In vivo persistent pigment darkening method: proposal of a new standard product for UVA protection factor determination

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Synopsis

The European Commission (EC) has recommended assessing the level of ultraviolet A (UVA) protection afforded by sunscreen products using the in vivo persistent pigment darkening (PPD) method or other methods giving equivalent results. In this context, the reproducibility of the in vivo PPD method is of importance. To check the validity of the UVA protection factor (UVAPF) tests, the Japanese Cosmetic Industry Association (JCIA) recommends using a standard product (JCIA standard) with an expected UVAPF 3.75 (SD 1.01). However, considering the increase in UVA efficacy of the new sunscreen products available in the market, with UVAPF up to 30, it seemed useful to develop a new standard product to be used when testing products with expected UVAPF ≥10. The PPD method was used in six centres to determine the UVAPF of the two products. Reproducibility of results was also studied by testing two batches of the new product at two different times. There was no statistical difference between the six centres with regard to the JCIA standard. The ring study showed that the mean value of UVAPF (4.3) was higher than that given by JCIA (3.75). These data enable the proposal of a new acceptance range for the JCIA standard product (3.4–5.2) derived from actual results from European laboratories. Whereas this range is different from that proposed by JCIA (2.74–4.76), there is an overlapping of the values. Data on the new standard product show that reproducibility is not influenced by the batches of this product. The mean UVAPF value obtained is 12.1. An acceptance range (9.6–14.6) is proposed for the new standard. Data presented here demonstrate that if an identical protocol is used, reproducible results can be expected and that the PPD method is reproducible and reliable.

Résumé

La Commission Européenne a recommandé d’évaluer la protection UVA apportée par les produits solaires en utilisant la méthode dite de pigmentation immédiate persistante (PPD) in vivo ou d’autres méthodes donnant des résultats équivalents. Dans ce contexte, la reproductibilité de la méthode PPD in vivo est très importante. Pour vérifier la validité des tests de facteurs de protection UVA (FPUVA), la JCIA recommande l’utilisation d’un produit standard de FPUVA attendu moyen de 3.75 (écart-type 1.01). Du fait de l’augmentation du niveau de protection vis-à-vis des UVA des nouveaux produits solaires disponibles sur le marché (FPUVA jusqu’à 30), il a semblé utile de proposer un nouvel produit standard à utiliser lors des tests de produits ayant un FPUVA attendu supérieur ou égal à 10. La méthode PPD a été utilisée pour déterminer dans 6 centres de
Introduction

A number of methods have been proposed to evaluate the effectiveness of sunscreens to protect against UVA in vivo [1, 2]. The endpoints used in these methods include the threshold for phototoxicity of psoralen-sensitized skin [3, 4], the threshold for immediate pigment darkening (IPD) [5], the threshold for erythema or pigmentation at 24-h post-exposure [6–8] and the threshold for a persistent pigment darkening (PPD) reaction at 2-h post-exposure [9–13].

Whereas the action spectra of UVA damage have not been fully established, it appears wide that the widest UVA spectrum should be taken into account and that any available in vivo method should be based on a measurable skin response with an action spectrum extending throughout the UVA waveband. PPD is an endpoint that is sensitive to the entire UVA spectrum [6–11], as the action spectrum of PPD in the UVA range decreases very slowly at longer wavelengths [9–11]. PPD is an endpoint proportional to the UVA dose received by the skin and it follows photo-biological reciprocity law within the usual range of testing flux rates (up to 130 mW cm$^{-2}$) unlike IPD observed immediately post-exposure [9, 10]. The PPD response requires doses, which are realistic. The minimal pigmenting dose is about 20 J cm$^{-2}$ (approximately 1-h exposure to midday summer sunlight), the stability of sunscreens is also challenged during a PPD test procedure [14]. PPD has been adopted as the endpoint to determine UVA effectiveness of sunscreens by the Japanese Cosmetic Industry Association (JCIA) [12, 13]. The PPD method is now recommended by the European commission (EC) as one of the methods to be used for determination of UVA protection level of sunscreen products [15]. However, development of in vitro testing methods which are also able to provide an equivalent degree of protection has been requested by the EC.

