HelioScreen

HelioNews

News about In Vitro Sun Protection Testing

HN 2015 N°20

In Vivo: How recent knowledge about In Vitro may enhance In Vivo reliability (read p2)

Contents

Congress & Events

in-cosmetics Barcelona
14-16th April 2015, Barcelona
Booth 6R37 Test

Conference by Dominique Lutz **«UVA protection: Facts and perspective for a real worlwhile harmonization»**

Sun Protection Conference
9-10th June 2015, London

Conference by Dominique Lutz **«In vitro SPF for label claim: Fact or Fiction?»**

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Editorial

The improvement and innovative solutions that our laboratory published and reported to the ISO SPF international expert group has been demonstrated by this international committee - after several checking - as the only solution, in the state of the art, to ensure reproducibility for In Vitro transmission measurements and go further on a solution for an In Vitro SPF method on which it is also our proposal which is now studied. Everything else including manual spreading ...in an expert manner as possible... has been sweep off.

As a consequence, for now more than a year, all practical international assays have to be only conducted in our facilities due to our innovative and adapted equipment.

This is what we also propose daily to our customers (both in EU and ASIA) in comparison with other institutes all over the world. Our level of equipment and know-how based on a huge research program (18 publications within the last 2 years) not to mention our quality certification has been audited several times by big companies and official health regulatory offices in EU.

Competition is normal on the market but it is always the responsibility of the customers to check about reliability of testing laboratories as it is for the sun protection a question of public health.

Our doors are open for each customer.

Dominique Lutz, CEO Scientist Manager

HelioScreen foothold in India

Last year HelioScreen firmed a subsidiary in Thailand with HelioScreen Asia Co., Ltd. borned via the joint venture with Chemico Inter Corporation Co., Ltd. for the distribution of In Vitro suncare testing with high quality for ASEAN and Asia countries.

Since last month there is a new step in the worldwide representation of the company with a new partnership firmed in Mumbai with C.L.A.I.M.S. Pvt. Ltd. for India which is a Clinical Research Organization that provides Safety, Efficacy and Sensory Testing services for Cosmetics, Cosmeceutical, Dermaceutical, OTC and Pharmaceutical products. C.L.A.I.M.S. Pvt. Ltd. also offers specialized Clinical Nutrition Research in the area of Foods, Nutraceuticals and Dietary supplements.

CLAIMS
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info@claimscro.com
Website: www.claimscro.com

With this new representation in India, D. Lutz will be present during HPCI exhibition in Mumbai the 4-5th March.

The Indian Care Chemicals Convention
HPCI EXHIBITION AND CONFERENCE
4–5 March 2015
BOMBAY CONVENTION & EXHIBITION CENTRE, MUMBAI
HOME AND PERSONAL CARE INGREDIENTS EXHIBITION & CONFERENCE INDIA

This new collaboration will help you in achieving your needs for sunscreen product claiming and development (SPF, UVA-PF, Critical Wavelength...) with updated modern and unique instruments allowing reliability and reproducibility for India market.

IN VITRO SUNCARE
OPEN DAYS 2015
JOURNEES PORTES-OUVERTES 2015

Be ready for the 5-6 October 2015!

Further information coming soon in our next HelioNews!

I. Challenging the In Vivo tests

I.a. Introduction

It's around 60's that the first time the concept of Sunstroke Protection as a scale to allow longer sun exposure before sunstroke, so called Sun Protection Factor (SPF) was introduced. It has become a worldwide standard for classification of the UV protection efficiency of the suncare products. At that time level of protection were very low (about around 6) and formulas quite basics.

It consists of comparing the UV radiation dose required with and without protection till the appearance of a skin redness due to UV induced erythema (or so called sunstroke) as a biological endpoint. In the 2000's, the COLIPA (now Cosmetics Europe), the CTFA SA, and the JCIA began discussions on the harmonization of the SPF measurement method, and reached a joint agreement of the test method in 2006. An international harmonized method was finally agreed at a worldwhile level through the ISO 24444:2010 standard without any further practical checking of the variability. So unless these attempts to harmonize there is a very high variability inter laboratories of In Vivo values (unless with variations as it is product dependant). It has never been checked and published before. But everyone would agree on this statement and all major companies have checked by themselves. As a matter of fact, this is for economic reason that no more checking have been made and there was only this to classify.

Nevertheless, some consumer associations check the relevance of the SPF claimed on sunscreen commercially available on the market with always the same conclusion in recent papers [1-4], some products could not reach the SPF claimed and health consumer safety could be challenged...

"...a poor reproducibility and repeatability for In Vivo SPF assessment which can lead to different SPF claimed (labelled) for Inter AND Intra labs."

