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## The sun protection factor (SPF) inadequately defines broad spectrum photoprotection: demonstration using skin reconstructed *in vitro* exposed to UVA, UVB or UV-solar simulated radiation

Wavelength specific biological damage has been previously identified in human skin reconstructed *in vitro*. Sunburn cell and pyrimidine dimers were found after UVB exposure, and alterations of dermal fibroblasts after UVA exposure. These damages permitted us to discriminate UVB and UVA single absorbers. The present study shows that these biological effects can be obtained simultaneously by a combined UVB + UVA exposure using ultraviolet solar simulated light (UV-SSR), which represents a relevant UV source. In addition, the protection afforded by two broad spectrum sunscreen complex formulations was assessed after topical application. These two formulations displayed the same sun protection factor but different UVA protection factors determined by the persistent pigment darkening (PPD) method. Dose response experiments of UVA or UV-SSR showed that the preparation with the highest PF-UVA provided a better protection with regard to dermal damage compared to the other formulation. Using an original UVB source to obtain the UVB portion of SSR spectrum, the preparations provided the same protection. This study strikingly illustrates the fact that the photoprotection afforded by two sunscreen formulations having similar SPF values is not equal with regard to dermal damage related to photoaging.

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Solar UV radiation reaching earth is a combination of UVB (280–320 nm) and UVA (320–400 nm) wavelengths. Acute as well as chronic sun exposure is well known to induce biological and clinical damage, such as sunburn, immunosuppression, skin cancers and photoaging. UVB radiation, which includes the most energetic photons, participates in all of these damages, and can induce direct DNA lesions such as cyclobutane pyrimidine dimers (CPD) and 6,4 pyrimidine pyrimidone [1]. Subsequent mutations are involved in the development of UV-induced skin cancers [2]. However, although UVA radiation is less energetic than UVB, it accounts for at least 95% of the solar UV irradiance received at ground level. Recent studies pointed out their role in immune-suppression [3, 4], photoaging [5, 6] and mutagenesis [7, 8]. For this reason, the new generation of sunscreens has to provide an efficient protection against UVA as well as UVB radiation [9]. The assessment of the

efficiency of a sunscreen is primarily based on the value of its sun(burn) protection factor (SPF), which reflects the protection against erythema [10]. In the skin erythema response, the actual contribution of UVA wavelengths represents only a small percentage [11]. In addition, numerous biological damages induced by sun exposure occur at doses lower than that inducing erythema, especially in repeated exposure conditions [4, 5, 12–14]. Therefore, the SPF value, which is still the only regulatory requested protection factor for sunscreen products, does not seem sufficient to reflect the efficiency of protection against all biological end-points induced by the entire solar UV spectrum. For example the protective effect of sunscreens on UV-induced immune suppression has been largely assessed [15–17]. The evaluation of products filtering both UVB and UVA radiation have been shown to afford a better protection with regard to photoaging markers, than preparations that absorb mostly in the UVB range [18, 19]. It has also been suggested that the development of basal cell carcinoma in sunscreens users may be due to poor absorption of the sunscreens used in the UVA waveband [20].

Human skin reconstructed *in vitro*, composed of both a living dermal equivalent and a fully differentiated epidermis, permitted the identification of tissue specific damages

**Abbreviations:** UV, ultraviolet, 290–400nm; UVB, 280–320 nm; UVA, 320–400 nm; UV-SSR: ultraviolet-solar simulated radiation; FITC, fluorescein isothiocyanate; SBC, sunburn cell; BED, biologically efficient dose; MED, minimal erythema dose; SPF: sun protection factor; CPD: cyclobutane pyrimidine dimers.

induced by either UVB or UVA radiation [21, 22]. Epidermal keratinocytes were preferentially targeted by UVB, while UVA induced major alterations in the dermal compartment. Taking advantage of these specific damages and the possibility of applying topical sunscreens on the skin surface, the efficiency of single absorbers has been tested [23]. Other studies using organotypic cultures confirm their usefulness in the evaluation of photoprotection [24, 25]. However these studies were based on general parameters related to cytotoxicity.