The PPD method for testing sunscreen products has been shown to be appropriate for testing products with very high UVAPF (>20) [16]. Reported data show that if an identical protocol is used, it is reasonable to expect that tests results from different laboratories will be consistent.

Despite the UVAPF test method being standardized, there is still the potential for test outcome differences because of the fact that, like the sun protection factor (SPF) test, the UVAPF test is a biological assay, not an analytical one. To ensure the reproducibility of the results, a thorough protocol has been described by the experts of the Agence Française de sécurité sanitaire des produits de santé (Afssaps) sun protection group [15].

To validate PPD testing, the JCIA method proposes the use of a standard product. The mean UVAPF value of the standard product shall be in the range 3.75 ± 1.01 (1.01 is equal to 1 SD) otherwise the product tested is rejected.

Since 1995, the UVA protection level of sunscreen products has increased dramatically, and it is now possible to reach UVAPF higher than 20. Thus, it seemed necessary to us to develop a second standard control product for testing products with higher UVAPF (UVAPF ≥10).

The purpose of the study reported here was to demonstrate the reproducibility of results on the two standard products in six independent test centres in Europe and to define a UVAPF range within which the mean PPD test value should fall for the data to be valid. The two standard products have been tested twice using different batches, first in April/May 2006 (Part 1) and second in November 2006 (Part 2). One of the six centres (C4) did not participate in Part 2 of the study.
Materials and methods

The protocol used for UVAPF determination is based on the JCIA method, but some specifications were added to improve the reproducibility of results between laboratories as already published [16].

Subjects

One hundred and sixteen subjects were included in the study. They were normal subjects with no history of photosensitivity, not under any medication and have not been exposed to UV light or sunlight in the previous 2 months. They were included following informed consent. Men and women were included with Fitzpatrick skin types II, III and IV [17]. In addition, colorimetric measurements were carried out and skin colour categories were defined as [Arc Tangent ((L*−50)/b*)] 180° 13. 14159 ITA° (Individual Typology Angle) values [18, 19] in the CIE (1976) L*a*b* colour space [20]. It was decided to only include volunteers with ITA° from 20° to 41° to avoid the development of UVA erythema rather than PPD on very fair skin (ITA°>41°). Individuals with dark skin (<20°) were not included because of the lack of contrast between current and induced skin pigmentation. The colorimetric criteria for inclusion of subjects are not described in the JCIA method.

UVA source for testing

The UVA source used in each test centre was a xenon arc solar simulator (Multiport 601 150 or 300 Watts, Solar Light Company, Philadelphia, PA, USA) filtered with a dichroic mirror, a Schott WG335, 3-mm thick short cut-off filter to minimize the UVB contribution and a UG11, 1-mm thick filter to minimize the visible and IR contributions to the spectral output. The exposure of the source was equipped with six liquid light guides (diameter 8 mm). The output irradiance from each successive light guide was adjusted to be 25% less than that of the adjacent guide. The maximum UVA irradiance in the testing centres was ranging from 62 to 113 mW cm⁻² as measured by radiometry depending on the laboratory UVA source. The time used for exposure of the unprotected area was from 5 to 9 min depending on the laboratory UVA source. All six laboratories participating to the studies complied with the requirements of the JCIA standard (12): the ratio of emitted UVA II (320–340 nm) was from 8% to 20% of total UVA.

PPD test method procedure for in vivo UVA-PF determination

Before performing the study, compliance of the complete procedure with the protocol described by the Afsasss Sun protection group [15] was checked in each laboratory.

