

# *In vivo* and *in vitro* evaluation methods: a comparison

The sun delivers energy as electromagnetic radiation, characterised by specific wavelengths. The spectrum of the electromagnetic radiation emitted by the sun and able to reach the Earth's surface ranges between 290 and 300 nm.

In particular, the electromagnetic radiation (EMR) with photo-biological interest are detailed in Table 1.

Despite the innumerable and beneficial effects of sun rays on our physical and psychological state, the role of UV rays in the adverse effects at systemic, ocular and skin level is becoming more and more evident. In the past, dermatologists and cosmetic formulators used to focus their attention on UVB protection, as UVB radiation was held responsible for immediate and evident skin damages, such as erythema or sunburn.<sup>1</sup> However, both the biological damage caused by UVB radiation on the DNA of keratinocytes and by the longer wavelength UVA radiation on the dermis, are asymptomatic, tend to increase over time and show their effects after many years. UVB radiation induces carcinogenesis too.

After penetrating the dermis, UVA radiation causes the release of free radicals which damage nuclear DNA, cell membranes, functional and structural protein of skin cells and causes the onset of elastosis as well as possible cancerous changes.

UVA radiation is also the main concomitant cause of skin photosensitisation and phototoxicity. Considering that the incident radiation is constituted by approximately 95% UVA radiation, we can easily work out how important it is to make an appropriate skin protection also from this constituent of the solar spectrum, which is particularly dangerous as it cannot be screened by either plastic or glass.

As regards UVC rays, they are extremely harmful but they do not reach the surface of the Earth because of the screening action of the ozone layer. All these considerations have generally led to a more cautious and controlled sun exposure. Moreover, the formulation of sunscreen products has increased and widened



Figure 1: Solar simulator (ISPE srl).

their protection range and information to consumer has become complete and clear.

## Evaluation of UVB sunburn protection factor (SPF)

The SPF, Sunburn Protection Factor, conceived over 40 years ago, is an index of the protection potential of the product from UVB rays. It was globally adopted and is today written on the labels of all sunscreen products, included the daily-use cosmetic products against skin photoageing.

Up to 1994, in the international context, different methods for *in vivo* SPF assessment on informed human volunteers coexisted: the FDA method from the US,<sup>2</sup> the German DIN method,<sup>3</sup> the Australian,<sup>4</sup> the Japanese<sup>5</sup> and the South African<sup>6</sup> ones (Table 2).

The different assessment procedures and the instruments used, including the UV ray source as a solar simulator, resulted in disagreeing numeric values, thus creating confusion among the consumers.

This is the reason why in 1990 Colipa (European Cosmetic Toiletry and Perfumery Association – now Cosmetics Europe) Task Force set up a work team called 'Sun Protection Measurement', made up of

experts from the scientific and industrial world, whose task was to harmonise the existing methods so that just one method could be generally accepted.

In 1994 the European Colipa Method for the assessment of the Sunburn Protection Factor<sup>7</sup> was drawn up. The Colipa Method introduced some important innovations when compared to the former methods as the definition of the UV-source emission spectrum, the criteria for the selection of the volunteers and the utilisation of standard products with a known SPF.

Further harmonisation was achieved with the next revision in 2003, when Colipa issued the International SPF Test Method CTFA-SA/Colipa/JCIA 2003<sup>8</sup> and its subsequent revision in 2006,<sup>9</sup> which included the European, Japanese and South African measurement systems.

In November 2010 Colipa, in collaboration with ISO (International Organization for Standardization), issued ISO 24444:2010 – Sun Protection test methods – *in vivo* determination of the Sun Protection Factor (SPF).<sup>10</sup> This ISO method was adopted by Europe, Australia and New Zealand, India, Canada, South Africa, Mexico, Chile, Russia, Japan, Mercosur and Asean.

Currently, in 2014, there are only two standard methods for the determination of the sun protection factor: ISO 24444:2010 and the US FDA 2011.<sup>11</sup> The two methods mainly differ by three points, as discussed further below. However, notwithstanding the differences, the SPF values obtained with both methods are comparable.