The present study was conducted using experimental conditions drawing nearer to more realistic situations. Two prototype sunscreen formulations were prepared with combinations of different commonly used physical and chemical absorbers. These two well controlled formulations displayed specific characteristics in order to be compared. They could be classified as « broad spectrum » products according to the modified Diffey method [26]. They had similar SPF values but different UVA protection factors as measured using the PPD method [27]. The efficiency of these products was evaluated with regard to their ability to protect from biological damage induced by various UV radiations, such as UVA alone, UV-Solar simulated radiation (UV-SSR), or UVB radiation. The latest is totally original and rigorously reflects the UVB portion of the SSR spectrum. The results showed that the skin equivalent model permitted the assessment, after topical application, of the different protection afforded by sunscreens with regard to dermal damage induced either by UVA radiation alone or by UV-SSR. However, no difference between the sunscreens could be found when samples were exposed to UVB radiation alone or UV-SSR, and evaluated for sunburn related parameters.

## Material and methods

### UV sources

UV-SSR, UVA and UVB irradiations were performed using a 1000 Watts Xenon lamp equipped with a dichroic mirror (Oriel, Les Ulis, France) filtered by a UG5, 2 mm thick (Schott, Clichy, France). UVA radiation alone was obtained by inserting a WG335 (3 mm) Schott filter. The measured irradiance of the UV-SSR source obtained with a WG 320 (1.5 mm) Schott filter, complied to the European Cosmetic and Perfumery Association [11] UV-SSR criteria. The relative cumulative erythemal efficacy, which is the key parameter to assess the relevance of the spectrum, was equal to 56.7% for the 290-310 nm waveband (respectively 56.4% for the standard sun defined in the Colipa SPF test method), 86.9% for the 290-320 nm waveband (84.2%), 92.5% for the 290-330 nm waveband (90.3%), 94.7% for the 290-340 nm waveband (91.1%) and 96.3% for the 290-350 nm waveband (95.1%). The standard erythemal dose (SED) which characterizes the erythemal efficacy independently of the individual sensitivity has been recommended by the Commission Internationale de l'Éclairage [28]. The dose rate of the UV-SSR source was 46 SED/ hour, compared to the standard sun dose rate corresponding to 7.8 SED / hour. The UVB spectrum was obtained using a custom made filter obtained by deposition of thin layers on fused silica (Micro-Module, Le Plessix Paté, France). Specifications on the transmission of the filter were to absorb more than 50% of the incident light at 320 nm, to let pass a much light as

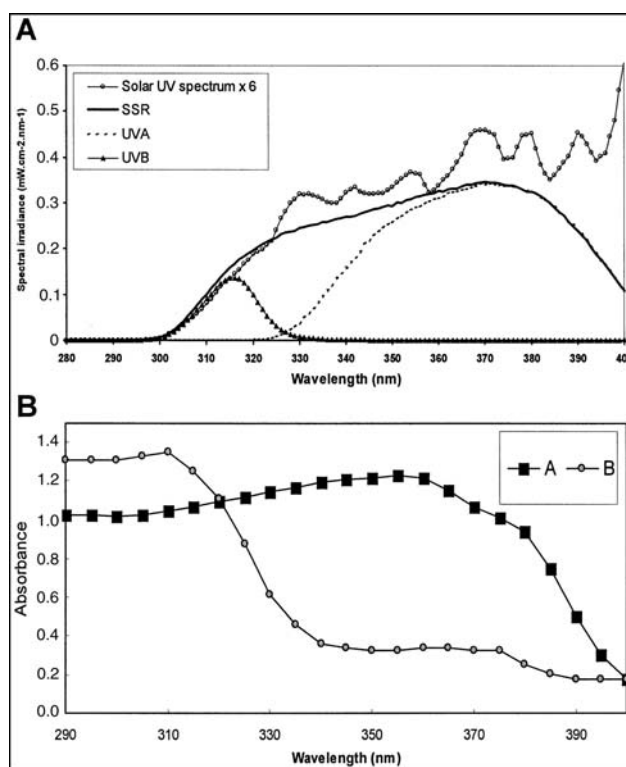


Figure 1. Spectral irradiance of UV sources at skin level and absorption profiles of sunscreen formulations.

**A:** UV-SSR, UVA or UVB radiation were all obtained from a Xenon lamp (1000 watts) equipped with a dichroic mirror and a Schott filter UG 5/2 mm. UV-SSR spectrum was obtained using an additional WG 320/ 1.4 mm Schott filter. UVA spectrum was obtained using an additional WG335/ 3 mm Schott filter. UVB spectrum was obtained using a custom-made filter (see text for detail). The sunlight spectrum is the standard reference spectrum defined in the Colipa SPF test method. Its spectral irradiance was multiplied by 6, which corresponds to the ratio of the dose rates in SED, in order to make the UVB part of the spectra match.

