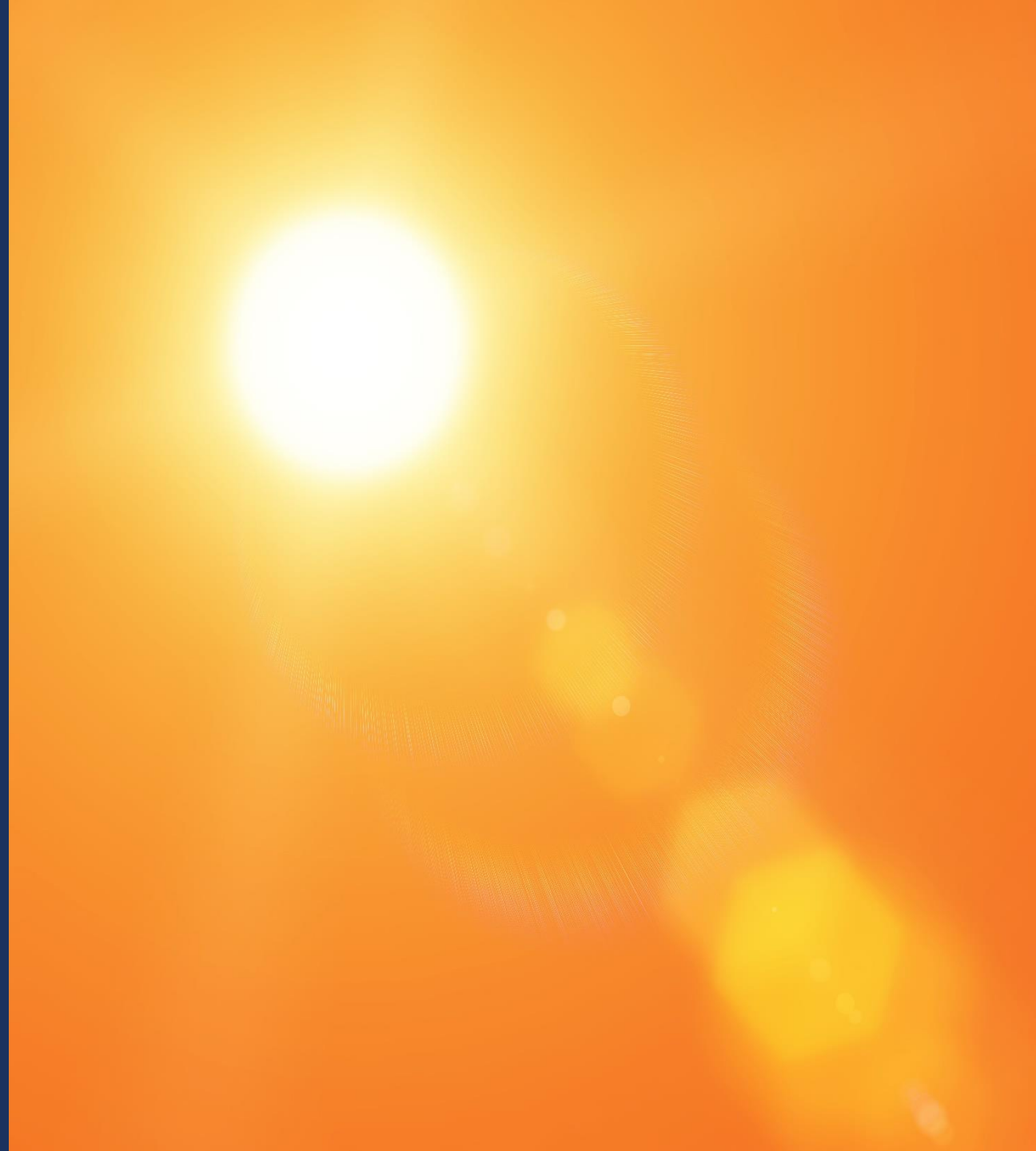
New Approach Methodologies for Photosafety of Fragrance Ingredients: A brief overview and case study

Webinar Series on *In Vitro* Phototoxicity Testing

Co-organized by IIVS and PETA Science Consortium



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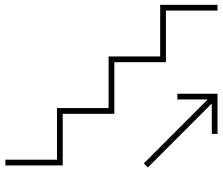
This presentation focuses on photosafety and collaborative efforts towards new approach methodologies (NAMs) for photoallergy.