## ISO 24444:2010 – *in vivo* determination of SPF

The ISO method is very similar to the 2006 Colipa method; we can examine this in detail. The method involves the induction of an erythema spot on the back of at least 10 selected subjects by means of an arc xenon lamp having an emission spectrum ranging between 290 nm and 400 nm and able to reproduce fairly well the ultraviolet band of the solar spectrum (Fig. 1).

At the beginning of the test, the skin

**Table 1: EMR having photo-biological interest.**

EMR	Wavelength (nm)
UVC	100-280
UVB	208-315
UVA	315-400
Visible	400-760
Infrared	>760

**Table 2: SPF assessment methods before 1994.**

Country	SPF method	Issue date
United States*	FDA	1978 ('93)
Australia/New Zealand	AS/NZS	1983
Germany	DIN	1984
Japan	JCIA	1992
South Africa	SABS1557	1992

\*First standard method

**Table 3: Classification of skin types according to Fitzpatrick.**

Type 1	Always burns, never tans
Type 2	Usually burns, tans with difficulty
Type 3	Sometimes burns, sometimes tans
Type 4	Burns minimally, always tans
Type 5	Rarely burns, tans profusely
Type 6	Never burns, deeply tans

**Table 4: SPF classification.**

Protection level	SPF value
Low protection	6, 10
Medium protection	15, 20, 25
High protection	30, 40
Very high protection	50+

sensitivity to sunburn of each subject is determined by means of the minimum erythral dose (MEDu) on unprotected skin. This is defined as 'the lowest dose of UV rays which induces a visible skin reddening (erythema) 16-24 hours after exposure'.

A standardised amount of the product (2.00 + 0.05 mg/cm<sup>2</sup>) is applied on a skin site measuring between 30 cm<sup>2</sup> and 60 cm<sup>2</sup>, located on the back of the subject between the waist and the shoulder line. A correct and homogeneous spreading of the product is very important in order to obtain reliable and reproducible results.

After 15-30 minutes the application site is exposed to a progressive dose of UV radiation, calculated on the basis of the expected SPF.

The minimal erythral dose of the protected skin (MEDp) is visually assessed 16/24 hours after UV exposure in sufficient and uniform illumination (450 lux). The SPF is the ratio between the minimum erythral dose (MEDu) obtained on the skin protected by the sunscreen product and the one obtained on the unprotected skin. In theory, it represents a ratio between two energy amounts. In practice, the SPF indicates how long an applied sunscreen product is able to extend the exposure time to UV rays before the onset of an erythema compared to the unprotected skin site. This derives from the assumption of direct proportionality between exposure time and released energy. The product sun protection factor (SPF) is calculated as the arithmetical mean of the individual SPF obtained from the total number of subjects used. The 95% confidence interval of the mean SPF value must fall within the interval+ 17% of the SPF value found.

A reference sunscreen product with known SPF (indicated by the method) is

tested at the same time and used as a control.

The test is performed on subjects belonging to phototypes I, II or III according to the Fitzpatrick classification (Table 3), on the basis of the fact that these skin types are more prone to sunburn. The skin colour is determined by the measurement of the Individual Typology Angle ITA° (>28°).

### Recommendations

#### The Recommendation of the European Commission 2006/647/CE of 22 September 2006 and the Colipa Recommendation n. 23 (2009)

Recommendations are issued at regular intervals by the Colipa aiming to regulate the cosmetic industry with respect to methodologies, labelling, and new rules. Recommendations are not legally binding but serve as guidelines for the companies in the cosmetic field and attempt to unify the presentation of sunscreen products as well as to protect the consumer's health.

In order to standardise and to simplify the way sunscreen products were tested and labelled in Europe, the European commission issued the recommendation 2006/647/EC.<sup>12</sup> The subsequent Colipa recommendation n. 23 was issued in 2009<sup>13</sup> and proposed important information to be reported on the label, as already indicated in the recommendation of the European commission.

According to these recommendations, a sunscreen product should satisfy the following requirements:

- Claims such as 'sunblock' or 'total protection' are banned as being misleading and deceitful for the consumer of sunscreen products.
- The method ISO 24444:2010 should be used to determine *in vivo* UVB protection.

