IN VITRO MODELS TO PREDICT PHOTOTOXICITY: STRATEGIES BASED ON THE CELLULAR MECHANISMS INVOLVED

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Facultat de Farmàcia
i Ciències de l'Alimentació

1. Introduction

- Solar light: Benefits and pathogenesis
- Phototoxicity
  - ❑ Photoirritation
  - ❑ Photoallergy
- Models in phototoxicity

2. Methodology

3. Results and Conclusions

4. More in vitro models in phototoxicity

PRESENTATION

Secció de Fisiologia.
Departament de Bioquímica i Fisiologia

Cellular
Response to
Xenobiotics
(CEREX)

SGR
Bioquímica Integrativa

Research

- Evaluation of the antioxidant power of products of natural origin.
- Genotoxicity studies adapted to the evaluation of the potential photoprotective effects of products of natural origin.
- Nanotoxicology in vitro
- **Development of in vitro techniques for studies of (photo) irritation and (photo) dermal sensitization**.

1. INTRODUCTION

SOLAR LIGHT BENEFITS

Vitamin D

Regulates body temperature

Maintains Circadian Rhythm

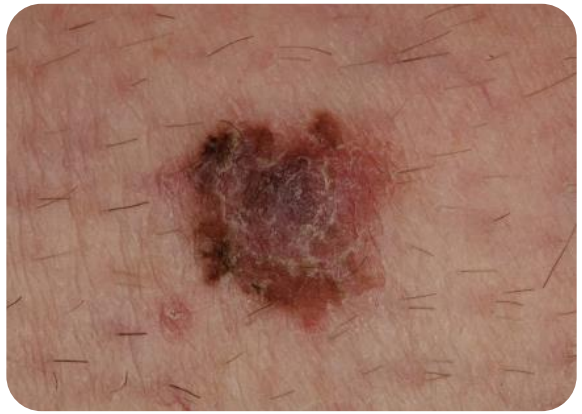
Fights Stress and Insomnia

Serotonin

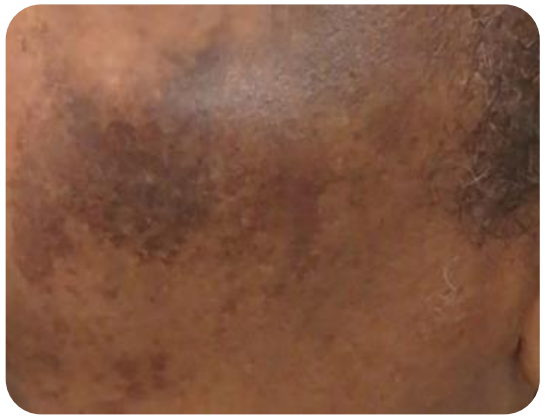
Melatonin

Improves Mood

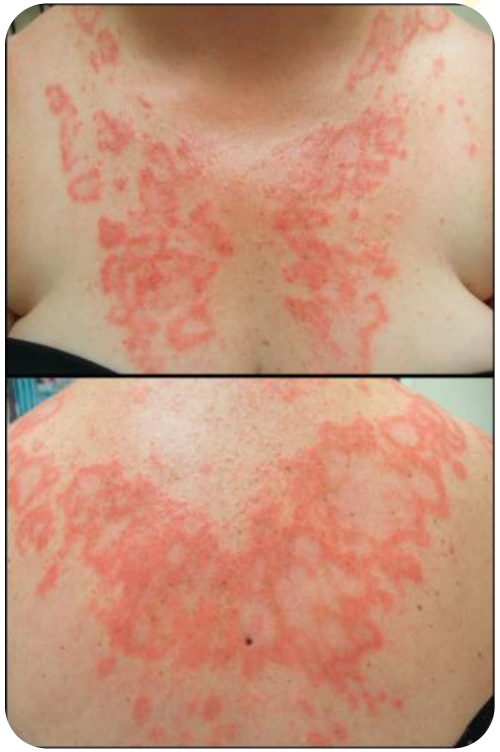
SOLAR LIGHT PATHOGENESIS



Skin Cancer

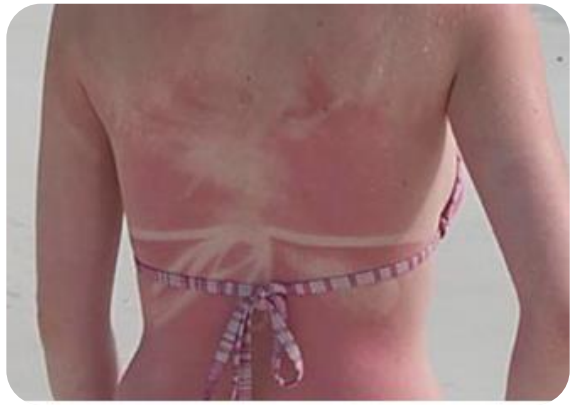


Hyperpigmentation

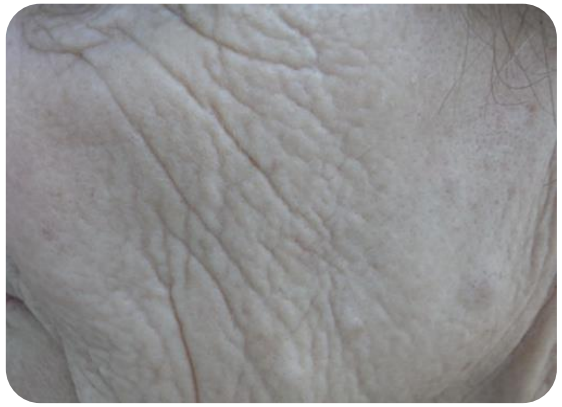


Photosensitivity

(Caused by photoactive molecules in skin)

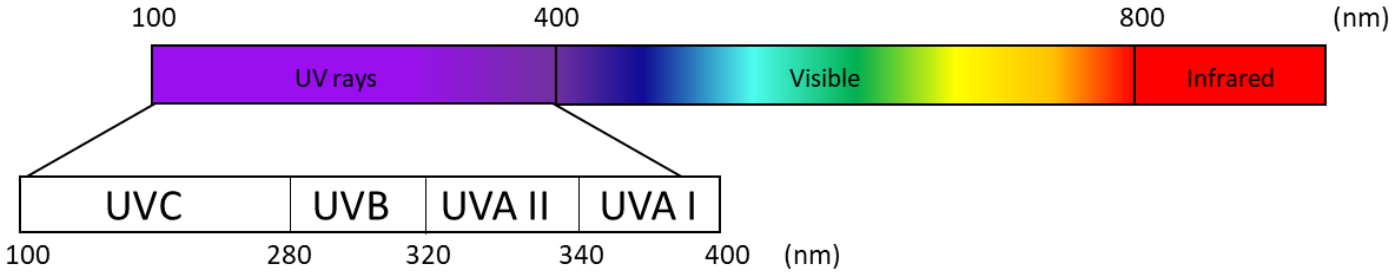


Redness

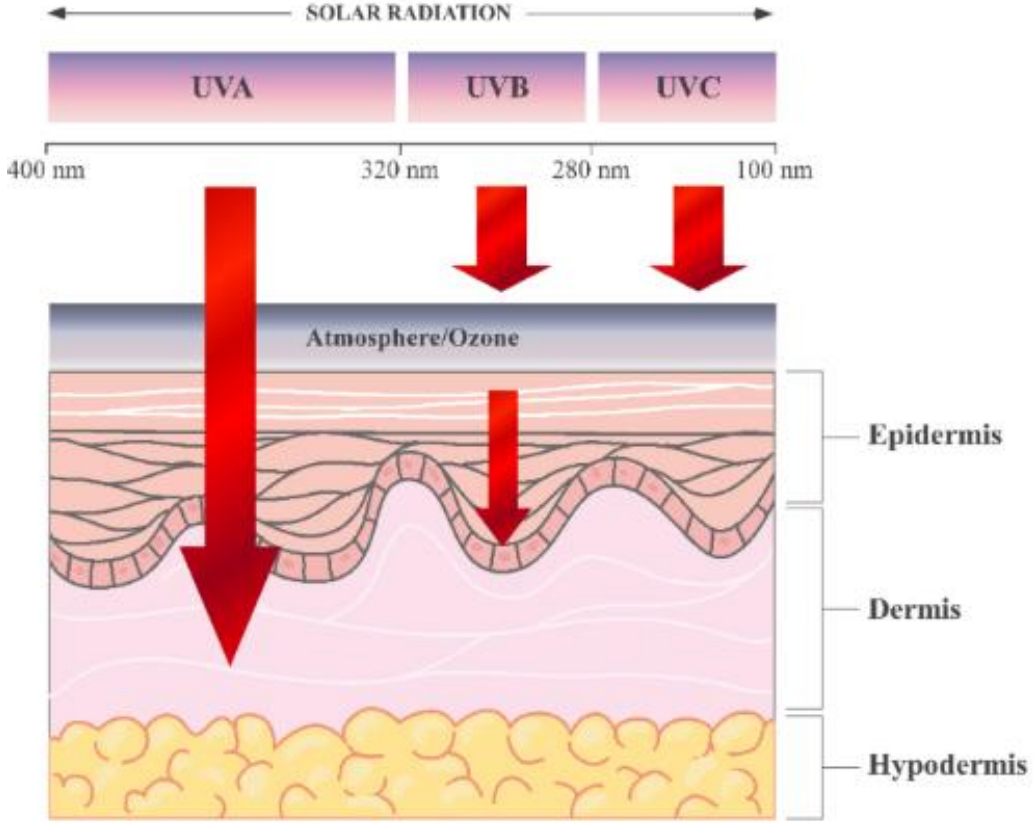


Photoaging

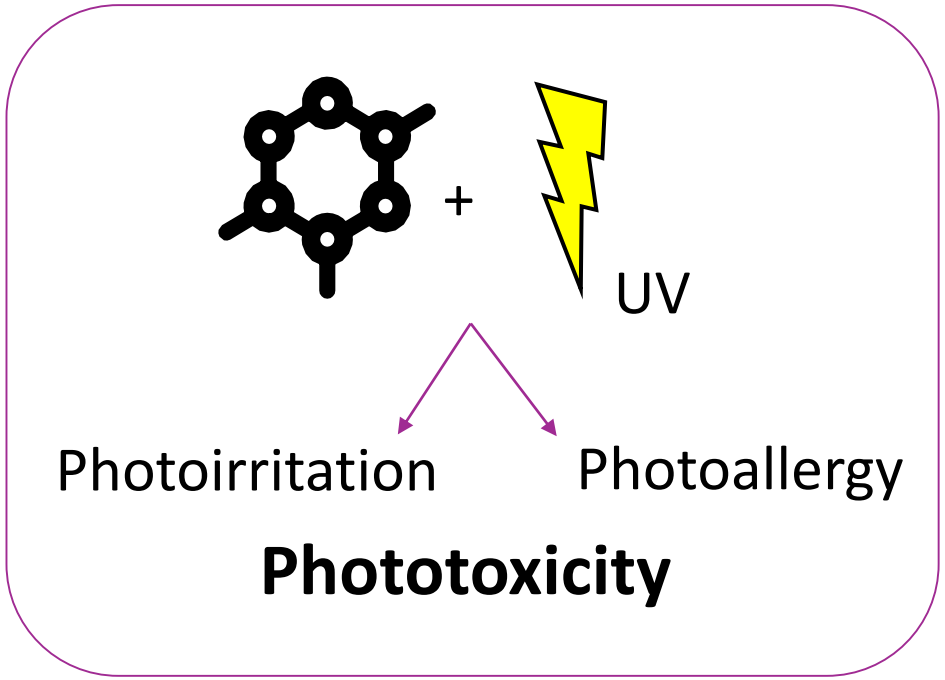
Driffey 2017, Krueger and N. Elbuluk 2021, Nou et al. 2015