History and evolution of photosafety at RIFM



Tiered approach for photoirritation

Photoallergy Research Collaborations

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Photosafety is an important component of RIFM’s fragrance material safety assessments.

Historically, photosafety has been an issue for fragrance materials, cosmetics, and consumer products.

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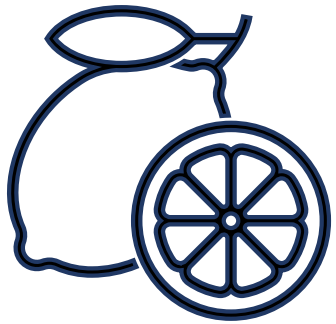
Photoirritation and photoallergy are separate and distinct endpoints with different risk management strategies.



Photoirritation

- Skin irritation + UV
- Furocoumarins
- Manage risk with concentration limits

Photoallergy

- Skin sensitization + UV
- Musk ambrette
- Manage risk with ingredient ban

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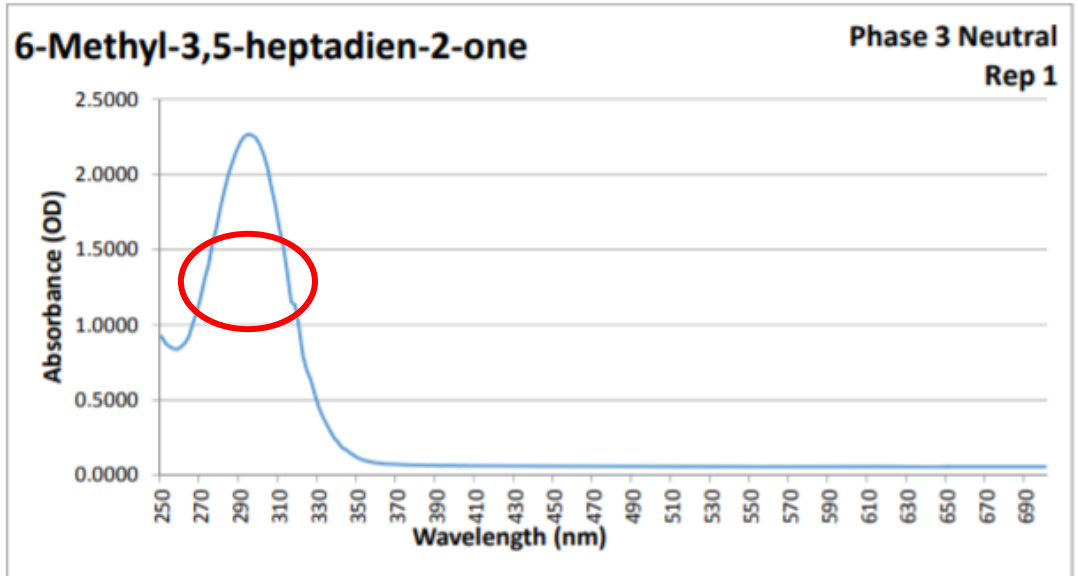
PHOTOSAFETY EVALUATION

UV absorbance is calculated on a molar basis and compared to a benchmark value.



OECD TG 101

Sample Description	6-Methyl-3,5-heptadien-2-one				
Physical State	Liquid				
Condition	$c_{i\ (mol/L)}$	λ (nm)	A	d (cm)	ε ($M^{-1}cm^{-1}$)
Neutral	2.87E-04	295	2.35	1	8191.93

Beer-Lambert Law

Benchmark comes from Henry et al., 2009
$=1000\ L \cdot mol^{-1} \cdot cm^{-1}$

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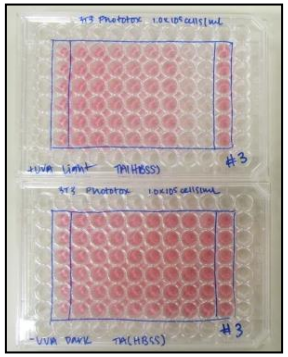
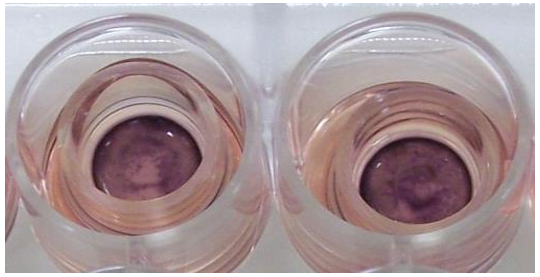
PHOTOIRRITATION

Photoirritation can be addressed by 3 assays which are used in a tiered approach.



