



Suncare Research Laboratories, LLC

2518-B Reynolda Road
Winston Salem, NC 27106 USA
(336) 725-6501
www.suncarelab.com

(336) 725-6503 fax
jstanfield@suncarelab.com

HUMAN PHOTOTOXICITY AND PHOTOALLERGENICITY TEST

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Protocol Number:

SRL2006-XXX

Title:

Human Phototoxicity and
Photoallergenicity Test

Objective:

The objective of the test is to assess the potential of a product to produce contact irritation, phototoxic, photoallergic and/or contact allergic reactions in normal use by the consumer population.

Test Product:

Subjects:

A sufficient number of healthy adult volunteers will be enrolled to complete 25 subjects of either sex.

Sponsor:

Investigator:

Introduction

Contact dermatitis is a local inflammatory response on skin without involvement of the immune system. Acute contact dermatitis can be caused by a single application of a cutaneous irritant, while cumulative irritation results from repeated exposures to a cutaneous irritant. Allergic contact dermatitis is an inflammatory reaction that results from an immune response to an antigen, which develops during an induction phase generally lasting from 10 to 21 days. After re-exposure to the antigen the reaction is elicited after a typical delay of 12 to 48 hours.¹

Photosensitivity reactions are abnormal responses to ultraviolet radiation (UVR) or visible light. As with contact dermatitis and allergic contact dermatitis, the pattern and time course of these responses permit their identification and classification. Systemic and topically applied agents can cause contact dermatitis or interact with sunlight to cause phototoxic reactions. These reactions occur on first exposure to the product and usually resemble exaggerated sunburn.² Phototoxic reactions are characterized by a so-called decrescendo pattern in the severity of successive observations of the response.³

The phototoxicity test measures the potential of test products to produce phototoxic reactions by a single exaggerated exposure of human volunteer subjects to a test product, with and without UVR, followed by scoring of responses. Comparison of responses to test product alone, test product irradiated with UV radiation and an irradiated, untreated site will permit assessment of the potential for phototoxicity of test products.

More rarely, topically applied products may contain one or more ingredients that can be converted to a photoantigen by UVR. In certain individuals the photoantigen can then trigger an inflammatory response by the immune system and produce an allergic reaction upon subsequent exposure to the product in sunlight.² Photoallergic reactions are characterized by a crescendo pattern in the severity of successive observations of the response.³

The photoallergenicity test measures the potential of test products to produce allergic reactions in the presence of UVR. This study consists of a 24 hour duplicate occluded exposure to up to 6 test products on the backs of healthy volunteers, followed by an evaluation and irradiation of one set of sites with UVR and 5 additional applications

and irradiations of one set of sites over a 2 week Induction phase. After a 9 day resting phase, another 24 hour occluded exposure, irradiation of one set of sites and 24 and 48 hour evaluation constitute a Challenge phase. The inclusion of evaluations of 24 and 48 hour responses of sites treated with test products, with and without UVR, in the Induction Phase and again in the Challenge Phase, permits simultaneous evaluation of the potential for contact irritation, phototoxicity, contact allergy and photoallergy.

Objective

The objective of the test is to assess the potential of a product to produce contact irritation, phototoxic, photoallergic and/or contact allergic reactions in normal use by the consumer population.

Test Product:

Handling of Test Materials

The test product will be logged in, labeled, and stored as appropriate on receipt. Unless otherwise stated in writing by the sponsor, the test product will be destroyed within one month of acceptance of the final report.

Study Design

This is an open-label, controlled study, consisting of six duplicate, occluded exposures to the test product in Finn Chambers, followed by UVR administration to half the sites, over a two-week Induction Phase; a 9 day Resting Phase and a single 24 hour application of the test product in a Challenge Phase. In the Challenge Phase, the test product will be applied to sites not previously treated, one site will be irradiated with UVR and responses will be evaluated 48 and 72 hours after removal of patches. Both sites will be scored at each removal of patches.

Subjects

A sufficient number of healthy adult volunteers will be enrolled to complete 25 subjects of either sex.

Inclusion Criteria

- Signed, written informed consent in conformance with 21CFR part 50
- At least 18 and no more than 60 years old
- Fitzpatrick skin types I, II, or III²

- Good general health
- Willing to avoid sun exposure, tanning lamps, and use of any topical products on the test areas
- Willing and able to complete all study visits

Exclusion Criteria

- History of photosensitivity disease or any sensitivity to cosmetics or topical or systemic products
- Pregnancy or nursing a child
- Significant systemic disease, infection, cataracts, glaucoma, diabetes, or lupus
- History of psoriasis, atopic dermatitis, skin cancer, dysplastic nevi, or other skin pathology
- Sunburn, excessive tan, uneven skin tones or blemishes of the mid-back, or use of tanning lamps or beds within 3 months before enrollment
- Use of systemic or topical drugs that might affect responses to UVR, or interfere with responses to test products. These drugs include, but are not limited to: corticosteroids, thiazides, tetracyclines, and NSAIDs
- Loss of more than 2 patches during the induction phase
- Participation in a clinical study within the 30 days before study enrollment
- Any other condition which might increase the risk of study participation or compromise study results

In addition, subjects must report any medications, vitamins, or herbal preparations used during the study.

