3649/P131

Evaluation and Transferability of a New Approach Methodology to Address Photoallergy Potential

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Introduction

Currently, there are no standardized non-animal test methods to evaluate photoallergy potential. To address this need, a collaboration between the Research Institute for Fragrance Materials (RIFM), and two laboratories, Shiseido and the Institute for *In Vitro* Sciences (IIVS), was established to evaluate and transfer new technologies. These new technologies established assays used to evaluate photoirritation (TG 432) and skin sensitization (TG 442D) with modifications. A photo-KeratinoSens assay was used to evaluate the induction of Antioxidant Response Element (ARE) genes associated with skin sensitization after exposure to 5 J/cm^2 of UVA and visible light. The methodology incorporated the work presented by Tsujita-Inoue, *et al.* (2015).

Test materials that absorb light may have potential to elicit photoirritant or photoallergic responses. Photoirritation occurs when an applied or ingested substance becomes activated and produces a sunburn-like reaction on the skin within minutes to hours of exposure and goes away when the offending chemical is removed. This response is different from photoallergy, which produces an immune-mediated response after exposure to the test substance and UV irradiation. This type IV delayed hypersensitivity response occurs after sensitization, wherein repeated exposure to the photoallergen, or in some cases, the sun, can lead to skin sensitization responses (*e.g.,* allergic contact dermatitis) over the course of weeks or months, even after the offending compound is removed (Maibach H. & Honari G., 2014).

When the collaboration was first established in early 2020, Shiseido planned to travel to IIVS to provide hands-on training, and then each team evaluate selected test materials with the ultimate goal of transfer of the technology into IIVS' laboratory in compliance with Good Laboratory Practices (GLPs). However, due to the global pandemic, in-person training was cancelled which resulted in some variations in procedures between the laboratories. IIVS experiences with the skin sensitization and photosafety assays provided a solid foundation, while Shiseido continued to provide guidance during the transfer of the assay.

Twelve reference test materials were selected by RIFM and evaluated in the photo-KeratinoSens assay by Shiseido and IIVS labs. The results were evaluated for sensitivity, specificity, and accuracy.

Experimental Design

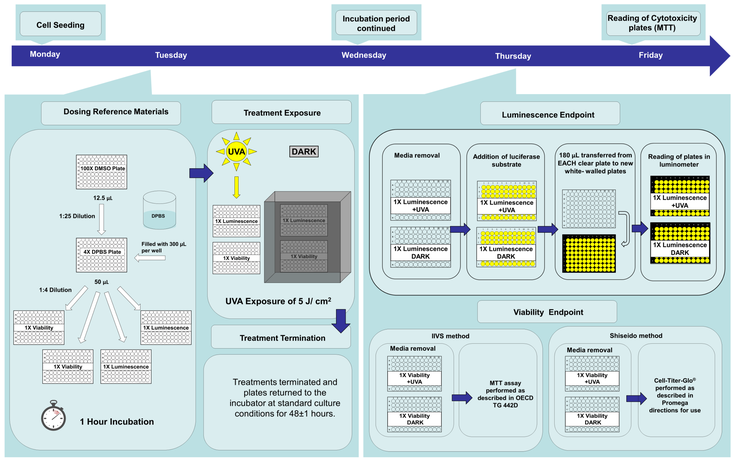


Figure 1. Summary Flowchart of Assay Steps

The procedures performed were adapted from OECD TG 442D (ARE-Nrf2 Luciferase Test Method) and OECD TG 432 (*In Vitro* 3T3 NRU Phototoxicity Test), as well as those presented by Tsujita-Inoue, *et al*. (2015). The assay setup for each laboratory was highly similar, with some exceptions, including the reagents used for the luciferase and viability endpoints. For example, the Promega Steady-Glo® and Cell Titer-Glo® were used by Shiseido, and the Promega One-Glo™ and MTT were used by IIVS for luciferase and viability endpoints, respectively. The differences were not expected to impact the outcome of the assays.

A preliminary dose range finding assay was performed to establish concentration ranges for the definitive assays. The maximum concentrations attempted were 5000 µg/mL (Shiseido) or 2000 µM (IIVS). At least two definitive assays were performed for each test material. Each definitive assay plate included a solvent control (1% DMSO in DPBS, 6 wells) and a positive control (6-MC prepared at 5 concentrations up to 2000 µM in a single well per concentration). A general overview of the procedures performed for a single definitive trial is presented in **Figure 1**.

