CLINICAL TRIAL MANAGEMENT & TESTING ASSOCIATES, INC.

Title: Safety Evaluation Report - Human Repeat Insult Patch Test, Barrier Function Test and Scarification Test among Sensitive Skin Panelists (Reference Code: 2020/Clintest/R0012)

Coordinator: Skin Tree Center (002673980-A)

Sponsor: Craftiviti Sdn Bhd

Principal Investigator: Gertrude P. Chan, M.D., M.Sc

Date: 5 October 2020

1. Introduction

The standard protocol for the clinical assessment of irritation potential is the patch testing. Patch Testing is a well- recognized diagnostic tool for allergic and irritation potential to specific allergens and test products. It will allow us to assess the primary irritation potential of cosmetic-finished products and raw materials. The protocol is based on the standardized Routine Patch Testing provided by the International Contact Dermatitis Research Group (ICDRG).

The repeat insult patch testing is intended to measure the extent of the skin damage (irritation) resulting from repetitive exposure to the product. It can detect weak irritants which require multiple applications to cause skin irritation. These reactions are due to direct damage to the epidermal cells different from immunologic or allergic mechanism. This procedure may also detect so called "fatiguing substances" which are mild irritants that cause more strongly positive reaction with successive multiple skin exposure.

The damage is visually measured and subjectively quantified after each exposure. The skin readings are scored according to the scale in Appendix 1.

All the work in this clinical study will be conducted in accordance to Good Clinical Practice and in accordance with the guidelines by COLIPA (Walker A.P. et al: Test Guidelines for Assessment of Skin Compatibility of Cosmetic Finished Products in Man. Food and Chemical Toxicology 34 1996, 651-660). Because it is a study in humans, it will be carried out in accordance with the Declaration of Helsinki (2000) and subsequent revisions.

2. Testing Facility:

The clinical study was carried out at the Clinical Trial Management and Testing Associates facility at the 2301 Civic Place Bldg, Unit 1207, Civic Drive, Filinvest Corporate City, Alabang, Muntinlupa City. The study was conducted from 9 Sept to 2 October 2020.

The dermatologists in charge were Dr. Gertrude P. Chan, Primary Investigator and Dr. Heidi Chan, Assistant Investigator.

3. Test Products

Leave On:
A5 – Yein&Young ORGANIC LOTION
Positive Control: SS22 (0.5% SLS)
Negative Control: SS21 (Water)

All the investigative products were coded accordingly by the sponsor. The codes were unknown to the investigator /evaluator and to the participants before and during the actual testing.

4. OBJECTIVES:

4.1. To evaluate the capacity of cosmetic products to elicit adverse skin reaction under exaggerated consumer use condition.

4.2. To substantiate the manufacturer's claim, as nonirritant, and safe by extending the standard 48-hour Finn Chamber irritation assay to 21 days. This involves continuous occlusive exposure of the skin of the panelists for 21 days with replacement of the test materials every 48 hours. The

longer period is more discriminating because with mild irritants the inflammatory response may just be starting by 7 to 10 days. When the response to the product stay the same and does not intensify or even diminishes, the product can be considered safe and can be used by consumers with sensitive skin.

4.3. To determine the subclinical/early damage that may develop after the application of mild irritant test product compared to the positive control or reference control product on SLS compromised skin. (Barrier Function Test)

4.4. To determine the actual tissue toxicity of a test product as compared to a positive control or a reference control product, without the stratum corneum as a barrier, thereby increasing the sensitivity of the skin. (Scarification Test)

5. METHODOLOGY

5.1. Subject Selection

Thirty-five (35) volunteers, who belong to sensitive skin type, were recruited, for which they were clearly informed, verbally and in writing, regarding the nature of the study, the timetable, constraints and possible risk. They gave their written informed consent before participating in the study. All of the panellists passed the inclusion and exclusion criteria which are as follows:

Inclusion Criteria:

1. Individuals 18-55 years of age;
2. Individuals belonging to sensitive skin type category.
3. Test area should have a healthy skin;
4. Individuals free of any systemic or dermatological disorder which, in the opinion of the investigator would interfere with the study results or increase the risk of adverse reaction;
5. Individuals with uniform-colored skin on the infra scapular area of the back which would allow a discernable erythema;
6. Individuals who has completed a patch study Medical Screening form as well as Personal History form;
7. Individuals who have read, understood, signed and dated an inform consent agreement.