3T3-Neutral Red Uptake Phototoxicity Assay
OECD TG 432



Reconstructed Human Epidermis (RhE) Phototoxicity Assay
OECD TG 498

Human Photoirritation Test

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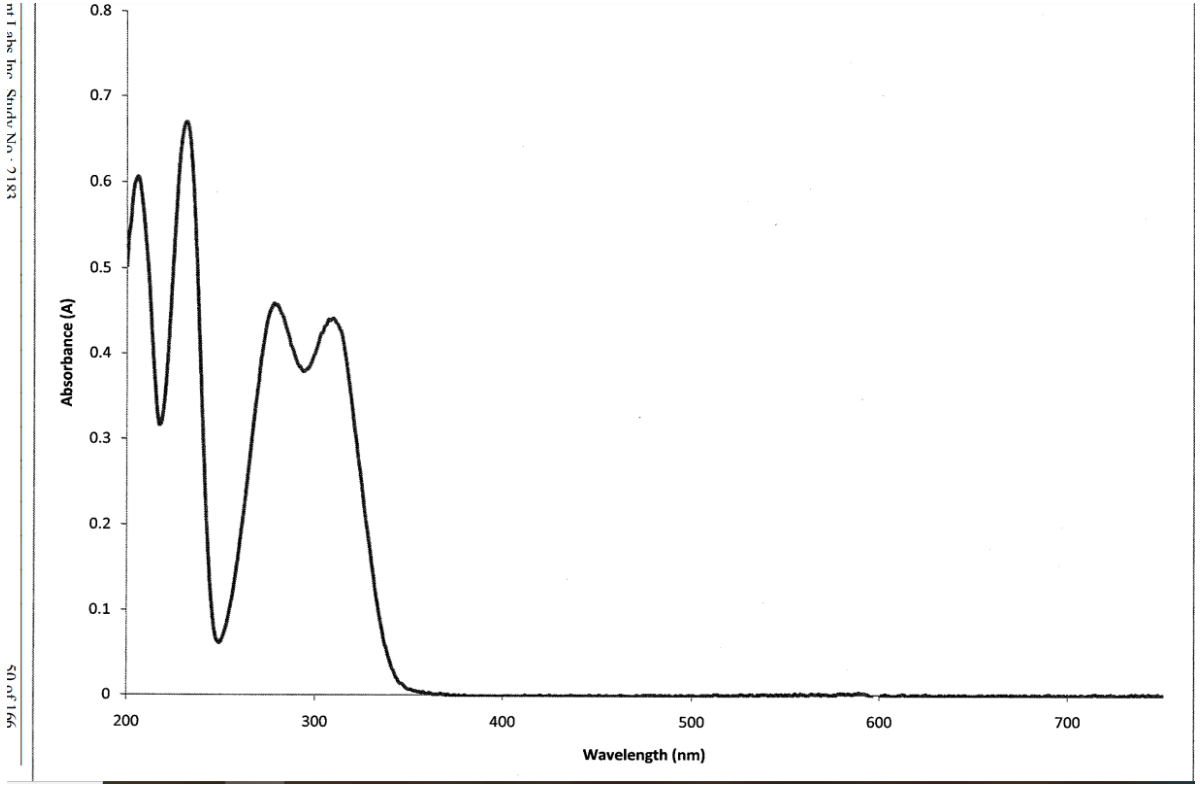
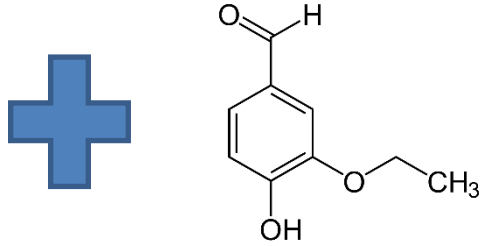
CASE STUDY: ETHYL VANILLIN

