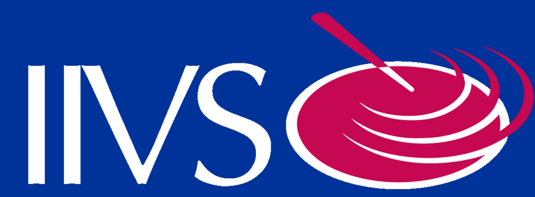

Abstract/Poster 3648/P130

Modifications in the RhE Phototoxicity Assay to Evaluate Long-Lasting Cosmetic Products

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Introduction

The reconstructed human epidermis (RhE) model evaluates phototoxicity potential (i.e., photoirritaion) through the use of a 3D model comprised of stratified human derived epidermal keratinocytes and a functional stratum corneum (OECD TG 498). The model utilizes a topical application for evaluation of various types of materials including complex cosmetic formulations. These cosmetics may include "long lasting" formulations intended for durable wear over extended periods of time, and by design, are intended to remain on the target tissue, and by definition would be difficult to remove. When evaluating long lasting cosmetics in the RhE model, residual test material may produce interference with the assay due to its dark color and difficulty in removal, which can impact the UV exposure, prolong the exposure to the tissues, and interfere with the assay endpoint which utilizes the MTT vital dye.

Further refinements of assay protocols were investigated for long lasting materials, and modifications in procedures including reduced dosing volumes, use of glass rod during application to spread the material, warmed buffer for rinsing, and surface swabbing for removal of material were performed.

Two blind-coded lipstick formulations, provided by Avon Products, Inc., were evaluated. One lipstick formulation (AB19) was designated as long-lasting (and thereby would be difficult to remove) and one lipstick formulation (AB20) was designated for standard use. Each lipstick formulation was tested under two different conditions: as is (neat) and spiked with several concentrations of a known photoirritant, chlorpromazine. The lipsticks were evaluated in the RhE Phototoxicity test using the EpiDerm™ model (MatTek Corporation, Cambridge, MA, USA) to determine if the modifications to the protocol impacted the ability to evaluate phototoxicity potential of the spiked treatment groups.

Materials & Methods

The procedures for testing were similar to those presented in OECD TG 498 with modifications as presented in **Figure 2.** All treatment groups were evaluated in duplicate tissues per +Irr or –Irr condition. Briefly, tissues were exposed to each treatment for ~24 hours at standard culture conditions (5% ± 1% CO_2, 37° ± 1 ° C in humidified air) (SCC), and then the treatments were removed. Half of the tissues in each treatment group were exposed to 6 J/cm² of UVA/visible light delivered by a SOL3 solar simulator (UVATEC) equipped with H1 filter, and the remaining half retained in the dark. The tissues then received an overnight incubation at SCC, after which the viability was assessed using MTT.

Test Materials

Two lipstick formulations provided by Avon, referred to as AB19 and AB20, were evaluated under different conditions: as is (neat) and spiked with three concentrations of a known photoirritant, chlorpromazine (CPZ). A photograph of the formulations in stock containers is presented in **Figure 1a**.

Spike Preparations

Spiked treatments of AB19 and AB20 were prepared by combining a stock CPZ solution in DMSO with each neat lipstick (**Figure 1**). The lipstick and CPZ were mixed between syringes attached via stopcock (**Figure 1b**).

Killed Controls

Killed control (KC) tissues, or previously viable tissues that were frozen, thawed, and no longer metabolically active, were used to account for possible MTT interference due to the dark color. KC tissues were handled the same as the viable tissues. Net MTT values of the KCs were subtracted from the relevant viable treatment group to account for direct MTT reduction or potential interference by the test material alone.

Swabbing of Tissues

The treated tissues were designated into "swabbing" and "no swabbing" groups to investigate potential impacts on treatment removal. At the time of treatment termination, sterile cotton swabs moistened with Calcium and Magnesium Free Dulbecco's Phosphate Buffered Saline (CMF-DPBS) were used to remove the treatments from the surface of the tissues designated for swabbing.

Assay Modifications

Modifications to the dosing procedure included reduced volumes of 20 µL and the use of a sterile glass rod to spread the treatments across the surface of the tissues. The treatments were removed prior to the irradiation/dark exposure (+Irr/-Irr) using CMF-DPBS warmed at 37±1°C. Groups designated as "no swabs" were rinsed only and no further removal attempts were made.