The test sites (30–54 cm²) were delineated on the back of the subjects. They were located between the waistline and scapulae and lateral to the midline. To improve the reproducibility of the UVAPF results, it has been recommended to use the application procedure as described in the International SPF test method and demonstrated on a CD Rom [21]. The test products were applied at the rate of 2.00 ± 0.04 mg cm⁻² and spread uniformly over one test area using a finger-cot except in one laboratory (C5), where no finger cots were used for Part 1 or Part 2 of the testing. The products were allowed to dry for 15–30 min before starting the UVA exposure. The exposure doses were calculated using a geometric series, each exposure dose being 25% less than the previous one. The series of six UVA doses chosen on unprotected skin according to the experience of the laboratory were multiplied by the expected UVA protection factor (UVAPF) of the products in the protected sites. The time delay prior to observation was calculated from the end of the exposure of the last area. It is the recommended practice to expose the unprotected area after protected areas. The minimal pigmenting dose (MPD) was assessed when PPD response was stable, i.e. 2–4 h after the end of UVA exposure. Visual evaluation was performed in bright (at least 500 lux) and uniform Northern-daylight equivalent illumination.

The minimal PPD dose for unprotected skin (MPDu) and that for protected skin (MPDp) were visually determined. MPDu and MPDp were defined as the amount of radiant energy required to produce the first unambiguous pigmented reaction. The UVAPF of each product for each subject was then calculated based on the ratio of the minimum threshold PPD dose (MPDp) on protected site divided by the threshold PPD dose (MPDu) on unprotected site.

\[
\text{UVA protection factor} = \frac{\text{MPDp}}{\text{MPDu}}
\]
The PPD UVAPF for each product was calculated as the arithmetic mean of the individual UVAPF obtained from at least 10 subjects. According to the JCIA method [12], the mean UVAPF value is accepted if the standard error (SE) is <10% of the mean measured UVAPF value. Otherwise the number of subjects shall be supplemented so that the SE falls within 10%.

**JCIA standard control product (coded S1)**

The standard control product recommended by JCIA method [12] is water in oil cream with 3% ethylhexylmethoxycinnamate as a UVB filter and 5% butylmethoxydibenzoylmethane (Avobenzone, Givandan Roure, Vernier, Switzerland) as a UVA filter. The mean UVAPF value of the control product should be in the range 3.75 ± 1.01 according to the JCIA measurement standard. The participating centres used an expected UVAPF value of 4 for defining UVA dose of exposure. In Part 1, they each used a sample from same batch of the standard and in Part 2 their own batch.

**New control sunscreen standard (coded S2)**

This product (developed by Coty-Lancaster research) is a water in oil cream containing 3% ethylhexylmethoxycinnamate, and 3% octocrylene as UVB filters, 5% butyl methoxydibenzoylmethane (Avobenzone) and 2% BEMT (bemotrizinol, Tinosorb S™) as UVA filters. An expected UVAPF value between 10 and 14 was indicated to the testing centres, which tested one batch of the product in Part 1 and a second batch in Part 2.

**Statistics**

One way ANOVA and comparison by Tukey’s test ($P < 0.05$) were used for laboratory comparisons in each part of the study. $t$-test was used for independent samples ($P < 0.05$) for comparison between the two parts of the study. Pearson’s correlation test was used for ITA° versus UVAPF ($P < 0.05$).

**Results**

**Phototypes and ITA° of the subjects**

The distribution of phototypes and ITA° value range in each centre for each part of the study is given in Table I. A majority of phototypes III and IV and few phototype II were included. There is no significant ($P > 0.05$) influence of skin colour (ITA° value) on the results. These data confirm that there is no relationship between skin types and UVAPF values (1.12).

**JCIA standard product (S1)**

Tables II and III show the data from the six laboratories for JCIA standard.