Procedures (See Table 1. Typical Schedule of Study Activities)

Study Enrollment

Prospective subjects will report to the laboratory and receive a complete explanation of study procedures. If they desire to participate and agree to the conditions of the study, subjects will sign a written, informed consent and provide a medical history. The back, between the belt line and shoulder blades, will be examined for uneven skin tones and blemishes using a Woods Lamp. The technician will complete the Subject History Form and will enroll qualified subjects.

Minimal Erythema Dose (MED)

Within one week after enrollment, each subject's MED will be determined using a calibrated 150-watt xenon arc solar simulator with a WG320 UVC-blocking filter and a UG-11 visible and infrared-blocking filter. A series of six timed doses of UVR (full spectrum UVA and UVB), increasing in 25% increments will be administered to the upper back as follows:

UVR doses to determine MED (seconds)					
1	2	3	4	5	6
6	8	10	13	16	20

Responses to these doses will be evaluated 16 to 24 hours after exposure. The lowest UVR dose which produces mild erythema with defined borders will be considered the MED.⁴ Typical MEDs for subjects with skin types I, II and III range from 8 to 16 seconds (10-20 effective mJ/cm²). Subjects whose MEDs are less than 6 seconds or who have no MED response at 20 seconds will not be included in the study.

Induction Phase

1. Within one week after MED determination, the test product will be applied. Duplicate applications of approximately 20 mg of the test product will be placed in Finn Chambers (0.5 cm²) and the chambers will be applied to the designated locations on the mid-back and secured with Scanpor® tape or equivalent.
2. After 24 ± 2 hours, the chambers will be removed. Test sites will be lightly wiped and examined. The investigator or a designee will evaluate and record any reactions using appropriate descriptive terms and the grading scale shown in Table 2. In addition, tape reactions or other adverse experiences will be recorded and appropriate treatment administered.
3. After examination of test sites, approximately 2 µl/cm² of the test product will be reapplied directly to the previously treated sites. Then the sites treated with the test product and an untreated control site will be irradiated with 10 Joules/cm² of UVA using a solar simulator with a Schott WG-345 filter in place, then with 0.5 MEDs of UVA/UVB radiation (WG-345 filter removed). Test sites

will again be examined and any immediate reaction will be evaluated and recorded.

4. After 24 ± 2 hours, the sites will be evaluated and graded according to the grading scale shown in Table 2.
5. After 24 ± 2 hours (a total of 48 ± 4 hours), the sites will be evaluated and graded according to the grading scale shown in Table 2. Then duplicate applications of approximately 20 mg of the test product will be placed in Finn Chambers and the chambers will be applied to the designated locations on the mid-back, as before.
6. After 24 ± 2 hours (a total of 72 ± 4 hours), the sites will be evaluated and approximately $2 \mu\text{l}/\text{cm}^2$ of the test product will be reapplied directly to both of its designated sites. Then one site for the test product will be irradiated with 3 MEDs. Test sites will be examined after irradiation and any reaction will be evaluated and recorded.
7. The process in 5. And 6. Above will be repeated for a total of 4 additional sets of applications with reapplication and UV doses of 3 MEDs 24 ± 2 hours later, within a period of 3 weeks.

Resting Phase

After the sixth application/UV exposure, a 9 to 14 day untreated, unexposed Resting Phase will follow.

Challenge Phase

1. Following the Rest Period, approximately 20 μl of the test product will be applied in Finn chambers to two untreated sites adjacent to the sites used in the Induction Phase. A third, empty Finn Chamber will be applied to an untreated site to serve as a control. Sites must be at least 2.5 cm apart.
2. After 24 ± 2 hours, the chambers will be removed. Test sites will be lightly wiped and examined. Any reactions will be evaluated and recorded using appropriate descriptive terms and the grading scale shown in Table 2., if applicable. In addition, tape reactions or any other adverse experience will be recorded and appropriate treatment will be administered.

3. After examination of test sites, approximately $2 \mu\text{l}/\text{cm}^2$ of the test product will be reapplied directly to one of the sites where test material was applied the day before. After a 15-minute drying period, the site treated with the test product and the untreated, control site will be irradiated with $10 \text{ joules}/\text{cm}^2$ of UVA using a solar simulator with a Schott WG-345 filter in place, then with 0.5 MEDs of UVA/UVB radiation (WG-345 filter removed). Test sites will again be examined and any immediate reaction will be evaluated and recorded.
4. Irradiated and unirradiated challenge sites will be evaluated 48 ± 2 hours later and again 72 ± 2 hours later, using the grading scale shown in Table 2.
5. At the conclusion of the Challenge Phase, subjects may be recalled for diagnostic follow-up testing as considered appropriate by the Investigator and Sponsor.