Viability Calculations

The relative viability for each treatment group was calculated to its respective control +Irr and –Irr:

$$\% \text{ Viability} = \frac{\text{corrected } OD_{550} \text{ of +Irr or -Irr Exposure Matched Treatment Group}}{\text{corrected } OD_{550} \text{ of +Irr or -Irr Exposure Matched Solvent Control}} \times 100$$

Prediction of Phototoxicity Potential

Phototoxicity was evaluated by comparing the viabilities of the tissues +Irr to the tissues –Irr within the same treatment group, as described in OECD TG 498. A ≥ 30% difference in viability +Irr and –Irr indicated phototoxicity potential. When the –Irr tissue viability was <35%, the test material was classified as cytotoxic. The % decreases in the viability of each treatment group in +Irr as compared to -Irr are presented in **Table 1.**

REFERENCE: OECD (2021), *Test No. 498: In vitro Phototoxicity - Reconstructed Human Epidermis Phototoxicity test method*, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, https://doi.org/10.1787/7b2f9ea0-en.

Experimental Design

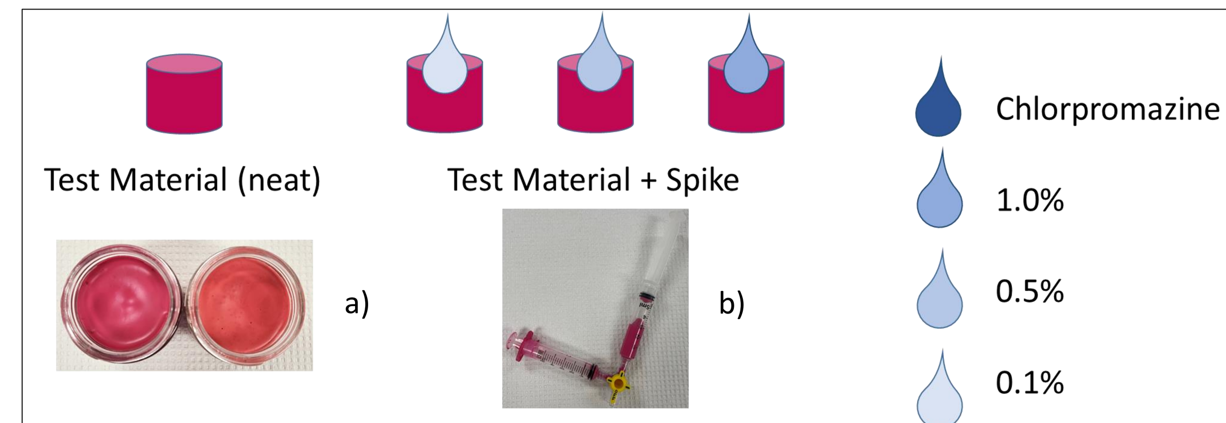

Figure 1. Treatment Conditions. a) Test material as is (neat: AB19 left and AB20 right), and b) preparation of the test material spiked with CPZ in syringes affixed with stopcock for mixing

Tissues dosed with 20 µL for ~24 hours at SCC
Lipstick spread over tissue using sterile glass rod for full coverage

Rinsed with warmed (~37°C) CMF-DPBS
Designated groups swabbed to remove treatments

Figure 2. Demonstration of Procedural Modifications

Test Article Removal: Swab and KC Impacts

Test article removal is a critical step for this assay as opaque or dark colored materials may physically block the light exposure to the tissues. Further, failure to remove test article at treatment termination results in an extended exposure to the tissues throughout the overnight post-exposure period. Sterile cotton swabs moistened in CMF-DPBS can be used as an additional measure in removal of test material. Care must be exercised as the use of swabs may induce mechanical damage to the tissues, and in some cases cause tissue detachment. Consideration was weighed for long lasting products which may not be able to be easily removed.

Minimal variability was observed in the use of swabs for lipstick AB19 (data not shown). However, KC tissues were included and highlighted how the use of swabs increased the test article removal. At neat and with 0.5% spike, the net KC OD was <9% of solvent control (with swabs) compared to a net KC OD of 20-23% of solvent control (without swabs).

Our data showed more variable results and reduced viability when swabs were used to remove lipstick AB20 (**Figure 3**). There was minimal, if any, MTT reduction/interference with the KC treated with AB20 neat and with spike (with or without swabs).

