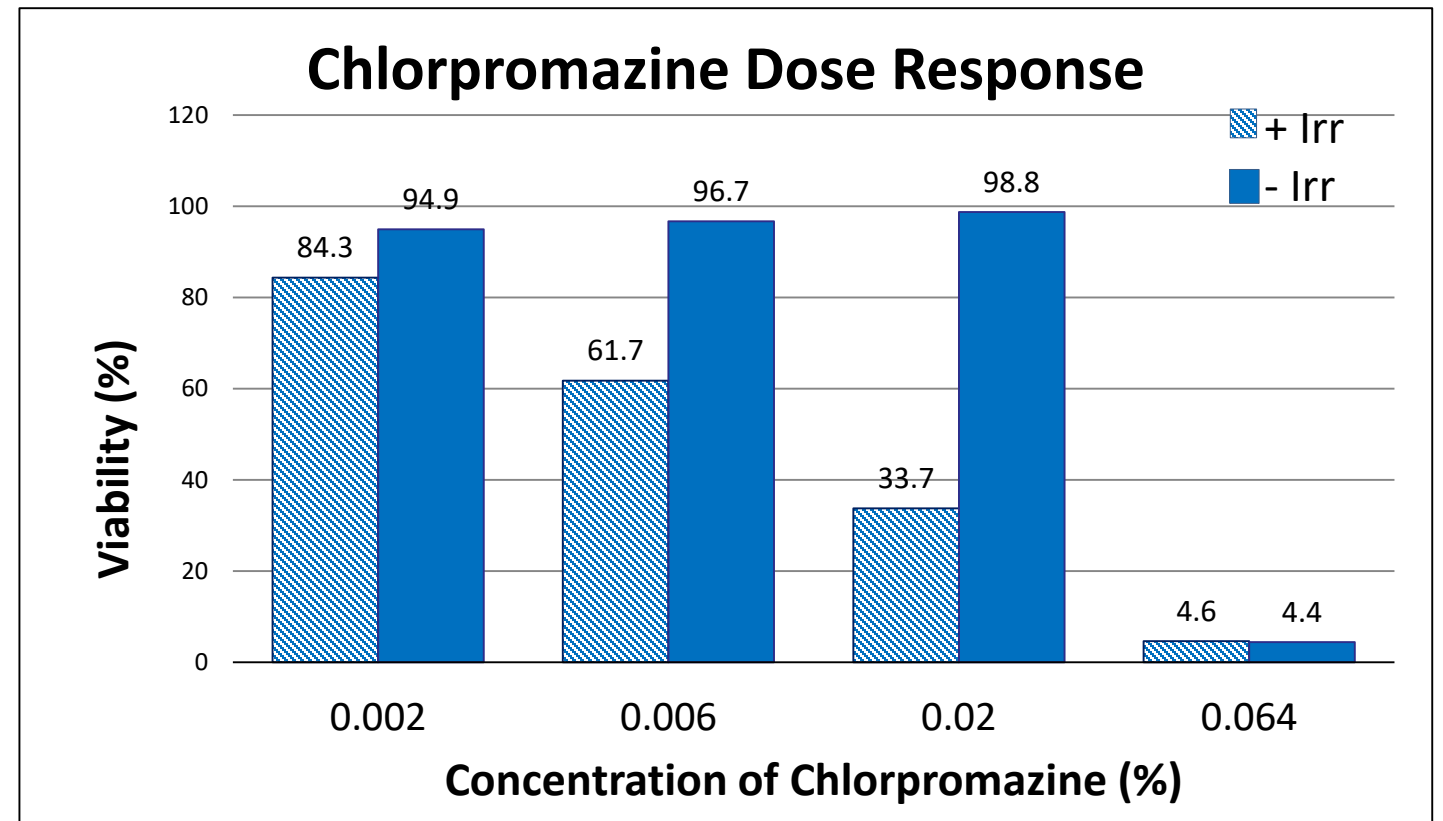


Phototoxicity Potential

# RhE Assessment of Phototoxicity Potential

## Prediction According to TG 498:

- Viability sufficient (*e.g.*, >35%) –Irr up to maximum concentration of 10%
- At least 1 concentration has  $\geq 30\%$  difference in tissue viability between +Irr & - Irr = **Phototoxicity Potential**
- Borderline: If no concentrations result in phototoxicity, BUT at least 1 concentration  $30 \pm 5\%$  difference, consider additional runs



# QC, Assay Controls, & Valid Test

- **Test system (*i.e.*, tissue model)**

- Negative/vehicle control OD (EpiDerm™ optical density (OD) = 0.8-2.8)
- Barrier function check (using benchmark, *e.g.*, SLS or Triton)
- QC check from developer