<table>
<thead>
<tr>
<th>Part 1</th>
<th>Part 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall mean</td>
<td>Overall mean</td>
</tr>
<tr>
<td>$n$</td>
<td>11</td>
</tr>
<tr>
<td>Mean UVA PF</td>
<td>4.9</td>
</tr>
<tr>
<td>SD</td>
<td>0.8</td>
</tr>
<tr>
<td>SE</td>
<td>0.3</td>
</tr>
<tr>
<td>10% of the mean</td>
<td>0.49</td>
</tr>
</tbody>
</table>

SD, standard deviation; SE, standard error of the mean.

**Table I** Phototypes and ITA° of the subjects

<table>
<thead>
<tr>
<th>Phototype Part 1</th>
<th>Phototype Part 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>C2</td>
</tr>
<tr>
<td>5 III, 7 IV</td>
<td>5 III, 5 IV</td>
</tr>
<tr>
<td>Mean ITA° Part 1</td>
<td>36.8</td>
</tr>
<tr>
<td>Mean ITA° Part 2</td>
<td>32.1</td>
</tr>
</tbody>
</table>
the tests were performed on 10–12 subjects. However, the acceptance criterion [12] (SE within 10% of the mean UVAPF) was fulfilled with 10 subjects. One lab (C1) found a mean value (4.9) out of the acceptance range of the JCIA method in Part 1 of the ring study. The mean values range from 3.8 to 4.9 in Part 1 and from 3.7 to 4.5 in Part 2.

**New standard product (S2)**

Tables IV and V show the data on the new proposed standard S2.

All of the tests were performed on 10–12 subjects. However, the criterion (SE within 10% of the mean UVAPF) was fulfilled with 10 subjects. The mean values range from 9.1 to 14.5 in Part 1 and from 11.4 to 12.6 in Part 2.

In Part 1, the result is significantly lower in laboratory C2 compared to laboratories C3, C4, C5 and C6 (P < 0.05). In Part 2, the results between the laboratories are not significantly different (P > 0.05).

**Table IV** Comparison of (UVAPF) mean results between laboratories for the new standard product S2 Part 1 of the study

<table>
<thead>
<tr>
<th></th>
<th>C1</th>
<th>C2</th>
<th>C3</th>
<th>C4</th>
<th>C5</th>
<th>C6</th>
<th>Overall mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>11</td>
<td>12</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>63</td>
</tr>
<tr>
<td>Mean UVA PF</td>
<td>11.8</td>
<td>9.1</td>
<td>14.2</td>
<td>13.1</td>
<td>12.2</td>
<td>14.5</td>
<td>12.2</td>
</tr>
<tr>
<td>SD</td>
<td>1.4</td>
<td>1.8</td>
<td>3.7</td>
<td>2.4</td>
<td>1.8</td>
<td>2.3</td>
<td>2.8</td>
</tr>
<tr>
<td>SE</td>
<td>0.4</td>
<td>0.5</td>
<td>1.2</td>
<td>0.8</td>
<td>0.6</td>
<td>0.7</td>
<td>0.4</td>
</tr>
<tr>
<td>10% of the mean</td>
<td>1.2</td>
<td>0.9</td>
<td>1.4</td>
<td>1.3</td>
<td>1.2</td>
<td>1.5</td>
<td></td>
</tr>
</tbody>
</table>

**Table V** Comparison of UVA mean results between laboratories for the new standard product S2 Part 2 of the study

<table>
<thead>
<tr>
<th></th>
<th>C1</th>
<th>C2</th>
<th>C3</th>
<th>C5</th>
<th>C6</th>
<th>Overall mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>11</td>
<td>12</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>53</td>
</tr>
<tr>
<td>Mean UVA PF</td>
<td>12.3</td>
<td>11.4</td>
<td>12.4</td>
<td>12.6</td>
<td>11.9</td>
<td>12.1</td>
</tr>
<tr>
<td>SD</td>
<td>2.8</td>
<td>2.6</td>
<td>2.0</td>
<td>0.3</td>
<td>2.2</td>
<td>2.2</td>
</tr>
<tr>
<td>SE</td>
<td>0.9</td>
<td>0.7</td>
<td>0.6</td>
<td>0.1</td>
<td>0.7</td>
<td>0.3</td>
</tr>
<tr>
<td>10% of the mean</td>
<td>1.2</td>
<td>1.1</td>
<td>1.2</td>
<td>1.3</td>
<td>1.2</td>
<td></td>
</tr>
</tbody>
</table>