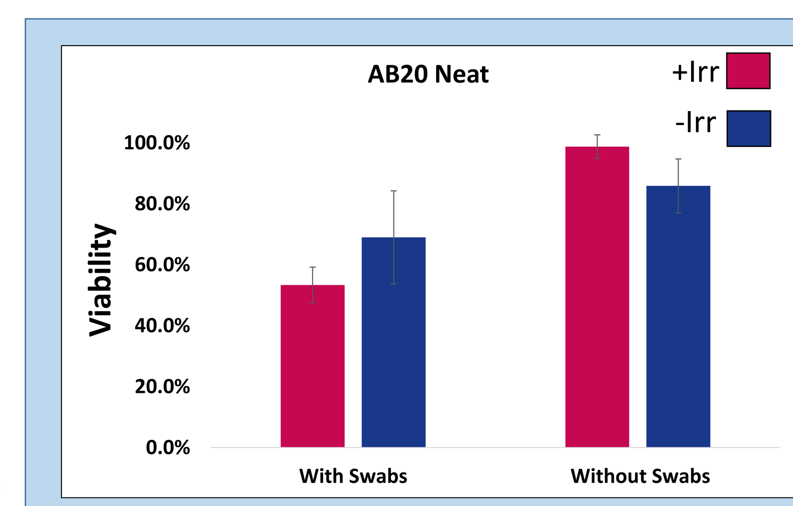

Figure 3. Viability of AB20 Neat, with and without the use of swabs during test article removal