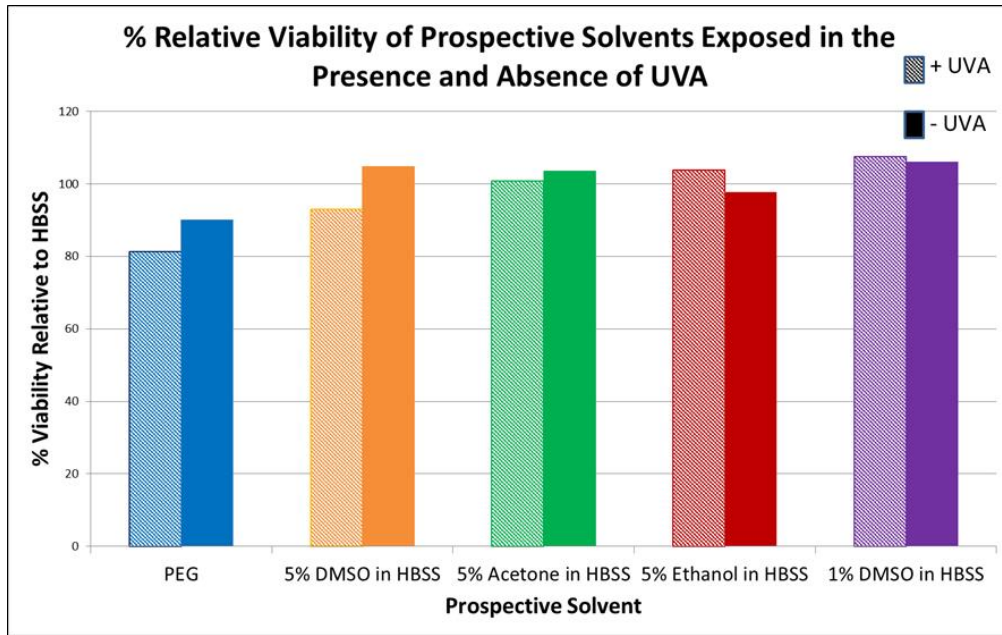
- **Assay Controls**

- ❖ Positive (chlorpromazine 0.01%-0.02%)
- ❖ Negative/vehicle (*e.g.*, HBSS, sesame seed oil)

- **Valid Test**

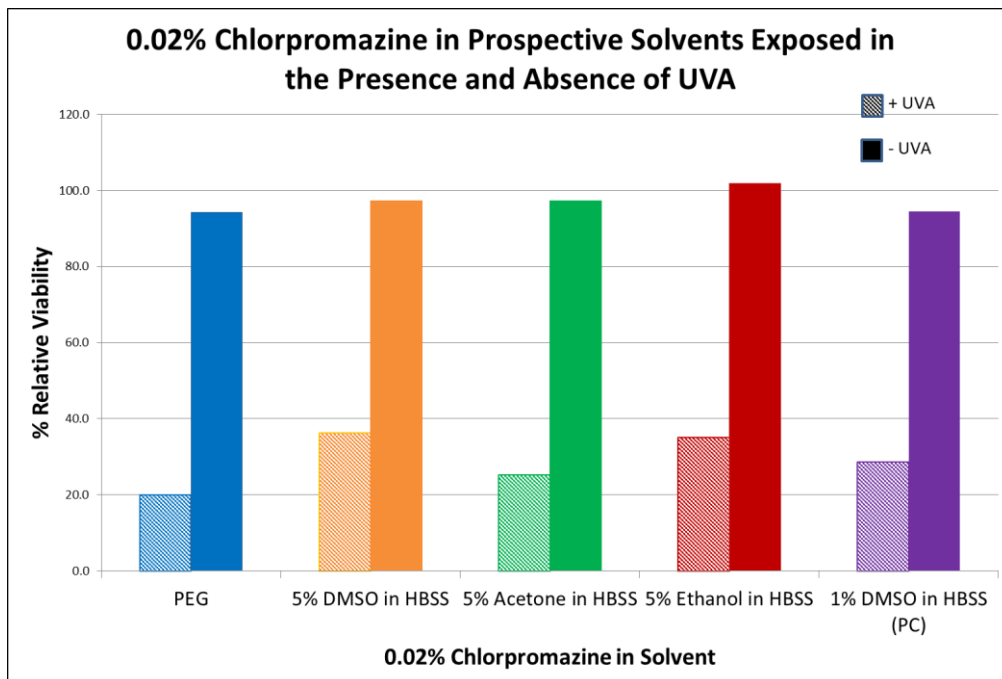
- ✓ Control tissue replicates  $\pm 20\%$
- ✓ Negative (or vehicle) control within acceptable OD (0.8-2.8)
- ✓ Control tissues mean OD  $+1\sigma \geq 80\%$  compared to  $-1\sigma$
- ✓ Positive Control = positive prediction