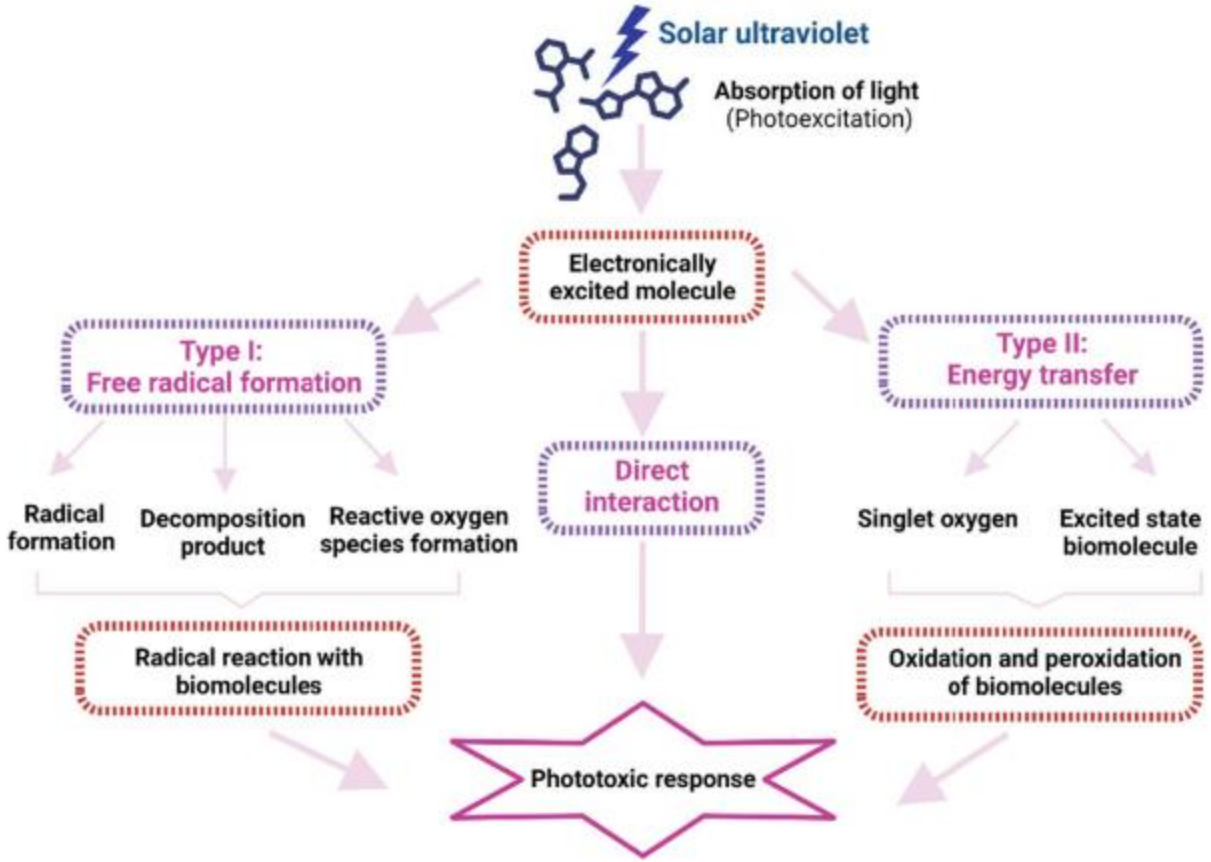
Solar Radiation Spectrum



UV penetration into the layers of the skin.
Pérez-Sánchez et al. 2018



PHOTOTOXICITY: PHOTOIRRITATION (PI)

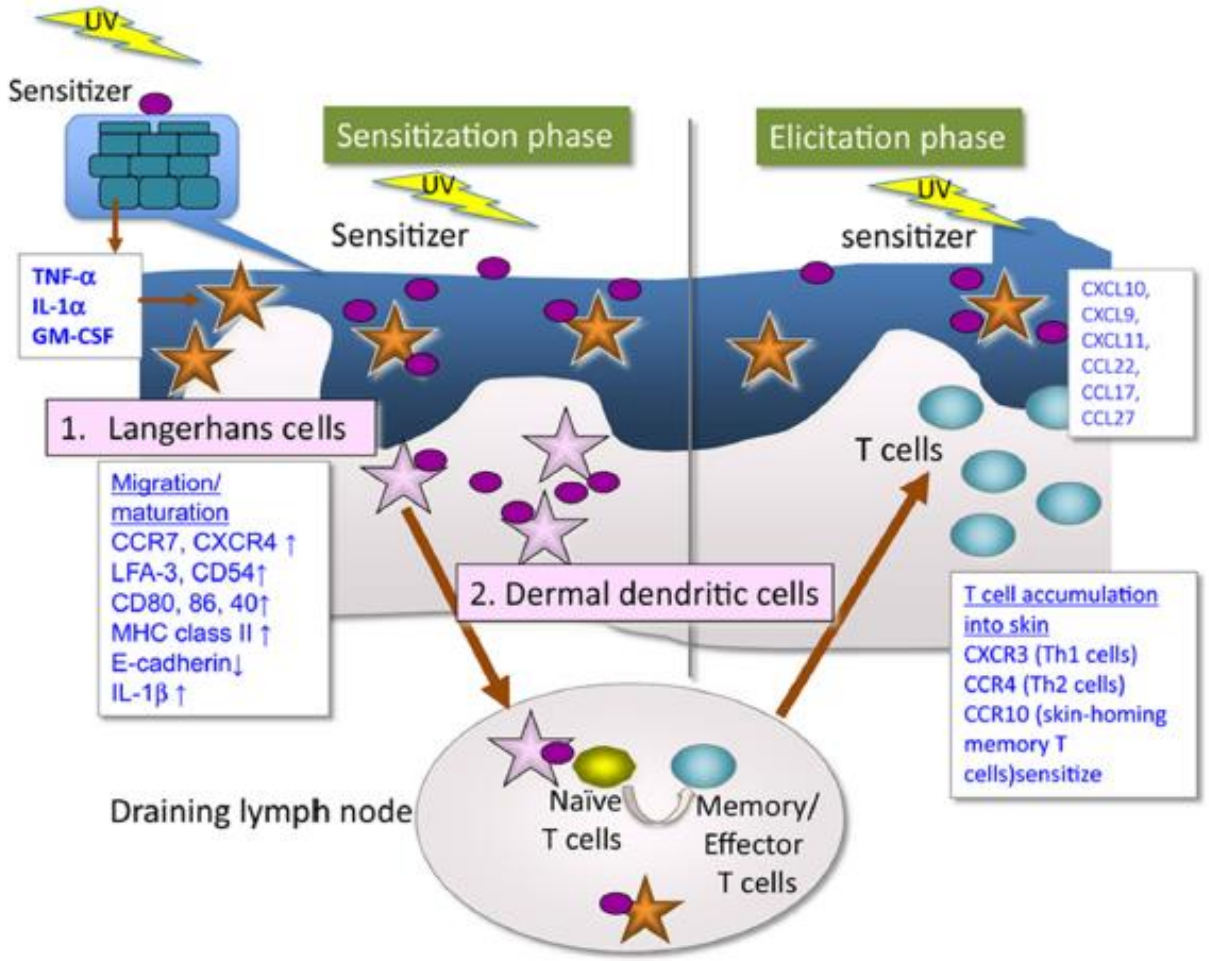


- Common, dose-dependent
- Often exaggeraed sunburn, erythema
- Histopathology: Necrotic keratinocytes, minimal inflammation
- Local manifestation

Calitxo et al. 2016

PHOTOTOXICITY: PHOTOALLERGY (PA)

- Uncommon, not dose-dependent
- Usually dermatitis
- Histopathology: Spongiotic dermatitis with eosinophils
- Can extend beyond



Tokura et al. 2018

PHOTOPATCH TEST

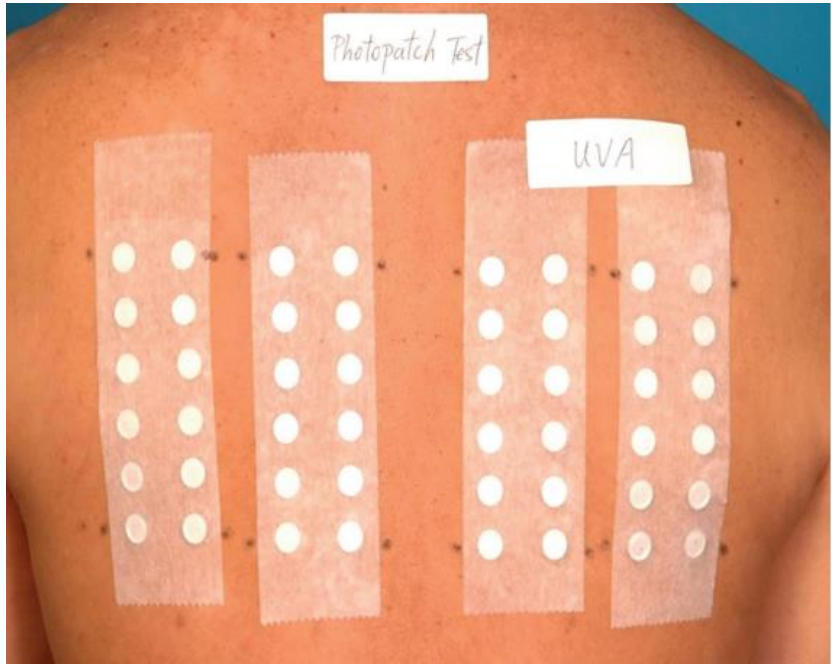


Table 3 Interpretation of photopatch test results

Reading 1		Reading 2 or 3			
No UV	UVA	No UV	UVA	Test results	Interpretation of positive reactions
–	++[a]	–	–	Immediate reaction	Photocontact urticaria
–	–	–	+ to +++	Positive photopatch test	Photoallergy or phototoxicity
+	+	++	++	Positive patch test	Contact allergy
+	+	+	++ or +++	Photoaggravated patch test	Photo-augmented contact allergy/or contact allergy+photoallergy
++	++	++	– or +	Photo-inhibition[b]	

[a]Immediate urticarial reaction after irradiation. Do not consider faint erythema occasionally observed with chemicals with more phototoxic potential

[b]The meaning of this type of reaction is not completely understood

Chong et al. 2017

PHOTOTOXICITY: PRECLINICAL STUDIES IN RESEARCH

Animal methods

In vivo

- Photo-local lymph node assay

Non-animal methods

In silico

- QSAR
- Toxtree

In chemico

- TG 101: UV-VIS absorption spectrum
- TG 495: ROS Assay for photoreactivity

In vitro

- TG 432: In vitro 3T3 NRU phototoxicity test
- TG 498: RHE phototoxicity test method

Why are *in vitro* models important?

