HelioScreen

HelioNews

News about In Vitro Sun Protection Testing

HN 2013 N°14

Contents

Congress & Events

2013 April 16-18th, Paris

We will be **Stand G18**

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Editorial

Please slow down… we are in a hurry!
Reliability of **In Vitro SPF method** has been now challenging for years. It is not only within ISO committee that it has been demonstrated correlation with In Vivo is not yet accomplished. It could be a perennial debate for years but we must be honest to recognize unless we need quickly a method, we should slow down to focus on repeatability. It is not acceptable to get different results for the same product and protocol either inter or intra laboratories. Nothing is magical.
If it is the case it is because we do not yet master every thing in the process. We know it is the case within ring tests in which we only focus on correlation on too few products (as it is product dependant). Difficult for me to accept it. It has been our goal to study, step by step the operative conditions included ones we did not think about before. Check with video and ask people to proceed strictly in front of a screen to apply. And it works! We have now «normally» no variability in our laboratory. Now it doesn't mean we have correlation all the time. It is another problem. How about the reliability of the In Vivo measurements? How about problems due to the transmission method we use In Vitro which need a film forming as regular as possible. We must go on. Clearly some improvements in respect of the conditions have been done with the publication of the ISO 24443 first In Vitro method. But sure most of the laboratories maybe don't know…. This is why we want to focus on then in this paper. Hope it will help you to be aware of the important criterias of In Vitro UV testing to select partners or to perform by yourselves.

Dominique LUTZ

I. IN VITRO PHOTO-PROTECTION ASSESSMENT OF SUNSCREEN PRODUCTS

I.a. Evolution of the In Vitro methods

The first use of sunscreen products was reported in 1930's-1940's through four different inventors who have been credited as being the first to invent sunscreen products. But it's Franz Greiter who introduced for the first time the concept of **Sun Protection Factor (SPF) in 1962** which has became a worldwide standard for classification of the UV protection efficiency of the suncare products against erythema. Already at this time, 2.0 mg of product by cm² was used for SPF assessment. This 2.0 mg/cm² application rate was only chosen to improve reproducibility of this In Vivo method and allows giving a relative classification of the UV protection efficiency. For several years, the In Vivo SPF method was not clearly defined with a continuous modification of legislation. **In 2000**, the European Cosmetic, Toiletry and Perfumery Association (Colipa), the Cosmetics, Toiletry and Fragrance Association of South Africa (CTFA SA), and the Japan Cosmetic Industry Association (JCIA) began **discussions on the harmonization of the SPF measurement method**, and reached a **joint agreement** of the test method **in 2006**. Nowadays, although an international application rate is agreed, a number of studies have shown that consumers apply much **less than 2.0 mg/cm²**, typically between 0.5 and 1.0 mg/cm². Furthermore, current debates on the evolution of the sunscreen products compound question the real protection of the product if the factor erythema is biased. Its sound that In Vivo SPF measured may be not really representative of real life effectiveness but a relative classification.

In parallel, as in most other fields, industrial laboratories and health authorities require that such In Vivo methods be substantiated by In Vitro methods for **ethical, economical and practical reasons**. The determination of the In Vitro SPF by means of a spectrophotometer was initially described by **B.L. Diffey and J. Robson in 1989** with the well-known Transpore. Then PMMA (Polymethyl methacrylate) sandblasted plates were introduced in 1999 but with a reproducibility roughness issue which increased In Vitro SPF variation. Different studies have been published to found better method but without an universal solution. Although, correlation issues are not yet resolve with these two previous substrates, **PMMA moulded plates have been proposed in 2008** with **high reproducibility** on several topographic parameters which allows reducing In Vitro SPF variation. …