Table 1. Typical Schedule of Study Activities

WEEK 1	Monday	Written Consent, History, MED Determination	ENROLLMENT
	Tuesday		
	Wednesday		
	Thursday		
	Friday		
	Saturday		
	Sunday		
WEEK 2	Monday	Apply	INDUCTION PHASE
	Tuesday	Evaluate Irradiate	
	Wednesday	Evaluate	
	Thursday	Evaluate Apply	
	Friday	Evaluate Irradiate	
	Saturday	Rest	
	Sunday	Rest	
WEEK 3	Monday	Apply	
	Tuesday	Irradiate	
	Wednesday	Rest	
	Thursday	Apply	
	Friday	Irradiate	
	Saturday	Rest	
	Sunday	Rest	
WEEK 4	Monday	Apply	
	Tuesday	Irradiate	
	Wednesday	Rest	
	Thursday	Apply	
	Friday	Irradiate	
	Saturday	Rest	
	Sunday	Rest	
WEEK 5	Monday	Rest	RESTING PHASE
	Tuesday	Rest	
	Wednesday	Rest	
	Thursday	Rest	
	Friday	Rest	
	Saturday	Rest	
	Sunday	Rest	
WEEK 6	Monday	Apply	CHALLENGE PHASE
	Tuesday	Irradiate	
	Wednesday	Rest	
	Thursday	Evaluate	
	Friday	Evaluate	

The Investigator or a designee will have the option to discontinue a subject or a test product if reactions become too severe to continue or when two consecutive grade 2 or 3 reactions occur. Application of a discontinued test product may be resumed if the reaction subsides within 48 to 72 hours. If the reaction persists or worsens, the product may be discontinued, with appropriate evaluation and documentation.

Subjects who miss a scheduled application will be allowed to make up the application within 24 hours. Upon a third missed application or evaluation, the subject will be dropped from the study. Subjects who repeatedly present loose patches (3 or more) will also be dropped from the study.

Responses will be scored using the scale in Table 2.

Table 2. Grading Scale

- 0 = No reaction
- 1 = Mild Erythema
- 2 = Moderate Erythema
- 3 = Erythema with Edema
- 4 = Erythema with infiltration, raised, spreading beyond borders, with or without vesiculation.
- 5 = Large vesiculo-bullous reaction.

Interpretation of Results

The Investigator, in consultation with the Sponsor, will interpret results and diagnose reactions as primary irritant, contact allergy, phototoxicity, photoallergy or other, as appropriate. Additional investigations involving test product or test product ingredients may be conducted outside this protocol by agreement between the Sponsor and Investigator.

Study Report

Within 2 weeks of study completion, Suncare Research Laboratories, LLC, will provide a final study report, summarizing subject demographics, methods, materials, and results. This report will include tabulations and appropriate analyses of results.

Informed Consent

Subjects will provide written, witnessed informed consent in conformance with 21CFR part 50.

Adverse Experiences

Any adverse experience will be documented in the subject file using the Adverse Experience Form and immediate medical attention will be obtained as appropriate. Any serious adverse experience defined as life threatening or requiring emergency measures, whether related to product use or not, will be reported to the Sponsor within 24 hours.

Indemnification

The Sponsor agrees to indemnify, defend, and hold harmless Suncare Research Laboratories, LLC, from any demands, costs, or judgements arising out of or connected with the non-negligent use of the test material or performance of activities to be carried out pursuant to this Protocol. Suncare Research Laboratories, LLC shall notify the Sponsor within 10 working days after receipt of notice of injury, claim or lawsuit.

Quality Assurance

This study will be conducted under Good Clinical Practices Guidelines, applicable regulatory requirements, and in compliance with Suncare Research Laboratories, LLC Standard Operating Procedures. Suncare Research Laboratories, LLC Quality Assurance Personnel will audit this study. Data and the Final Report will be examined for completeness, accuracy, and proper documentation. The Sponsor may conduct an on-site inspection and audit at any time during the course of the study.

Record Retention

Record Retention: All study documents will be retained on file at Suncare Research Laboratories, LLC for a minimum of five (5) years, or as otherwise required by law.

Protocol Approval

For Sponsor

Date

For Suncare Research Laboratories, LLC

Date

References

1. Emmett EA. Toxic responses of the skin. In: Casarett and Doull's Toxicology. 4th Ed. New York, McMillan 1991.
2. Gould JW, Mercurio MG, Elmets CA. Cutaneous Photosensitivity Diseases Induced by Exogenous Agents. *Journal of the American Academy of Dermatology*, 1995; 33:551-573.
3. Murphy, GM. Investigation of photosensitive disorders. *Photodermatol Photoimmunol Photomed* 2004; 20 305-311.
4. U. S. Food and Drug Administration. Sunscreen Drug Products for Over-the-Counter Human Use; Final Monograph; 21CFR Parts 310, 352, 700, and 740. *Federal Register* 64 (98) May 21, 1999. 27666-27693.