

Cytotoxicity and DNA Damage Biomarkers in the Assessment of Photogenotoxicity

Teixidó E¹, Maddaleno AS², Guardia-Escoté L¹, Vinardell MP², Mitjans M²¹ Department of Pharmacology, Toxicology and Therapeutic Chemistry, Universitat de Barcelona, Spain (eteixido1511@ub.edu)² Department of Biochemistry and Physiology, Faculty of Pharmacy and Food Sciences, Universitat de Barcelona, Spain

INTRODUCTION & AIM

Phototoxic reactions:

- Are relevant for **pharmaceuticals, cosmetics, and consumer products** that absorb UV-visible light.
- Can lead to **skin damage** and increased **cancer risk**.

⚠ Current validated assays (3T3 NRU, RHE):

- ✓ Detect **photocytotoxicity**
- ✗ Do not assess **photogenotoxicity**

Why it matters

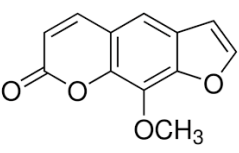
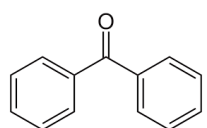
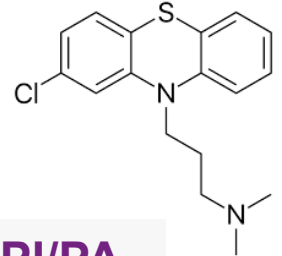
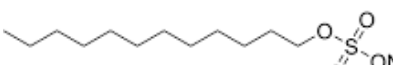
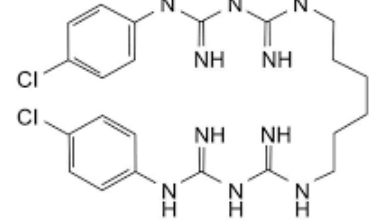
Photogenotoxicity is a critical endpoint for evaluating long-term health risks.



Develop and evaluate an alkaline comet assay in HaCaT keratinocytes as an ethical in vitro screening method

METHOD

Chemicals selected for preliminary profile exploration:

	8-methoxypsoralen (8-MOP)	Benzophenone (BZ)	Chlorpromazine (CPZ)
	 PI	 PA	 PI/PA
	Sodium dodecyl sulfate (SDS)	Chlorhexidine (CHX)	p-Phenylenediamine (PPD)
	 Non-phototoxic	 Non-phototoxic	 Non-phototoxic