- The SPF value obtained experimentally by means of the *in vivo* test must be written on the label after approximation by defect to the next whole number reported in the SPF Classification Table (Table 4). Eight values only should be claimed on the label as well as the relevant protection level. The aim of such recommendations is to limit the chaos of the past years, when the labelled values were not significantly different from one another. It is important to underline that the minimum suggested labelled SPF must be 6 and the maximum 50+ (corresponding to a measured SPF value ≥60). Furthermore, the SPF value must be followed by the qualitative description (low, medium, high or very high protection). The desired aim is to limit the wide variety of numbers used on labels for indicating the sun protection factor that does not support the making of simple and meaningful claims.
- Finally, the recommendation highlights that a sunscreen product must ensure appropriate protection from UVA rays.

In detail, sunscreen products are required to develop a protection factor in the UVA band equal to 1/3 of the UVB protection factor. For example, an SPF 15 product should at least ensure a UVA protection factor equal to 5. Furthermore, the critical wavelength must be at least equal to 370 nm. 'Critical wavelength' means the wavelength for which the section under the integrated optical density curve starting at 290 nm up to 370 nm is equal to 90% of the integrated section between 290 to 400 nm. The compliance to both the requirements is shown on the label by the circled acronym UVA.

Furthermore, according to the Colipa recommendation n. 23, 2009,<sup>13</sup> the

following warnings for the consumers must be reported on the label:

- Do not stay too long in the sun, even while using a sunscreen product.
- Keep babies and young children out of direct sunlight.
- Over-exposure to the sun is a serious health threat.
- Reducing the quantity of sunscreen product applied will lower the level of protection significantly.
- Apply sunscreen products before sun exposure.
- Apply generously.
- Reapply frequently to maintain protection, and especially after perspiring, swimming or towelling.

Based on several studies, the International Agency for Research on Cancer of the World Health Organization has emphasised the importance of the link between the correct application of sunscreen products and the efficacy of the sun protection factor claimed. In particular, frequent re-application of sunscreen products is crucial. Moreover, in order to reach the protection level indicated by the sun protection factor, sunscreen products have to be applied in quantities similar to the ones used for testing, i.e. 2 mg/cm<sup>2</sup>, which equals 6 teaspoons of lotion (approx. 36 grams) for the body of one average adult person. This quantity is by far higher than that usually applied by the consumers. Applying a smaller quantity of sunscreen product leads to a disproportionate reduction in protection. For example, if the quantity applied is reduced by half, protection may fall by as much as two-thirds. These considerations have led to a more cautious and controlled sun exposure and more detailed use recommendations.

### Evaluation of the sun product water resistance

In December 2005 Colipa published the guidelines for determining the water resistance of a sunscreen product<sup>14</sup> (*Colipa Guidelines for evaluating Sun Product Water Resistance 2005*) and the next year the Colipa recommendation n. 16 on 'Water Resistance labelling, 2006'.<sup>15</sup> These suggest how to evaluate the SPF (using the method ISO 24444:2010) before and after water immersion of the skin site where the sunscreen product has been applied.

For this purpose, a spa-pool fitted with a water recirculation device is utilised. This contains water at constant temperature between 27 °C and 31 °C.

For sunscreen products claiming to be 'water resistant' the SPF is measured after two 20 minutes immersions (40 minutes in total). While, if they claim to be 'very water

resistant', four 20 minutes immersions will be required (80 minutes in total).

A sunscreen product can claim to be 'water resistant' or 'very water resistant' if the SPF value after immersion is equal to more than 50% of the value found before immersion (90% lower unilateral confidence limit for the mean percentage of water resistance retention  $\geq 50\%$ ).

To give an example, a 30 SPF product can claim to be water resistant if it keeps its SPF value higher than 15 after immersion in water.

### In vitro method for the determination of SPF

The *in vitro* test cannot be considered an alternative to the *in vivo* method, but it can be useful as screening for the selection among the products, even if there is a poor correspondence between the *in vivo* and *in vitro* SPF values (especially for high SPF values).

The method, developed by Diffey and Robson in 1989,<sup>16</sup> is based on the assessment of UV transmittance through a thin film of sunscreen sample spread on a roughened substrate (polymethylmethacrylate – PMMA plate).