3R Principle

- Replacement
- Reduction
- Refinement

Regulation and ethical guidelines

- REACH, Pharmaceuticals...
  Promotion alternatives
- Banning of animal testing for cosmetics
  (EU & Other countries)

- No *In vitro* models available to discriminate PI & PA
- Proposes to identify PA based on the AOP of skin sensitisation

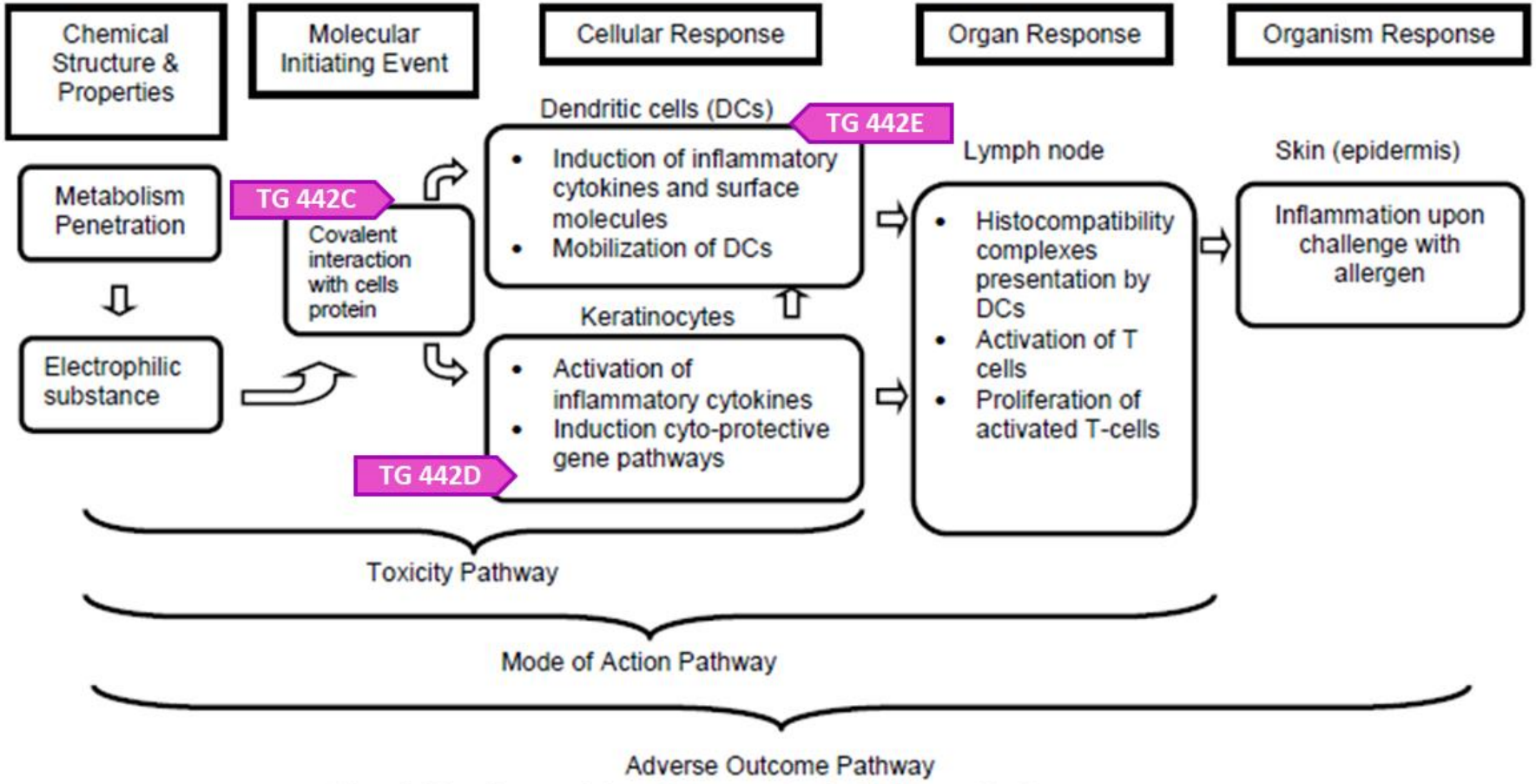
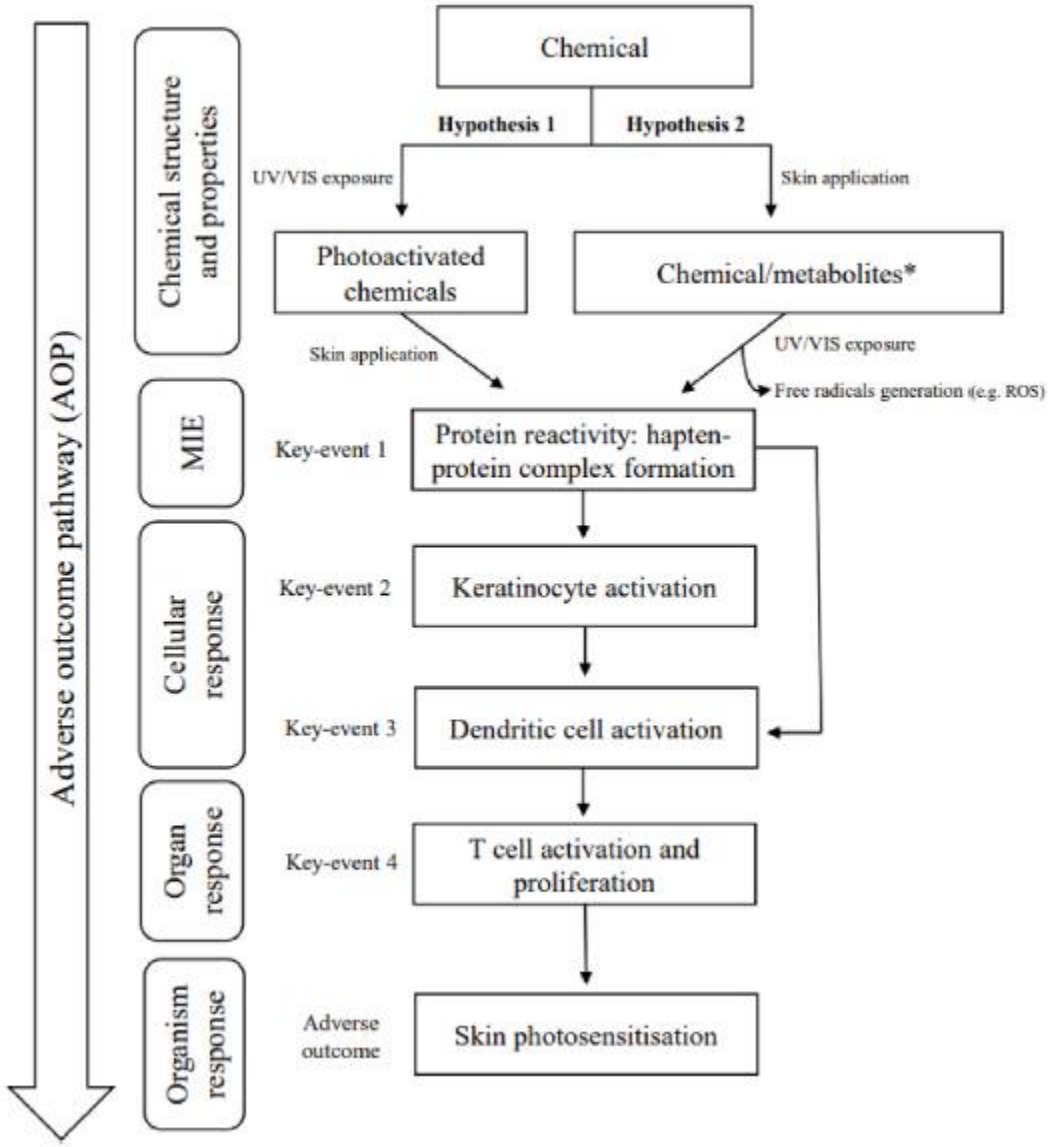


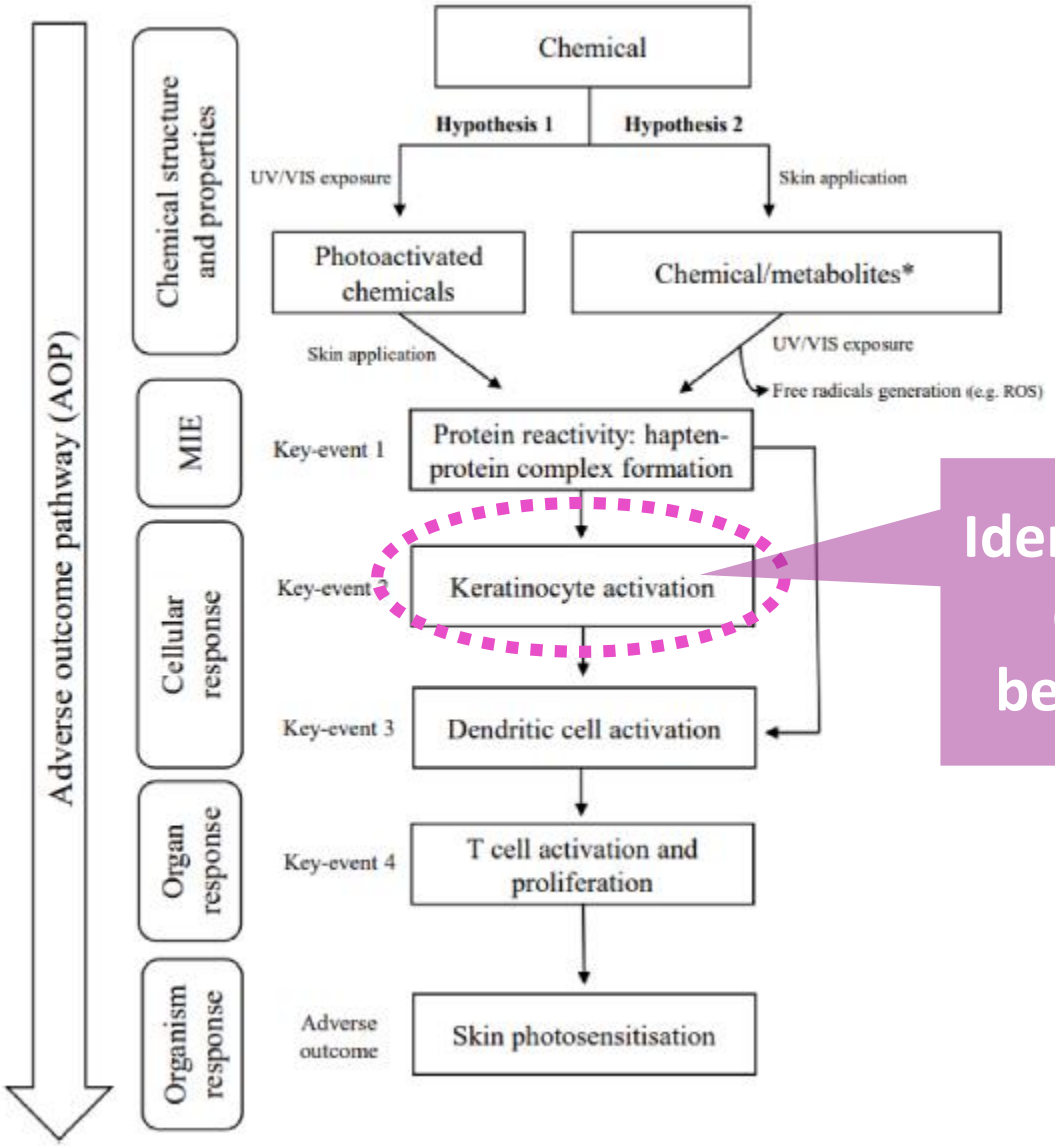
Figure 3. Flow diagram of the pathways associated with skin sensitisation.



Schematic overview of the of the proposed Adverse Outcome Pathway (AOP) key events (KEs) of skin photoallergy.