Data Analysis

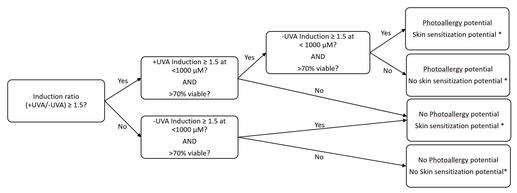
The data for fold induction and % viability was calculated with individual test well results relative to the solvent controls, and then used to calculate the $EC_{1.5}$ value (*i.e.,* concentration inducing > 1.5-fold luciferase induction) and IC_{30} value (concentration resulting in 30% reduction of viability), similar to calculations described in OECD TG 422D. *Note: RLU = Relative Light Units; OD = Optical Density*

$$\% \text{ Cell Viability} = \frac{\text{Corrected } OD_{570} \text{ of Test or Control Article}}{\text{Mean Corrected } OD_{570} \text{ of Solvent Control}}$$

$$\text{Fold Induction} = \frac{\text{Corrected } RLU_{570} \text{ Value of Test or Control Article Concentration}}{\text{Mean Corrected } RLU_{570} \text{ Value of Solvent Control}}$$

A fold induction ratio comparison of +UVA and –UVA was calculated for each concentration and used to evaluate photoallergy potential as presented in **Figure 2.**

$$\frac{\text{Fold induction at concentration (+UVA)}}{\text{Fold Induction at concentration (-UVA)}}$$



* - results to evaluate skin sensitization potential should be carefully considered since all requirements outlined in TG 442D not incorporated into this approach

Figure 2. Evaluation of Test Results Flowchart

Results

Reference Chemical	CAS #	Photoallergy Potential	IIVS prediction	Average highest fold induction ratio (+UVA/-UVA)	Shiseido prediction	Average highest fold induction ratio (+UVA/-UVA)
6-Methylcoumarin	92-48-8	Positive	Positive	4.01	Positive	2.42
8-MOP	298-81-7	Positive	Positive	41.0	Positive	15.6
Anthracene	120-12-7	Negative	Negative	1.52[a]	Negative	1.35
Acridine	260-94-6	Negative	Negative	1.46	Negative	1.26
Dichlorophene	97-23-4	Positive	Negative	1.30	Negative	1.24
Hexachlorophene	70-30-4	Positive	Negative	2.88[b]	Negative	1.44
Fenticlor	97-24-5	Positive	Positive	2.78	Positive	2.29
Isoniazid	54-85-3	Positive	Negative	1.24	Positive	2.09
Amiodarone HCl	19774-82-4	Positive	Negative	1.27	Positive	2.04
Ketoconazole	65277-42-1	Positive	Negative	1.31	Negative	1.32
Musk Ambrette	83-66-9	Positive	Positive[c]	1.52	Negative	1.31
TCSA	1154-59-2	Positive	Positive	1.77	Positive	2.21

Table 1. Test Results.
Summary results of 12 reference materials and photoallergy potential, as presented in the literature. The fold induction ratios present the average of the highest induction comparison ratio of at least three valid trials with exception of Musk Ambrette, which was evaluated in two trials by Shiseido. A negative result for photoallergy potential is highlighted in green and a positive result for photoallergy potential is highlighted in orange.

[a] - Anthracene (IIVS) produced > 1.5-fold induction ratio (+UVA/-UVA), but $EC_{1.5}$ (+UVA) was not > 1.5-fold.

[b] - Hexachlorophene (IIVS) produced > 1.5-fold induction ratio (+UVA/-UVA) but viability < 70% at concentrations of fold induction > 1.5.

[c] - For Musk Ambrette, 1 of 3 trials with > 1.5-fold induction ratio (2.29), but viability < 70%; average of the 2 trials with viability > 70% at determining dose presented with > 1.5-fold induction ratio (1.52)

Representative graphic responses from both labs in **Figures 3-5** show induction (left graphics) and cytotoxicity (right graphics) +UVA and –UVA for test materials that area predominantly photoallergen (6-Methylcoumarin), a photoirritant (Acridine), and a photoallergen predicted as a false negative at IIVS (Isoniazid). The cytotoxicity dose responses may show photoirritation potential (*i.e.,* increased toxicity in the presence of irradiation as compared to the absence of irradiation). Further comparison of IC_{50} values (-UVA/+UVA) and a comparison of dose responses across the range of concentrations +UVA and -UVA, described as the Photo Irritancy Factor (PIF) and Mean Photo Effect (MPE), respectively in TG 432 can provide insight.