### In vivo determination of UVA photoprotection

In the past, in order to determine the UVA protection factor, the PPD (persistent pigment darkening) *in vivo* test was employed.<sup>17</sup> The method currently used is ISO 24442:2011.<sup>18</sup>

The biological endpoint of this *in vivo* method is the onset of persistent grey-blue pigments on the back of at least 10 subjects exposed to UVA rays (320 nm-400 nm) by means of a xenon arc lamp. The selected subjects must have a good pigmentation capacity (phototypes II, III and IV according to the Fitzpatrick's classification).

The method ISO 24442:2011 was adopted by Europe, Canada, Mexico, South Africa, Japan, Mercosur and Asean.

The drawbacks of the *in vivo* UVA test are, first of all, the poor reproducibility, because of the difficulty of reading of the induced pigmentation. Secondly, the subjects need to be exposed to UVA rays for very long times and this is not considered ethically correct.

### In vitro determination of UVA photoprotection

In 2007 Colipa issued the *in vitro* evaluation method of the UVA protection factor and of the critical wavelength.<sup>19</sup> After two subsequent revisions, in 2009<sup>20</sup> and in 2011<sup>21</sup> the Colipa method was substituted by the ISO 24443:2012 method.<sup>22</sup>

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This method is based on the transmittance through a layer of sunscreen product applied on a standard substrate (PPMA polymethylmethacrylate Plexiglas), before and after controlled UV exposure. The sunscreen sample is exposed to an irradiation dose proportional to the initial UVA protection factor UVAPF<sub>0</sub>, calculated from the adjusted absorbance data of the non-exposed sample.

This method permits to determine the UVA protection factor (UVAPF) of the tested sunscreen product, the ratio between the labelled SPF protection factor, which was determined by the above-described *in vivo* test, the UVAPF and the critical wavelength.

### FDA 2011 final rule

The FDA (1978) was the first *in vivo* method created for the evaluation of SPF on informed human volunteers. It was revised in 1993, 1999 and 2011.

The last revision issued on 17 June 2011 by the Federal Register<sup>11</sup> is very similar to the ISO 24444:2010 method. In fact, both are grounded on the International Sun Protection Factor (SPF) test method, Colipa, 2006.

The slight differences between the ISO and FDA methods have no influence on the results. Therefore, the SPF values obtained with the two methods are comparable.

The main differences between the two methods are the following:

- The calibration times of the UV source (12 months for the FDA and 18 months for the ISO).
- The reference sunscreen products (P2 for the FDA and P2, P3 or P7 for the ISO).
- The progressions of exposure times used.

Furthermore, with regard to the labelling of the sunscreen products:

- The FDA method establishes that the maximum SPF value to be reported on the label is 50+.
- The UVA protection can be indicated on the label as a 'broad spectrum' UVA protection only for sunscreen products having SPF  $\geq 15$  and a critical wavelength of at least 370 nm.

The water resistance claims allowed to be reported on label are the following:

- 40-minute water resistance, followed by the SPF value measured after the immersion.
- 80-minute water resistance, followed by the SPF value measured after the immersion.

Lastly, in the US sunscreen products are not considered cosmetic but over-the-counter (OTC) products.



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### Australia sunscreen standard AS/NZS 2604:2012

Issued in 2012,<sup>23</sup> it indicates ISO 24444 and 24443 as methods for the determination of *in vivo* SPF and *in vitro* UVA protection factor. Sunscreen products are classified in the following way.

#### Therapeutic sunscreens

These are regulated by TGA (Therapeutic Goods Administration), i.e. sunscreen products with SPF  $\geq 4$  having the primary function of protecting from UV rays and protective products such as insect repellent (SPF  $\geq 4$ ) or skin care products with SPF  $> 15$  having the secondary function of protecting from UV rays.

#### Cosmetic sunscreens

These are regulated by NICNAS (National Industrial Chemicals Notification & Assessment Scheme), i.e. protective products having the secondary function of protecting from UV rays (skin care products with SPF  $\leq 15$  and size  $< 300$  mL, lip balm and lip stick with solar filters, makeup products with solar filters).

The method AS/NZS 2604:2012 indicates 11 SPF values to be reported on the label:

- Low protection: 4-6-8-10
- Medium protection: 15-20-25
- High protection: 30-40-50
- Very high protection: 50+

All the products must have on the label the indication 'broad spectrum' UVA protection, apart from the coloured products with SPF  $< 30$ .

