AFTER-SUN CLAIMS SUBSTANTIATION: EXPERIMENTAL CRITERIA TO ASSESS THE IN VIVO EFFECTS OF SUN CARE PRODUCTS UNDER CONTROLLED-USING CONDITIONS

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Summary

Cosmetic claim substantiation is a new exciting reality although some complexities involving methodological guidance and technical sophistication justifies some reserve from the industry. Nevertheles, this can also be a beneficial opportunity for all the agents involved, as long as science-based demonstration of the above mentioned claims is achieved. The present study is a practical demonstration of this possibility regarding the claim substantiation of “after-sun” products. Ten healthy women 35 to 65 (44.5 ± 8.7) years old, following informed written consent, were selected after specific inclusion criteria. The procedure involved irradiation of both legs (antero-lateral) in laboratory for 6 sequential days using an indoor solarium-type as the UV source. Efficacy assessment endpoints were defined from the product’s typical claims. Thus, methodological strategies included (a) sensorial (clinical) evaluation, by a trained observer, (b) biometrical measurements involving the assessment of Hydration (epidermal “capacitance”) biomechanics (Ua and Uf descriptors obtained from the Cutometer suction method) and Erythema and Melanin indexes (Mexameter MX16) and, (c) a user’s preference questionnaire. Results have shown that, regarding the clinical evaluation, discrete but, nevertheless positive appreciation was detected on the scored parameters. This was further confirmed by the biometrical assessment showing that both evaluation zones were significantly different, by the end of the study, in terms of hydration and biomechanical descriptors. The biometrical assessment of erythema and melanin served only to confirm the procedure’s safety and efficiency regarding the irradiation phase. Finally, the “consumer’s preference” questionnaire concluded on an absolute favourable opinion on the product’s characterisation regarding the previewed claims. Thus, this study design and protocol illustrates how the predefined endpoints can meet claims under a scientifically based perspective, contributing to the definition of experimental criteria for “cosmetic efficacy” substantiation.
After-sun claims substantiation: experimental criteria to assess the in vivo effect of sun care products

Riassunto

La pubblicizzazione dell’efficacia documentata di un determinato prodotto cosmetico rappresenta una stimolante novità anche se la difficoltà delle metodologie utilizzate, spesso molto sofisticate, giustifica alcune riserve da parte dell’industria. Questi studi documentati, rappresentano comunque una opportunità per tutti gli studiosi in grado di dimostrare tale efficacia con ricerche scientificamente ineccepibili.

Questo studio rappresenta una dimostrazione: il controllo che può essere effettuato su di un prodotto cosmetico “dopo sole”.

Sono state sottoposte a sperimentazione controllata 10 donne volontarie di età variabile tra i 35 ed i 65 anni (44,5 ± 8,7), irradiando in laboratorio entrambe le loro gambe antero-lateralmente, e per 6 giorni consecutivi mediante l’uso di un solarium.

La metodologia di studio si è basata su (a) osservazioni chimiche soggettive, (b) misurazioni biometriche e biomeccaniche che hanno rilevato l’idratazione cutanea, l’elasticità (cuteometer), l’indice di eritema e di melanizzazione (Mexameter MX16) e (c) la stesura di un questionario di preferenze. Sia le rilevazioni cliniche/soggettive che i controlli biomeccanici oggettivi, hanno rivelato una differenza significativa nei confronti dei controlli ed una sicurezza di impiego di tali metodologie. Infine, il parere dei consumatori si è rivelato positivo nei confronti dei messaggi pubblicitari utilizzati per il prodotto.

Il protocollo sperimentale utilizzato dimostra come sia possibile coniugare attività ed efficacia dei prodotti cosmetici definendola con criteri sperimentali corretti.
INTRODUCTION

The proof of the effect claimed for the cosmetic product is a known reality born from the progressive adoption of the Council Directive 93/35/CEE of June 1993within the European Union.

From a practical point of view, although determined “by the nature of the effect or product”, the manufacturer (or his representative) is now strongly encouraged to avoid unnecessary claim disputes adopting a proactive posture and focusing the claims as a function of what can be defined as a sufficient proof of efficacy from experienced professionals.