I.b. General procedure

The In Vivo SPF assessment is carried out by measuring the Minimal Erythemal Dose (MED) which consist of comparing the UV radiation dose required with and without sunscreen product protection for the appearance of a first unambiguous biological endpoint, in this case skin redness. The final SPF is the arithmetic mean of all valid SPFi values (e.g. each human volunteer). In the In Vivo SPF assessment, four key steps must be respected with:

- Balanced volunteer panels with a majority of I, II and III skin type response.
- Careful rate application and spreading method at 2.0 mg/cm² and light pressure.
- Controlled light source with calibrated UV SSR source.
- Reproducible MED reading as described for the first redness sawn by human eyes after training.

I.c. The limits

It seems that people think that the In Vivo SPF test is closed to the real condition and is more representative of biological effect for every human but:

(i) It is interesting to show that not all skin type response (in total VI) are represented for In Vivo evaluation but only a minority (I, II and III). Unless the institute keep with these rules already huge for selection of skin types, they are still great differences due to geography, volunteers history, etc.

(ii) Before evaluation, a target value is necessary to adjust UV radiation doses but how about with a different target value? Clearly, it is impossible to take the risk of over irradiation for volunteers but at the same time it may induces some deviation when a target value is already known which is not the case as an example for In Vitro.

(iii) Although an internationally application rate is agreed, we shouldn't forget that the quantity of 2.0 mg/cm² has been established many years ago (about 50 years!) with former formulas quite basics (how many?) and very low SPF. The reasons? This quantity was only chosen as convenient as to allow best spreading as possible and just give a relative classification of the UV protection efficiency. But it is well known that now the products have higher SPF, formulas are more complicated, and that the reproducibility of results is product dependant. Perhaps, the quantity of product should be modified in order to reduce the variability with recent products more representative of the market and why not closer to real condition by consumers (e.g. typically 0.5 to 1.0 mg/cm²).

(iv) Current debates on the evolution of the sunscreen products compound question the real protection of the product if the factor erythema is biased. Does most of the so called SPF booster does act on an improvement of the UV protection or the delay of the end point (erythema reaction)?

(v) Concerning light source used for In Vivo SPF assessment, the UV SSR source (mainly constituted of UVB and short UVA radiations) is not as representative of real life condition as Midday midsummer sunlight (UVB and UVA radiations) but it was necessary to reduce skin damage of volunteers. Nevertheless, it was demonstrated that UVA radiations have a part in erythema for about 10 to 20% and could lead to different In Vivo SPF results in real condition. Unphotostability is taken into account as a result when the final dose is obtained but never as an unlimited dynamic chemical process. Means degradation can go on and we have never information on this important parameter about the product using In Vivo testing.

(vi) Besides these exogenous parameters, we should also consider that endogenous parameters can lead to different In Vivo values. Based on criterions put in evidence in different In Vitro studies, it seems logical to transpose key parameters from in vitro methods to In Vivo methods. Thus, according to body part, we can observe different physical-chemistry skin properties which could influence In Vivo results such as roughness, surface energy or temperature not to mention application.

Anyway, beyond these different highlighted points, we attempt to estimate with few datas an example of the variability of In Vivo SPF afforded by sunscreen products and there consequences. For that, we gather In Vivo SPF ± Standard Deviation values from different testing laboratories (Inter) and in a same testing laboratory (Intra). The **Figure 1** shown different results according to the products tested.

First of all, the variability is clearly product dependent and we could have a great reproducibility for Intra and Inter laboratories as for the Product P1 within the lab C and P4

between the lab B & G. Nevertheless, in some cases such as the products P1-P2-P3, we can observe a poor reproducibility and repeatability for In Vivo SPF assessment which can lead to different SPF claimed (labelled) for Inter AND Intra labs.

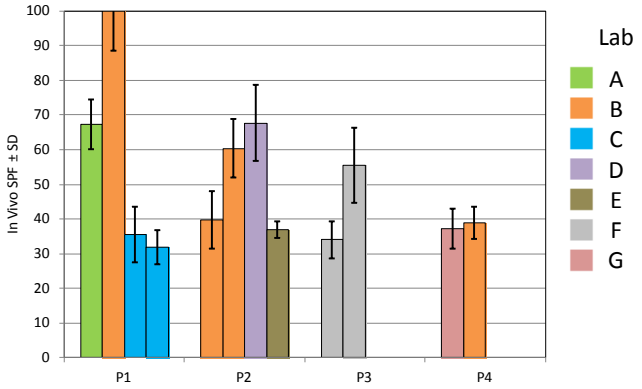

Figure 1. In Vivo SPF ± SD intra and inter laboratories

According to these results, the following part summarizes the different possibilities of SPF claimed (following for example the European Recommendation 2006):

P1: SPF 30 - SPF 50+
P2: SPF 30 - SPF 50 - SPF 50+
P3: SPF 30 - SPF 50
P4: SPF 30

"Without any unambigous, the In Vitro method leads to better reproducibility of SPF compared to In Vivo method."