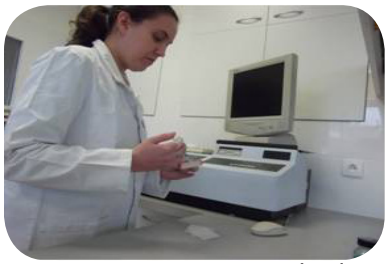

Figure 1. Previous method

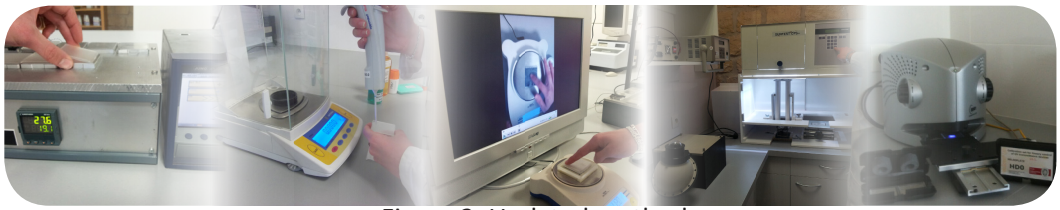

Figure 2. Updated method

Table 1. In Vitro tests evolution

Concern	Previous method	Updated method	Why this evolution?
Substrate	Sandblasted PMMA	Moulded PMMA with topography parameters controlled (HD6 for example)	Improve the reproducibility and correlation
Spectrophotometer	No controlled	Controlled with several conditions	
Product application	No controlled	Several tiny equal drops	
Spreading method, pressure and temperature	No controlled	Controlled	
Product pre-conditioning	No controlled	Controlled	
UV exposure	No controlled	Controlled	
Training	No realized	Realized	

… Besides substrates evolution, the In Vitro method has really evolve during these last years (see Figure 1 and 2) by identifing many others parameters which influence In Vitro values. For example: **quantity of product, spreading method, interfacial properties between substrate and product*, choice of spectrophotometer*, temperature of substrate surface*, etc**. The table here above (see Table 1) shows the evolution of In Vitro tests. By means of continual research and innovation, the In Vitro method passed from an uncontrolled method to a method controlled and soon an harmonized method. For that, different task force meeting work on a norm for In Vitro SPF assessment and should be able to define a method. Although, we trust that In Vitro methods are the future, it's really a challenge to change mind about the objective and performance of these tests. Nevertheless, people take conscience that In Vivo values are not so reproducible and that correlation is only due to the fact that In Vivo values are targeted because of their historical background…

An example of In Vitro method success was the **norm ISO 24443 for In Vitro UVA-PF assessment**. At beginning, studies in 1990's have shown that UVA radiation can cause several negatives effects in human skin. Impact evaluation of UVA radiation was first evaluated by the Immediate Pigment Darkening (IPD), Persistent Pigment Darkening (PPD) and UVA erythema skin response. But it's the PPD skin response which has been selected for development of a standardized in vivo method in 1990's because of it is fulfills the different requirements for selecting a method. But such as said before, an In Vitro method was preferred for ethical, economical and practical reasons. For that, a lot of work has been realized during these last 7 years to **establish a reliable harmonized method**, the ISO 24443 norm created **in 2012**. This In Vitro method is based on the assessment of UV transmittance through a thin film of sunscreen sample spread on a roughened substrate (normative Helioplate HD6) with exposure to a controlled dose of UV radiation from a defined UV source. Through this norm, it's proof of desire to replace in vivo by In Vitro methods.

* demonstrated by HelioScreen Labs

Table 2. How to control In Vitro tests laboratory according to norm ISO 24443 requirements

Concern	Description	Range or Value	How to control
Substrate	**Topographic parameters strictly described** and must be respected.	Ra = 4.853 ± 0.318 µm Rv = 13.042 ± 0.628 µm Rdq = 11.122 ± 1.289 ° A1 = 239.750 ± 44.510 µm²/mm SSc = 0.033 ± 0.013 L/µm Vw = 1.044E-6 ± 6.192E-7 mL/m²	Ask for Substrate Certificate (HD6 for example)
UV spectrophotometer	Regular intervals, at least every month, c**alibration by threefold test requirement** with reference materials: 1. Wavelength accuracy (Holmium Oxide Filter) 2. Dynamic range 3. Linearity test	1. Peak at 361 ± 1 nm 2. Minimum limit 2.2 AU 3. Minimum limit 85%	1. Ask for Holmium Certificate and results 2 & 3. Ask for Substrate Certificate (HD0 for example) and results
UV exposure source	Check every 18 months or after 3000 hours of **lamp running**: 1. Total UV irradiance 2. Irradiance ratio of UVA and UVB 3. Temperature during UV exposure 4. After 20min warm up, UV exposure does not switch off while placing samples under the lamp.	1. 40 – 200 W/m² 2. 8 – 22 3. 25 – 35 °C 4. *NB: For example with the SUNTEST CPS+, the safe system automatically switches off UV exposure when door is open.*	1 & 2. Ask for Control Certificate and results 3. Ask for results 4. Ask more details about testing conditions
Spreading method	The **spreading method** is very important to assure reproducibility and correlation: 1. Without finger cot 2. First phase, small circular motions, minimal pressure 3. Second phase, linear strokes, moderate pressure 4. Drying time	1. – 2. Less than 30 sec 3. About 20 – 30 sec 4. 30 min at same temperature during UV source exposure conditions	1. Ask for procedure 2 & 3. Ask details about control pressure and movement time 4. Ask details about temperature control
Calculation method	To establish the protection aspects of the test sample, different criteria must be respected to valid interpretation: 1. Min 4 plates and max 10 plates according to CI95% value 2. Correction factor C	1. Less than 17% 2. 0.8 – 1.6	1 & 2. Ask for results
Standard S2 product	The method is controlled by the use of **standard product S2** with criteria to respect: 1. UVA-PF results 2. Min 4 plates and max 10 plates according to CI95% value 3. Correction factor C	1. 10.7 – 14.7 2. Less than 17% 3. 0.8 – 1.6	1, 2 & 3. Ask for S2 Certificate and results