Despite the obvious advantages implied, for the industry and regulatory authorities as well as for the consumer’s information on the basis of which cosmetic expectation and satisfaction are built, several difficulties are apparent. Primarily because it is necessary to support any experimental demonstration on the basis of what may be called a “good scientific practice” and, simultaneously, to demonstrate the relevance of the claim substantiation procedure facing the product itself. Detailed information and guidance on methodologies and other technical aspects to meet those purposes has not been produced by the European Union nor by the national authorities. The European scientific authority (SCCNFP, Scientific Committee for Cosmetic Products and Non-Food Products) published some notes regarding skin compatibility and is expected to produce complementary opinion on the efficacy issue. Meanwhile, the Danish authority produced some notes on the subject and COLIPA (the European Cosmetics Toiletry and Perfumery Association) formed a special task-force to face the efficacy-testing problem, publishing from 1997 a few guidelines and some general information for the cosmetic’s efficacy evaluation. Finally, emphasis is due to the work produced by the EEMCO (the European Group for the Efficacy Measurement of Cosmetics and Other Topical Products), an independent European expert group formed to endorse many of the relevant issues implied. Several reviews on technical assessment of efficacy-interesting variables have been published which should be looked upon as being technical advisory keynotes meant to offer scientifically based assistance to meet the requirements of the Council Directive.

From a practical standing, several papers, especially addressing methodological aspects of the analysis, have been produced definitely contributing to build up a scientific domain where the claim substantiation is the central motivation. Basically principles that are generally accepted include (a) clear and objectively defined test hypothesis, (b) adaptation of the level of experimental sophistication to the final endpoint in the trial, which in turn should be supported by (c) an adequate study design and protocol. These are crucial aspects which should be permanently considered prior to any study and regarded as good scientific procedure.

In order to contribute to this new (conceptual) reality, the present protocol was designed to specifically address “after-sun” formulation claims, from the cosmetological properties referred on the product labelling and packaging analysis. This provides a good example to test the main principles which should be attended regarding study design of cosmetic claims substantiation, since obvious variability factors are present. Moreover it is clear that search for after-sun products is a fast growing market, mostly resulting from a poorly established sun-protection awareness. Official data is missing but practice reveals that exaggerated sun exposure is taking place probably as a consequence of over-expectation / misinformation regarding the use of sun-protection products.

Claims were assessed from the biological impact of the product in the normal human skin
and taking into account the user’s appreciation (consumer’s preference) regarding the product. This way, the authors aims to contribute to an objective definition of experimental criteria involving the efficacy assessment of this class of products, thus contributing to a better management of solar skin care.

**MATERIAL AND METHODS**

**Panel**

Experimental data was obtained from healthy women selected after informed written consent, according with previously defined inclusion criteria. Volunteers (n=10) were aged from 35 to 65 years (44.5 ± 8.7), phototype II to III (Fitzpatrick classification) referring regular or occasional use of “after-sun” products, clearly expressing their availability to be exposed to the experimental procedure involving irradiation at laboratory for 6 sequential days.

Specific non-inclusion criteria included:
- cutaneous marks at the evaluation area which may influence or interfere with the effects appreciation (pigmentation disturbances, scars or scarring elements, hirsutism, ephelides and/or naevi high density distribution);
- allergy or special reactivity to body care products, including solar cosmetics;
- cutaneous sensitivity to sun exposure, including solar urticaria and idiopathic photodermatitis,
- treatment by any photosensitising medication within 1 month before the study
- treatment by acid Vitamin A or its derivatives within 3 months before the study;
- treatment by UVA or UVB within 1 month before the study

**Irradiation Challenge**

All experimental procedures were taken at laboratory, involving the permanent control of room temperature and humidity, in accordance with the usual recommendations regarding the application of this type of technologies5-7,21-28. Prior to all measurements volunteers were left in the room for at least 20 minutes in order to allow skin’s adaptation to room’s temperature (21 ± 1°C) and humidity (40-60%) conditions.

The irradiation challenge was initiated with a reduced dose of radiation (Table I) in order to avoid any late form of erythema development. Following sessions took place 72H later, and proceeded for 5 sequential days (Table II).