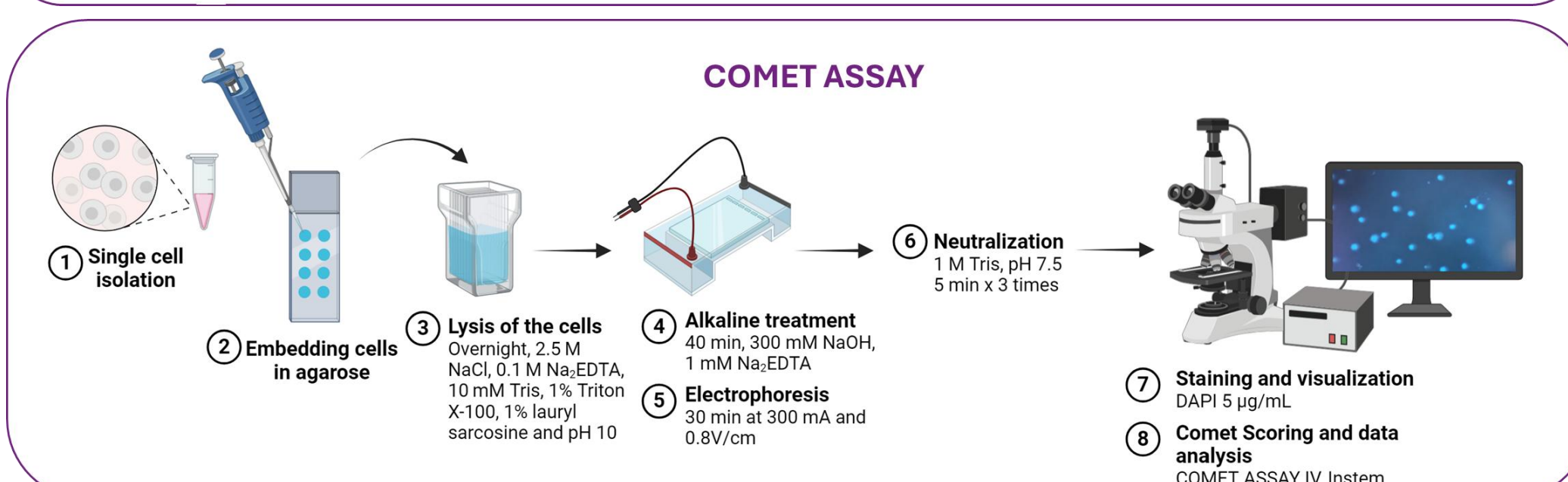
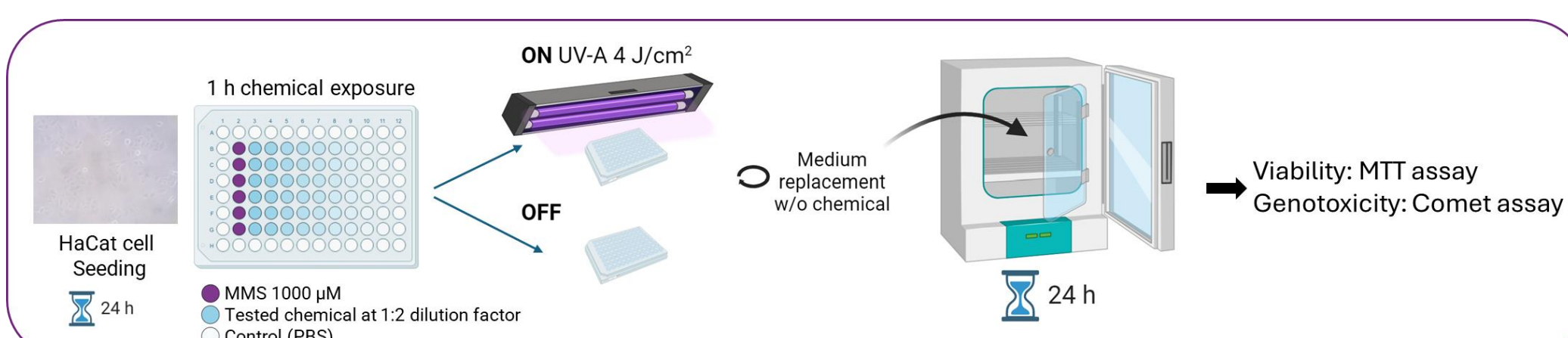
PI: Photoirritant
PA: Photoallergent

Phototoxicity determination

Photoirritation factor (PIF) using cell viability of MTT assay:

$$\text{PIF} = \text{IC}_{50}(\text{No UV}) / \text{IC}_{50}(\text{UV})$$

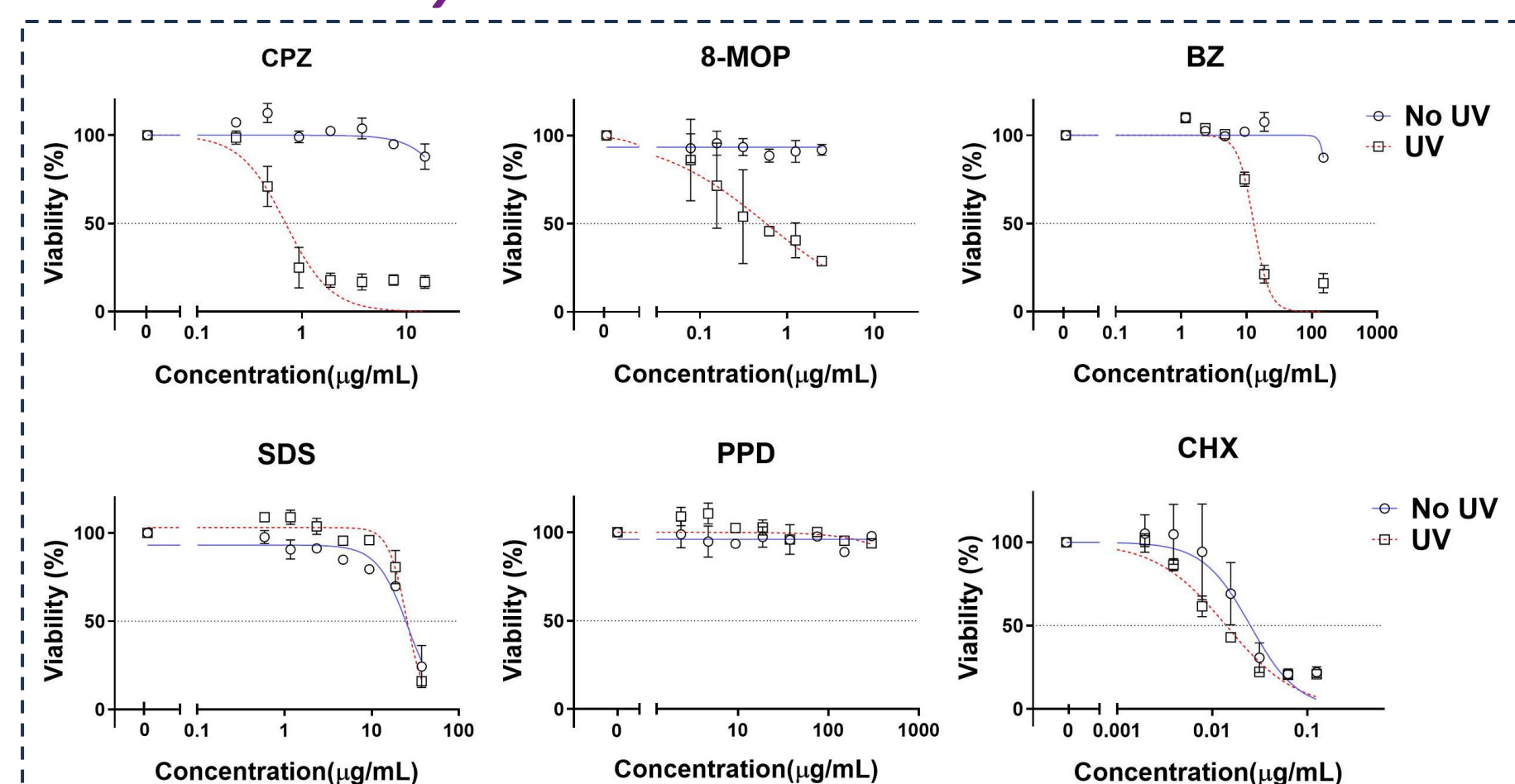
Mean Photo Effect (MPE): Phototox 2.0



RESULTS & DISCUSSION



Photocytotoxicity



Chemical	IC ₅₀ No UV (µg/mL)	IC ₅₀ UV (µg/mL)	PIF	MPE Mean
8-MOP	>2.5	0.58	>4.3	0.23
CPZ	35.08	0.70	50.1	0.62
BZ	>150	12.93	>11.6	0.27
SDS	26.10	25.19	1.03	-0.05
CHX	0.025	0.014	1.78	0.12
PPD	>300	>300	-	-0.05