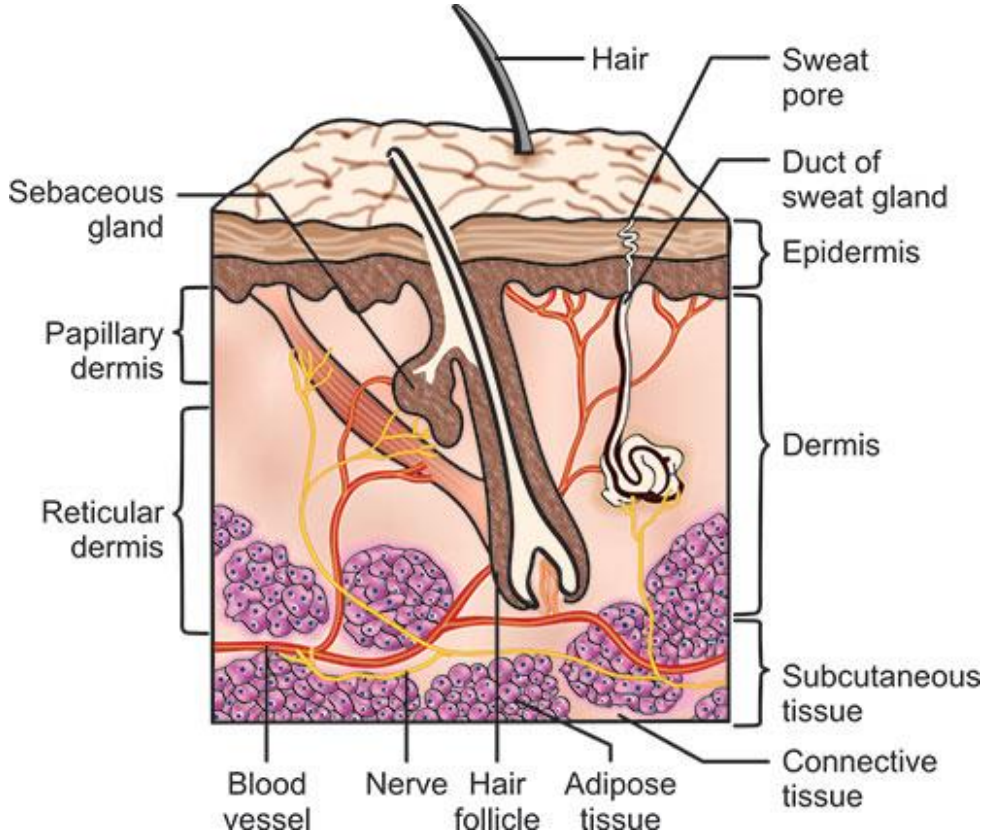
Ávila et al. 2023

MAIN GOAL OF THE PROJECT



Identify markers to discriminate between PI & PA

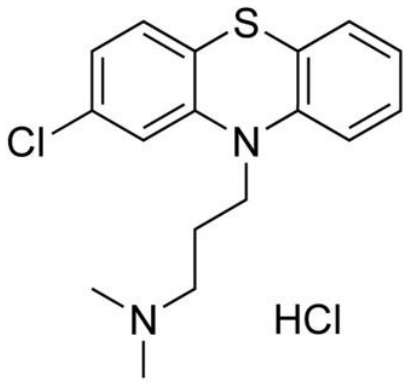
2. METHODOLOGY



Layers of the skin. Sarabahi, S. and Bajaj, S.P. (2010) .

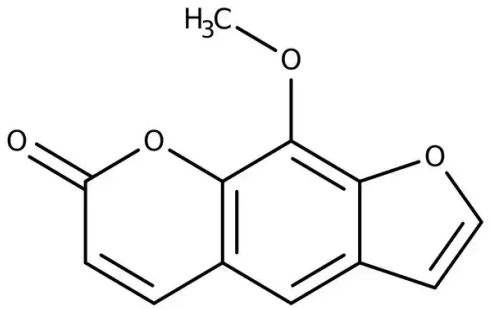
Development of an *in vitro* model using keratinocytes

Epidermis: **Keratinocytes**, Melanocytes, Langerhan cells...



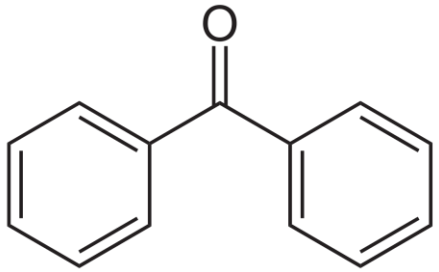
Chlorpromazine HCl (CPZ)

PI/PA



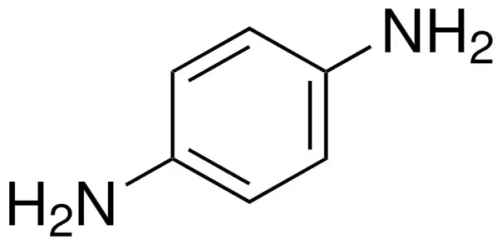
8-methoxypsolaren (8-MOP)

PI



Benzophenone (BZ-F)

PA

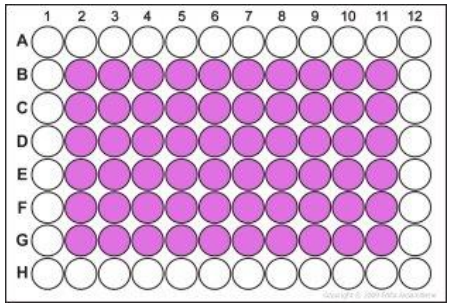


P-Phenylendiamine (PPD)

A

Experimental design

- Protocol based on OECD TG 432
- Keratinocytes instead of fibroblasts (BALB/c 3T3)



Human keratinocytes (HaCaT)

→ 24h, 5% CO_2, 37ºC

Incubation with compounds in PBS

→ 1h, 5% CO_2, 37ºC

UVA irradiation 4 J/cm2

→ 24h, 5% CO_2, 37ºC

Cellular viability

- IC50 Dark
- IC50 UVA

↓

PIF

↓

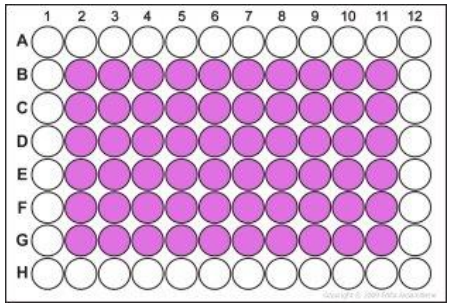
CV80

↓

Next study

PIF	Classification
<2	Non-phototoxic
2-5	Probable phototoxic
>5	Phototoxic

Supernatants collection



Human keratinocytes (HaCaT)

24h, 5% CO_2, 37ºC

Incubation with compounds* in PBS

***[ ] >CV80**

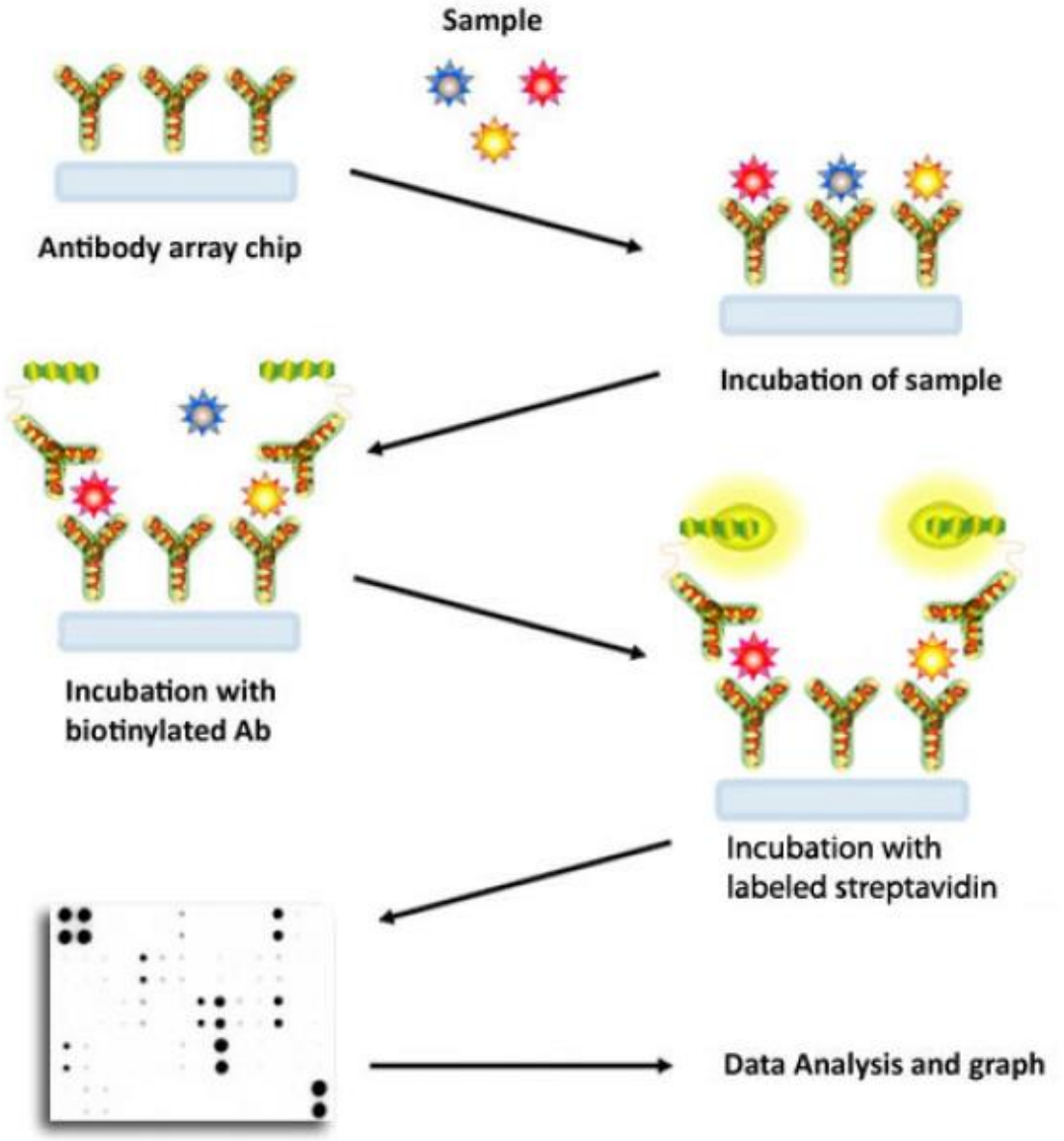
1h, 5% CO_2, 37ºC

UVA irradiation 4 J/cm2

24h, 5% CO_2, 37ºC

Semi-Quantitative study

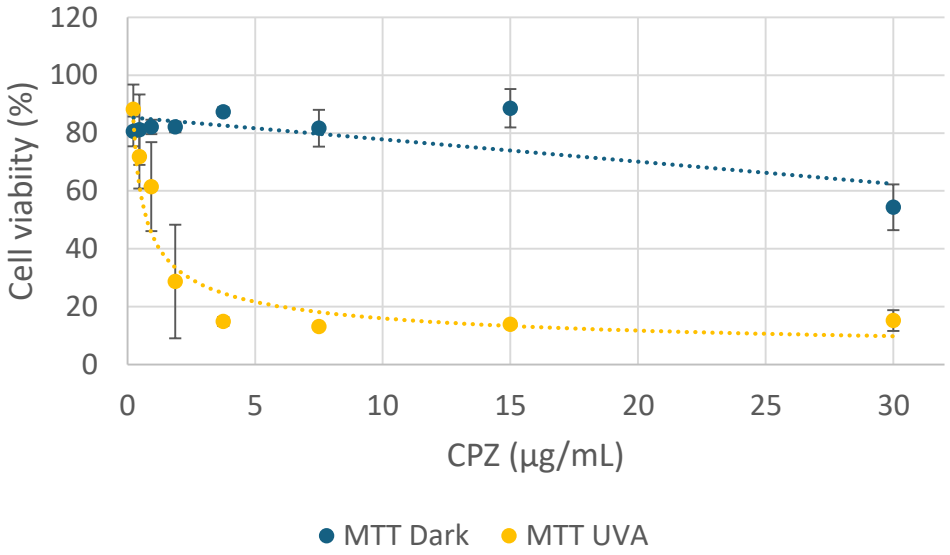
- Anti-inflammatory cytokines
- MMPS



RayBiotech.com

3. RESULTS AND CONCLUSIONS

CPZ (PI/PA)



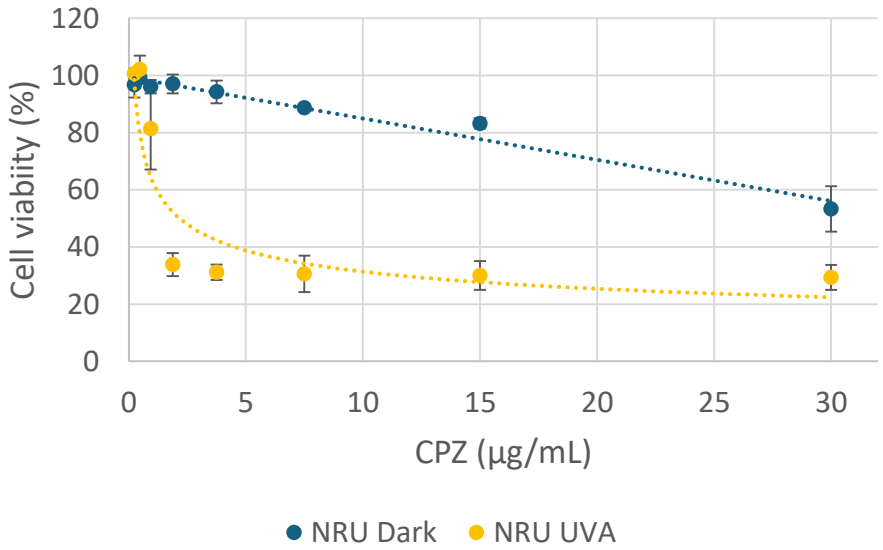


Figure 1. Cell viability of keratinocytes obtained through MTT and NRU assays, treated with different concentrations of CPZ exposed to 4 J/cm2 . The percentage of viable cells was calculated relative to cells not treated with CPZ (darkness and UVA control). The results are expressed as the mean ± standard deviation of at least 3 replicates.