In order to ensure full protection of the volunteers, the irradiation challenge was performed under permanent control involving the following measures:

(a) Irradiation was achieved through the system Sunny HB-406 Solarium, from Phillips; this system includes a 50Hz Carrying grip Compact UV lamp (type: Cleo HPA 400), corresponding to safety standard referred by the European CENELEC requirements (Insulation class II, UV-type III), and uses a specially shaped reflector of high-grade anodised aluminium and a UV-A filter glass

(b) The use of the Sunny system allowed to select the irradiation areas; in the present case only the evaluation and control anatomical areas (antero-lateral faces of both legs) were irradiated, always at a fixed distance (50cm).

(c) the irradiance was controlled through a radiation meter (Waldmann UV meter, France) which allows a detailed detection of UV-A and UV-B (long and short range) radiation

(d) all volunteers wore protective eye goggles (type: HB 072) while irradiated

(e) The mean UV radiation exposure dose or fluence (in the respective SI units – J/cm²) achieved for the present study is shown in Table I
Table I

Mean UV radiation exposure dose or fluence (J/cm²) achieved during the irradiation procedure. The second session (Day 4) took place 72H after Day 1 session in order to detect any late form of cutaneous reactivity to UV radiation (see text).

<table>
<thead>
<tr>
<th></th>
<th>Day 1 session</th>
<th>Day 4 session</th>
<th>Day 5 session</th>
<th>Day 6 session</th>
<th>Day 7 session</th>
<th>Day 8 session</th>
<th>Day 11 (no session)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV A</td>
<td>7.8</td>
<td>11.7</td>
<td>15.6</td>
<td>15.6</td>
<td>15.6</td>
<td>15.6</td>
<td>0</td>
</tr>
<tr>
<td>UV B</td>
<td>0.024</td>
<td>0.036</td>
<td>0.048</td>
<td>0.048</td>
<td>0.048</td>
<td>0.048</td>
<td>0</td>
</tr>
</tbody>
</table>

Table II

Schematic presentation of the sequential experimental calendar adopted according with the proposed study design. Clinical and biometrical evaluations preceded the irradiation sessions where indicated. Assessments at Day II were taken to evaluate the effect’s persistence 72H after the last irradiation session (see text).

<table>
<thead>
<tr>
<th></th>
<th>Day 1 session</th>
<th>D 4 (OS-72H) session</th>
<th>D 5 session</th>
<th>D 6 session</th>
<th>D 7 session</th>
<th>Day 8 session</th>
<th>D 11 (OS-72H) session</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical evaluation:</td>
<td>X</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Skin Biometrics:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mexametry</td>
<td>X</td>
<td>-</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Capacitance</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cutometry</td>
<td>X</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Self-perceptive evaluation (Questionnaire):</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Evaluation Methodology

The evaluation methodology was defined to support the biological efficacy eventually claimed by the manufacturer. Under this perspective, the global evaluation of the product’s properties and the respective in vivo efficacy were investigated through three complementary approaches as follows (Table II illustrates the sequential calendar of the experimental methodology):

The “Clinical” Assessment

This evaluation was performed by a trained evaluator and involved the sensorial (visual and tactile) investigation of each volunteer’s skin characteristics at the application and control zones. Considering the reference literature on the subject, sensorial parameters considered relevant for the study purposes were

- Desquamation (scaling)
- Roughness
- Cracks (fissures)

All these parameters were scored by the beginning and end of the study, and results expressed through semi-quantitative analogue scales (see ahead);

The Biometrical Evaluation

Skin properties were assessed by several variables obtained through non-invasive technologies considered specially relevant for the present study purposes. These were:

- Epidermal hydration obtained by the Corneometer CM825™ system (Courage±Khazaka GmbH, Koln, Germany); this system is actually the most frequently and referenced technological tool used to assess skin surface hydration 14-16 expressed in Arbitrary Units (AU’s);
- Skin Biomechanics assessed through "cutometry" a well known method using the Cutometer SEM474™ system (Courage±Khazaka GmbH, Koln, Germany); this method allows to define several biomechanical descriptors from the skin deformation obtained after applying a negative (suction) pressure on skins surface through an appropriate probe. In the present case an 8mm aperture diameter probe was used in all measurements in order to ensure adequate signal resolution at the anatomical site chosen. Relevant descriptors for the study purposes were:

- \( \text{Uf} \) - Total extensibility of the skin (expressed in mm), and
- \( \text{Ua} \) - Total deformation recovery at the end of the stress-off period (expressed in mm);

The Melanin and the Erythema indexes obtained with the Mexameter MX16™ (Courage±Khazaka GmbH, Koln, Germany), used as a complementary assessment tool to follow up two biological expressions resulting from the experiment: the erythema and the melanogenic response following irradiation. These systems are based on the diffuse remittance spectrometry principle; the absorbance of a volume of tissue at specific wavelengths is measured and the concentration of the absorbing pigment is estimated and given as a relative index (in Arbitrary Units, AU’s);

**The Self - Perceptive Cosmetic Evaluation Assessment (the "in use" Consumer’s Preference Questionnaire)**

The self-perceptive Cosmetic Evaluation Assessment corresponding to the volunteer’s "in use" preference questionnaire, included the evaluation of the most important product - related aspects emerging from its regular use. These aspects were defined according to the product’s "vocation", the manufacturer’s claims (see ahead), and the foreseen consumer’s expectation regarding the product’s utilisation.

Tested aspects and respective options were:

- (a) "Cutaneous comfort after application" - (A: Agreeable; W: Without any changes; D: Disagreeable)
- (b) "The Calming Effect" - (V: Very satisfactory; S: Satisfactory; I: Insufficient; M: Very Insufficient)
- (c) "The Refreshing Effect" - (V: Very satisfactory; S: Satisfactory; I: Insufficient; M: Very Insufficient)
- (d) "The Moisturising (hydrating) Effect" - (V: Very satisfactory; S: Satisfactory; I: Insufficient; M: Very Insufficient)

Results were quantified and expressed in percentage referred to each qualitative scale adopted.

**Methodological remarks**

Other relevant aspects to consider were:

- the quali-quantitative composition of the after-sun formulation was irrelevant for the study purpose, chosen for being referred to present moisturising properties with soothing and revitalising benefits, helping to replace lost moisture and to feel cool.
- biometrical evaluation took place every day from the beginning of the study to the last day of essay (D11) 72H after the irradiation sessions (Table II); measurements were taken after the volunteer’s adaptation to room condition, before irradiation
- application took place in one hemipart of the body, the contralateral hemipart serving as negative (intra-individual) control; performed by the volunteer, in both legs, after each session, there was no quantity restriction regarding daily application; additionally, each volunteer was allowed to take the formulation with you and to apply it, until a maximum of 3 applications/day
- each volunteer was also encouraged:

(a) not to apply similar products ("après soleil") in the tested zones;
(b) to strictly respect the application conditions specified by the protocol
(c) to maintain the regular hygiene and skin care (including make-up) habits

Statistics

Paired t student test or the non parametric Wilcoxon paired-test and Mann-Whitney (for independent variables comparison) were chosen according with a normal or asymmetric data distribution. Data normality was tested, for both reference and application zones, through the Kolmogorov-Smirnov Test and through the distribution analysis (Normal Q-Q Plot and Detrended Q-Q Plot tests), and variance analysis performed through the Kruskall-Wallis test. A 95% confidence level was adopted (p < 0.05).

RESULTS AND DISCUSSION

The experimental criteria adopted intended to reduce the many different variability factors which are known to determine this type of studies, being recognised as the main source for experimental bias and inconclusive data. Therefore, these criteria involved the previous identification of experimental variability sources related with the evaluation methodology chosen. Following the panel profile definition, assumed to represent the consumer’s reference profile, it was crucial to define the “stimulus nature”. In the present case this corresponded to the radiation source exposure in order to mimetise with adequate reproducibility, the “using” conditions justifying the use of this class of products. The variability introduced by the natural exposure to the sun (in the beach or at the sea-side) added to the risk involved in a whole-body solarium (tann-centres) were sufficient arguments to exclude them as alternatives. The UV stimulators used for solar testing and the phototherapy systems used for UVA/UVB therapy could also be regarded as alternatives but it is necessary to attend to the implied specificities and cost. Thus, the in-door solarium alternative assured a suitable radiant light source providing:
1. control of the irradiation process, crucial for the study appreciation and validity, assuring that all individuals received the same UV radiation doses during the experiments;
2. selective irradiation of the chosen part of the body, contributing to measurement standardisation
3. full control of the experimental methodology including each individual safety conditions
4. low cost