**B:** Absorbance curves obtained from the two sunscreen formulations A and B. Spectra of the products were generated spectro-radiometrically.

possible under 320 nm and to absorb as much UVA light as possible. The spectral irradiances were carefully measured with a spectroradiometer (Macam Photometrics, Livingston, UK) calibrated against traceable standard lamps (National Physics Laboratories, Teddington, UK) (Fig. 1A).

### Sunscreen formulations

Two prototype formulations, A and B, were prepared in the same simple oil-in-water vehicle. Formulation A contained 7% of UVB absorber octocrylene (Uvinul N539, BASF, Germany) and 3% of UVA absorber butyl-methoxydibenzoylmethane (Parsol 1989, Givaudan-Roure Vernier, Switzerland). Formulation B contained 3.75% of UVB filter octyl methoxycinnamate (OMC or Parsol MCX, Givaudan Roure Vernier, Switzerland), and 7.5% of zinc oxide (Z-Cote, Sunsmart, USA). Both formulations were characterised by their SPF, UVA-PF and absorbance curves. SPF values were determined in 20 human volun-

teers according to the COLIPA SPF test method. The values of the SPF were  $7.4 \pm 1.5$  for formulation A and  $7.5 \pm 1.6$  for formulation B. UVA protection factors were determined using the *in vivo* method based on persistent pigment darkening (PPD) [29]. The values of UVA-PF were  $7.2 \pm 1.8$  for formulation A and  $2.8 \pm 0.8$  for formulation B. The transmission spectra of the sunscreen products were obtained using a Diffey and Robson method [30], through a roughened quartz plate (Fig. 1B).

### Reconstructed skin *in vitro*

Dermal equivalents were prepared as previously described [31] using a collagen-fibroblast mixture containing  $10^6$  fibroblasts. After contraction, human keratinocytes were seeded on this support. The culture was maintained during 7 days in immersed conditions and raised at the air-liquid interface for another 7 day period to obtain a complete differentiation process. The same strains of cells were used for the whole study. Normal human keratinocytes were isolated from breast skin (age 24) obtained after mammary reduction and used at the first passage for skin reconstruction. Normal human dermal fibroblasts were isolated after spreading from mammary skin explants, and cultured in DMEM 10% fetal calf serum. Dermal fibroblasts were used at passage 7 for skin reconstruction. Each condition was performed in duplicate in an experiment. Each experiment was repeated at least three times.

### Irradiation procedure

Reconstructed skins on grids were irradiated without medium, 10 minutes after topical application of  $\sim 2 \text{ mg/cm}^2$  (checked by weighing) of the vehicle or sunscreens preparation [23]. Formulations were applied using a curved sterile Paster pipette. Samples were rinsed after irradiation using 3 times 7 ml of PBS before adding fresh medium under the grid. Samples were fixed for classical histology or frozen in liquid nitrogen for immunolabelling, 24 h or 48 h after UV exposure. During the post irradiation period, samples were maintained at  $37^\circ\text{C}$  and 5%  $\text{CO}_2$ , fed by capillarity with the medium.

### Immunostaining

Immunostaining was performed on  $5 \mu\text{m}$  cryostat sections as described previously [22]. Mouse monoclonal antibodies (Mab) were against cyclobutane pyrimidine dimers (CPD) (clone H3) [32], human vimentin (Monosan, The Netherlands). Fluorescein isothiocyanate (FITC)-conjugate rabbit anti-mouse immuno-globulins (Dako, Denmark) were used as second antibodies.

## Results

### Determination of the protective effect of both sunscreen formulations after UVA irradiation

The Biologically Efficient Dose after UVA exposure (UVA-BED) has been previously described in reconstructed skin as  $25 \text{ J/cm}^2$  [22]. The effects corresponded to dermal alteration characterised by the disappearance of fibroblasts in the superficial area of dermal equivalent 48 hours after exposure. The disappearance of dermal cells is due to an apoptotic process occurring during this period, and which could be visualized using the TUNEL

technique as soon as 6 hours after exposure. Epidermal alterations located in the granular layers occurred when UVA doses were increased above the BED (Fig. 2).