Only C2 found a significantly higher UVAPF in Part 2 compared to Part 1 (P = 0.02). The results from the other laboratories are not different (P > 0.2) between the two periods of testing on the two different batches of product.

There was no adverse event related to the product throughout the test on 116 subjects.

**Influence of finger cot**

There is no statistical difference (P > 0.05) between the results obtained by laboratory C5, which did not use a finger cot for application of the two products in the two parts of the study compared with those obtained by the other laboratories except C2 in Part 1. Because C2 results are significantly different from those of other laboratories (C3, C4, C6), we believe the difference between C2 and C5 is not linked to the use of the finger cot. Globally, the variability between subjects is lower in laboratory C5 compared with that of others. C5 has declared that the application of the product S2 is easier without than with finger cot.

**Calculations for acceptance ranges of future UVAPF tests**

**JCIA standard S1**

To calculate the ranges for acceptance of future UVAPF tests, the global mean including all individual values collected through the two parts of this study was calculated. The total number of individual values was 116. The overall mean is equal to 4.3 with an SD of 0.9. The variability (SD/ Mean) is 20.9%. Therefore, the acceptance range of values proposed for S1 is based on the Mean ± 1 SD, i.e. (3.4 – 5.2).
New standard S2 for UVAPF ≥ 10

The overall mean ± SD covering 116 individual data was calculated and is equal to 12.1 ± 2.5. The variability (SD/mean) is 20.7%. Therefore, the acceptance range of values proposed for S2 is 9.6–14.6.

Discussion

The data reported in Tables II and III for the JCIA standard S1 show that there is no statistical difference between the six laboratories involved in the study and within each laboratory at two different times of testing with a gap of 6 months in-between. In Part 1, the laboratories used the same batch and in Part 2 their own batch. The data show that data and reproducibility are not influenced by the batches of this standard product. The acceptance criterion as proposed by the JCIA method (SEM within 10% of the mean UVAPF) is fulfilled with 10 subjects in each series even if some laboratories included more subjects. One laboratory found a mean value (4.9) in Part 1 of the study out of the acceptance range of the JCIA method, even if this value was not significantly different from those obtained in the other laboratories involved in this study. This ring study shows that the mean value of the testing laboratory’s mean values (4.3) is higher than that given by JCIA (3.75). This finding leads to the proposal of a new acceptance range for S1. Using all individual data (116 subjects) collected in this ring test, the acceptance range of values proposed based on the overall mean ± 1 SD (4.3 ± 0.9) is (3.4–5.2). All mean UVAPF results in the ring study are compliant with this range except laboratory C2 in Part 1.

The variability calculated as SD/overall mean for S1 and S2 is close to 20%, which can be considered as acceptable because of the fact that UVAPF testing is a biological essay. Results from the two ring tests after a space of 6 months demonstrate that the PPD method is able to provide reproducible results.

The data illustrate that if an identical protocol is used, it is reasonable to expect consistent results. The UVAPF values obtained for the JCIA standard S1 showed the necessity to propose a new acceptance range for validation of the test data. A new standard product S2 with a mean UVAPF 12.1 is proposed as support for validation of UVAPF evaluation tests on products having an expected UVAPF ≥10. The ring studies have proven that it is possible to obtain results within a reasonable range of variability on two different batches. The acceptance range of UVAPF values proposed enables non-consistent data to be excluded. Therefore, the inclusion of this new standard S2 in a future ISO standard for UVA protection evaluation should be recommended.

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