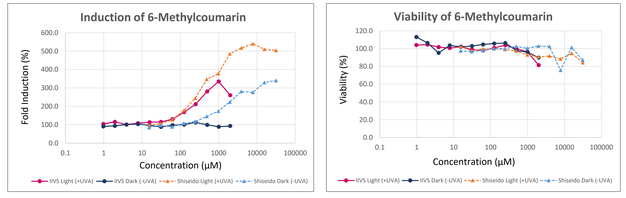


Figure 3. 6-Methylcoumarin (6-MC) Induction and Viability. 6-MC, a chemical that is predominantly a photoallergen showed increased luciferase induction +UVA and a > 1.5-fold induction ratio (+UVA/-UVA).

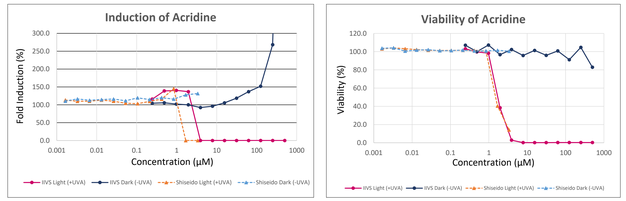


Figure 4. Acridine Induction and Viability. Acridine, a photoirritant, produced high levels of cytotoxicity +UVA as compared to –UVA. Further, these results suggest skin sensitization potential since an $EC_{1.5}$ value was produced at higher concentrations with viability > 70% (-UVA).

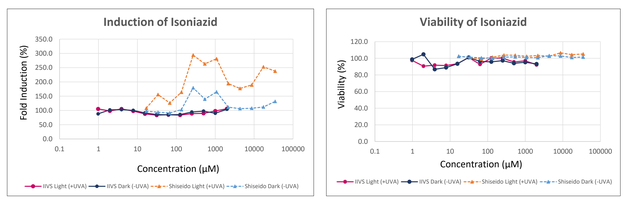


Figure 5. Isoniazid Induction and Viability. Isoniazid, a chemical that is predominantly photoallergen, was not correctly predicted at IIVS (the results showed no increases in luciferase induction + or - UVA up to the highest concentration tested), unlike Shiseido results showing increases in luciferase induction (+UVA)**.**

Conclusions & Future Directions

- Our collaborative work is the foundation for establishment of a future battery of test methodologies that address predicting photoallergy potential and differentiating it from phototoxicity.
- Overall similar results for both labs with accuracies of 66.7% (8/12) (Shiseido) and 58.3% (7/12) (IIVS), and a high specificity of 100% (2/2). The sensitivities were lower but remained similar between IIVS (50%, 5/10) and Shiseido (60%, 6/10).
- The 2 photoirritants (Anthracene and Acridine) were correctly predicted as negative for photoallergy potential, and the 3 reference compounds identified as predominantly photoallergens (Dichlorophene, Hexachlorophene, and Ketoconazole) were incorrectly predicted as negative by both labs.
- One possible source of the difference between results between the labs is the concentration range and dilution steps tested
- The global pandemic impacted transfer of assay, which resulted in some variations in approach, however, the assay was still successfully established.
- This assay also provides insight into photoirritation potential and skin sensitization potential; however, results for these endpoints should be interpreted cautiously since the photo-KeratinoSens assay includes modifications from the standard test guidelines.
- A photo-DPRA assay, already established in both laboratories, the 3T3 NRU Phototoxicity assay (OECD TG 432), and a photo-h-CLAT assay (established by Shiseido) may elucidate further photoallergy potential in combination with these assay results.
- For future direction- should a "2 of 3 approach", similar to skin sensitization potential, be considered for a full assessment of photoallergy potential?
- Further understanding of the type of photo response (*i.e.,* irritant, allergen, or both) using the larger dataset may elucidate the mechanism and applicability of different test platforms.

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