Considering the study global objectives, evaluation analysis involved complementary approaches, meant to provide the maximum quantitative information about the product’s biological impact on in vivo healthy human skin. These were defined having in mind several aspects regarding the most frequently referred claims found in this class of products31-34 including:
- Hydration (replacement / reinforcement)
- Skin nutrition
- Calming effect
- Refreshing effect
- Fast “vapouring” effect
- Pleasant feeling (after application on the skin) / lubricating /
- Easy to handle and to apply
- Non - “gras” / non “tacky”
These determined the nature of the analysis involving the three complementary approaches as follows:

Sensorial (clinical) Assessment

Sensorial evaluation of both areas allowed to confirm that the same skin condition was present in both legs. Scaling (small scales only, surface lightly dull in colour) was present in 6 / 10 volunteers at the beginning of the study. By the end of the study, scaling was absent in 8 / 10 volunteers on application area while at the reference area this condition worsened in 4 volunteers.
Roughness, detected by tactile evaluation was also present, slightly irregular and scratchy on tangential tactile evaluation, in 4 / 10 volunteers at the beginning of the study. By the end, this condition still persisted in 3 volunteers, in the application area. In the control area, this condition worsened in 5 / 10 volunteers.

Single and superficial cracks were also presented in the skin evaluation area in 2 / 10 volunteers, disappearing in one of them only in the application area, by the end of the study. Skin redness and pigmentation although not related with the product’s efficacy, were also evaluated to follow the adequacy of the methodological procedure. At the beginning of the study, redness was detected in one volunteer who presented small areas of minimal / diffuse faint redness. The end of the study revealed no change on this parameter, thus not related with the product. Regarding the pigmented reaction no changes were detected whatsoever.

Results obtained from the present approach stresses the relative usefulness of the sensorial evaluation even when normal (non-pathological) skin is involved. Taken as indicators of the product’s impact on normal in vivo skin only slight but, nevertheless, clear changes can be observed between the first and the last day of the study. Although reflecting a positive evolution in all the hydration – related variables, there’s no dramatic evidence of significant differences between these two evaluation moments. Some significant differences, regarding skin dryness could still be found from the independent variables analysis between reference and application zones (p< 0.05). However, considering that the scores itself, being non-continuous variables, may evoke major distribution changes around the mean, these results should be carefully interpreted and complemented with data from biometrical measurements.

**Biometrical Assessments**

Erythema and melanin indexes were determined by the need to ensure that the experimental procedure was adequately developed, meaning to avoid any acute response to the UV exposure and, at the same time, to confirm that the pigmented reaction was effectively occurring. Therefore, these results are rather meant to confirm the efficacy of the experimental procedure than to support any other claim related to the product’s properties.

As suggested in Figure 1 significant differences
UA's in D0 and 519.4 ± 6.78 UA's in D11 for the control zone and 486.7 ± 3.08 UA's in D0 and 516.5 ± 4.89 UA's in D11 for the application zone. These results suggested the efficacy of the pigmentary reaction induced through the chosen experimental procedure.

Regarding the skin’s surface hydration, statistically significant differences were immediately found from D4 on, following the application procedure (Table III). These seems to result directly from the product’s hydration capacity since a clear improvement of the epidermal “capacitance” is apparent in the application area, while in the control zone, no differences are detectable. Also noteworthy is that these differences are progressively marked form Day 6 on (Table IV). This is further confirmed by the variance analysis (Kruskall-Wallis test) which shows significant differences for D7 (p< 0.05), D8 (p< 0.05) and D11 (p< 0.005) as a consequence of the product’s impact on the biological elements of human skin. Data from reference zone shows a distinct evolution including progressive dryness in the last days of the study. Regarding the biomechanical assessment, a special careful analysis is recommended having in mind the many complexities involved in this present approach, specially for claim substantiation purposes. For the chosen method, data results from analysis of the curves obtained by applying a negative perpendicular pressure to the skin’s surface (creep). Deformation of the analogue signals is an obvious factor to attend when choosing the appropriate parameters. Additionally, a clear-cut relationship between those variables provided by the system and the respective biophysical equivalents, is absent. Therefore, considering the various biomechanical descriptors available, those whose interpretation depend less from the curve deformation, should be chosen. This is the case for descriptors Uf corresponding to the total extensibility of the