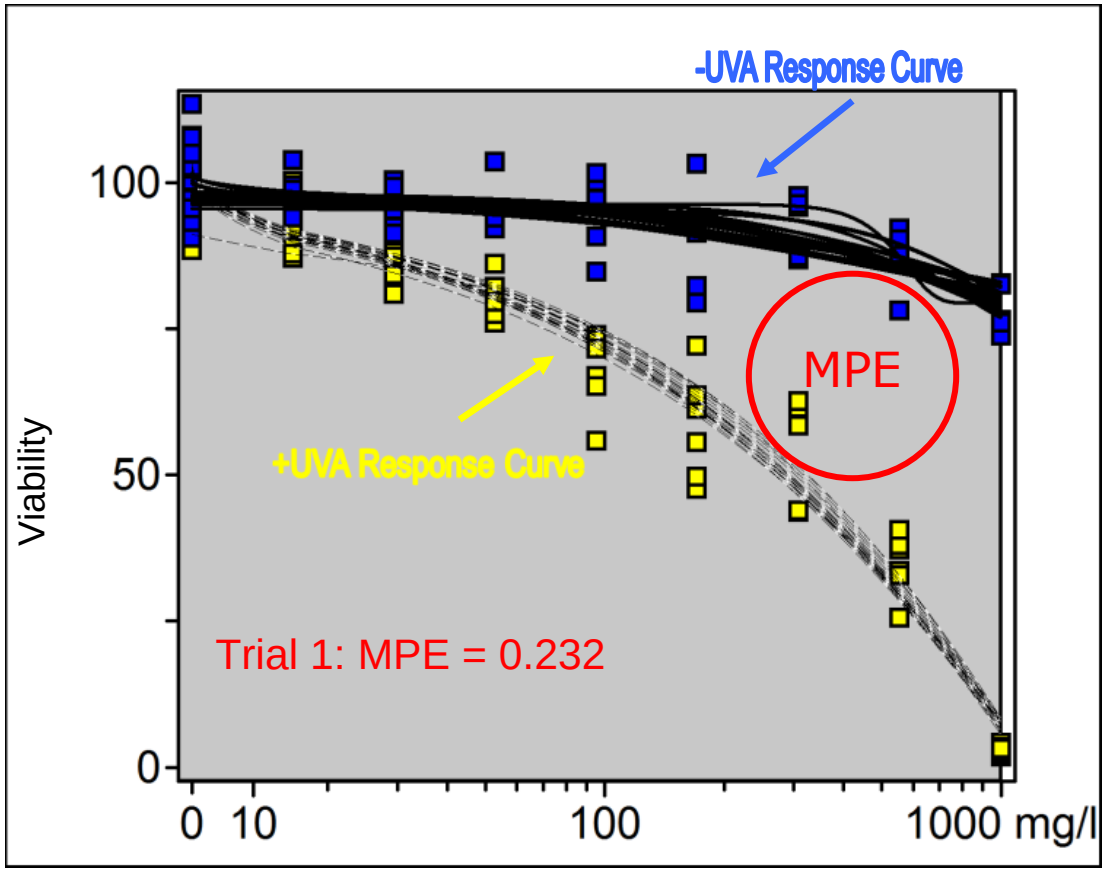
Ethyl vanillin absorbs UV light, indicating the potential for photoactivation.