Established from MTT data of at least 3 independent assays

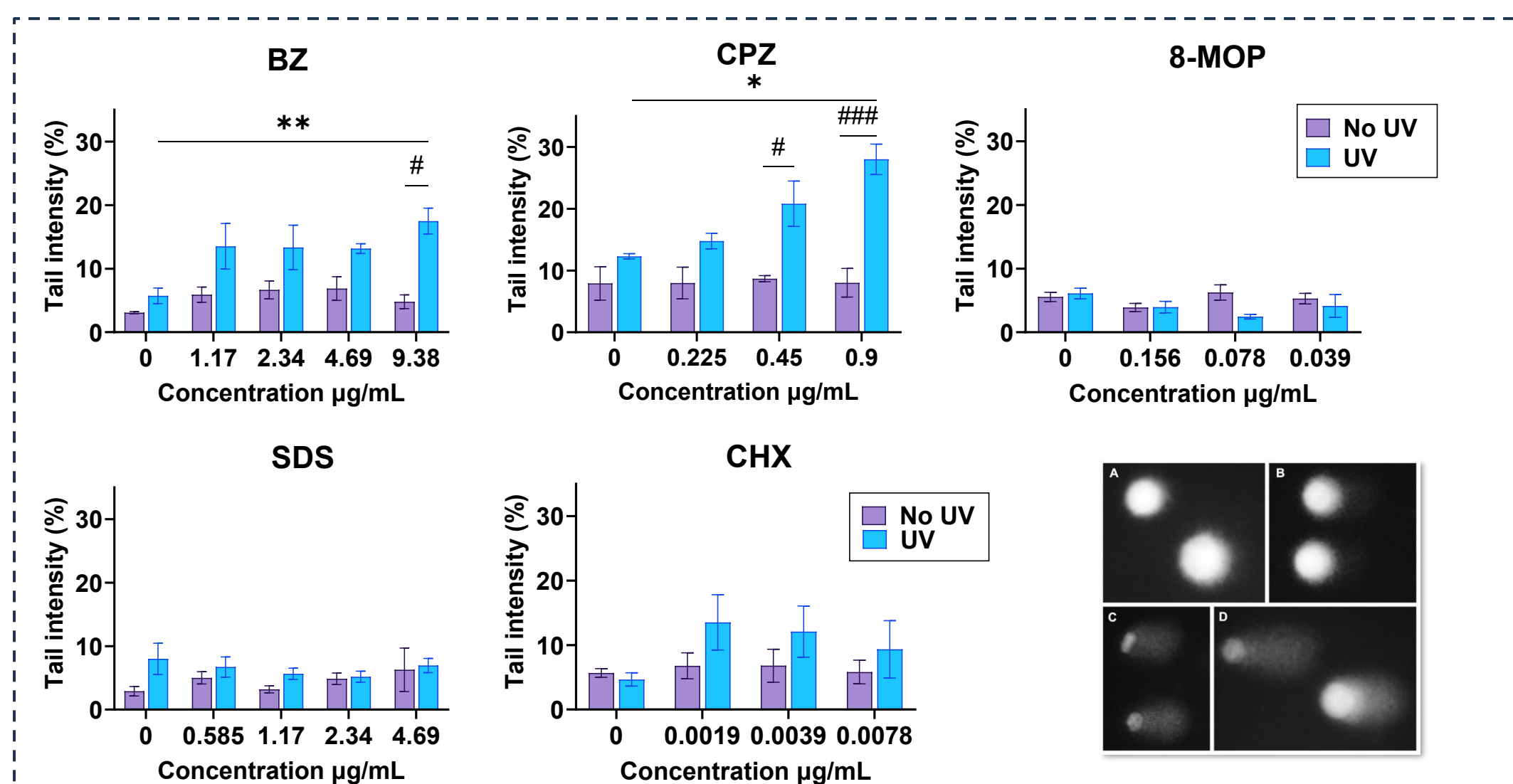
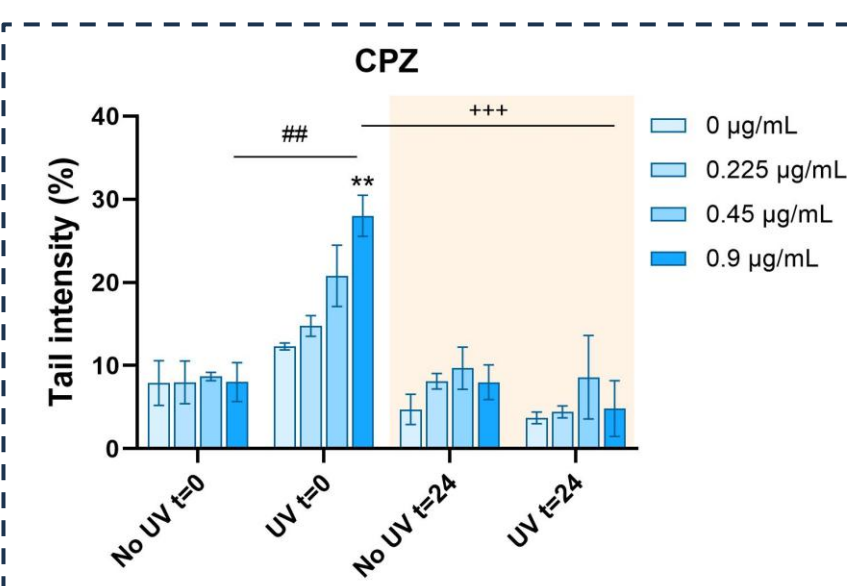


Photogenotoxicity

DNA damage and repair following irradiation

DNA damage was measured immediately (t=0) and 24h (t=24) after irradiation

* indicates differences with the corresponding control, + differences between time points and # differences between no UV and UV. Response values in the graphs are mean values ± SEM (n=3, 50 cells each)

CPZ, BP-3 → Significant DNA strand breaks
8-MOP → ✗ No strand breaks (likely due to crosslinking)
SDS, CHX → ✗ No DNA damage

CONCLUSION

Timing is critical in **photogenotoxicity assessment**

HaCaT photo-comet assay: rapid and ethically aligned tool



Next steps: expand tested substances for validation



Mechanistic insight: include complementary assays (e.g. lesion-specific repair enzymes, γH2AX phosphorylation)

FUTURE WORK / REFERENCES