Two  $\text{mg/cm}^2$  of the sunscreen preparations and vehicle were topically applied. From the BED, the UVA dose was progressively increased by  $5 \text{ J/cm}^2$  increments. Visualization of fibroblasts within the dermal equivalent 48 hours after UVA exposure was performed by classical histology (Fig. 2A-E) and vimentin immunostaining (Fig. 2F-J). From  $50 \text{ J/cm}^2$  the protection afforded by sunscreen formulations could be distinguished. The skin treated with formulation A still showed a normal epidermal differentiation and the presence of numerous dermal fibroblasts. The skin treated with formulation B exhibited clear alterations of the granular layers and the disappearance of superficial fibroblasts.

To determine the upper limit of protection obtained with formulation A, UVA doses were increased to  $250 \text{ J/cm}^2$  (data not shown). At  $100 \text{ J/cm}^2$  UVA, a good morphology of both epidermis and dermal equivalent was obtained. At  $200 \text{ J/cm}^2$  UVA, the number of fibroblasts in the lattice was decreased, showing typical UVA-biological damage. At  $250 \text{ J/cm}^2$ , drastic alterations were observed, both dermal and epidermal. Therefore the upper dose was determined at the dose able to induce the disappearance of fibroblasts i.e.  $200 \text{ J/cm}^2$ .

### Determination of protection afforded by both sunscreen formulations after UV-SSR irradiation

We wondered if the differential protective efficiency of both sunscreens after UVA irradiation was still relevant after more realistic UV exposure. Actually, obtention of pure UVA radiation required the use of Schott filter WG 335/3mm. However, this led to a low level of short UVA wavelengths within 320 to 340 nm (Fig. 1A). The two sunscreens were then tested using UV-SSR. In addition, since both sunscreens display a similar SPF, the question of their ability to protect similarly against UV-SSR damage was important.

The first experiments were focussed on the determination of the UV-SSR biological efficient dose (Fig. 3) based on the UVB-BED previously determined as the dose able to induce SBC 24 hours after exposure [21] and the UVA-BED determined as described above. A dose response experiment was performed and samples were taken after 24 and 48 hours.

24 hours after UV-SSR exposure, samples displayed typical SBC within the epidermis at a dose of  $5.4 \text{ J/cm}^2$  UV ( $0.44 \text{ J/cm}^2$  UVB +  $4.96 \text{ J/cm}^2$  UVA) (Fig. 3A-B). Increasing the UV dose led to an increase in the number of SBC. At that dose, typical DNA lesions such as thymine dimers were induced, as shown on samples taken immediately after irradiation. Fig. 3D-E show positive nuclei in all epidermal keratinocytes. Analysis of dermal fibroblasts within the dermal equivalent showed that the dose of  $5.4 \text{ J/cm}^2$  UV-SSR induced the disappearance of fibroblasts 48 hours after exposure (Fig. 3A,C, F,G). Higher UV-SSR doses led to more drastic damage including alterations of epidermal morphology (not shown).

The UV-SSR-BED was thus determined at a dose of  $5.4 \text{ J/cm}^2$  UV, which corresponds to 3.1 SED.

In order to evaluate the protection afforded by the different formulations, the UV-SSR dose was progressively increased from the UV-SSR-BED up to  $98 \text{ J/cm}^2$  UV ( $90 \text{ J/cm}^2$  UVA +  $8 \text{ J/cm}^2$  UVB). The samples were taken

