SAFETY INVESTIGATION

SINGLE-BLIND RANDOMIZED CONTROLLED CLINICAL STUDY OF SKIN IRRITATION AND SENSITIZATION POTENTIAL OF SKIN CLEANSING AND PROTECTION PRODUCT

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1. INTRODUCTION

The cosmetics industry has grown considerably in the last few years, as well as its interest in the development of safe and effective products. The establishment of the Code of Consumer Protection, the requirements of the Ministry of Health's National Sanitary Surveillance Agency (ANVISA), and the competition itself have led the industry to be more cautious with respect to the action and benefits of its products and to try to associate its representations with scientific works.

According to ANVISA's Cosmetic Product Safety Assessment Guide, a safety assessment must be conducted prior to the introduction of cosmetic products in the market.

The industry's awareness as well as consumer and regulatory agency requirements have led cosmetics manufacturers to adopt procedures that provide them with better knowledge of their products: they are conducting clinical tests on safety and effectiveness coordinated by doctors and experts before placing their products in the market. These procedures provide companies with more safety, credibility and consumer trust.

The cosmetics industry is increasingly concerned with avoiding adverse events among users of its products. Once a cosmetic product is freely available to the consumer, it must be safe under normal or reasonably predictable conditions of use. (According to ANVISA'S Cosmetic Product Safety Assessment Guide).

According to Good Clinical Practices, an Adverse Event is any untoward medical occurrence in a clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with the treatment (International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use – ICH).

Skin contact with products for topical application, such as cosmetics, may cause different types of reactions. Skin reactions caused by cosmetics include contact eczematous dermatitis, urticaria, acne and spots (SAMPAIO & RIVITTI, 2000). In general, contact dermatitis occurs as a result of two mechanisms: by primary irritation through the action of irritating substances, or by sensitization.

The irritating potential of a product depends on a series of variables: formula components, ingredient concentration, absorption, applied amount, skin condition, mode and frequency of application, and cumulative effect (DOOMSGOOSSENS, 1993).

Skin permeability varies according to the region of the body, with greatest absorption in the folds and on the face. When a product is applied on the skin, percutaneous absorption is greater or lesser depending on the product's concentration, type of vehicle used, surface skin area and skin contact time (ZATZ,1993). Thus, some regions of the body are more susceptible to irritation than others.

Investigations involving human beings are regulated by very rigid laws designed to protect and safeguard them. These laws vary from country to country. In Brazil, these investigations are allowed if they are guided by protocols approved by a Medical Ethics Committee and follow the precepts of the Helsinki Declaration and CNS Resolution No.

196/96 (NATIONAL HEALTH COUNCIL, 1996).

Investigations of Use are conducted with the finished product, prior to its introduction in the market. (BARAN & MAIBACH, 1994).

In addition to safety, these investigations can also assess a product's sensory characteristics by identifying additional complaints and comments regarding its performance.

Through clinical tests, the company becomes aware of any considerations and complaints that may emerge once its product is sold and may develop strategies in those respects, including specific training for the Client Services Department (SAC) prior to product launch (BARAN & MAIBACH, 1994).

2. OBJECTIVE

The objective of this study was to examine the SKIN CLEANSING AND PROTECTION product provided by the COMERCIAL NACIONAL DE PRODUTOS HOSPITALARES LTDA company by applying patch tests in order to assess its potential for primary skin irritation, cumulative skin irritation, and skin sensitization.

3. SUBJECTS AND METHODS

3.1. POPULATION AND SAMPLE / SELECTION OF VOLUNTEERS

Up to 70 (seventy) volunteers were selected in order to complete the investigation with at least 50 (fifty) volunteers who were approved based on the inclusion and exclusion criteria.

- **INCLUSION CRITERIA**

1. Age group: 18-70 years old;
2. Both genders;
3. Phototypes I, II, III and IV;
4. Skin integrity in the test region;
5. Signing of the Free and Informed Consent Form (FICF).

- **EXCLUSION CRITERIA**

1. Skin marks in the test area, which might have interfered in the assessment of possible skin reactions (pigmentation disturbances, vascular malformations, scars, increased pilosity, freckles and nevus in great quantity, sunburns);
2. Active dermatoses (local or disseminated) that might have interfered in study results;
3. Pregnancy or breastfeeding;
4. History of allergic reactions, irritation or intense discomfort caused by topical use products: cosmetics or medications;
5. Volunteers with a history of allergy to the material used in the study;
6. History of atopia;
7. History of pathologies that were worsened or triggered by ultraviolet radiation;
8. Carriers of immunodeficiency disorders;
9. Exposure to intense sunlight or tanning session up to 15 days prior to initial assessment;
10. Planned exposure to intense sunlight or tanning session during the period of the study;
11. Planned sea, pool, or bathtub bathing during the study;
12. Volunteers who practiced water sports;
13. Dermographism;
14. Use of the following topical or systemic medications: immunosuppressors, antihistamines, anti-inflammatory drugs, and corticoids up to 2 weeks prior to selection;
15. Treatment with Vitamin A acid and/or its derivatives administered orally or topically up to 1 month prior to the start of the study;