The water resistance claims to be reported on the label are:

- SPF from 4 to 7: no water resistance properties
- SPF from 8 to 14: maximum 40-minute water resistance properties
- SPF from 15 to 29: maximum 2-hour water resistance properties
- SPF  $> 30$ : maximum 4-hour water resistance properties

### Formulation solutions

It is quite simple to understand that, when taking into account all the described parameters (SPF, UVA, critical wavelength, water resistance) to be respected, the formulation of a sunscreen (especially at high SPF) becomes a hard path. Moreover, one should consider other variables, like the solubility and stability of some UV filters and, being a cosmetic, the spectrum of sensorial aspects.

Usually the best start point is the selection of the theoretical right combination of UV filters able to reach the required UVA and UVB protections. This can be done in different ways: starting from an old formula, already tested; following the suggestions of the suppliers and/or the use of a sunscreen simulator freely available online ([www.sunscreensimulator.basf.com](http://www.sunscreensimulator.basf.com)). Of course, the mixture of a fair number of UV filters at low percentage is better than a combination of two or three, both for safety concerns and also to achieve a synergic combination of effects. Ideally the aim is to reach the highest possible protection, with the minimum amount of UV filters. Moreover, a high amount of sunscreen ingredients is often associated with a bad (greasy or waxy) final perception on the skin.

Some good combinations could be:

- Diethylamino hydroxybenzoyl hexyl benzoate and ethylhexyl triazone in 3:1 ratio.
- Octocrylene and butyl methoxydibenzoylmethane in a ratio of more or less 3:1.
- Octocrylene + butyl methoxydibenzoylmethane + bis-ethylhexyloxyphenol methoxyphenyl triazine.

Moreover, the addition of small amount of inorganic UV filters helps to boost the SPF values, because solids are able to scatter the UV rays and increase their probability to hit molecules of 'chemical' UV filters.

This important phase requires also considerations concerning:

- The type of final users. The use of octocrylene, ethylhexyl methoxycinnamate and PABA derivatives is not suggested for sensitive skin or for baby products.<sup>24</sup>
- The market: Europe, Japan, US, etc; similarly to what happens for the test methods, also the number of available sunscreen ingredients is different according to the different legislations. In the US (where sunscreen products are sold as OTC and not as cosmetic products) the available number of UV filters is extremely limited in comparison to Europe. Also their maximum allowed percentages are different in many cases (ethylhexyl methoxycinnamate allowed at 10% in Europe, at 20% in Japan).
- If a water resistant formula is requested, the use of hydrosoluble UV filters (phenylbenzimidazol sulfonic acid) could be a bad idea.

In the second step, the perfect solution of solid UV filters (butyl methoxydibenzoylmethane, ethylhexyl triazone, etc.) must be guaranteed. This aspect is often neglected during the formulation process, with the consequence that, during its shelf life, the product slowly decreases its protective capacity due to the precipitation of filters. Therefore, this step asks for the selection of different oils (usually esters like ethylhexyl benzoate, dibutyl adipate, diisopropyl sebacate are good solubilisers) able to keep well dissolved the filters. A suggestion could be to prepare the oil phase of the formula (without waxy ingredients) and put it in the fridge in order to verify the stability of such a solution.

The third step is the selection of the emulsifiers and their combinations. This is linked, of course, to the desired or requested characteristics and functionalities: W/O or O/W, water resistant, fluid or creamy product. As an example, to achieve a water resistant product the best

solution, of course, is a W/O emulsion, but in order to make the formula sprayable, probably O/W formulas are more suitable. One dilemma could be the request of a sprayable emulsion with water resistant efficacy; one solution is the stabilisation of the emulsion using a little amount of emulsifiers helped by the introduction of polymers (like acrylates derivatives). The choice of O/W formula requires, also, the introduction of a film-former. On the market, the most used are the acrylates/octylacrylamide copolymer and the VP/eicosene copolymer.

The following steps are dedicated to the selection of sensorial modifiers able to improve the skin feel of the formula. Probably in the past the bad feel of a high SPF product was tacitly accepted. On the contrary, in recent years, where the premium brands founded their success on amazing sensorial performances, the request for a more and more pleasant texture is increasing also for this kind of product.