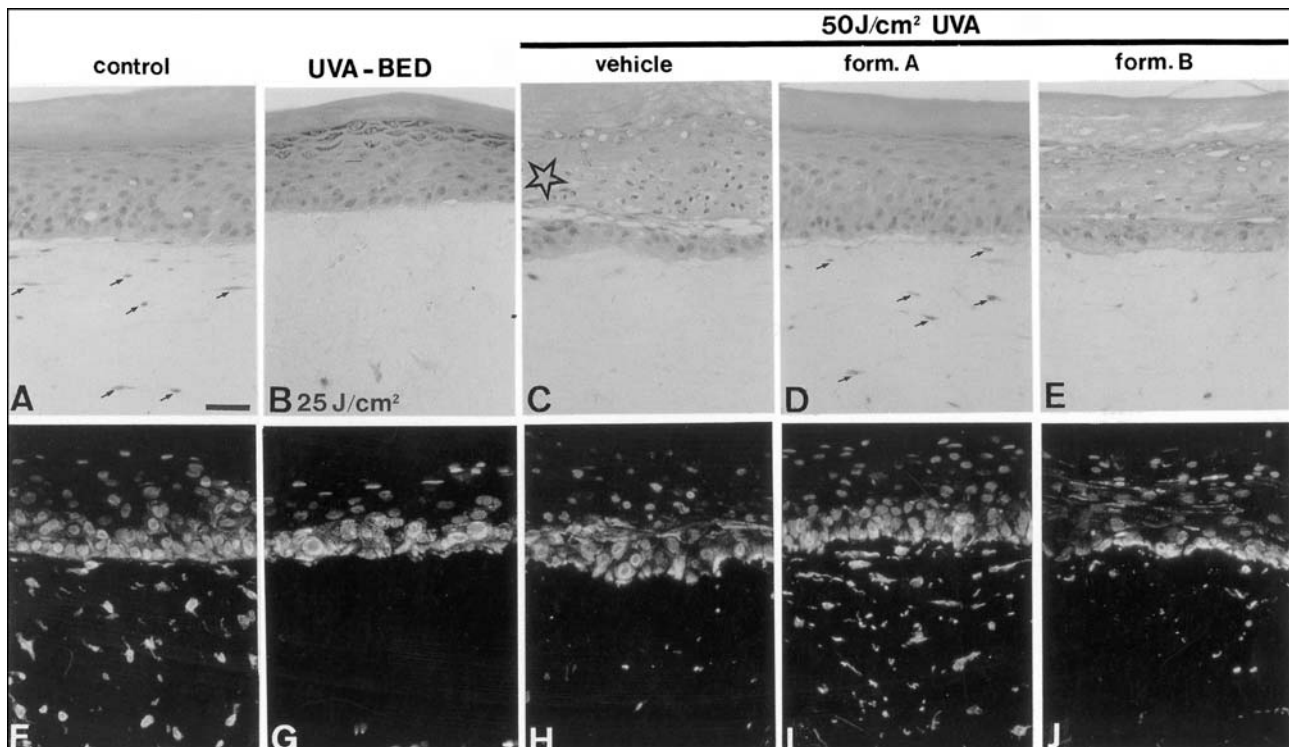


Figure 2. Evaluation of sunscreens protection in skin samples exposed to UVA radiation. **A-E**: Histology of skin reconstructed *in vitro* 48 hours after UVA exposure. **E-H**: vimentin immunostaining obtained 48 hours after UVA exposure. **A, F**: control sham-irradiated sample. **B, G**: sample exposed to  $25\text{ J/cm}^2$  UVA (UVA-BED). **C, H**: sample applied with vehicle prior to exposure  $50\text{ J/cm}^2$  UVA. **D, I**: sample treated with formulation A prior to exposure to  $50\text{ J/cm}^2$  UVA. **E, J**: sample treated with formulation B prior to exposure to  $50\text{ J/cm}^2$ . Note that superficial fibroblasts (arrows) are numerous in control sample and disappeared at the BED ( $25\text{ J/cm}^2$ ). Epidermal alterations occurred at higher UVA doses, mostly in the granular layers (star). Topical application of vehicle or formulation B prior UVA exposure, could not prevent the alterations. In contrast the application of formulation A led to avoid UVA-induced damages. bar:  $50\mu\text{m}$ .

24 or 48 hours after UV-SSR exposure, and analysed for SBC formation and fibroblast disappearance.

