Milan, December 22nd 2014

# SKIN IRRITATION POTENTIAL EVALUATION through 48 HOUR OPEN PATCH TEST

METHOD: Ref. T20C

CUSTOMER: **IVAS INDUSTRIA VERNICI S.p.A.**
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PRODUCT: **VITA Therm**

**Ref. ISPE: 263/14/03 – 459/14**

STARTING DATE OF THE STUDY: 13/11/2014

COMPLETION DATE: 20/11/2014

## ETHICAL AND QUALITY CRITERIA

The current study was carried out in compliance with the quality assurance system requirements, according to the principles of good laboratory practice (GLP) and good clinical practice (GCP), as well as the principles established by the World Medical Association in the Declaration of Helsinki.

## REFERENCES

The data given in this report are exclusively related to the tested sample. This report can be only in full reproduced.

ISPE s.r.l.
Director of Laboratory
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## INDEX

## 1. SAMPLE DATA SHEET

SAMPLE REF.: **VITA Therm**

**Ref. ISPE: 263/14/03 – 459/14**

SAMPLE ARRIVAL DATE: 12/11/2014

PRODUCT:

- Physical Form: fluid
- Colour: white

QUALITATIVE FORMULA:

- Known: / yes /
- Other information: / /

OTHER INFORMATION RELATED TO THE PRODUCT SAFETY:

None.

FILE: 1 sample with the code number **Ref. ISPE: 263/14/03 – 459/14** and the study findings will be kept filed in our archives for one year and for ten years respectively. After these periods, the sample and the findings report will be discarded, unless otherwise required by the client.

## SKIN IRRITATION POTENTIAL EVALUATION through 48 HOUR OPEN PATCH TEST (Ref. T20C)

## 2. AIM OF THE STUDY

Used as an alternative to the occlusive-type test, the non-occlusive epicutaneous test is employed to test finished cosmetic products or new formulations containing substances with a high irritating potential, volatile substances and peculiar solvent mixes. This type of test allows to prevent either stronger irritating effects or apparently positive reactions due to the occlusion of the tested substance.

The method consists in a non-occlusive application of the product on the back and/or on the forearm of 20 selected subjects. The skin irritation potential of the product is visually evaluated at three control times:

- ✓ 30 minutes ($T_1$) after the product application complying with "open" mode,

*to evaluate the Immediate Skin Irritation Potential (**ISIP**);*

- ✓ 48 hours ($T_2$) after the product application complying with "open" mode and
- ✓ 24 hours after the removal of the product ($T_3$)

*to evaluate the Skin Irritation Potential (**SIP**).*

## 3. SELECTION OF THE VOLUNTEERS

### 3.a. Criteria for recruitment and admission

The test is carried out according to the Declaration of Helsinki, on 20 volunteers (17 females and 3 males), average age 46.3 years.

Subjects are informed of the nature, purpose and risk of the study and give their written consent before participating in the test.

The selection is carried out according to the following criteria:

### 3.b. Inclusion Criteria

- Race Caucasian.
- Female and male subjects, 18 - 70 years old, in general good health.
- Subjects able to follow all study directions and to commit to all follow-up visits for the duration of the study.
- Subjects who complete the informed consent process.
- Subjects who avoid the exposure to UV radiation and the use of tanning beds for the duration of the study.

### 3.c. Exclusion Criteria

- Pregnant or nursing females.
- Subjects with a history of unusual skin reactions to skin care toiletry products, cosmetics, or sensitivity to any of the test article components.
- Subjects who are taking topical or systemic drugs that could affect the results of the test (anti-inflammatory agents, corticosteroids, etc).
- Subjects showing systemic diseases or skin disorders (such as eczema, psoriasis, severe acne, etc.) that may affect the evaluation of the test articles or increase risk to the subject.
- Subjects who underwent epicutaneous tests within a period of 4 weeks prior to admission in this study.