# Additional Considerations: Solvents



- ✓ No phototoxicity
- ✓ Minimal, if any, cytotoxicity

Compare with established controls



- ✓ Ability to detect photoirritant

Sheehan D, Pidathala A, Hilberer A. Evaluation of New Solvents for the Use in the Multi-Dose Reconstructed Human EpiDermis (RhE) Phototoxicity Assay. In:2016 Annual Meeting Abstract Supplement. Society of Toxicology. Abstract no 3908



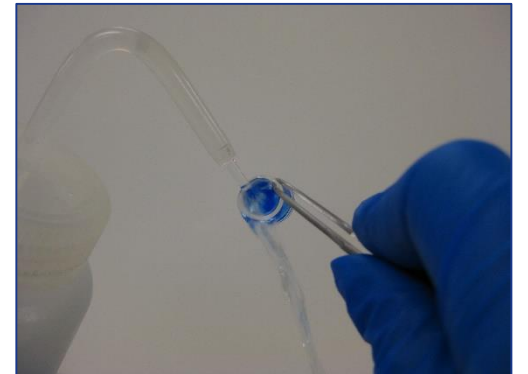
# Additional Considerations

## Preliminary Screening Assay

- Cast wide net -> hone on concentrations of interest
- Adjust for definitive assay

## Rinsing Procedures-Potential interference?

- Consider pre-rinse if material is opaque, dark colored
- Overnight exposure sufficient for penetration into tissue



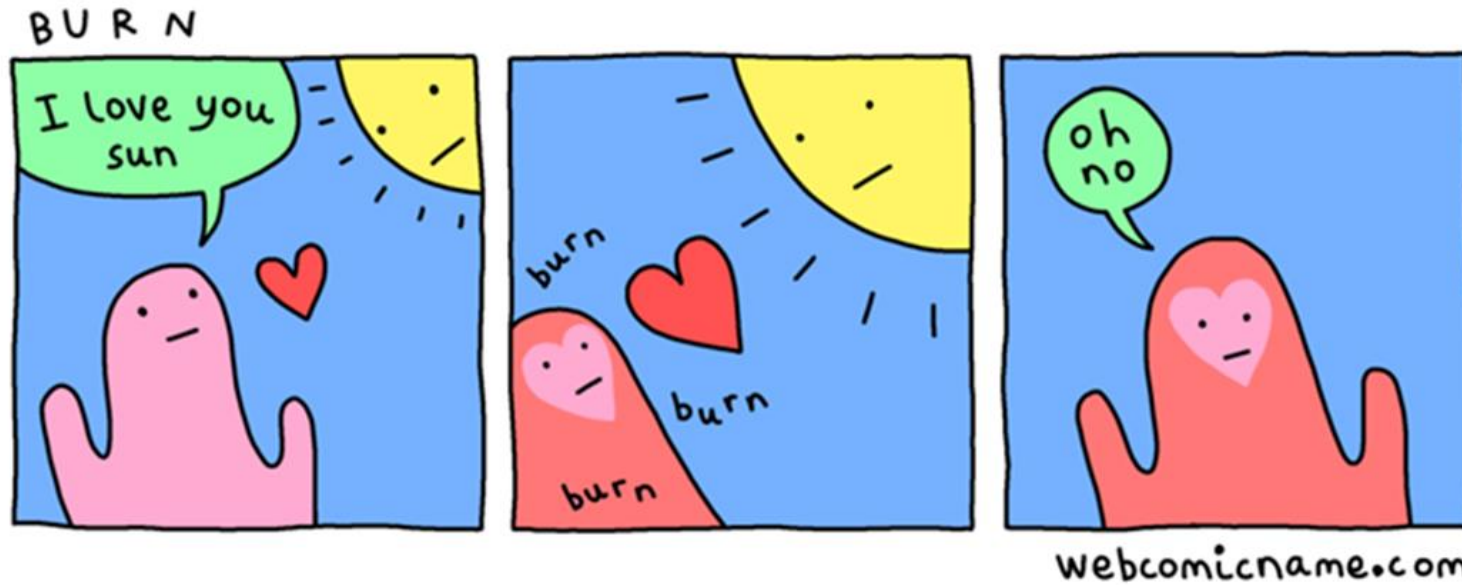
# Testing Mixtures & Formulations

- RhE Phototoxicity pre-validation in late 90s
- Non-regulatory application for decades (e.g., cosmetics)
  - Multi-dose approach (consider relevant concentrations)
  - Single-dose approach (final formulation)
- Pharmaceutical industry – ICH S10 update (2015)
- Risk assessment (or part of tiered approach)
  - No Effect Levels (NOELs)



<https://www.bing.com/images>

# Thank you for your attention!



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