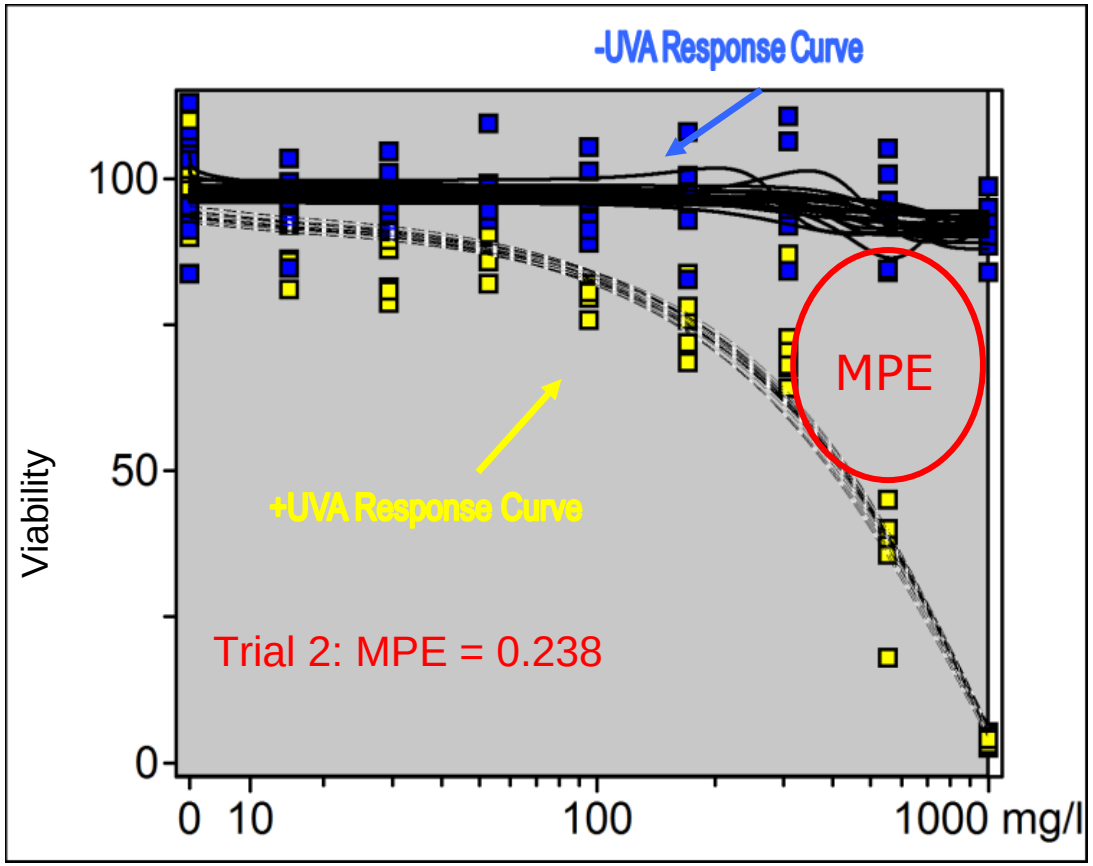


Absorbance = 7,328 L · mol^{-1} · cm^{-1}



The 3T3-NRU Phototoxicity Assay, compares cytotoxicity (+UV/-UV) to assess photoirritation.





Mean Photo Effect (MPE) compares -UV/+UV dose response curves. MPE for both trials was ≥ 0.150, which predicts that Ethyl Vanillin is photoirritating.