### Table III

<table>
<thead>
<tr>
<th>Application zone</th>
<th>Basal</th>
<th>D4</th>
<th>D5</th>
<th>D6</th>
<th>D7</th>
<th>D8</th>
<th>D11</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean</strong></td>
<td>44,4</td>
<td>49,7</td>
<td>49,6</td>
<td>52,2</td>
<td>54,6</td>
<td>53,8</td>
<td>54,7</td>
</tr>
<tr>
<td><strong>St. deviation</strong></td>
<td>8,81</td>
<td>7,56</td>
<td>7,09</td>
<td>8,35</td>
<td>8,71</td>
<td>7,05</td>
<td>7,07</td>
</tr>
<tr>
<td><strong>p</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control zone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>44,0</td>
<td>44,2</td>
<td>44,7</td>
<td>44,9</td>
<td>45,7</td>
<td>42,8</td>
<td>43,5</td>
</tr>
<tr>
<td><strong>St. deviation</strong></td>
<td>8,63</td>
<td>6,76</td>
<td>7,07</td>
<td>6,98</td>
<td>7,57</td>
<td>6,14</td>
<td>5,17</td>
</tr>
<tr>
<td><strong>NS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NS non significant differences found, * p < 0.05; ** p < 0.005
skin, and \( U_a \) corresponding to the total deformation recovery at the end of the stress-off period.

Regarding the total extensibility of the skin descriptor (\( U_f \)), as shown in Table V, very significant differences are detected at the end of the essay (D8 and D11) in the application zone as opposed from the control zone. Failing to demonstrate significant differences between control and application zones (Man-Whitney test, Table VI) may result from some data dispersion around the mean. This may be justified by an eventual low sensitivity of the system to detect small changes (around the mean), evoking enough dispersion to transform the mean in a non-significant “\( p \)” for the present confidence interval. In any case, the border-line “\( p \)” value obtained should be noted (Table VI).

### Table IV

Man-Whitney tests for independent data regarding the hydration changes obtained in the different phases of the experimental protocol in both experimental areas. Results clearly detect progressively marked differences between both zones, which can be attributed to the after-sun formulation impact on skin’s surface.

<table>
<thead>
<tr>
<th>Application v. Control zone</th>
<th>Basal</th>
<th>D4</th>
<th>D5</th>
<th>D6</th>
<th>D7</th>
<th>D8</th>
<th>D11</th>
</tr>
</thead>
<tbody>
<tr>
<td>( p )</td>
<td>0.970</td>
<td>0.069</td>
<td>0.130</td>
<td>0.064</td>
<td>0.045</td>
<td>0.004</td>
<td>0.003</td>
</tr>
<tr>
<td>Non-significant differences found</td>
<td>* ( p &lt; 0.05 ) ; ** ( p &lt; 0.005 )</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Biomechanical related improvement is further confirmed by the \( U_a \) descriptor, which shows again, at the application zone, very significant differences in D8 and D11; while, for the reference zone no differences are detected (Table V and Table VI).

### Table V

Skin biomechanical changes, expressed in terms of the biomechanical descriptors \( U_f \) and \( U_a \) (mm) obtained in the beginning and in the end of the experimental phases (D8) in both experimental areas. Assessments at Day 11 were taken to evaluate the effect’s persistence 72H after the last irradiation session (see text). Results from comparative statistics (Wilcoxon) are also shown, illustrating a clear significant difference at the application site when compared with basal (see text).