Vehicle treated samples showed numerous SBCs and a total absence of dermal fibroblasts at a dose of  $19.7\text{ J/cm}^2$  ( $19.1\text{ J/cm}^2$  UVA +  $0.6\text{ J/cm}^2$  UVB) (Fig. 4 A-B). Samples topically applied with both sunscreen formulations displayed a normal morphology up to a dose of  $27.6\text{ J/cm}^2$  UV ( $26.7\text{ J/cm}^2$  UVA +  $0.86\text{ J/cm}^2$  UVB). From that dose, sunscreen formulation B did not prevent the disappearance of dermal fibroblasts within the dermal equivalent (Fig. 4 C-F). Higher doses led us to emphasise the differential efficiency between sunscreens (Fig. 4 G-H). Anti-vimentin immunostaining also revealed the poor protection of dermal fibroblasts afforded by formulation B (Fig. 4 I-J). The damages were similar to those obtained with pure UVA radiation (see fig. 2). In addition, at the latter dose ( $76.3\text{ J/cm}^2$  UV), no SBCs were observed in samples pre-treated with either one or the other sunscreen formulation. Higher UV-SSR doses were then tested to analyse SBCs. However, higher UV-SSR dose  $87.1\text{ J/cm}^2$  UV ( $80\text{ J/cm}^2$  UVA +  $7.1\text{ J/cm}^2$  UVB) led to total destruction of the tissue when samples were treated with sunscreen B (not shown). As a result, the two sunscreens could not be compared with regard to this specific biological parameter.

#### Analysis of the protection using a UVB source

Since it was not possible to assess SBCs using UV-SSR exposure, samples were exposed to pure UVB radiation

corresponding to the UVB part of UV-SSR spectrum (Fig. 1A). This might avoid the destruction of the tissue at high UV-SSR doses, resulting from a high level of UVA.

A dose response experiment was performed to determine the BED.  $0.85\text{ J/cm}^2$  UV dose ( $0.62\text{ J/cm}^2$  UVB +  $0.23\text{ J/cm}^2$  UVA), corresponding to 4 SED, led to the formation of typical SBCs at 24 hours (Fig. 5 A-B) and numerous DNA lesions in all the epidermal layers immediately after exposure (Fig. 5 E-F).

Comparison of both sunscreens was conducted at UVB doses calculated from the proportion of UVB contained in UV-SSR dose resulting in the destruction of the tissue, i.e.  $87.1\text{ J/cm}^2$  UV (UVB =  $7.1\text{ J/cm}^2$  +  $80\text{ J/cm}^2$  UVA). Therefore the UVB doses chosen were  $7.7\text{ J/cm}^2$ ;  $8.5\text{ J/cm}^2$  UV and  $10.25\text{ J/cm}^2$  UV (5.6; 6.1 and  $7.45\text{ J/cm}^2$  UVB respectively).

These experiments showed that even at the highest UVB dose, the two sunscreen preparations prevent SBC and pyrimidine dimer formation equally (Fig. 5 C-D, G-H). No damage was observed compared to sham-irradiated samples. These data support and confirm the hypothesis that the absence of protection in samples treated with formulation B and exposed to UV-SSR essentially resulted from UVA-induced damage.