HelioScreen Labs has recently acquired new equipments

The HelioScreen laboratory is an important actor of In Vitro tests and try to be always pioneer. For that, we allocate an important part in research to improve and create the In Vitro test of tomorrow. In this logic, we recently acquired several new equipments.

1. Treatment Plasma

It is a plasma generator Femto (see Figure 3) from Diener Electronic which allows to modify surface energy of substrate by a plasma treatment. By means of this equipment, we are **now able to modify surface energy of substrates** (see Figure 4) and afforded better In Vitro test in several cases. We recently published an article about these elements (see here below).

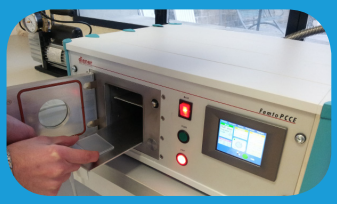

Figure 3. Plasma generator

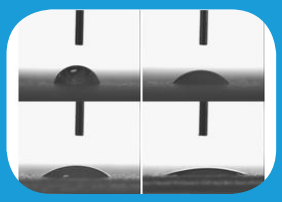

Figure 4. Wettability

2. UV Source Control

It is a spectroradiometer OL-754 (see Figure 5) from Optronic Laboratories which allows measuring UV source of different equipments. By mean of this equipment, we can **calibrate UV exposure source to determine total UV irradiance, and UVA/UVB ratio** according to Colipa rev. 2011 and ISO 24443 norms (see Figure 6).

Figure 5. Sprectoradiometer

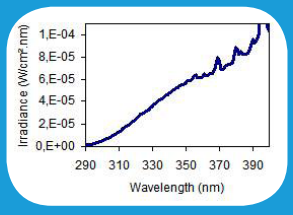

Figure 6. UV source

3. UV Source Generator

It is a generator of UV source Research Arc Lamp Sources (see Figure 7) from Oriel Instruments. By means of this equipment, we can **illuminate small targets by generating UV source**.

Figure 7. UV source generator

4. Surface topography measurement

It is a profilometer Altisurf500 (see Figure 8) from Altimet which allows **measuring topographic parameters** with high resolution. By means of this equipment, we provided a new kind of service and discovering new research subjects (see Figure 9).

Figure 8. Profilometer

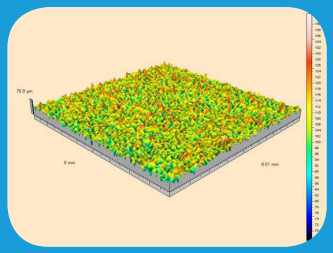

Figure 9. Surface topography

I.b. How to check the reliability of laboratories to follow norms?

The fact that measures of UV protection are more and more realized by In Vitro methods led industries to propose this kind of tests. But the real danger comes that even if norms exist for strong and reliable In Vitro tests, nothing not guaranteed that these laboratories respect it. Obviously, it seems logical to have **a certification** which assures the respect of these specific norms but this procedure is long to create. We present in our previous HelioNews 2012 N°13, the news rules for In Vitro UVA-PF assessment but without ways to verify if norm is respected. So, the table according to you (see Table 2) suggests a non exhaustive check-list of the elements to guarantee the relevance of the results of **In Vitro tests laboratory**. For simplification, we only take an example with the latest ISO 24443 norm requirements obligatory. It is very important for you to have certificates which proof the norm is correctly following for your own guarantee if your products are controlled.