Exclusion Criteria

1. Individuals with any visible skin disease at the study site, which, in the opinion of the investigative; personnel, would interfere with the evaluation;
2. Individuals receiving systemic or topical drugs or medication which, in the opinion of the investigative personnel, would interfere with the study result;
3. Individuals currently under oral steroid treatment for asthma or other medical conditions;
4. Individuals with psoriasis and/or active atopic dermatitis/ eczema;
5. Individuals with a known sensitivity to cosmetics, skin care products, household products or topical products related to products being tested;
6. Females who are pregnant, planning pregnancy or nursing a child.

5.2 Study Material Application

The test materials were applied to the back of each panelists, with the positive and negative controls left for 48 hours. After 48 hours, reapplication of the test and control materials was done on the same sites, and left again for 48 hours. Before reapplication the test sites were evaluated for skin irritation based on the scoring scale (Annex 1). If an irritation score of "2" was observed at any time during this period, application of the sample to the affected test site was discontinued and a score of "2" was entered for all the subsequent scoring dates.

Treatment sites were assessed for the presence of irritation by a trained evaluator using a 4 point scoring scale at 48 hours (30 minutes after patch removal) and every other day for 3 consecutive weeks (9 applications).

5.3 Patch Definition

Occlusive: Finn Chambers or IQ Chambers or the equivalent Non-porous, plastic film adhesive bandage with a 2cm x 2cm Webril pad, affixed with Hypo allergenic tape (Micro pore) as needed.

5.4 Site Definition

Patches (Study materials and 0.5% SLS as positive control and Distilled water as negative control) were applied to the infra scapular area of the back.

Generally, 30-50 uL of each test material or a sufficient amount to fill up the Finn Chambers were used. Sodium lauryl sulfate 0.5% aqueous solution served as the positive control and water served as negative control. To occlude and affix the Finn Chambers on the skin, the hypoallergenic tape (Micro pore) was used. The chambers with the corresponding test materials and controls were applied on the infrascapular area of the back and the spatial order of the patches on the back noted or label accordingly. The chambers were separated from each other by 1-2 centimeters.

6. Results:

Refer to the attached overall clinical evaluation report.

6.1 Validation of the test

To validate the clinical testing, positive control, 0.5% SLS (**SS22**) and the negative control, water (**SS21**), were run at the same time with the test products.

The Primary Irritation Index after 21-day RIPT were as follows.

SS22 (+Ctrl) = 1.09
SS21 (-Ctrl) = 0.00

Statistical analysis utilizing the Wilcoxon Signed Ranks Test at 0.05 level of significance, was done. The results showed **SS22** was statistically more irritating than **SS21** with a p value of 0.000.

6.2 21-Day Cumulative Irritancy Assay

The Primary Irritation Index (PII) was derived using the responses observed by the trialist. It is a value depicting the average response of the panel as a whole. It is calculated by adding the final irritation scores and divided by the total number of test subjects. This value represents the cumulative exposures.

When the 35 panelists were exposed topically, daily for 21 consecutive days to the product, the Primary Irritation Index (PII) were as follows:

SS22 = 1.09
A5 = 0.39

Referring the PII values to Table 1 (Intact Skin only),

issued by the National Institute for Occupational Safety and Health Interpretation of Skin Ratings, states that under these conditions, the test product **A5** was considered to posses an acceptably low potential to produce cumulative irritation under anticipated use condition. Hence, the product is nonirritant: probably safe for intact human skin contact. Annex 2

6.2.2.Data analysis

To compare the irritation potential of the test product **A5** to the positive control **SS22**, Wilcoxon Signed-ranks Test was utilized and the results were as follows: (p value equal or less than 0.05 was considered significant).

Table 1: Data Analysis comparing the products with the positive control

Products versus SS22 (0.5% SLS)	21-day-RIPT	Barrier Function Test	Scarification Test
A5	SS22 significantly more irritating, p=0.000	SS22 significantly more irritating, p=0.000	SS22 significantly more irritating, p=0.000

Test product **A5** was significantly less irritating than the positive control after the 21-day Repeat Insult Patch Test. (Table 1)

6.3 Barrier Function Test for Subclinical Damage and Transepidermal Water Loss

When the same pre-selected panelists were exposed with 3% sodium lauryl sulfate pretreatment before the application of the products under occlusive dressing for 48 hours, the PII values 96 hours after the removal of the patches were as follows:

SS22 = 0.94
A5 = 0.29

Early damage of the skin by mild cosmetics is subclinical and may occur several days before it can be actually perceived by the eyes. A simple test to measure subclinical damage is by the use of an irritant substance such as sodium lauryl sulfate (SLS) which will result in weakening of the skin barrier and renders the skin more penetrable to chemicals, provoking an inflammatory response. With application of 3% SLS, the threshold of the skin to mild irritants is lowered to threefold or fourfold after 2-3 days of application.