### 3.d. Drop-out

The following reasons were considered sufficient cause for interrupting the subject's participation in the study:

- free choice of the subject;
- medical reasons not correlated with the treatment (ex. onset of disease, surgical operation);
- reasons correlated with the treatment.

Details of any cases of drop-out are anyway included.

### 3.e. Restrictions

The following restrictions are imposed on the subjects for the whole duration of the study:

- to use products or detergents on the patch test area;
- to wet the strips;
- to do sports;
- to expose to UVA and UVB rays.

## 4. METHOD

### 4.a. Accomplishment method

The test samples are placed on a special strip made up of filtering paper discs applied onto a stretch adhesive tape (Curatest®).

In case of hair-products to use after mixing with a development solution (for instance hair-dyes to mix with peculiar oxidants), the product and its oxidant are mixed immediately before application according to the ratio specified by the client.

The matrix only is applied in case of products diluted with water or dispersed with vaseline.

The tape application is performed within few minutes after filling, to avoid evaporation or drying of the product.

Two filtering paper discs for each product are used: one disc is applied on the forearm (to establish ISIP value), the other is applied on the back (to establish SIP value). The discs are applied on the skin previously cleaned from sebum.

### 4.b. Normalisations and controls

In case of products diluted with water or dispersed with vaseline a filtering paper disc soaked with the matrix is also applied in the same test conditions.

If there is a positive reaction in this area, the result of the subject is excluded from the test.

### 4.c. Recording the results

30 minutes after application, half of the adhesive tape is removed and the **Immediate Skin Irritation Potential** (**ISIP, $T_1$**) is evaluated.

The **Skin Irritation Potential** (**SIP**) is evaluated on the basis of the cutaneous reactions observed 48 hours after the application (**$T_2$**) and 24 hours after patch removal (**$T_3$**).

### 4.d. Data collection

The visual assessment is performed 30 minutes after the removal of the products, in order to allow possible skin redness, caused by the stretch adhesive tape, to disappear.
The onset of an evident redness strictly limited to the application site is considered as a sign of irritation caused by the product.

The observed reactions are evaluated on the basis of the following scale:

| | |
|---|---|
| *-* | ***no erythema*** |
| *+/-* | ***doubtful reaction*** |
| *+* | ***homogeneous pink-red erythema of minimum degree*** |
| *++* | ***sharp bright-red erythema, of moderate degree, clear limits, possible faint homogeneous oedema*** |
| *+++* | ***strong erythema and spread oedema; possible vesicles and/or follicular pustules*** |

Moreover possible ***allergic reactions*** are considered. They differ from irritative reactions in the following characteristics:

a) eczema-like oedema and vesiculation;
b) reaction is getting to overflow product application area.

An allergic reaction, that appears during the test, is considered as subjective allergic reaction to one or more of the components of the product.

## 5. CALCULATION of ISIP and SIP

The **Immediate Skin Irritation Potential** (**ISIP**) and the **Skin Irritation Potential** (**SIP**) are separately evaluated. The irritation potential of the product, expressed as percentage of irritation, is evaluated considering the amount and the intensity of the reactions occurred in the total number of the subjects.

The following reactions are considered in detail:

*a) positive reactions (from + to +++), observed at product removal ($T_2$);*
*b) positive reactions (from + to +++) that appears at $T_3$ (not detectable at product removal - $T_2$).*

The total irritation cases are calculated by summing the number of reactions observed for each type of reactions. Then the irritation percentage is obtained by means of the following mathematical calculation:

$$\textit{Irritation Percentage} = \frac{\textit{(total irritated cases number)}}{20} \times 100$$

Moreover possible allergic reactions, that differ from irritative reactions in the characteristics reported at paragraph 4.d, are reported in the table, although they are not considered in the calculation of irritative potential.