Results

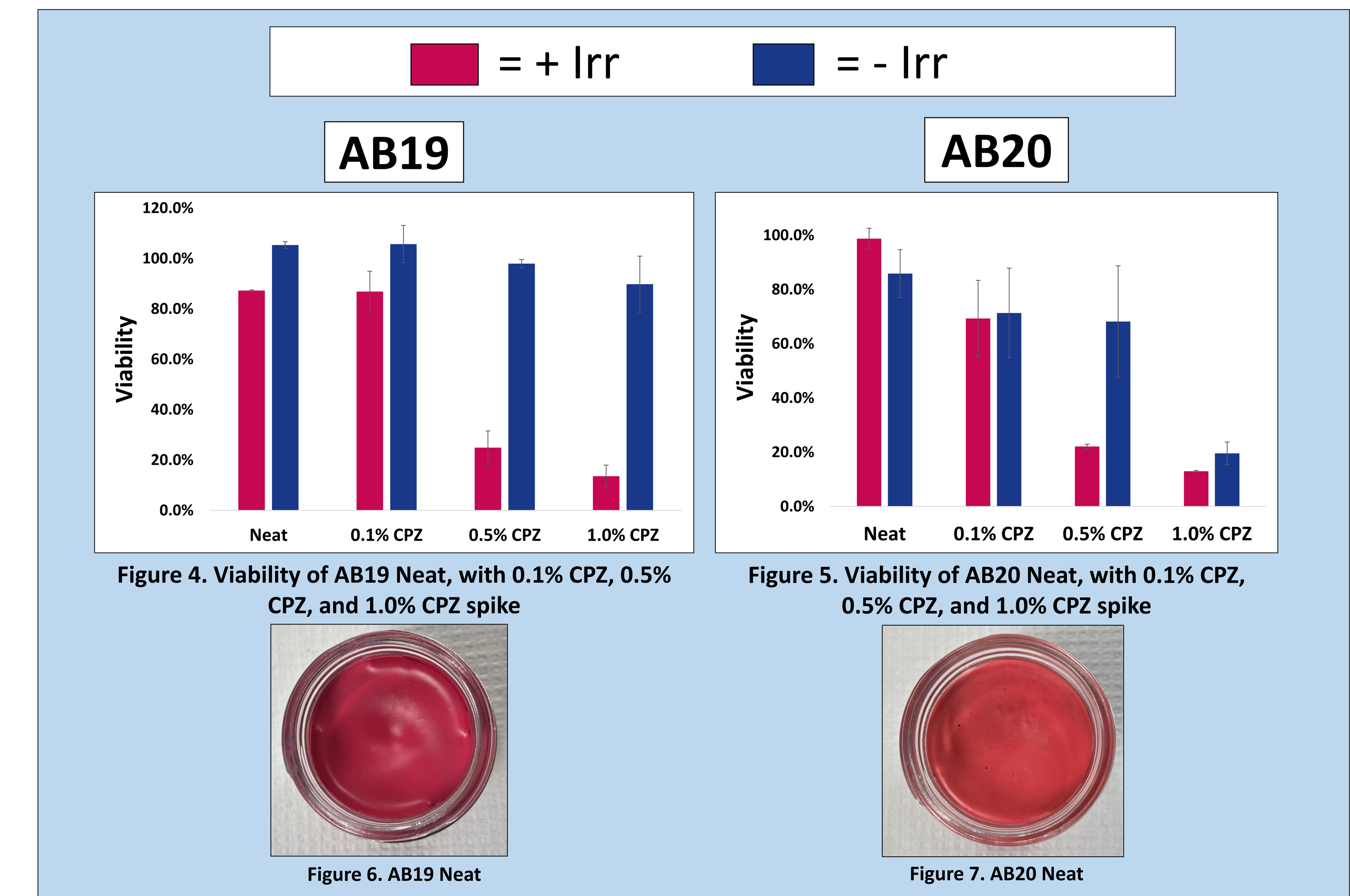

Figure 4. Viability of AB19 Neat, with 0.1% CPZ, 0.5% CPZ, and 1.0% CPZ spike

Figure 5. Viability of AB20 Neat, with 0.1% CPZ, 0.5% CPZ, and 1.0% CPZ spike

Figure 6. AB19 Neat

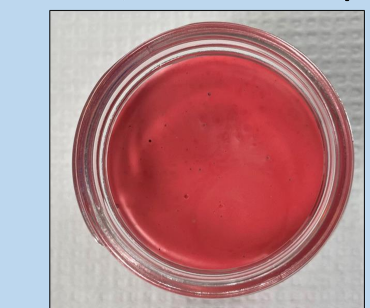

Figure 7. AB20 Neat

Table 1. Summary of % Difference in Viabilities and Prediction of Phototoxicity Potential.

Test Material	Chlorpromazine "Spike" Conc. (w/v)	% Difference in Viabilities (+Irr compared to -Irr)	Phototoxicity Potential*
AB19	0% (Neat)	18.1	Non-Phototoxic
	0.1%	18.8	Non-Phototoxic
	0.5%	73.1	Phototoxic
	1.0%	76.2	Phototoxic
AB20	0% (Neat)	-12.9	Non-Phototoxic
	0.1%	2.1	Non- Phototoxic
	0.5%	46.1	Phototoxic
	1.0%	6.6	Cytotoxic^

^ - –Irr was <35% viable; phototoxicity potential could not be evaluated

* - >30% difference between +Irr and -Irr was considered to have phototoxic potential

Conclusions & Future Directions

The modifications in dosing volume, application, and removal were appropriate to evaluate the phototoxicity potential of long-lasting cosmetics, or products that may be difficult to remove. The lipstick formulations as is (Neat) did not show phototoxicity potential nor cytotoxicity; however, when the chlorpromazine spikes were added at sufficient concentrations, phototoxic responses were evident.

The lipsticks (Neat) were not fully removed with the rinsing methods and prolonged the total exposure to the tissues up to ~45 hours. However, there was minimal cytotoxicity observed despite the extended exposure, which supports their extended use.

Lipstick formulation AB20 showed increases in cytotoxicity with increasing concentrations in chlorpromazine in the presence and absence of light, demonstrating that this formulation acted as a good carrier vehicle of the CPZ.

The use of cotton swabs as a technique to assist in test article removal produced variability **(Figure 3**) and may be expected when removing long lasting products. However, when cotton swabs were not utilized, evaluation of phototoxicity potential was still accurately predicted. To ensure viability is not overestimated when residual test article remains, killed control assessments should be performed for any test articles that are dark colored or directly reduce MTT, and viability values adjusted as needed.

Our results demonstrate the benefit of the RhE model in final formulation testing, and its ability to assess phototoxicity potential if the offending chemical within the formulation is at sufficient concentration to elicit a safety concern.

These modifications to the standard protocol allow for phototoxicity assessment of long-lasting cosmetic materials and can also provide essential data for companies operating in Latin American markets which require supporting data for SPF marketing claims of long-lasting products.