If someone thinks that this is already a big hurdle for the formulator, now, the new request from the market is for pigmented, water resistant, high SPF (50 or 50+), sprayable sunscreen with a velvety feel. This means that in addition to all steps already listed, the difficulties to realise a pigmented emulsion (stability, sedimentation, colour homogeneity, etc.) have to be taken into consideration.

## Conclusion

In conclusion, effective photoprotection represents the best way to keep our skin healthy in sun exposed environments, even if the cosmetic protection does not have to induce to an imprudent and prolonged exposure to the sun's rays. The gradual harmonisation of the evaluation methods of the efficacy of sunscreens will lead to greater safety for all consumers. Furthermore, the several warnings described on the labels may help the consumers to better protect their own health, increasing their awareness about the risks of prolonged and thoughtless sun exposure.

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## References

- 1 Bonfigli A. Misura del fattore di protezione solare, in *Manuale del Cosmetologo. Tecniche Nuove* 2007: 455-63.
- 2 Department of Health and Human Services, FDA, USA. Sunscreen drug products for over-the-counter human use; tentative final monograph; proposed rule. *Federal Register* 1993; **58** (90): 28194-302.
- 3 Deutsches Institut für Normung (DIN). *Experimental evaluation of erythema protection of external sunscreen products for the human skin*. Revision of DIN 67501 (Draft), 5/1993.
- 4 Standard Association of Australia. *Sunscreen products, evaluation and classification*. AS 2604-1986.
- 5 Japan Cosmetic Industry Association (JCIA). *Standard SPF Test Method*, 1991.
- 6 South African Bureau of Standards: *Standard specifications, sunscreen products*. SABS 1557:1992.
- 7 Colipa Sun Protection Factor Test Method. Ref. 94/289 October 1994.
- 8 International Sun Protection Factor (SPF) Test Method 001-2003 (dated February 2003). Proposed by CTFA South Africa, COLIPA and JCIA for methodology.
- 9 International Sun Protection Factor (SPF) Test Method Colipa, CTFA South Africa, JCIA and CTFA, 2006.
- 10 International Standard ISO 24444:2010. *In vivo* determination of the sun protection factor (SPF).
- 11 Department of Health and Human Services, FDA, USA. Labelling and effectiveness testing; sunscreen drug products for over-the-counter human use. *Federal Register* 2011; **76** (117): 35620-65.
- 12 Commission Recommendation of 22/09/06 on the efficacy of sunscreen products and the claims made relating thereto, document C(2006) 4089, (2006/647/EC).
- 13 Colipa Recommendation N°23-24 – February 2009.
- 14 Guidelines for Evaluating Sun Product Water Resistance, proposed by COLIPA December 2005.
- 15 Colipa Recommendation N°16 – July 2006.
- 16 Diffey BL, Robson J. A new substrate to measure sunscreen protection factors throughout the ultraviolet spectrum. *J Soc Cosmet Chem* 1989; **40**: 127-33.
- 17 Determinations on volunteers of the UVA protection factor of cosmetic product(s) according to Japanese cosmetic industry association method (dated 15/11/1995).
- 18 International Standard ISO 24442:2011. *In vivo* determination of sunscreen UVA protection.
- 19 The European Cosmetic Toiletry and Perfumery Association, Colipa (2007). *Method for the in vitro determination of UVA protection provided by sunscreen products*. Ref: 2007a.
- 20 *In vitro* method for the determination of the UVA protection factor and 'critical wavelength' values of sunscreen products. Colipa Guidelines, June 2009.
- 21 *In vitro* method for the determination of the UVA protection factor and 'critical wavelength' values of sunscreen products. Colipa Guidelines, March 2011.
- 22 International Standard ISO 24443:2012. *Determination of sunscreen UVA photoprotection in vitro*.
- 23 Australian/New Zealand Standard. AS/NZS 2604:2012. *Sunscreen products – Evaluation and classification*.
- 24 Pigatto et al. Photopatch tests: an Italian multicenter study from 2004 to 2006. *Contact Dermatitis* 2008; **59**: 103-8.