The PII value of the test product after pretreatment with 3% sodium lauryl sulfate was compared with the positive

control **SS22** utilizing the Wilcoxon Matched-pairs Signed Rank test.

Test product **A5** was significantly less irritation than the positive control, **SS22** after pre-treatment with 3% sodium lauryl sulfate. (Table 1)

Referring the PII value to Table 1 (Mixed Reaction)suggest that test product **A5** under an exaggerated conditions, with 3% Sodium Lauryl Sulfate pretreatment, is safe for human skin; and may be safe for abraded skin contact when protection is maintained.

6.4 Scarification Test (abraded skin)

When the skin of the same pre-selected panelists were superficially scarified with a fine gauge 30 needle before the application of the test products under occlusive dressing for 48 hours, the PII values 96 hours after the removal of the patches were as follows:

SS22 = 0.83
A5 = 0.20

The rationale for the scarification test is to make sure the product reaches the dermis wherein lie the blood vessels that express the inflammatory response through leakage of fluids (edema/swelling) and cells (erythema).

To compare the irritation potential of test product **A5** with the positive control **SS22**, after superficial scarification with a fine gauge 30 needle Wilcoxon Matched-pairs Signed-ranks test was utilized and the results were as follows: (p value equal or less than 0.050 was considered significant).

Test product was significantly less irritating than the positive control **SS22** after superficial scarification with a fine gauge 30 needle. (Table 1)

Referring the PII value to Table 1 (Mixed Reaction)suggest that test product **A5** after the skin test site was scarified superficially with a disposable gauge 30 needle, is safe for human skin contact; and may be safe for abraded skin contact when protection is maintained.

To claim "mild and gentle" the product should pass the 21-day HRIPT, for intact skin, and the Barrier Function Test and scarification test for mixed reaction (intact and abraded skin). All the test product passed the 3 tests and can be considered "mild and gentle".

7. Conclusions:

21-Day Repeat Insult Patch Test

On the basis of the 21-Day Repeat Insult Patch Test, product **A5 – Yein&Young ORGANIC LOTION** compared to the positive control, **SS22** is considered to have low level of irritation potential, and safe for use by consumers with sensitive skin.

Barrier Function Test

On the basis of the Barrier Function Test conducted, product **A5** compared to the positive control, **SS22** is considered to have low level of irritation potential, and safe for use by consumers with sensitive skin.

Scarification Test

On the basis of the Scarification Test conducted, product **A5** compared to the positive control, **SS22** is considered to have low level of irritation potential, and safe for use by consumers with sensitive skin.

Product **A5** passed the 21-day Human Repeat Patch Test, Barrier Function Test and the Scarification Test and can claim "Dermatologist" Tested as safe, mild, and gentle to consumers with sensitive skin.

Submitted By:

Gertrude P. Chan, M.D.,M.Sc., FPDS
Principal Investigator.
5 Oct. 2020

OVERALL CLINICAL EVALUATION REPORT

5 Oct. 2020

Test Products coded as:

Leave On:
A5 – Yein&Young ORGANIC LOTION
Positive Control: SS22 (0.5% SLS)
Negative Control: SS21 (Water)

Test Procedures:

a) 21 - Day Repeat Insult Patch Test: (Induction Phase)

b) Barrier Function Test for Subclinical Damage with 3% Sodium Lauryl Sulfate
Pretreatment : Occlusive

c) Scarification Test (Abraded Skin)

d) Concentration: 100% concentration for the leave-on products
1 % concentration in aqueous solution for rinse-off products
1:1 dilution in water for powder products

e) Scores and Primary Irritation Index (PII):

a. 21-Day Repeat Insult Patch Test

TEST MATERIAL	#SUBJECT	IRRITANCY SCORE							
		0	+	1	1+	2	2+	3	PII
A5	35	13	19	2	0	1	0	0	0.39
SS22 (+ control)	35	0	10	17	0	8	0	0	1.09
SS21 (- control)	35	35	0	0	0	0	0	0	0.00

b. Barrier Function Test

TEST MATERIAL	#SUBJECT	IRRITANCY SCORE							
		0	+	1	1+	2	2+	3	PII
A5	35	17	16	2	0	0	0	0	0.29
SS22 (+ control)	35	0	13	16	3	3	0	0	0.94
SS21 (- control)	35	35	0	0	0	0	0	0	0.00

c. Scarification Test

TEST MATERIAL	#SUBJECT	IRRITANCY SCORE							
		0	+	1	1+	2	2+	3	PII
A5	35	22	12	1	0	0	0	0	0.20
SS22 (+ control)	35	0	14	19	2	0	0	0	0.83
SS21 (- control)	35	35	0	0	0	0	0	0	0.00