16. Aesthetic and/or dermatological treatment to the body up to 3 weeks prior to selection;

17. Planned vaccination during the study or up to 3 weeks prior to the study;

18. Participation in another clinical study then or less than 07 days before selection when the previous study was a study of use or 21 days when the previous study was to assess compatibility or investigate adverse reactions;

19. Any condition not mentioned above, which, in the investigator's opinion, might have compromised the study's assessment;

20. History of failure or unwillingness to adhere to the study's protocol;

21. Professionals directly involved in conducting this protocol and their family members.

- **PROHIBITIONS AND RESTRICTIONS**

1. No application of any other product on the test region (back);

2. No change in cosmetic habits, including hygiene;

3. No aesthetic or dermatological treatments to the body;

4. No change in dietary habits;

5. No change in hormone treatment;

6. No change in medicated contraception method;

7. No wetting patches: by taking pool or sea baths, sauna, or excessive sweating;

8. No removal of patches;

9. No use of tight clothing that might have removed the patch by friction or cause redness;

10. No exposure to prolonged and intense sunlight, and no artificial tanning;

11. No use of the following medications: corticoids, antihistamines, immunosuppressors, Vitamin A acid and derivatives, non-hormonal anti-inflammatory drugs for continuous use (sporadic use had to be assessed by the investigator for exclusion from the study);

12. Any and all aesthetic, medical cosmetic, and dermatological treatments were also prohibited during the study;

13. Whenever the therapeutic use of any medication listed above was required, the volunteer was excluded from the study.

Volunteers were submitted to an interview (Exhibit 1) and a dermatological examination (Exhibit 2).

Seventy-four (74) volunteers were selected, and one volunteer (139) was excluded from the investigation for presenting some of the exclusion criteria and/or failing to present some of the inclusion criteria – selection failure (FS).

The study sample (Exhibit 7) was composed of 73 volunteers, of which 65 were female and 08 were male, aged 19-70 years.

The table below shows the distribution of volunteers in the investigations:

INVESTIGATION	NUMBER OF VOLUNTEERS	GENDER		AGE	
		Female	Male	Minimum	Maximum
Primary Skin Irritation	73	65	08	19	70
Cumulative Skin Irritation/Sensitization (RIPT)	73	65	08	19	70

3.2. ETHICAL ASPECTS

The studies were conducted according to the principles of the Helsinki Declaration, applicable regulatory requests, including CNS Resolution No. 196/96, and in the spirit of Good Clinical Practices (Document of the Americas and ICH E6: Good Clinical Practice).

Prior to the start of the investigations, the subjects were informed of the study's objective, its methodology and duration, possible benefits, and attached restrictions. The volunteers signed a Free and Informed Consent Form prepared in accordance with the Helsinki Declaration, approved by the Committee on Ethics of Investigations of the Irmandade de Misericórdia de Campinas – Hospital Irmãos Penteado, and registered at the Commission on Ethics of Investigations (CONEP).

3.3. PRODUCT UNDER ASSESSMENT

PRODUCT	IDENTIFICATION	CODE
SKIN CLEANSER AND PROTECTION	Cleanser and Protection	11-27869-01

The product information, as provided by the sponsor, is described in Exhibit 5. A sample of the product was catalogued and is in our files, where it will be kept for a period of one year.

3.4. MATERIAL USED

The material used to conduct the tests was standard according to the POP 1.00 investigation protocol, consisting of:

- hypoallergenic patch;
- hypoallergenic semi-occlusive tape;
- sterile physiological solution used as control (NaCl 0,9);
- product samples (provided by the sponsor company).

3.5. STUDY PERIOD

- Primary Skin Irritation

The investigation started on May 9, 2011 and was completed on May 12, 2011.

- Cumulative Skin Irritation / Sensitization (RIPT)

The investigation started on May 9, 2011 and was completed on June 17, 2011.

3.6. APPLICATION TECHNIQUE

The product was tested as found. It was cut into squares approximately 2.0 x 2.0 cm, which were applied with hypoallergenic semi-occlusive tape.

The test product was identified by a letter corresponding to its position of application and an indication of the investigation.

A sterile physiological solution was used as control.

During the entire investigation, the test product and the control were always applied on the same location (the volunteer's right or left back).

3.7. ASSESSMENTS

3.7.1. Patch test

During the investigation, if an adverse reaction appeared, such as contact dermatitis, the volunteer was submitted to epicutaneous tests. The interpretation scale used was as recommended by the International Contact Dermatitis Research Group – ICDRG (FISHER, 1995).