<table>
<thead>
<tr>
<th>( U_f )</th>
<th>Application</th>
<th>Basal</th>
<th>D8</th>
<th>D11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>1.22</td>
<td>1.42</td>
<td>1.42</td>
<td></td>
</tr>
<tr>
<td>St. Deviation</td>
<td>0.23 **</td>
<td>0.31 **</td>
<td>0.30</td>
<td></td>
</tr>
<tr>
<td>( p )</td>
<td>** **</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>( U_a )</th>
<th>Application</th>
<th>Basal</th>
<th>D8</th>
<th>D11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>1.00</td>
<td>1.14</td>
<td>1.14</td>
<td></td>
</tr>
<tr>
<td>St. Deviation</td>
<td>0.23 **</td>
<td>0.27 **</td>
<td>0.27</td>
<td></td>
</tr>
<tr>
<td>( p )</td>
<td>** **</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>
Table VI

Analysis of independent data (Man-Whitney) performed for the chosen biomechanical descriptors in both experimental areas. A positive effect over the skin biomechanics is only shown from the Ua variable. The border line "p" detected for the Uf variable may indicate about some dispersion of results (see text).

<table>
<thead>
<tr>
<th>Uf Application v. Control zone</th>
<th>Basal</th>
<th>D8</th>
<th>D11</th>
</tr>
</thead>
<tbody>
<tr>
<td>p</td>
<td>0.940</td>
<td>0.064</td>
<td>0.059</td>
</tr>
<tr>
<td>NS</td>
<td></td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ua Application v. Control zone</th>
<th>Basal</th>
<th>D8</th>
<th>D11</th>
</tr>
</thead>
<tbody>
<tr>
<td>p</td>
<td>0.112</td>
<td>0.008</td>
<td>0.013</td>
</tr>
<tr>
<td>NS</td>
<td></td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

No significance differences between basal values and values obtained by the end of the essay could be detected at the reference (control, non-treated) zone. So, the present assessment suggests that, under the present experimental condition, the positive evolution of both biomechanical descriptors Uf and Ua can be attributed to the regular "treatment" foreseen in the experimental methodology.

THE SELF-PERCEPTIVE COSMETIC EVALUATION

Finally, regarding the self-perceptive evaluation of the product, an important tool useful to assess the consumer’s satisfaction (regarding the expectation generated by the product), a clearly positive appreciation of the product utilisation and characteristics resulted from the present evaluation. Again, it should be stressed that the self-perceptive evaluation questionnaire was constructed from the most frequently referred properties claims by the manufacturer for this special class of products. Excluding other aspects more related with the formulation characteristics, this assessments revealed an absolute positive appreciation (100%) regarding the "Cutaneous comfort after application", "The Calming Effect", "The Refreshing Effect", and "The Moisturising (hydrating) Effect" questions (Figure 2).

These results are in full agreement with data from the other assessments corroborating a global positive biological impact, under the present experimental conditions, on normal human skin.
After-sun claims substantiation: experimental criteria to assess the in vivo effect of sun care products

Figure 2. ("a" to "d"). Results from the self-evaluation questionnaire on the volunteer's opinion about the product qualities (efficacy claims) and the consumer's satisfaction. Results, expressed as a percentage of the obtained responses, are referred to two different moments of product's use – D1 and D11. A clearly positive appreciation is obtained from the regular use of the product under study (see text).
CONCLUSION

Previously defined endpoints and a clear definition of the study's rationale, are crucial elements for any scientifically based study. Keeping in mind the multiple factors which supervise the validity of biomedical trials involving human subjects and the critical determinants of non-invasive methodologies used for efficacy assessments, the present proposal illustrates how to involve the most frequently used evaluation tools—clinical, biometrical and consumer's in-use assessments, to establish a relevant relationship with the claim and the parameter, in order to reveal the main effects of the product. In spite of recognising that several experimental aspects can be improved, as it happens in the present example with the irradiation source, this approach also demonstrates how to meet under acceptable compromise (main goals-level of sophistication-cost), the predefined endpoints with the respective claims, in a scientifically based perspective.

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References


33. Sinclair, A., Remedies for common family ailments: Sunscreen creams and lotions, Prof Care Mother Child 4:5; 145-6 (1994)
34. Sun Care Formulary, Cosmet & Toill, 113; 2: 83-103 (1998)

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