	MTT	NRU
IC50 DARK	46.3	34.2
IC50 UVA	2.1	8.7
PIF	21.6	3.9

8-MOP (PI)

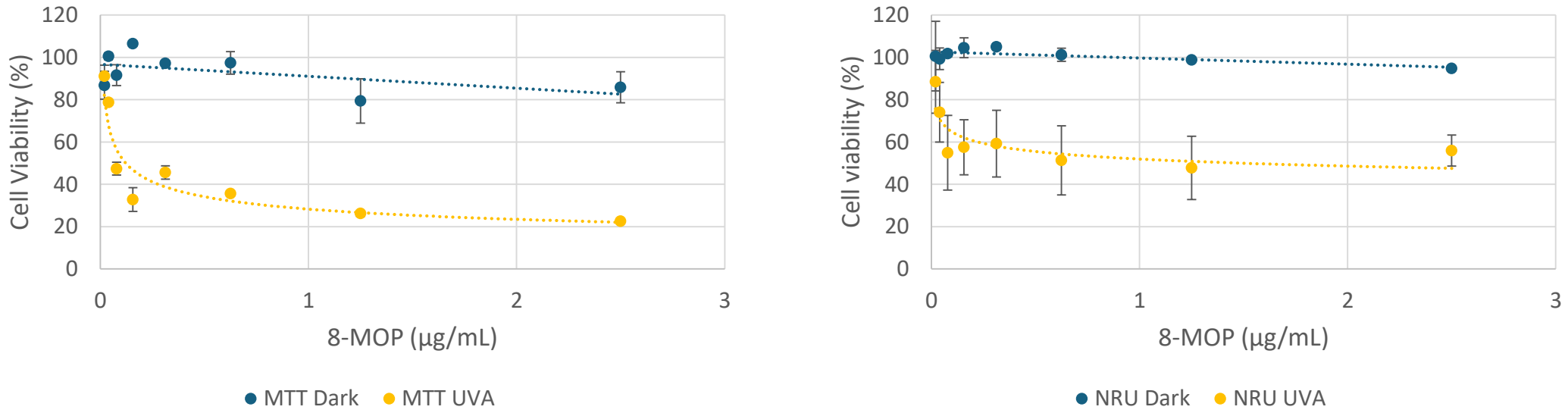


Figure 2. Cell viability of keratinocytes obtained through MTT and NRU assays, treated with different concentrations of 8-MOP exposed to 4 J/cm2 . The percentage of viable cells was calculated relative to cells not treated with 8-MOP (darkness and UVA control). The results are expressed as the mean ± standard deviation of at least 3 replicates.

	MTT	NRU
IC50 DARK	>2.5	>2.5
IC50 UVA	0.12	1.5
PIF	>20.5	>1.7

BZ-F (PA)

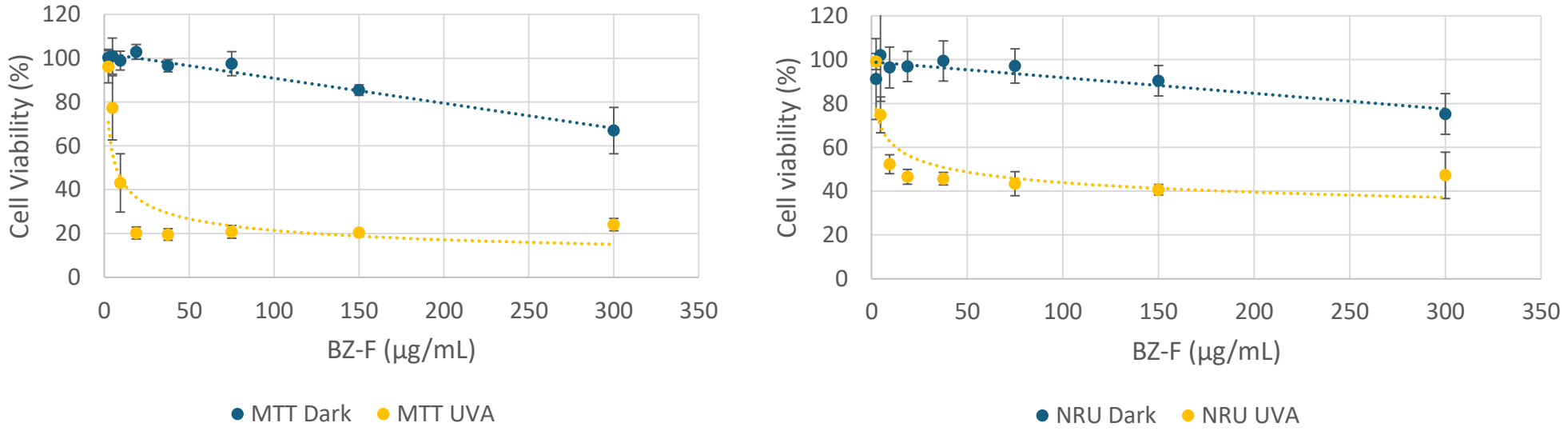


Figure 3. Cell viability of keratinocytes obtained through MTT and NRU assays, treated with different concentrations of BZ-F exposed to 4 J/cm2 . The percentage of viable cells was calculated relative to cells not treated with BZ-F (darkness and UVA control). The results are expressed as the mean ± standard deviation of at least 3 replicates.

	MTT	NRU
IC50 DARK	458.1	684.6
IC50 UVA	13.6	42.77
PIF	33.6	16.0

PPD (A)

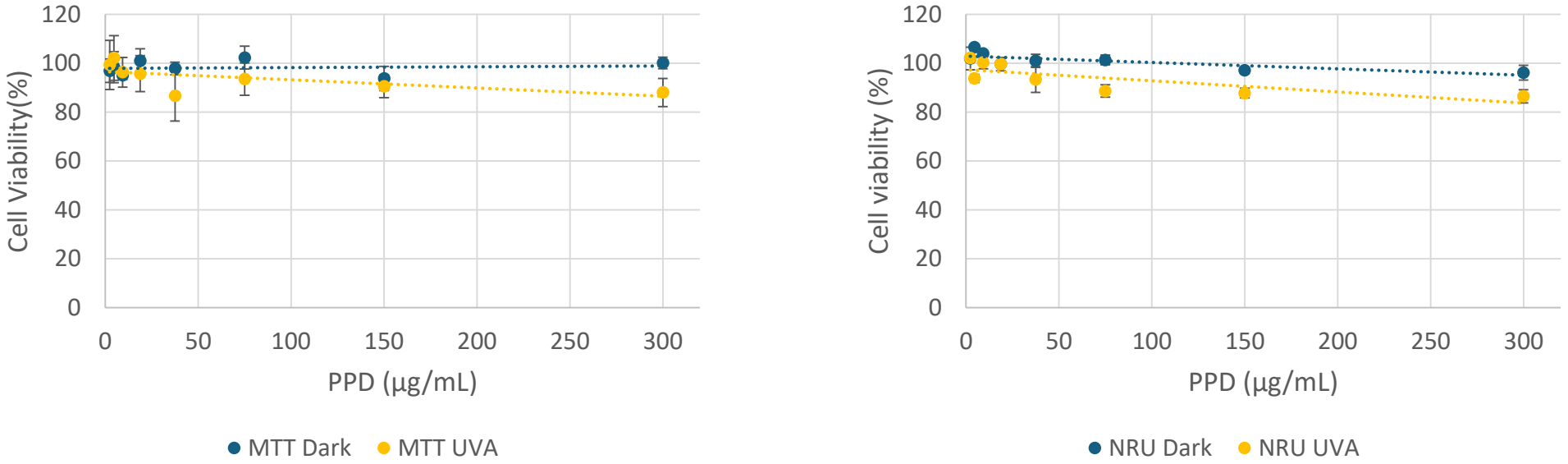
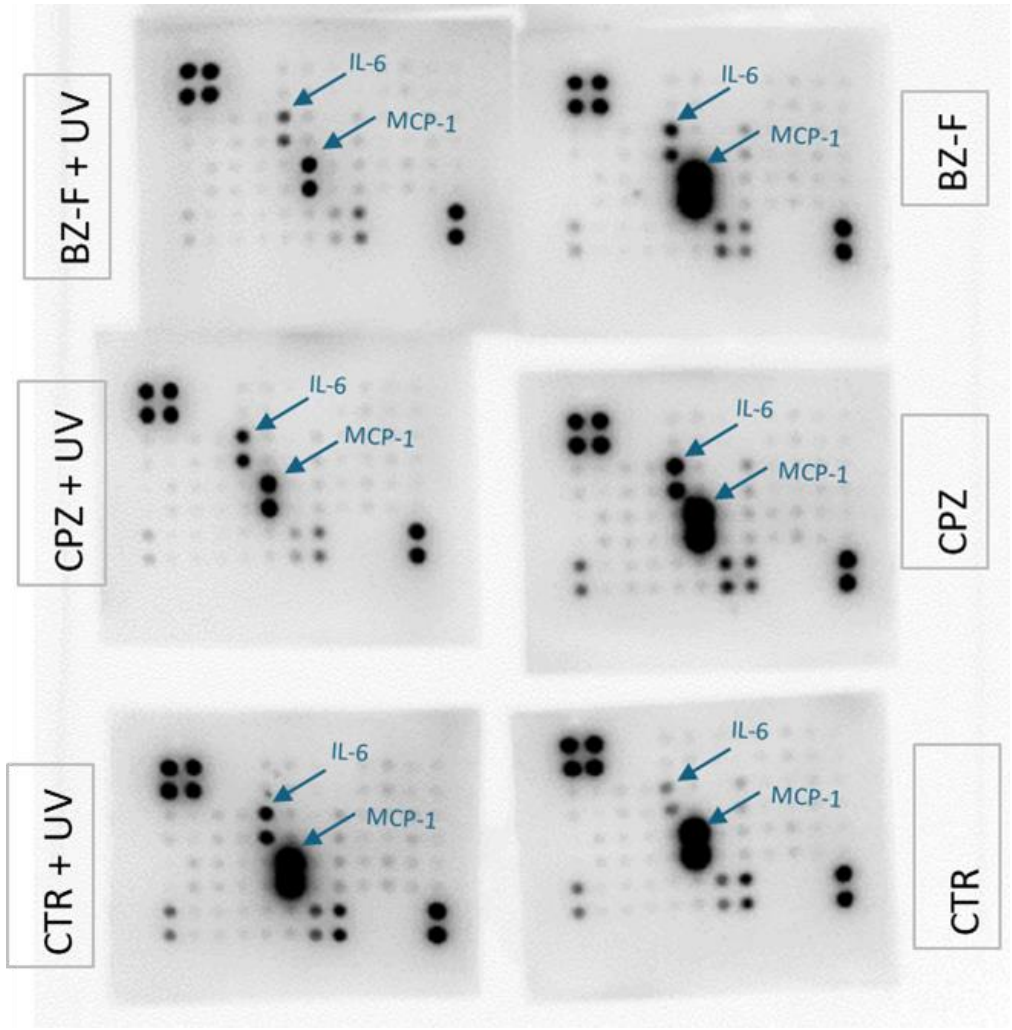
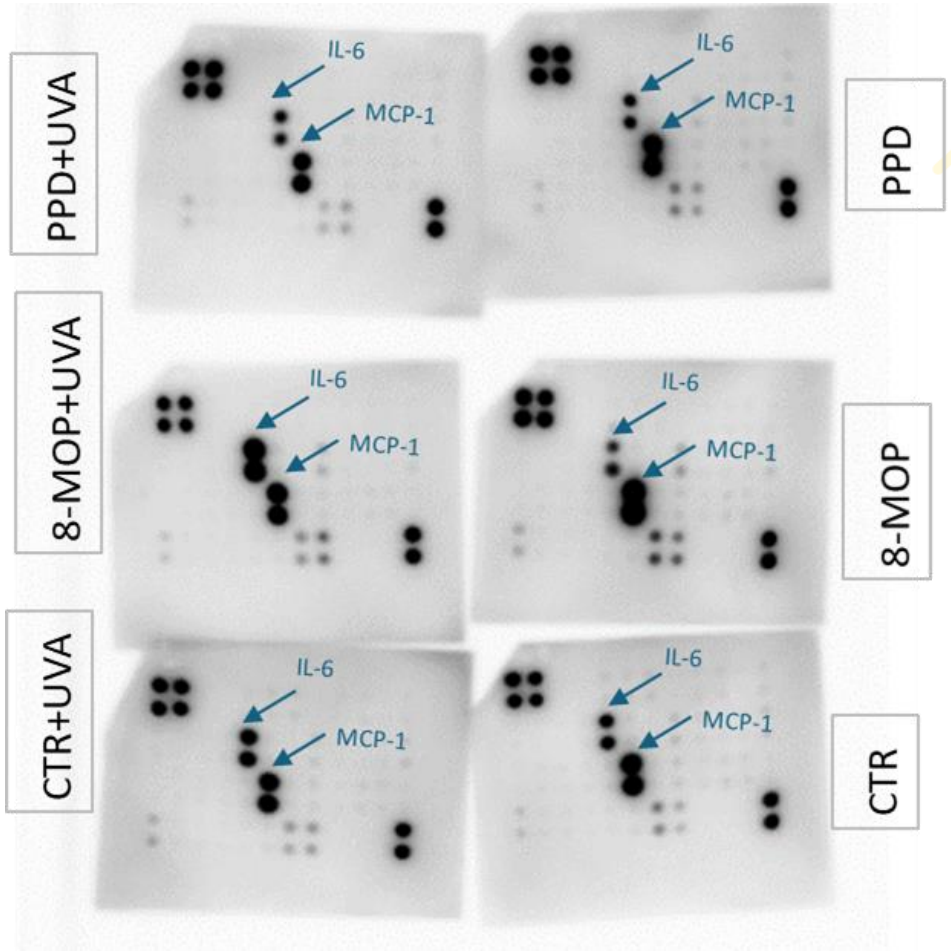