Indeed, as everyone knows this variability, we should be concerned about the methods (i.e. Transmission In Vitro) which are supposed to replace the existing method and qualified with their ability to correlate.

Just as an example, the same 4 samples previously presented in the present paper have been tested with the best way for reproducibility by means of a robotic spreading already published*. Of course, several key parameters have to been also controlled such as temperature at interface, substrate surface characteristics, spectrophotometer, UV irradiation... The **Figure 2** shown the Coefficient of Variation (CV%) between the In Vivo and In Vitro SPF results!

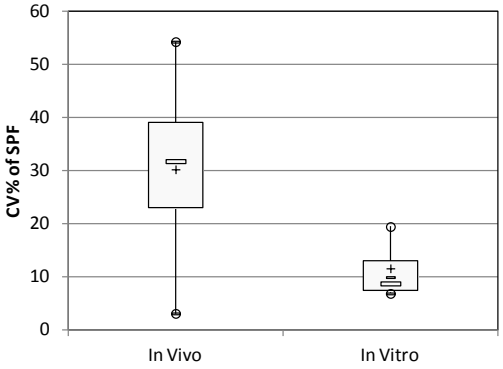

Figure 2. CV% for In Vivo vs. In Vitro

Without any unambigous, the In Vitro method leads to better reproducibility of SPF compared to In Vivo method.

Thus, why most of people attempts to try to improve In Vitro method on trying to mimic the In Vivo method? How about the contrary? Does the recent knowledges of compulsory parameters to follow to get reliable results with In Vitro could be also usefull for In Vivo method?

I.d. How knowledge for In Vitro could help to improve In Vivo

Based on our experience and knowledge in the sun protection assessment, we propose some ideas in order to improve the reproducibility as far as possible based on a logical approach for the four In Vivo key steps.

1. Selection of human volunteers

a - Beyond the skin phototype well determinated with ITA°, we shouldn't forget that the skin could be considered as a substrate. In this way, it has been worldwide recognized that the strict control of the roughness (more precisely the topography parameters) reduces variability of In Vitro results. By extrapolation, taking into consideration the skin's roughness with measurement during In Vivo test could reduce variability with for example a range of acceptable values.

b - Skin's properties are an important point for product affinity at the interface during product spreading. As we have a lot of differencies between volunteers, a simple pre-test could be done in terms of pH and hydration measurement and ranges could be recommended in order to reduce any source of variability.

2. Application of products

a - In the current methods, manual spreading is used with few explanation. In a recent In Vitro study, it has been demonstrated that the manual spreading leads to poor reproducibility even with strong experience. However, an innovative robotic spreading allows to improve considerably the reproducibility of results during In Vitro test and could be also help in case of In Vivo test.

b - Checking film forming by means of a Wood lamp gives visual information about the repartition of the product on the skin. A computer analysis of the photography could bring a percentage of homogeneity for acceptance of application.

3. UV irradiation source

a - During UV exposure, a geometrical progression for the npMED is performed in the ISO 24444:2012 with recommended 25% for unprotected skin and with 15% for protected skin. A worldwide harmonized progression at 10% could reduce the variability of the detection of the first redness (and of course of the SPF calculation) as for the unprotected skin than for the protected skin.

b - A compulsory appliance** with the ability to produce UVA or UVA+UVB from individual outputs with adjustment of the intensity of light in order to deliver various doses of UV from each output as well. This feature allows multiple tests to be performed in a shorter period of time and reduce variation of the beam uniformity.

4. End point reading

a - An important part of variation provides from biological response which is on a practical point of view expressed as the apparition of a reddish color. Indeed with a human eye, the determination of the first endpoint may be different according to the operator. In order to improve this step, we propose to perform a computer analysis of a photography (under standardized light) of the whole test area which take into consideration the different sub-sites (with a blank reference) and a calculation of the first visible redness which is detected by human.

Of course, these different proposals need to be checked and validated during In Vivo test but we used this same logical approach in order to improve the In Vitro test and we are now able to propose reproducible In Vitro results unless for years before no!

* S Miksa, D Lutz and C Guy, In Vitro UV Testing-Robot vs. Human Spreading for Repeatable, Reproducible Results, Cosm & Toil, 128(10) 742-752 (Oct 2013)
**the model 601 Multiport® SPF Testing 6 output Solar Simulator from Solar Light Inc. is a convenient tool.