I.c. Conclusion

After lot of years of In Vivo time, the In Vitro methods are more and more used for evaluation of protection of sunscreen products. The first In Vitro UVA-PF ISO 24443 norm change context around the In Vitro tests and we are in a turning point about future of In Vitro tests, especially about SPF assessment. To be sure this first norm is correctly following, don't forget to evaluate the different criterions described here above. What could be the same table for the future harmonized In Vitro SPF assessment norm?

II. INFLUENCE OF SURFACE ENERGY ON IN VITRO SPF

In previous HelioNews, we presented you the issue about the **relationship between surface properties and quality of spreading**. Now, we show you some results about the **influence of surface energy** on In Vitro SPF. This article is partly extracted from publication "Adjusting Substrate/Product Interfacial Properties to Improve In vivo/In vitro SPF Correlation" by S. MIKSA, D. LUTZ, J. ONGENAED, HelioScreen Labs and D. CANDAU, L'Oréal Research & Innovation. Cosmetics & Toiletries March 2013.

II.a. Introduction

Many studies have been published in which several parameters have been varied to improve the reproducibility and correlation of In Vitro SPF evaluations with in vivo methods. Although repeatability can be now be made relevant for very specific protocols, correlation with in vivo values is still a challenge for some products. This is clearly because a key parameter has not been considered: **the affinity between the plate and the product**, owing to the surface properties of the substrate. Therefore, a new method is explored in this paper based on the step-by-step **physical-chemistry modification of interfacial properties** through plasma treatments in order to improve the substrate/product affinity.

II.b. Materials and Methods

Plasma generator:

A low-pressure system Femto LF PCCE control from Diener Electronic was used to created a set of modified substrates according to different surfaces energies.

Surface energy:

To characterize polar and dispersive components of substrate, we used the sessile drop method by measuring the contact angle of two liquids (water and diiodomethane).

Substrate:

We used HD6 plates from our company. These plates are controlled and guaranteed for HD6 within the Colipa and ISO 24443 requirements.

Product:
Thirty-four sunscreen products covering various formulations were chosen with In Vivo SPF values ranged from 9 to 85.
Transmittance measurements:
The Labsphere UV-2000S was used to measure the UV transmittance through the thin product layer. Before, the linearity/ additivy was controlled by calibrated reference standard Helioplate HD0 PMMA plates and Holmium filter.
Procedure:
We applied the required quantity on the plate and spread product on whole surface by a specific protocol which guaranteed a high repeatability. After drying, each plate was measured and 2 plates per product.

II.c. Results

By means of our system, we guaranteed a graduated surface energy on HD6 with good repeatability of process. The plasma treatment allows reaching **three different surfaces energies required** for this study (see Figure 10). All products were spread on HD6 according to the different plasma treatment level. First, the In Vitro spreading method with the same operator was repeatable with a low average coefficient of variation.

Second, in order to obtain In Vitro SPF values closest to In Vivo SPF, majority of product required a plasma treatment. From data, the correlations between In Vitro and In Vivo SPF values were compared with and without plasma treatment (see Figure 11 and 12). **Results led to a correlation coefficient of 0.660 without plasma treatment**, a slightly better correlation coefficient of 0.720 with plasma treatment level 1, 0.698 with level 2 and finally a **high correlation coefficient of 0.763 with a plasma treatment level 3**.
Nevertheless, as expected, whatever the level of plasma treatment used, the In Vivo/In Vitro SPF correlation coefficient was improved by chosen the best plasma treatment according to In Vivo SPF value. Thus, it allows a better correlation coefficient of 0.812.

II.d. Conclusion

From this study, it appears that **the majority of product is sensitive to surface energy modification**. By changing substrate surface energy, we improve the correlation coefficient between In Vitro and In Vivo SPF values. But finally, the required plasma treatment level depended upon the product and we reach a correlation coefficient of 0.812 with an adapted plasma treatment. The next step in this process will be to define an eligibility criterion to select best plasma treatment level to predict In Vivo SPF.

New or updated services from HelioScreen

1. Training sessions

Training sessions in our laboratories or at customers are dispensed for several years in France or abroad. HelioScreen Labs is now registred as trainer following article L.6352-12 (code du travail).

2. Calibration of SUNTEST

ISO 24443 and Colipa method ask for conducting spectoradiometric irradiance on the UV exposure source over the UV range. Your UVA radiometer (compulsory for control at each irradiation) must be exposed in the same position for further irradiation. HelioScreen proposes now as a new service in your laboratories, first and compulsory step of calibration with its spectroradiometer.
Please contact us in case.