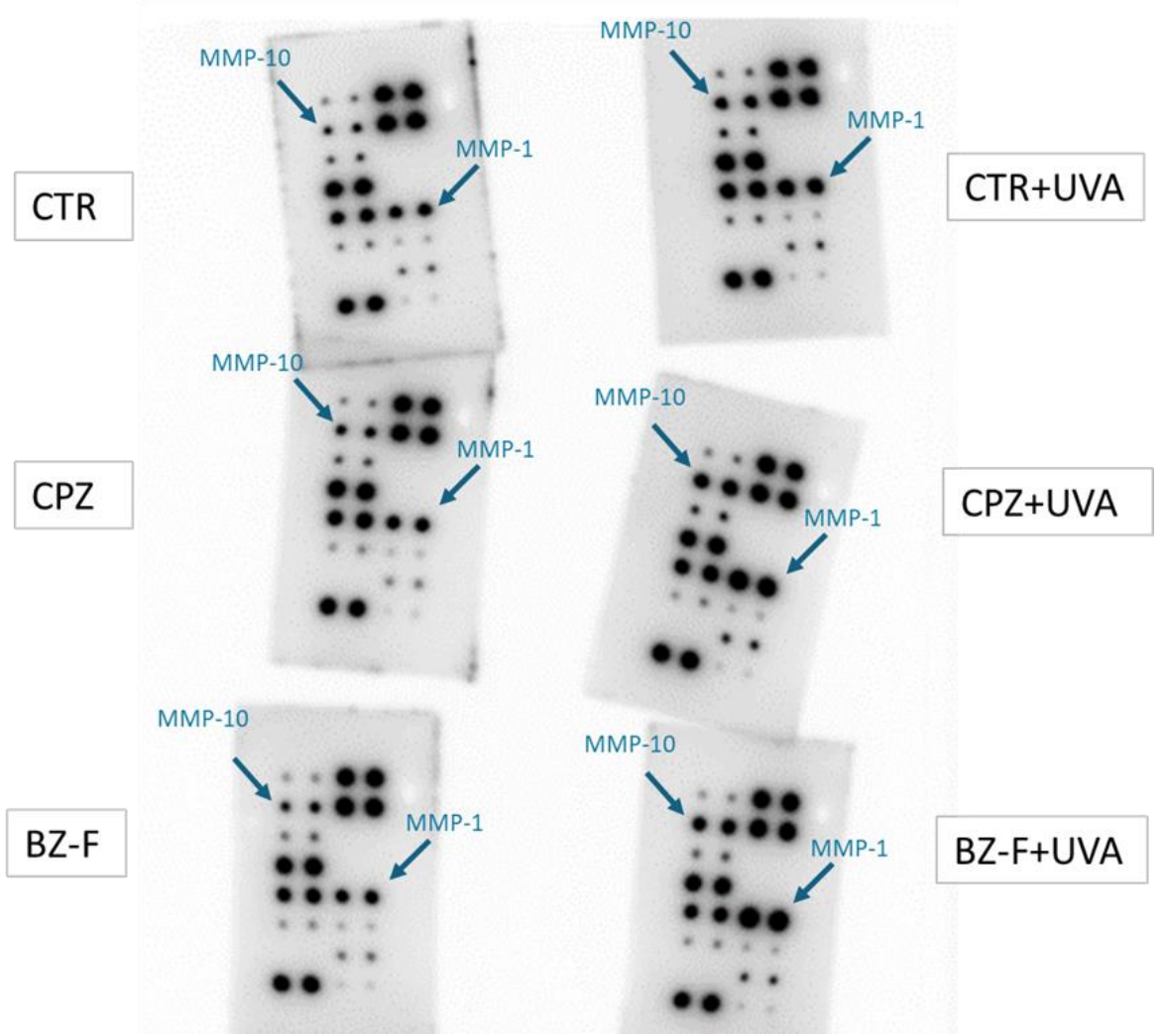
Figure 4. Cell viability of keratinocytes obtained through MTT and NRU assays, treated with different concentrations of PPD exposed to 4 J/cm2 . The percentage of viable cells was calculated relative to cells not treated with PPD (darkness and UVA control). The results are expressed as the mean ± standard deviation of at least 3 replicates.

