Phototoxicity

The reconstructed human epidermis (RhE) model evaluates phototoxic potential (i.e., phototoxicity) through the use of a 3D model comprised of stratified human-derived epidermal keratinocytes and a functional stratum corneum (OECD TS 448). 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, printing the exposure to the tissue, 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-scrubbing for removal of material were performed.

Two blind lipid formulations, provided by Avon Products, Inc., were evaluated. One lipid formulation [AB10] was designated as long-lasting and thereby would be difficult to remove, and one lipid formulation [AB20] was designated for standard use. Each lipid formulation was tested under two different conditions: a) as (neat) and spiked with several concentrations of a known phototoxin, Chlorpromazine (CPZ). The phototoxic potential was evaluated in the RhE model 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 and Methods

The procedures for testing were similar to those presented in OECD TS 448 with modifications as presented in Figure 2. all treatment groups were evaluated in duplicate tissues per 1 hr or 24 hr condition. Briefly, tissues were exposed to each formulation for 24 hours at standard visible conditions (40% UV, 60% visible) or 24° C and humidified (60%) CO2, and then the treatments were removed. Half of the tissues in each treatment group were exposed to 6.4 J/cm² of UV/visible light delivered by a SDL solar simulator (UVITEC) equipped with 415nm filter, and the remaining half retained the dark. The tissues then received an overnight incubation at SCC, after which the viability was assessed using MTT.

Test Materials

Two lipid formulations provided by Avon, referred to as AB10 and AB20, were evaluated under different conditions: a) as (neat) and spiked with three concentrations of a known phototoxin, chlorpromazine (CPZ). A photograph of the formulations in stock containers is presented in Figure 1a.

Spikes Preparations

Spiked treatments of AB10 and AB20 were prepared by combining a stock CPZ solution in DMSO with each neat lipid (Figure 1). The lipid 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 as the same as viable tissues. For 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 cultures. The treatments were removed prior to the irradiation/dark exposure (4 hr) using CMF-DPBS warmed at 37°C. Groups designated as “no swab” rinsed only and no further removal attempts were made.

Viability Calculations

The relative viability for each treatment group was calculated to its respective control +1 hr and -1 hr.

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\% \text{ Viability} = \frac{\text{OD (100)} - \text{OD (-1 hr)}}{\text{OD (+1 hr)}} \times 100
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Phototoxicity Potential

Phototoxicity potential was evaluated by comparing the viabilities of the tissues +1 hr to the tissues -1 hr within the same treatment group, as described in OECD TS 448. As a 30% difference in viability +1 hr and -1 hr indicated phototoxic potential. When the -1 hr tissue viability was >75%, the test material was classified as cytotoxic. The % decrease in the viability of each treatment group in -1 hr compared to +1 hr are presented in Table 1.

Results

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 lipid formulations as (neat) did not show phototoxicity potential nor cytotoxicity; however, when the chlorpromazine spikes were added at sufficient concentrations, phototoxic responses were evident.

The lipid formulations (not) were fully removed with the rinsing methods and prolonged the total exposure to the tissues up to ~40 hours. However, there was minimal cytotoxicity observed despite the extended exposure, which supports their extended use.

Phototoxicity formulation AB20 showed increased in cytotoxicity with increasing concentrations in chlorpromazine in the presence of 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 underestimated when residual test articles remain, 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 data showed more variable results and reduced viability when swabs were used to remove lipids AB20 (Figure 3). There was minimal, if any, MTT reduction/interference with the KC treated with AB20 and neat with (or without swabs).

Conclusions & Future Directions

The modifications to the RhE model in final formulation testing, and its ability to assess phototoxic potential if the offending chemical within the formulation is an unacceptable concentration, is 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 DPR marketing claims of long-lasting products.