I.e. An alternative to In Vivo tests

As already well known, the In Vivo test presents different inconvenients such as economical and practical reasons but one of the most deficits of SPF determination is due to ethical concerns as UV radiation is carcinogenic and melonoma is related to sunburn.

Recently, a new noninvasive hybrid method [5] has been presented and published with the DRS (Diffuse Reflectance Spectroscopy) for human part and In Vitro transmission. The DRS, sometimes known as Elastic Scattering Spectroscopy, is a non-invasie technique that measures the characteristic reflectance spectrum produced as light passed through a medium which consists of a white light source (low energy), a probe and a spectrometer.

This new hybrid approach allows now also the SPF determination by a combination of DRS and In Vitro method according to 5 different steps (see **Figure 3**):

1. In Vitro method (for UVB part)
2. DRS (for UVA part)
3. Adjust the In Vitro based on DRS results
4. Final DRS for UVA and UVB part
5. Calcul of the SPF value thanks to the spectrum

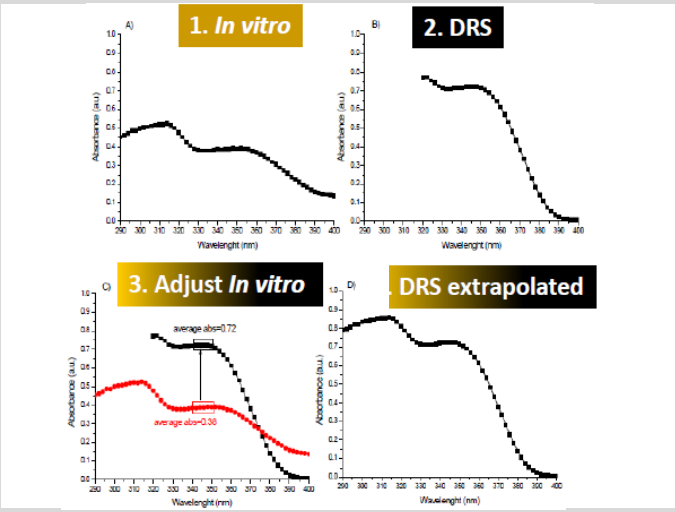

Figure 3. Different steps of hybrid method

In order to check the relevance of this hybrid SPF method, the authors tested 17 sunscreen products (only photostable). By using this hybrid method, the **Figure 4** shows the In Vivo SPF values correlation with the In Vivo-DRS/In Vitro-UVB SPF values

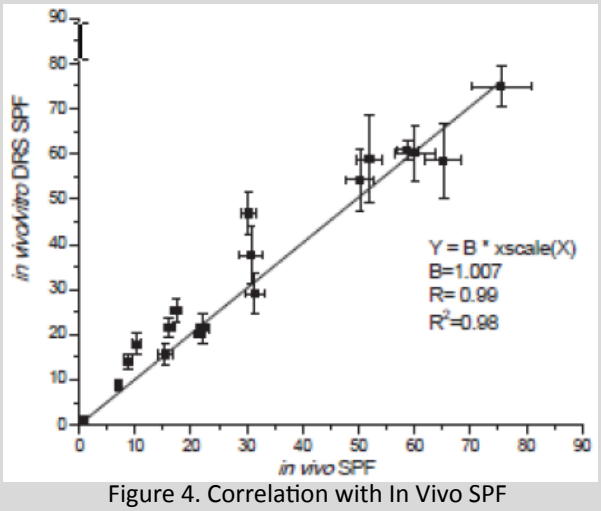

Figure 4. Correlation with In Vivo SPF

obtained from the results available in the publication [1].

To conclude, with the hybrid In Vitro method and DRS together, there is a realistic chance to replace the In Vivo SPF. Further works have to be done as the photostability part but also reproduciblity which is the prior condition for any methods. A group of scienticfs has already started to join their support for the DRS approach with the coordinator Uli Osterwalder (uli.osterwalder@basf.com).

I.f. Conclusion

The In Vivo SPF test is worldwide used but as previously shown, measurements of several sunscreen products applied on volunteers in different testing laboratories demonstrate a significant variability of In Vivo SPF. Indeed, it was shown and already well known a poor repeatability between the same laboratory and poor reproducibility between different laboratories for several products.

Through these results, it should necessary to maybe reconsider the In Vivo SPF value not such as a target value for development of other methods but as a relative value obtained by a different method. It is also of importance to never compare to a single value from one laboratory.

Perhaps, instead of working to obtain at any price good correlation between In Vivo and In Vitro values, at least for products used for the comparison we should start working for improvement of reproducibility of In Vivo methods first.

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