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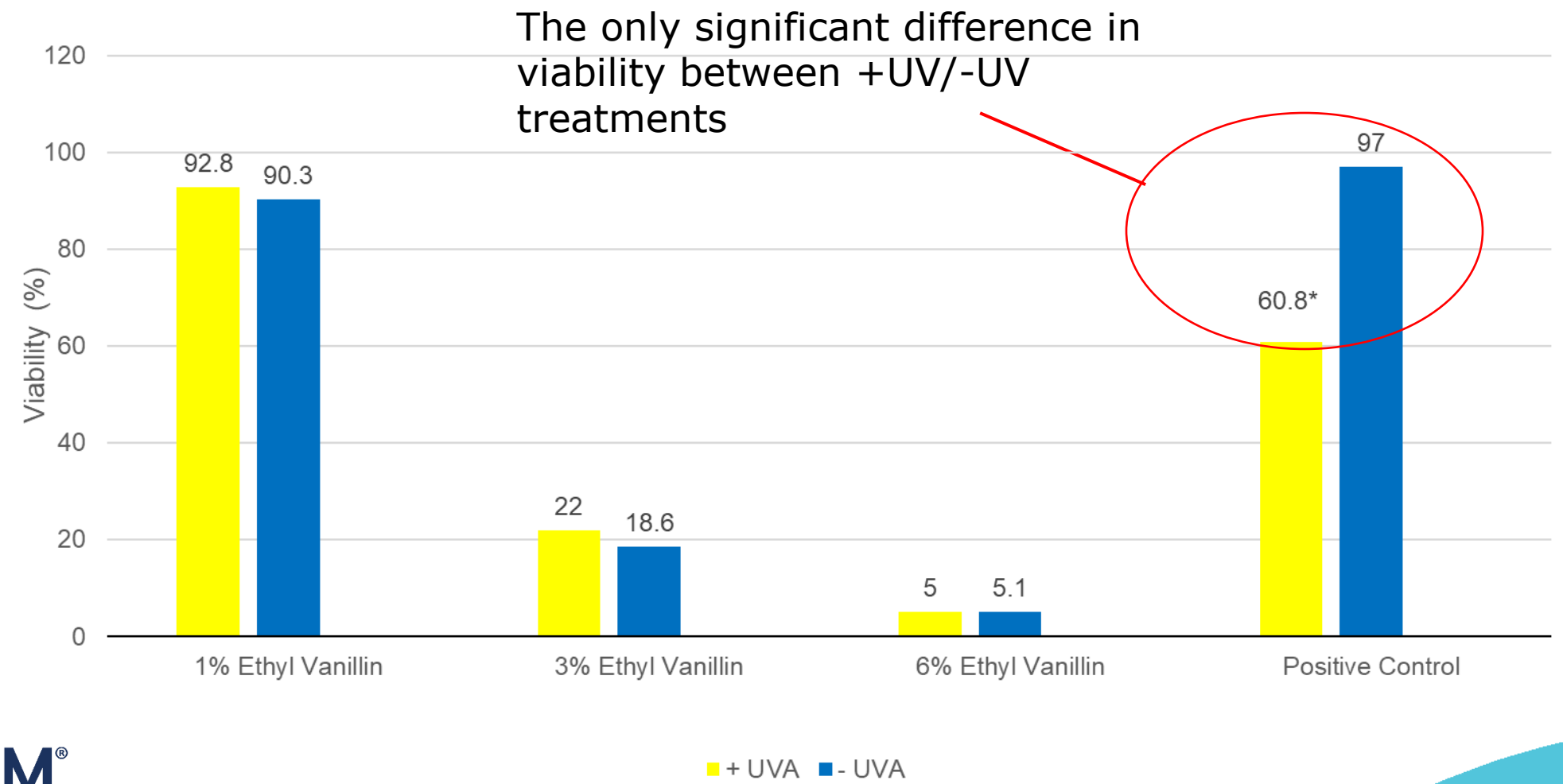
If photoirritation is predicted by the 3T3-NRU assay, a reconstructed human epidermis (RhE) phototoxicity test is conducted.



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The no-effect level for photoirritation found in the RhE test is then confirmed with a human photoirritation test.

	Irradiated			Non-Irradiated		
	24 h	48 h	72 h	24 h	48 h	72 h
1% Ethyl Vanillin	0 (27)	0 (27)	0 (27)	0 (27)	0 (27)	0 (27)
3% Ethyl Vanillin	0 (27)	0 (27)	0 (27)	0 (27)	0 (27)	0 (27)
6% Ethyl Vanillin	0 (27)	0 (27)	0 (27)	0 (27)	0 (27)	0 (27)
Vehicle Control	0 (27)	0 (27)	0 (27)	0 (27)	0 (27)	0 (27)
Saline Control	0 (27)	0 (27)	0 (27)	0 (27)	0 (27)	0 (27)

No photoirritant reactions were seen at irradiated or non-irradiated sites, at any dose of Ethyl Vanillin.