The trained technician visually assessed the volunteer's back based on the table below. This result was recorded on internal record forms at the time of the assessment.

REACTION	RESULT
0 – absent	negative (-)
1 – slight erythema	doubtful (?)
2 – clear erythema	positive (+)
3 - erythema + edema + papules	positive (++)
4 - erythema + edema + papules + blisters	positive (+++)

3.8. CLINICAL INVESTIGATIONS

3.8.1. Investigation of Primary Skin Irritation - IP (KLIGMAN & WOODING, 1967)

The test method used was a patch test.

Test application locations were the volunteers' properly protected right or left back.

The test patch was applied and removed after 48 hours of contact with the skin.

Assessments (readings) were made approximately 30 minutes (reading 48h) and 24 hours (reading 72h) after the removal of the test patch.

3.8.2. Investigation of Cumulative Irritation and Sensitization – RIPT (KLIGMAN & WOODING, 1967; MARZULLI & MAIBACH, 1975)

Both test-product and control applications were made every day, and the first application remained in contact with the skin for 48 hours. On the weekend, the test patch remained applied for 72 hours.

Induction period: The induction period corresponds to fourteen consecutive test-product and control applications.

During this period, the test product and the control were always applied on the same properly protected region (the volunteers' right of left back).

The test patch was applied and removed after 24 hours of contact with the skin.

Readings were made approximately 30 minutes (reading 24h) after the removal of the test patch.

Rest period: A rest period of at least 10 days after the induction period followed, when no patch was applied.

Challenge phase: After the rest period, a patch with the test product and the control were applied on the volunteers' right of left back, in a virgin area, that is, where no patch was applied previously.

The patch was removed by the investigators after 48 hours of contact with the skin.

Assessments (readings) were made approximately 30 minutes (reading 48h) and 24 hours (reading 72h) after the removal of the test patch.

3.9. STUDY DESIGN

The study was a single-blind, randomized, and controlled clinical trial.

3.10. CRITERIA AND PROCEDURES FOR THE EXCLUSION OF SUBJECTS FROM THE INVESTIGATION

The investigator may have excluded volunteers from the investigation for the following reasons:

- Volunteers not included in the investigation: subjects who signed the FICF but did not meet the investigation's inclusion and exclusion criteria;
- Volunteers who presented changes that affected their eligibility between signing FICF and randomization;
- Volunteers who presented, in the opinion of the investigator, any problem that prevented the continuation of product applications in any period of the study;
- Withdrawal of an investigation subject's consent, regardless of reason;
- Investigation subject's failure to adhere to the study. A subject's failure to appear at the center for assessments was considered to be a failure to adhere;
- Serious Adverse Event;

- Concurrent illness or treatment: any pathological process or treatment that may have occurred during the course of the study and that might have interfered in the product under study, such as a medication interaction, or masked the results.

Subjects removed from the study by the investigator received follow-up care when they presented any event that was possibly related to the study, even after the removal. Subjects removed due to the occurrence of an adverse event received follow-up care until the condition was fully resolved.

4. RESULTS

4.1. ADHERENCE TO THE STUDY

For the investigation of Primary Skin Irritation – Sixty (60) volunteers completed the study. Thirteen volunteers (103, 112, 114, 129, 131, 133, 136, 143, 145, 152, 161, 165, and 169) removed themselves from the study for personal reasons unrelated to the product.

For the investigation of Cumulative Skin Irritation / Sensitization (RIPT) – Fifty-nine (59) volunteers completed the study. Twelve volunteers (103, 112, 114, 131, 133, 136, 143, 145, 152, 163, 165, and 169) removed themselves from the study for personal reasons unrelated to the product.

Volunteers 129 and 134 presented irritation after continuous skin exposure to the semi-occlusive tape (surgical tape), probably due to individual predisposition. For this reason, applications were interrupted and the data was not considered in the investigation.

4.2. PATCH TEST ASSESSMENTS

No adverse reactions (erythema, edema, papules, or blisters) were detected in the product and control application areas during the period of the study.

The results are described in Exhibit 6.

5. CONCLUSION

Based on the methodology used to assess the skin irritation and sensitization potential for the **SKIN CLEANSER AND PROTECTION** product provided by the **COMERCIAL NACIONAL DE PRODUTOS HOSPITALARES LTDA** company, we were able to conclude the following:

In accordance with the test conditions and results, we conclude that the product did not induce a process of skin irritation in the study group.

Based on the results, we also conclude that the product did not induce a process of skin sensitization.

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Analytical Laboratory Accredited by ANVISA

See scope on ANVISA's website:
http://www.anvisa.gov.br/reblas/bio/anali/index.htm

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