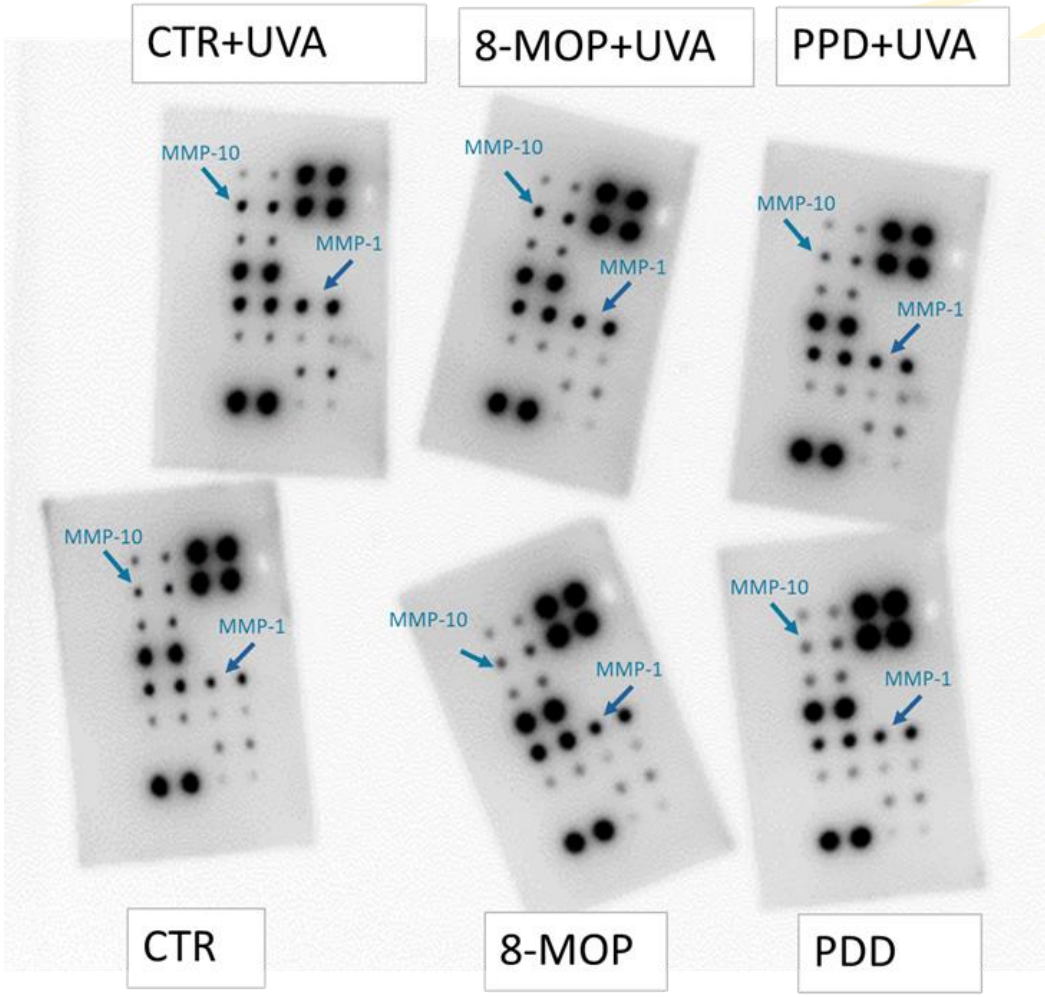




CPZ	0.5 µg/mL
8-MOP	0.02 µg/mL
BZ-F	5 µg/mL
PPD	10 µg/mL

IL-6, MCP-1





CPZ	0.5 µg/mL
8-MOP	0.02 µg/mL
BZ-F	5 µg/mL
PPD	10 µg/mL

MMP-1, MMP-10

Secretion regulation of cytokines and MMPs

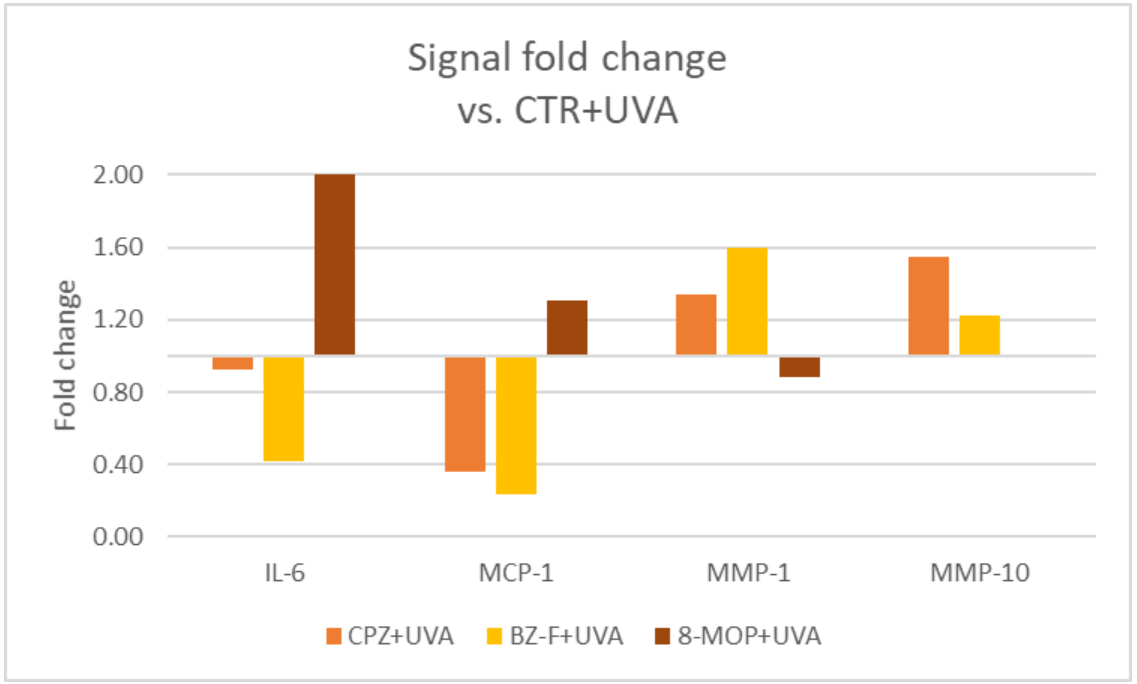
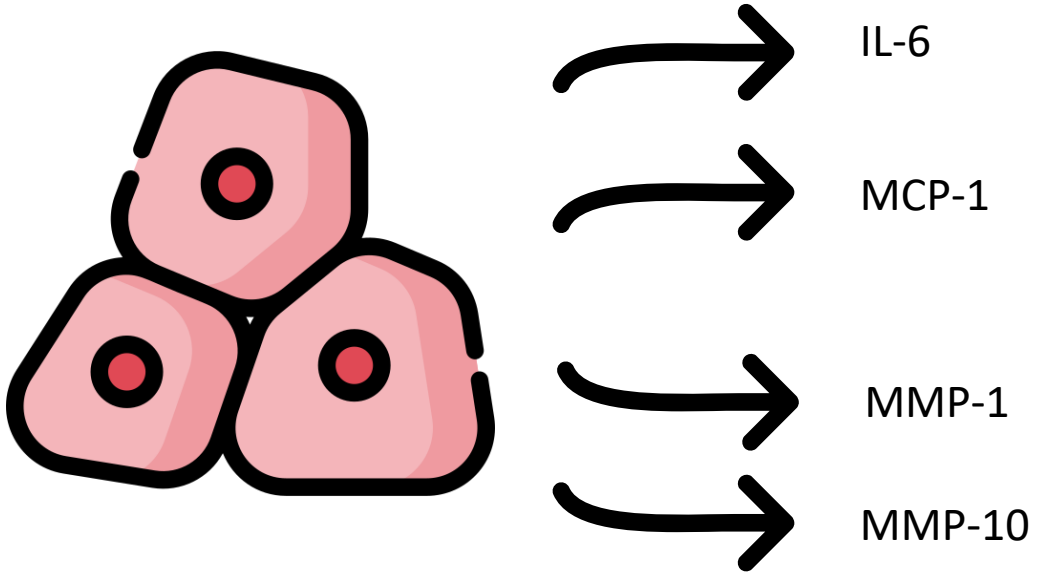


Figure 5. Signal fold expression of different cytokines and MMPs induced by different phototoxic compounds at 4J/cm2 of UVA respect to CTR+UVA.

- Photoallergens seem to upregulate secretion of MMP-1, MMP-10 and downregulate IL-6, MCP-1
- Photoirritants seem to upregulate secretion of IL-6, MCP-1

Conclusions

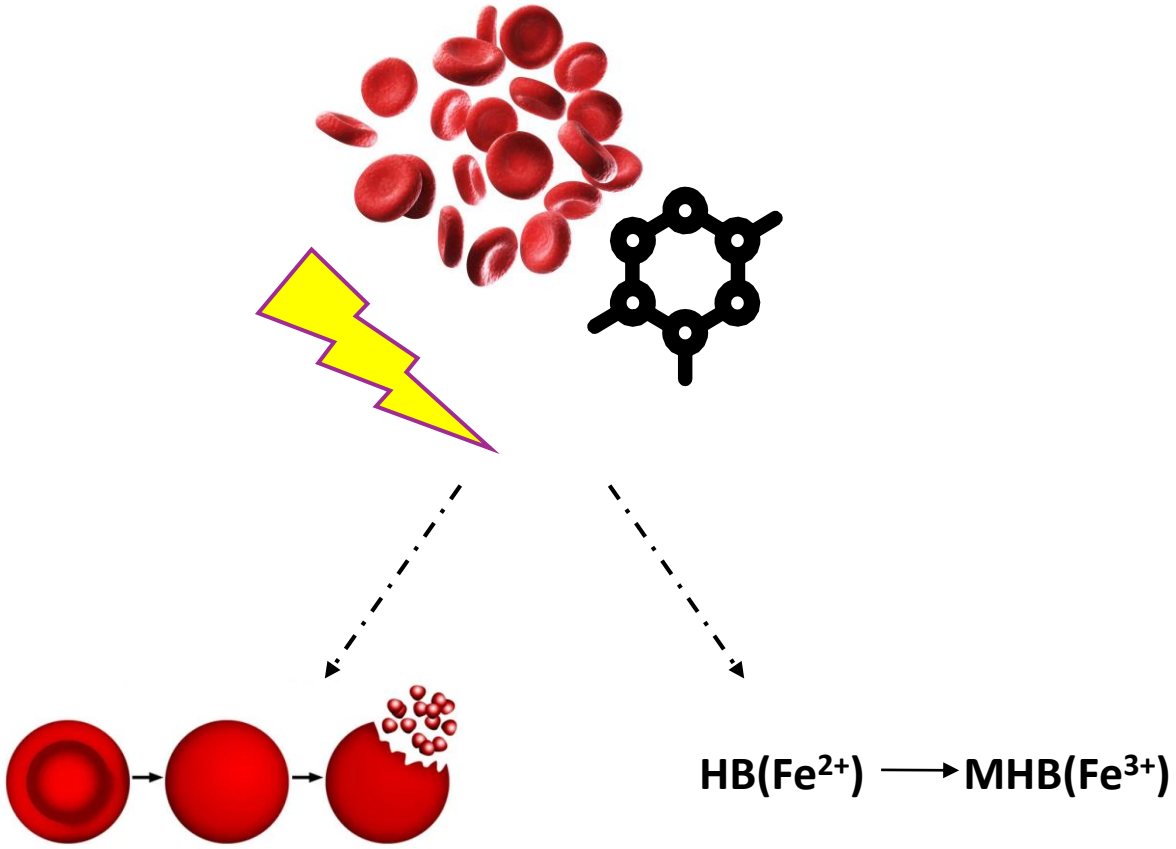


POTENTIAL BIOMARKERS FOR PHOTOALLERGY

Further studies are needed (Study of intracellular production of cytokines and MPPs, quantification by ELISA, determination in RHE…)

4. MORE IN VITRO MODELS IN PHOTOTOXICITY

RBC phototoxicity test



EURL ECVAM Database

- Haemolysis of erythrocyte membranes
- Oxidation of haemoglobin

Classification of phototoxic potential

Haemolytic factor >3*

and/or

MetHb formation** (OD +IRR – OD-IRR)

= 0.05 or greater

* HF = (concentration of 50% haemolysis - IRR/ concentration of 50% haemolysis +IRR)

** MetHb F.= (OD +IRR – OD-IRR)

Example 1: CPZ phototoxicity

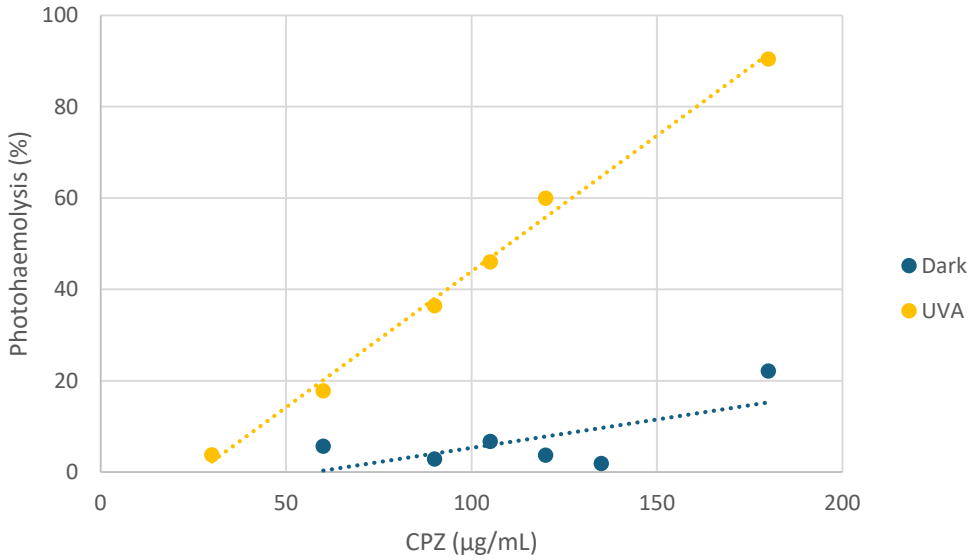


Figure 6. Photohaemolysis results of CPZ. Haemolysis induced by CPZ under UVA and dark conditions.

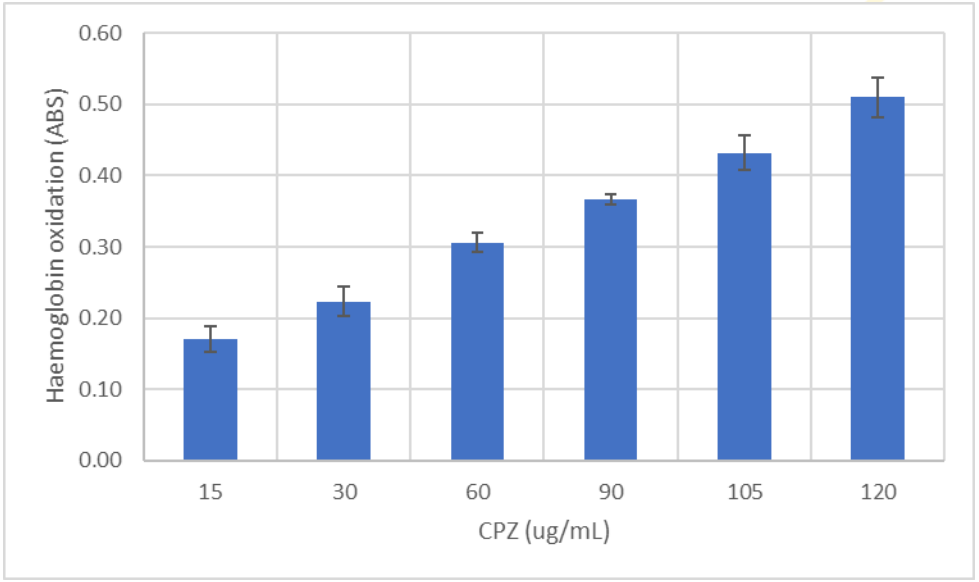


Figure 7. Haemoglobin oxidation by CPZ. Results are expressed as mean ± standard deviation of n=2. Significant haemoglobin oxidation when values of ABS are >0.05.