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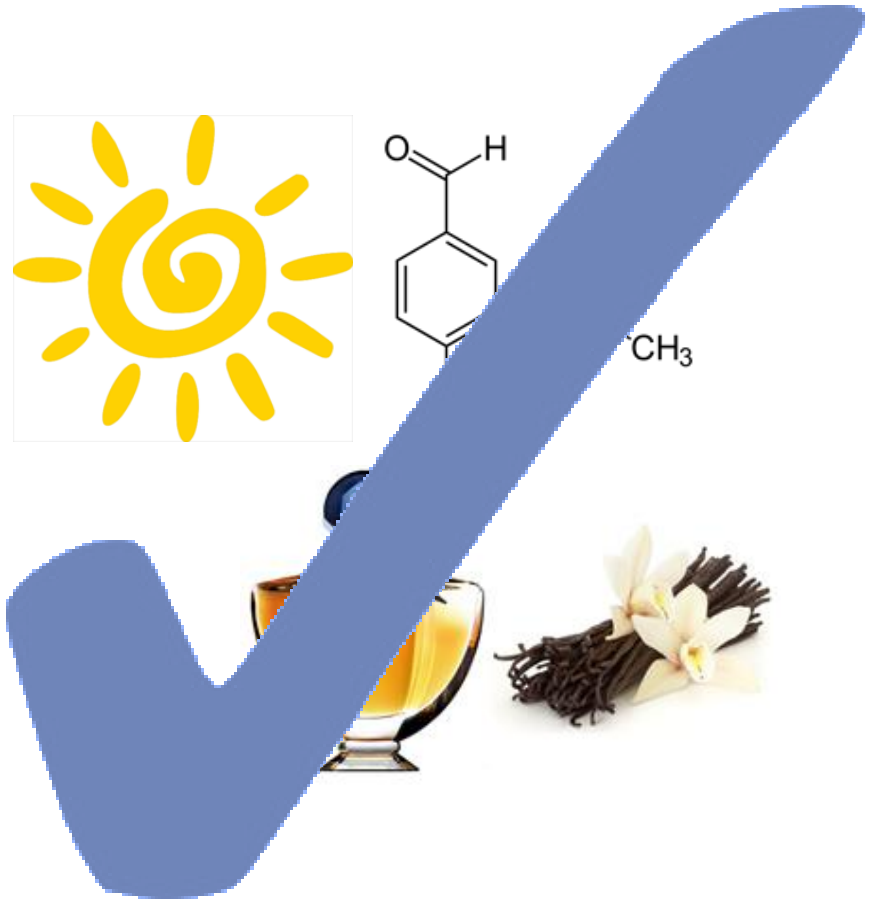
In summary, Ethyl Vanillin does not present a concern for photoirritation at concentrations up to 6%.

Physical-chemical property (UV absorbance) revealed a concern for photoactivation, *in vitro* hazard-based testing (3T3-NRU) suggested the potential for photoirritation

Risk-based testing (RhE, human photoirritation) resulted in a NOEL for photoirritation equal to the maximum concentration tested



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Our manuscript describing the tiered approach for photoirritation evaluation was recently published.

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Use of alternative test methods in a tiered testing approach to address photoirritation potential of fragrance materials

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ABSTRACT

The safety assessment of fragrance materials for photoirritation utilized by The Research Institute for Fragrance Materials has recently been modified and is described in detail. Materials demonstrating significant absorbance in the ultraviolet and visible light (UV/VIS) range (290–700 nm) may present a concern for photoirritation and require further investigation. If there are no photoirritation data or data are insufficient, then data on read-across materials are considered before a tiered approach for testing begins. The hazard-based 3T3-Neutral Red Uptake (NRU) Phototoxicity Test (OECD TG 432) is used as a first-tier assay; if it predicts photoirritation, it is followed by the reconstructed human epidermis (RhE) phototoxicity assay (OECD TG 498). The RhE phototoxicity assay is used to determine a No Observed Effect Level (NOEL) for photoirritation that is used in a confirmatory human photoirritation test. Data are presented on 108 fragrance materials exhibiting significant UV/VIS absorbance and evaluated in the 3T3-NRU Phototoxicity Assay. Twenty-one materials were predicted to be phototoxic; twenty were evaluated in the RhE Phototoxicity Assay to establish a NOEL. Fourteen materials were then evaluated in a confirmatory human phototoxicity test. The tiered testing approach presented represents a scientifically pragmatic method to minimize the likelihood of photoirritation from fragrance materials.

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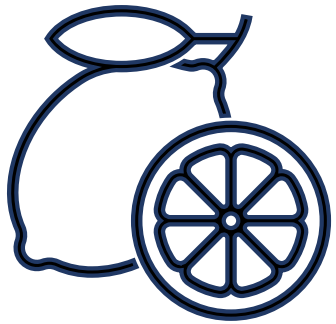
Photoirritation and photoallergy are separate and distinct endpoints with different risk management strategies.



