Determination of a Maximum Tolerated UVA Exposure for the Photo-Genotoxicity Test Method Using the Reconstructed Skin Micronucleus Assay

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ABSTRACT

To address a lack of suitable tools to screen novel ingredients in personal care products for mutagenic/clastogenic activity after solar light exposure, Cosmetics Europe and IFRA initiated a three-phase project to integrate established UVA/visible light (UVA/vis) photo activation techniques to the reconstructed skin micronucleus (RSMN) assay. The first phase was conducted to establish an appropriate photo irradiation schedule to identify a maximum tolerated UVA/vis exposure compatible with the repeat dose regimen of the 72-hour RSMN protocol. Reconstructed human epidermal (RhE) tissues were irradiated daily over 3 days with UVA/vis ranging from 1 to 6 J/cm^2 and compared to RhE tissues cultured without UVA/vis to determine the cytotoxicity and micronucleus induction potential. Two tissues in each group were assessed for binucleation frequency and induction of micronuclei, and two tissues were assessed for viability using the MTT viability assay. No UVA/vis-related effects on MTT metabolism were observed. The 3 J/cm^2 group induced a notable increase in the % binucleated cells (135%), while the 6 J/cm^2 group induced a decrease (64.9%), suggesting some toxic effects at the highest exposures. No significant increases of micronuclei induction in the binucleated cells were observed in any of the UVA/vis treatment groups. Accordingly, 3 J/cm^2 was determined to be a well-tolerated UVA/vis exposure, and will be utilized in the subsequent phases to evaluate test method performance in identifying promutagens requiring photoactivation.

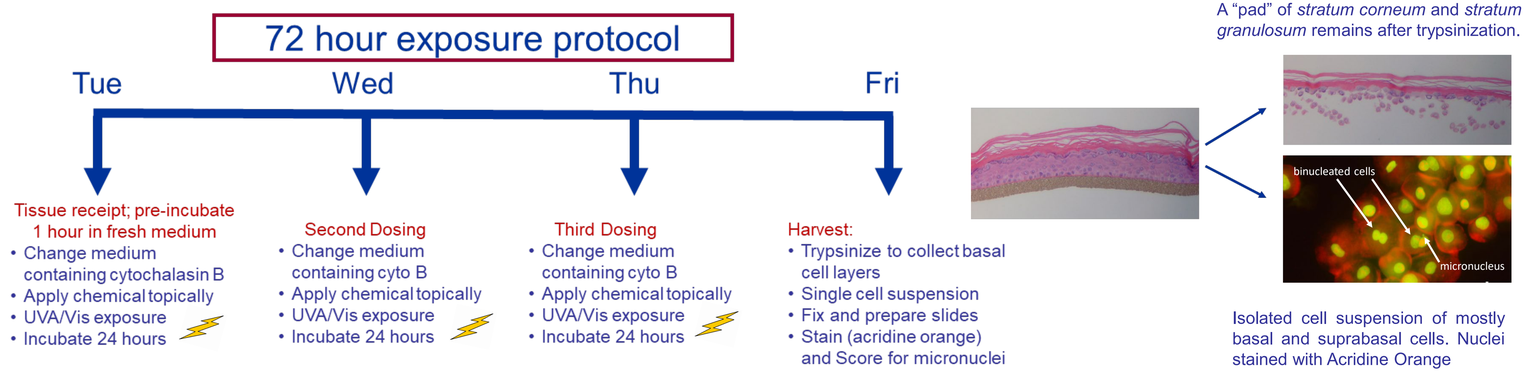
INTRODUCTION

An objective of the 3-phase project will be to generate preliminary data to determine whether the new methodology shows merit. If successful, the methodology is envisioned to be formally evaluated and submitted for regulatory acceptance for hazard identification purposes. After Phase 1, the following phases will be initiated:

Phase 2: Identify a known photo-genotoxic substance and conduct proof of concept trials of the test methodology. The ideal chemical will be non-mutagenic in the absence of photo activation, but should be known to induce micronuclei in replicating mammalian cells after photo irradiation.

Phase 3: Expand proof of concept testing to include additional photo-genotoxins, as well as mutagens that are not activated by UVA/visible light; the latter "negative control" will determine whether differences in micronuclei induction can be measured when comparing treated tissues in the presence and absence of UVA/visible light exposure.

MATERIALS AND METHODS

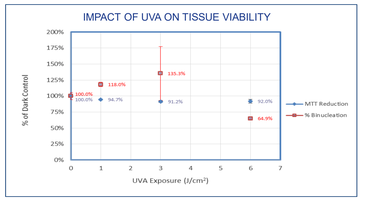


PHASE 1 STUDY DESIGN

Tissues were irradiated with a range of UVA/visible light exposures to determine the cytotoxicity and micronucleus induction potential.

- Tissues were treated with 10 µL of the standard solvent (acetone) at t=0, t=24, and t=48 hours, followed immediately by exposure in the presence or absence of UVA/visible light.
- A Dermalight SOL 3 solar simulator, equipped with a UVA H1 filter (320 to 400 nm) delivering 1 J/cm^2 per 10 minutes was used.
- Four photo irradiation treatment groups, each containing four tissues per group were tested:
  - 6 J/cm^2 – 60 minutes exposure per day
  - 3 J/cm^2 – 30 minutes exposure per day
  - 1 J/cm^2 – 10 minutes exposure per day
  - 0 J/cm^2 – 60 minutes, as a dark exposure control
- Two tissues in each group were harvested and assessed for binucleation frequency and induction of micronuclei.
- Two tissues in each group were assessed for viability using the MTT viability assay.

PHASE 1 RESULTS



MICRONUCLEUS INDUCTION

Tissue #	Treatment	% BN	% Survival by BN	MNBN	% MNBN	Avg. % MNBN
1	UVA 6J/cm^2	22.6%	64.2%	1	0.1%	0.10%
2		23.1%	65.6%	1	0.1%	
5	UVA 3J/cm^2	37.3%	105.6%	1	0.1%	0.20%
6		58.2%	165.0%	3	0.3%	
9	UVA 1J/cm^2	42.5%	120.5%	1	0.1%	0.15%
10		40.7%	115.4%	2	0.2%	
13	Dark	33.8%	96.0%	1	0.1%	0.15%
14		36.7%	104.0%	2	0.2%	

BN: Binucleated cells
MNBN: Binucleated cells with micronuclei

UVA-induced Cytotoxicity

- No UVA exposure-related effect on MTT metabolism was observed. Replicates showed good reproducibility
- 3 J/cm^2 induced a notable increase in the %BN cells with variability in the replicates (165% and 105%)
- 6 J/cm^2 induced a decrease in the %BN cells, suggesting an upper limit

Micronucleus Induction

- No significant increases in the %BN cells with micronuclei (%MNBN cells) was observed
- The %MNBN was in line with the historical controls for the lab

Conclusions

- 3 J/cm^2 was determined to be a well-tolerated UVA/visible light exposure
- Can proceed to Phase 2 studies to evaluate the test method using 3 J/cm^2 with 8–methoxypsoralen (8-MOP) which acts by DNA adduct formation and DNA intercalation after UVA exposure