HC50 Dark	459.1 µg/mL
HC50 UVA	110.2 µg/mL
HF	4.17

Photohaemolysis ✓
Haemoglobin oxidation ✓

CPZ PHOTOTOXIC

Example 2: BIT phototoxicity

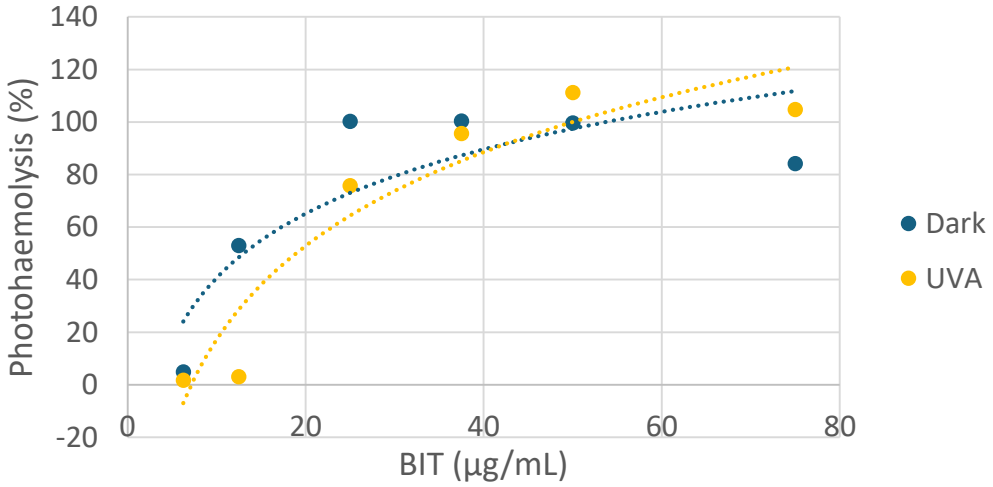


Figure 8. Photohaemolysis results of BIT. Haemolysis induced by BIT under UVA and dark conditions.

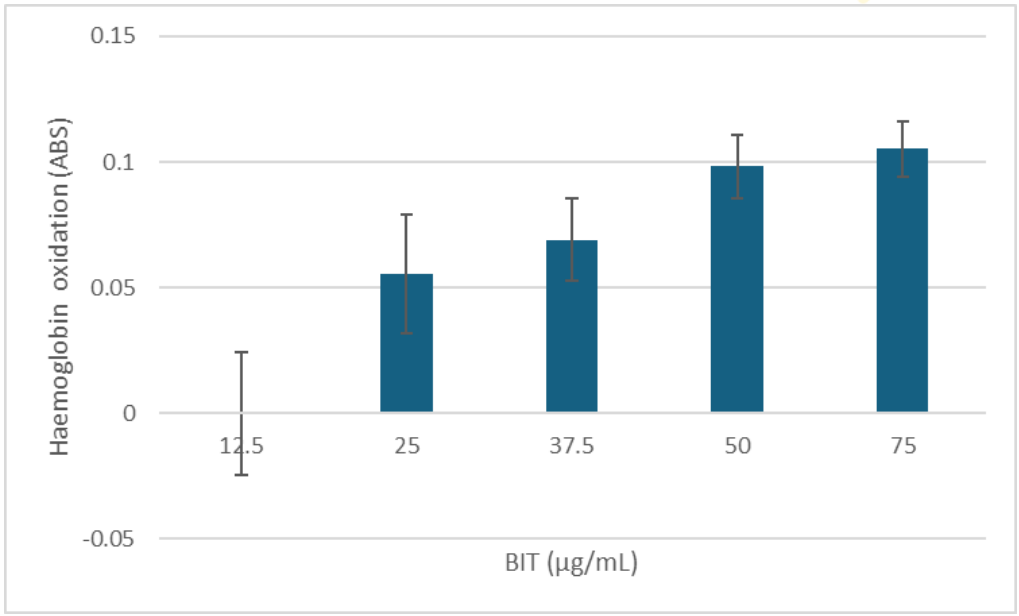


Figure 9. Haemolysis oxidation by BIT. Results are expressed as mean ± standard deviation of n=2. Significant haemoglobin oxidation when values of ABS are >0.05.

HC50 Dark	18.93µg/mL
HC50 UVA	13.03 µg/mL
HF	0.69

Photohaemolysis ✗
Haemoglobin oxidation ✓

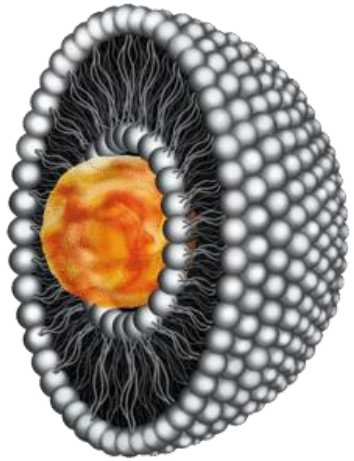
BIT PHOTOTOXIC

Bithionol (BIT)

Example 3: Phototoxicity study of Guarana encapsulated

Paullinia cupana

Guarana



Guarana encapsulated: protection of phototoxicity induced by CPZ?

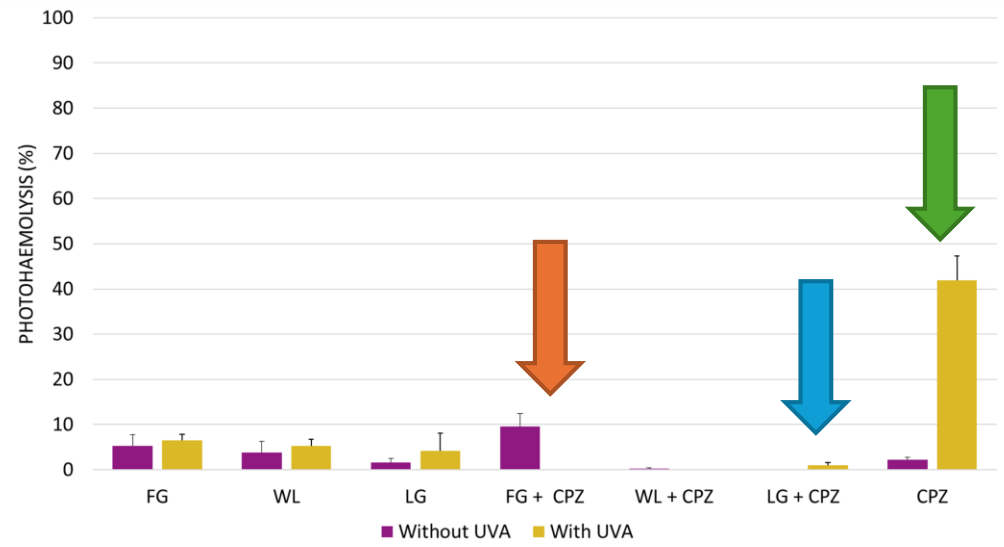


Figure 10. Photoprotective activity from free guarana and nanosomes with or without guarana. Results are expressed as mean ± standard error of n=3. The data indicated that both free and encapsulated guarana do not induce haemolysis when irradiated (5J/cm2) and protect from photohaemolysis induced by CPZ.

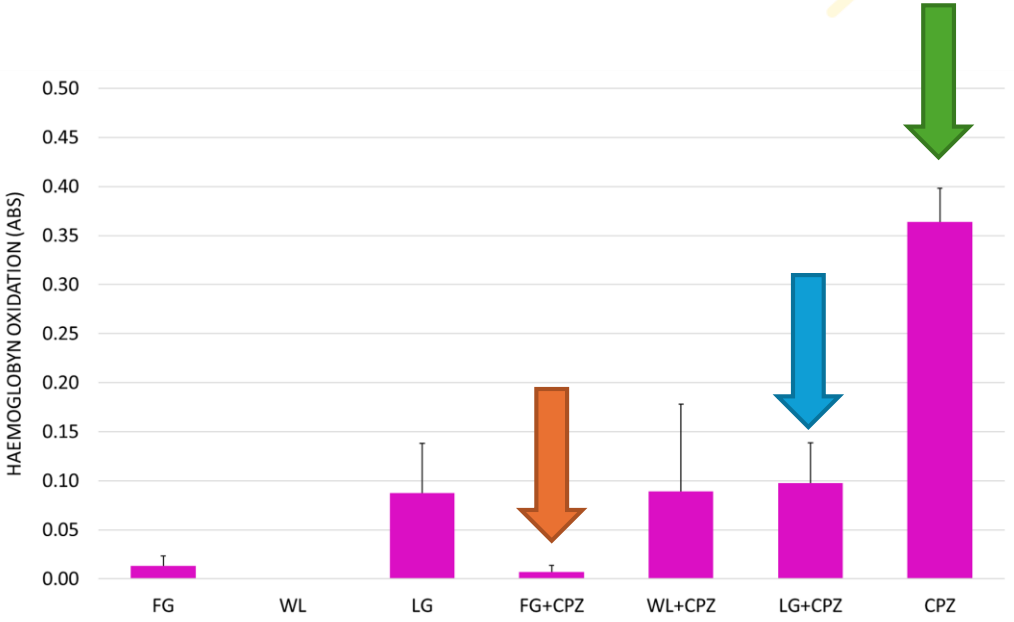


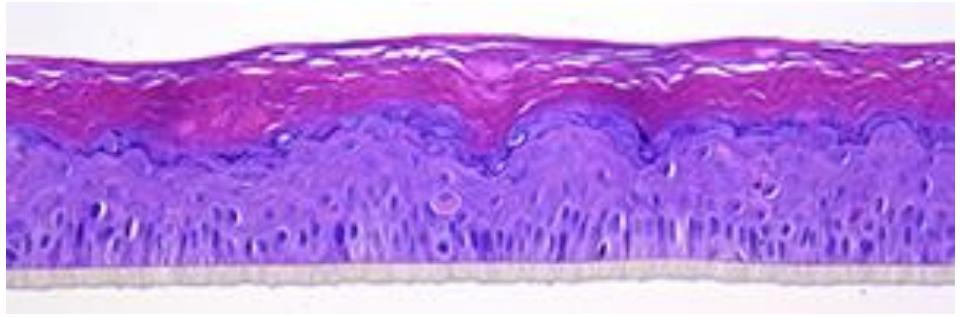
Figure 11. Protection of haemoglobin oxidation induced by CPZ with UVA. Results are expressed as mean ± standard error of n=3. The invittox algorithm, which is an indirect measure of metahaemoglobin production, indicates significant haemoglobin oxidation when values of ABS are >0.05.

FG: Free guarana

WL: White nanosome

LG: Nanosome with guarana encapsulated

TG No. 498 In vitro Phototoxicity: RHE phototoxicity test method



SkinEthic RHE (Episkin.com)

- Reconstituted Human Epidermis (RHE)
- Application of chemical or formulation (water/PBS, oil) overnight
- UVA Irradiation dose approx 6 J/cm2
- Redness, inflammation, cellular viability evaluation

Table 1. Proficiency Substances[1]

	Substance	CAS RN	In vivo[2]	Vehicle[3]	Typical phototoxicity ranges [% w/v or % v/v] (references)
	PHOTOTOXIC SUBSTANCES				
1	Chlorpromazine	69-09-0	PT	Water	0.003% – 0.01% (4)
2	Anthracene	120-12-7	PT	EtOH[4] or Acetone: Olive Oil (4:1)	0.01% – 0.03% (5)(30)
3	Bergamot oil[6]	8007-75-8	PT	Oil[5]	0.0316% – 3.16% (4)(8)
	NON-PHOTOTOXIC SUBSTANCES				
4	Sodium Dodecyl Sulphate	151-21-3	NPT	Water	Non-phototoxic up to highest conc. tested (1%) (4)
5	Octyl salicylate	118-60-5	NPT	Oil[5]	Non-phototoxic up to highest conc. tested (10%) (4)
6	4-Aminobenzoic acid (PABA)	150-13-0	NPT	Oil or EtOH	Non-phototoxic up to highest con. Tested (10%).(27)(30)

TAKE HOME MESSAGE

- Development of *In vitro* models is necessary (Cosmetic Industry,…)
- *In vitro* models to discriminate photoallergens are not available
- New methods addressed to KE of AOP are under development
- Protocols and oficial guides available in database for phototoxicity

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IN VITRO MODELS TO PREDICT PHOTOTOXICITY: STRATEGIES BASED ON THE CELLULAR MECHANISMS INVOLVED

Dra. Adriana S. Maddaleno

Secció Fisiologia

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UNIVERSITAT DE BARCELONA

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