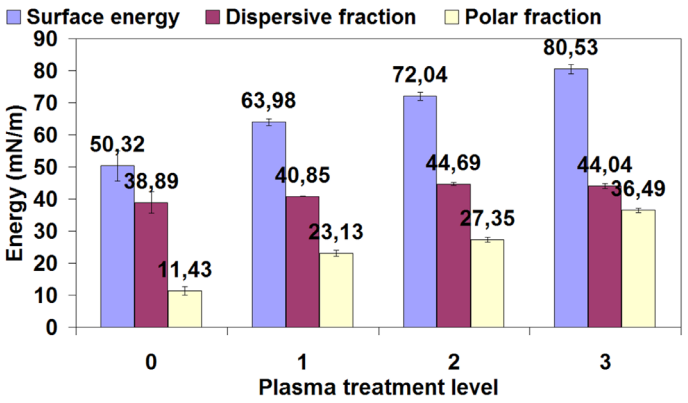

Figure 10. Surface energy

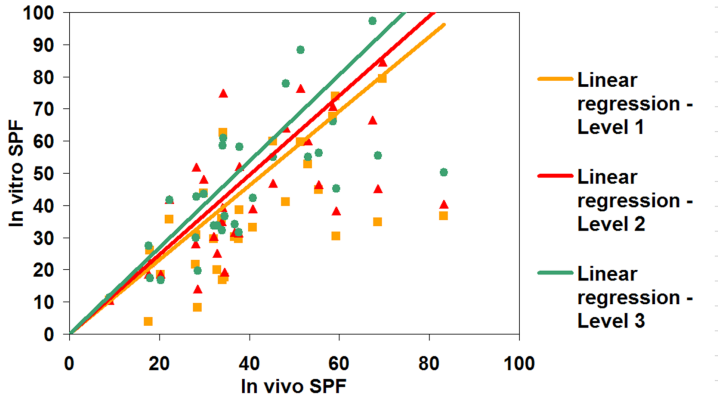

Figure 11. In Vivo/In Vitro SPF correlation

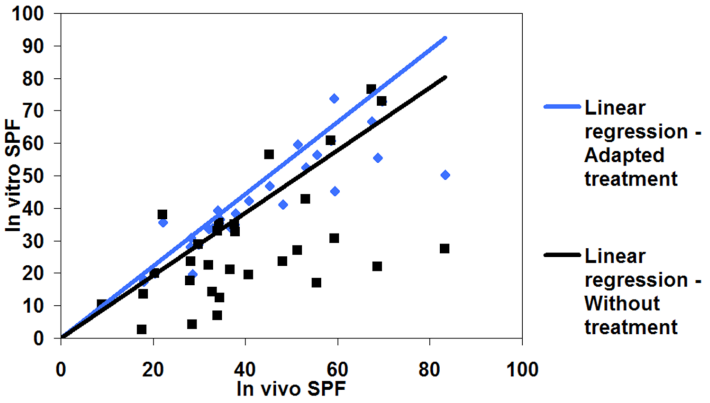

Figure 12. In Vivo/In Vitro SPF correlation

Scientific articles

Compendium on Sun care - Household and Personal Care Today - Vol. 7 nr. 3 July/September 2012:
- PIERETTI DA SILVA A., BILIBIO U., DE CARVALHO A.E., SOUZA FERREIRA V., GONCALVES TRINDADE M.A. *Electrochemical techniques. Analytical tools for quality control of sunscreens.*
- CARIOU N., LUTZ D. *Sunscreen in-vitro SPF determination inter and intra comparison tests between several measurement instruments.*
- CASALE G.R., SIANI A.M., COLOSIMO A. *Occupational exposure to solar UV radiation. A short review of relevant papers on the quantification of exposure to solar ultraviolet (UV) radiation of outdoor workers.*

Cosmetics & Toiletries, March 2013:
- MIKSA S., LUTZ D., ONGENAED J., CANDAU D. *Adjusting Substrate/Product Interfacial Properties to Improve In vivo/In Vitro SPF correlation.*

We will be present at these next events

- In-cosmetics, Paris, 16-18 april 2013
- Cosmetics & Beauty Expo, Osong Korea, 3-26 may 2013
- NYSCC Supplier's Day, New-York, 14-15 may 2013
- In-cosmetics, Bangkok, 29-31 october 2013