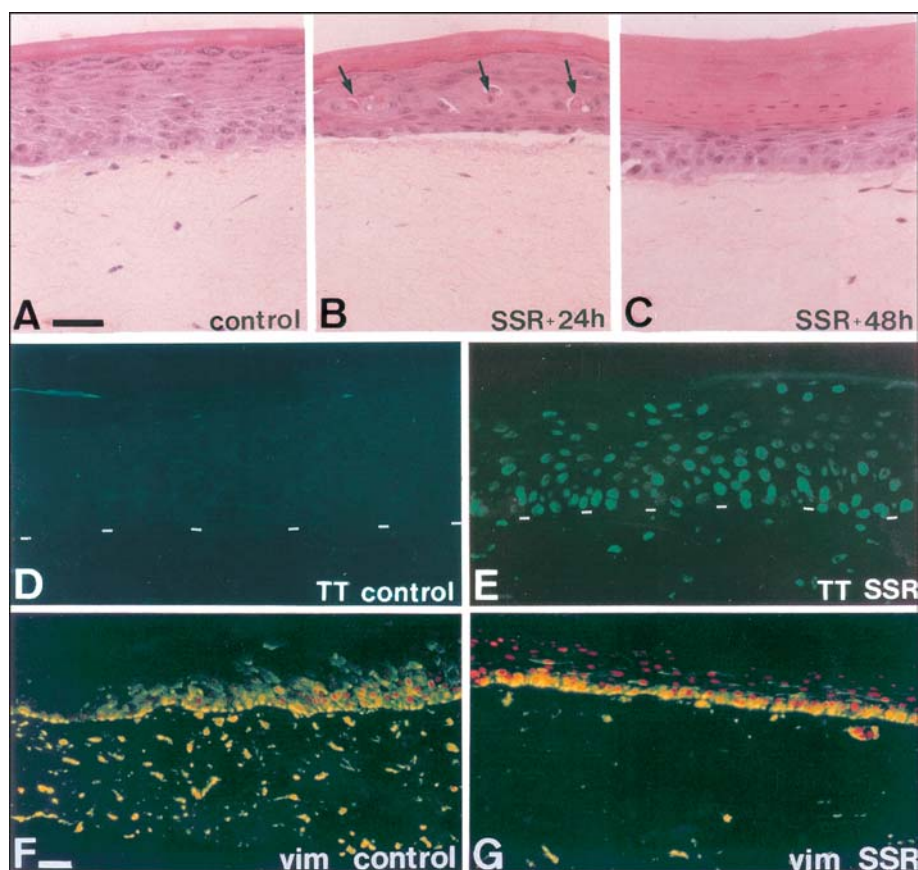


Figure 3. *Determination of UV-SSR Biologically Efficient Dose*

**A-C:** classical histology. **A:** sham-irradiated skin sample. **B:** skin sample taken 24 hours after exposure to 5.4 J/cm<sup>2</sup> (0.44 J/cm<sup>2</sup> UVB, 4.96 J/cm<sup>2</sup> UVA). **C:** skin sample taken 48 hours after exposure to 5.4 J/cm<sup>2</sup> UV. **D-E:** pyrimidine dimers detected using the H3 antibody immediately after UV-SSR exposure. **D:** sham irradiated sample, **E:** 5.4 J/cm<sup>2</sup> UV-SSR. **F-G:** anti vimentin immunostaining performed 48 hours after UV-SSR exposure. **F:** sham irradiated sample, **G:** 5.4 J/cm<sup>2</sup> UV. Note that 5.4 J/cm<sup>2</sup> UV-SSR led to the formation of SBC and DNA lesions. Exposure to the same UV-SSR dose also led to the disappearance of superficial fibroblasts at 48 hours. Bar: 50µm.

## Discussion

Relevant specific biological markers of UVB or UVA exposure have been identified in skin reconstructed *in vitro*. They made possible the evaluation of the efficiency of single absorbers in this model. The present study examines the relevance of the SPF value in broad spectrum photoprotection using more complex formulations and three relevant UV sources. Actually, sunscreen products usually combine chemical and/or physical absorbers [33] and solar UV radiation includes both UVB and UVA radiation with specific spectra [34]. The SPF value, which reflects the protection against sunburn and erythema, is the only protection value requested for commercial sunscreen products. Recent studies evidenced that the SPF does not systematically reflect the level of protection provided with regard to other biological or clinical damages [19, 35-37]. These studies also emphasize the role of UVA wavelengths in numerous biological effects. The two tested formulations in our study were designed to follow several criteria: i) rigorous similar SPF, ii) broad spectrum classification and iii) different profiles of filtration. This appeared important to perform a correct comparative analysis of two filtrating systems avoiding different vehicle or additional active components of commercial

products. Our results showed that the two formulations are different in their ability to protect dermal fibroblasts after UVA exposure. These alterations have been related to the photoaging process characterised by dermal drastic modifications of extracellular matrix [5, 6, 38]. Dose response experiments defined the upper UVA dose for both sunscreen formulations. Based on calculation of the value of SPF (ratio of MED with sunscreen/MED without sunscreen), we roughly estimate UVA protection factors in this model. The fibroblast alterations were taken as the referred biological end-point. Formulation A gave the value of 8 (200/25), and formulation B only 2 (50/25). These values are similar or at least of the same order of PPD values *in vivo* (PPD value for formulations A and B are 7.2 and 2.8 respectively).

These data also reinforce the fact that SPF values do not reflect the protection against various biological endpoints, even for sunscreens classified as "broad spectrum" [26]. To properly address that point, experiments were performed using more physiological exposure conditions, obtained with UV-SSR. Moreover, the determination of SPF value is recommended with this UV-spectrum [11, 39]. In addition, this study allowed the identification of the biological effects of UV-SSR on skin reconstructed *in vitro*. The formation of CPD and SBC could be mostly