Photoirritation

- Skin irritation + UV
- Furocoumarins
- Manage risk with concentration limits

Photoallergy

- Skin sensitization + UV
- Musk ambrette
- Manage risk with **ingredient ban**

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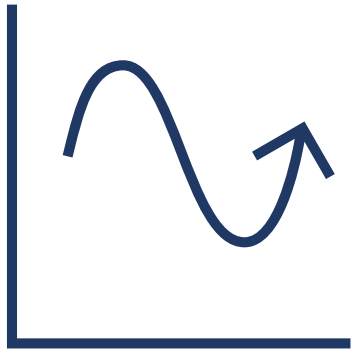
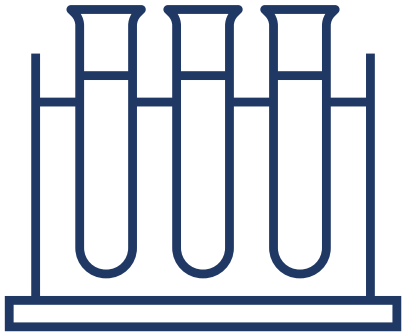
To address photoallergy, validated, guideline assay options are limited.



UV/Vis Absorbance
OECD TG 101



Reactive Oxygen Species (ROS)
OECD TG 495

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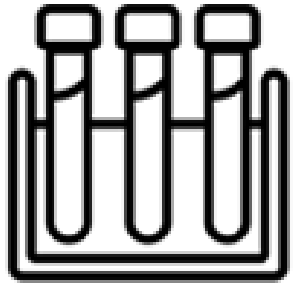
PHOTOALLERGY RESEARCH

Through collaborations, we are determining if *in chemico* or *in vitro* assays for skin sensitization can be modified to evaluate photoallergy.



Shiseido and IIVS

- **Photo-DPRA**
- **Photo-Keratinosens** *(SOT 2023)*
- **Photo-hCLAT**

SenzaGen

- **GARDskin Dose Response for photoallergy** *(SOT 2023)*

ImmunoSearch

- **Photo SENS-IS**

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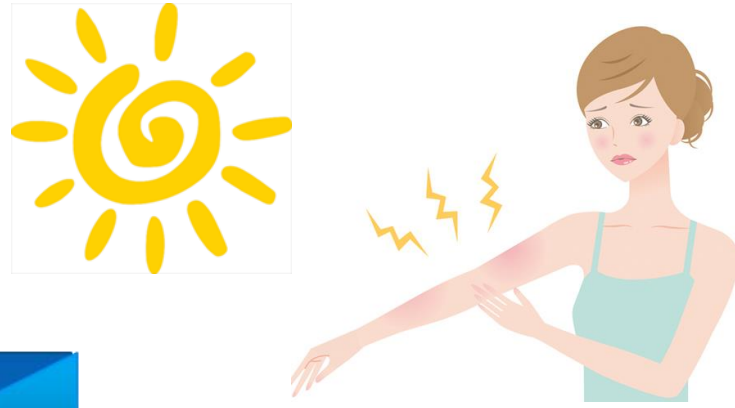
The assays address specific key events in the adverse outcome pathway.



Key Event 1: Protein Binding

Photo-DPRA

Key Event 2: Keratinocyte Activation

Photo-Keratinosens
Photo SENS-IS

Key Event 3: Activation of dendritic cells

Photo-h-CLAT

GARDskin

Key Event 4: T Cell proliferation

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Reference Test Materials

- "Predominantly" Photoirritant
- "Predominantly" Photoallergenic
- Both Photoirritant and Photoallergen

	Material
PHOTO IRRITANTS	8-Methoxypsoralen
	Dimethyl Anthranilate
	Methyl B-Naphthyl Ketone
	Anthracene
	Acridine
	Naproxen
	5-Methoxypsoralen
PHOTO ALLERGEN	6-Methylcoumarin
	Musk Ambrette
	Dichlorophene
	Fenticlor
	Hexachlorophene
	Isoniazid
Both	TCSA
	Amiodarone
	Ketoconazole

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Through our tiered testing approach, RIFM can effectively address photoirritation.

- **UV/Vis Absorbance (OECD 101)**
- **3T3-NRU (OECD 432)**
- **RhE Phototoxicity (OECD 498)**

Through collaborations with research partners, RIFM is working towards NAMs for photoallergy.

- **IIVS & Shiseido:** Photo-DPRA, Photo-Keratinosens, Photo-hCLAT
- **SenzaGen:** GARDskin Dose Response for Photosensitization
- **ImmunoSearch:** PhotoSENS-IS

Thank you for your attention!

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