On the basis of the data reported in literature and of the obtained irritation percentage, the tested product can be considered:

| | |
|---|---|
| **0% ≤ 5%** | **NON IRRITANT** |
| **> 5% ≤ 10%** | **MINIMUM** |
| **> 10% ≤ 30%** | **MILD** |
| **> 30% ≤ 50%** | **MODERATE** |
| **> 50% ≤ 80%** | **STRONG** |
| **> 80%** | **MAXIMUM** |

## 6. RESULTS

PRODUCT: **VITA Therm**

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### IMMEDIATE SKIN IRRITATION POTENTIAL (ISIP)
### (30 minutes after product application, T1)

TOTAL NUMBER of SUBJECTS: **20**

| | IRRITATED SUBJECTS NUMBER |
|---|---|
| **cases +** *homogeneous pink-red erythema of minimum degree* | 0 |
| **cases ++** *sharp bright-red erythema, of moderate degree, clear limits, possible faint homogeneous oedema* | 0 |
| **cases +++** *strong erythema and spread oedema; possible vesicles and/or follicular pustules* | 0 |
| **Total irritation cases** | 0 |
| ***Irritation Percentage*** | **0%** |

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## SKIN IRRITATION POTENTIAL (SIP)

**[48 hours after product application ($T_2$) and 24 after product removal ($T_3$)]**

| TOTAL NUMBER of SUBJECTS: **20** | |
|---|---|
| | IRRITATED SUBJECTS NUMBER |
| **cases +** *homogeneous pink-red erythema of minimum degree* | 0 |
| **cases ++** *sharp bright-red erythema, of moderate degree, clear limits, possible faint homogeneous oedema* | 0 |
| **cases +++** *strong erythema and spread oedema; possible vesicles and/or follicular pustules* | 0 |
| Total irritation cases **appeared at T3** | 0 |
| **Total irritation cases** | 0 |
| **Irritation Percentage** | **0%** |

| | |
|---|---|
| Allergic reactions | 0 |

## 7. CONCLUSIONS

All the selected subjects completed the test.

The application of the tested product complying with "open" mode for 30 minutes ($T_1$) on 20 healthy subjects, did not induce any reaction related to an Immediate Skin Irritation Potential.

The application of the tested product complying with "open" mode for 48 hours, on 20 healthy subjects, did not induce any reaction related to a Skin Irritation Potential, as assessed at the product removal ($T_2$) and 24 hours after the product removal ($T_3$).

No allergic reaction was observed.

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| | % of IRRITATION |
|---|---|
| ISIP | 0% |
| SIP | 0% |

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Dr. Fernanda Distante

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Test guidelines for the assessment of human skin tolerance of potentially irritant cosmetic ingredients - COLIPA guidelines. August 1997.

Cosmetic product test guidelines for the assessment of human skin compatibility - COLIPA Guidelines. August 1997.

## 9. REACTIONS TABLE

VOLUNTEERS (IN ORDER OF SELECTION):

| | CUTANEOUS REACTIONS | | |
|---|---|---|---|
| **SUBJECT** | **$T_1$** | **$T_2$** | **$T_3$** |
| **1** | --- | --- | --- |
| **2** | --- | --- | --- |
| **3** | --- | --- | --- |
| **4** | --- | --- | --- |
| **5** | --- | --- | --- |
| **6** | --- | --- | --- |
| **7** | --- | --- | --- |
| **8** | --- | --- | --- |
| **9** | --- | --- | --- |
| **10** | --- | --- | --- |
| **11** | --- | --- | --- |
| **12** | --- | --- | --- |
| **13** | --- | --- | --- |
| **14** | --- | --- | --- |
| **15** | --- | --- | --- |
| **16** | --- | --- | --- |
| **17** | --- | --- | --- |
| **18** | --- | --- | --- |
| **19** | --- | --- | --- |
| **20** | --- | --- | --- |

***EXPLANATION*** *(type of reactions):*

**$T_1$** = *30 minutes after product application complying with "open" mode*

**$T_2$** = *48 hours after product application complying with "open" mode (at the product removal)*

**$T_3$** = *24 hours after the product removal*