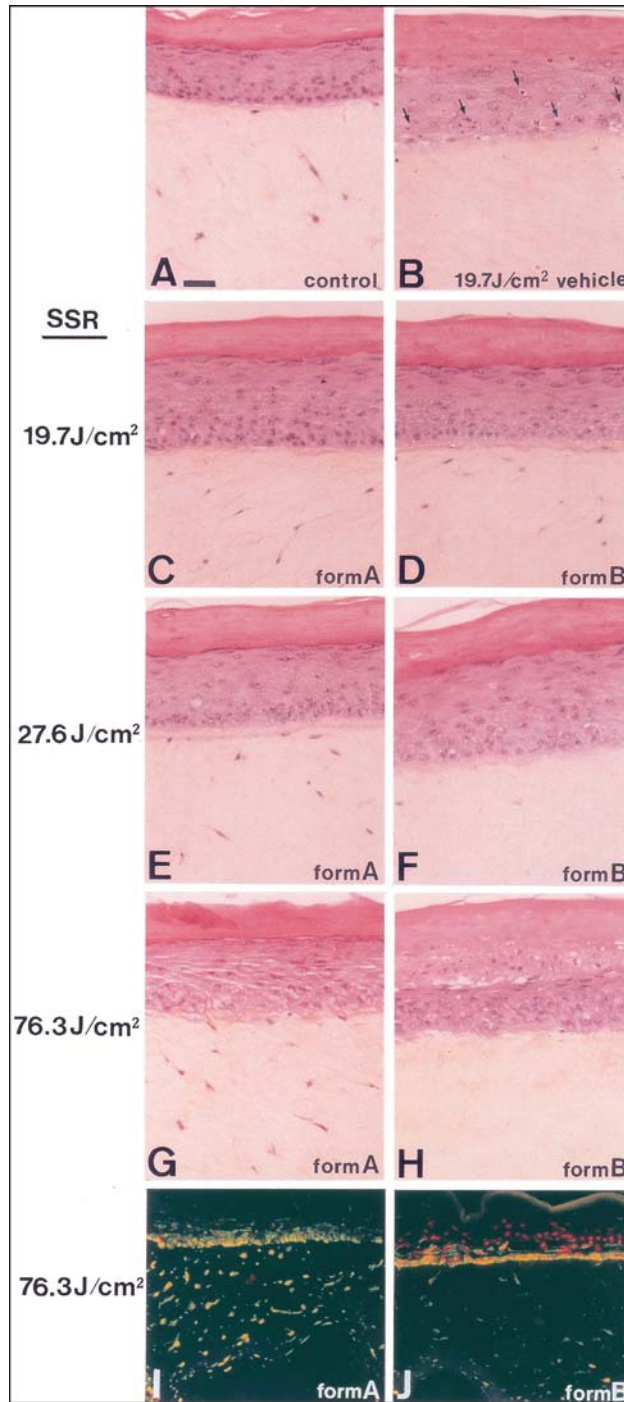


Figure 4. Determination of the protective efficiency of sunscreen formulations after UV-SSR exposure. **A-H**: histology. **A**: control sham irradiated sample. **B-D**: skin reconstructed in vitro exposed to 19.7 J/cm<sup>2</sup> UV (19.1 J/cm<sup>2</sup> UVA + 0.621 J/cm<sup>2</sup> UVB). **B**: vehicle, **C**: formulation A, **D**: formulation B. **E-F**: UV-SSR dose 27.6 J/cm<sup>2</sup> UV (26.7 J/cm<sup>2</sup> UVA + 0.87 J/cm<sup>2</sup> UVB); **E**: formulation A, **F**: formulation B. **G-H**: UV-SSR dose: 76.3 J/cm<sup>2</sup> UV (70 J/cm<sup>2</sup> UVA + 6.3 J/cm<sup>2</sup> UVB), **G**: formulation A, **H**: formulation B. **I-J** vimentin immunostaining of skin sample exposed to UV-SSR dose: 76.3 J/cm<sup>2</sup> UV, **I**: formulation A, **J**: formulation B. Note that both sunscreen preparations were efficient at the dose of 19.7 J/cm<sup>2</sup> UV-SSR. A differential protection was observed at the dose of 27.6 J/cm<sup>2</sup> UV-SSR, showing the absence of protection of dermal fibroblasts with formulation B. Even at higher doses such as 76.3 J/cm<sup>2</sup> UV-SSR, formulation A still protects the tissue while formulation B could not prevent dermal and epidermal alteration. However also note that no SBC could be detected in sunscreen treated samples. Bar: 50 μm

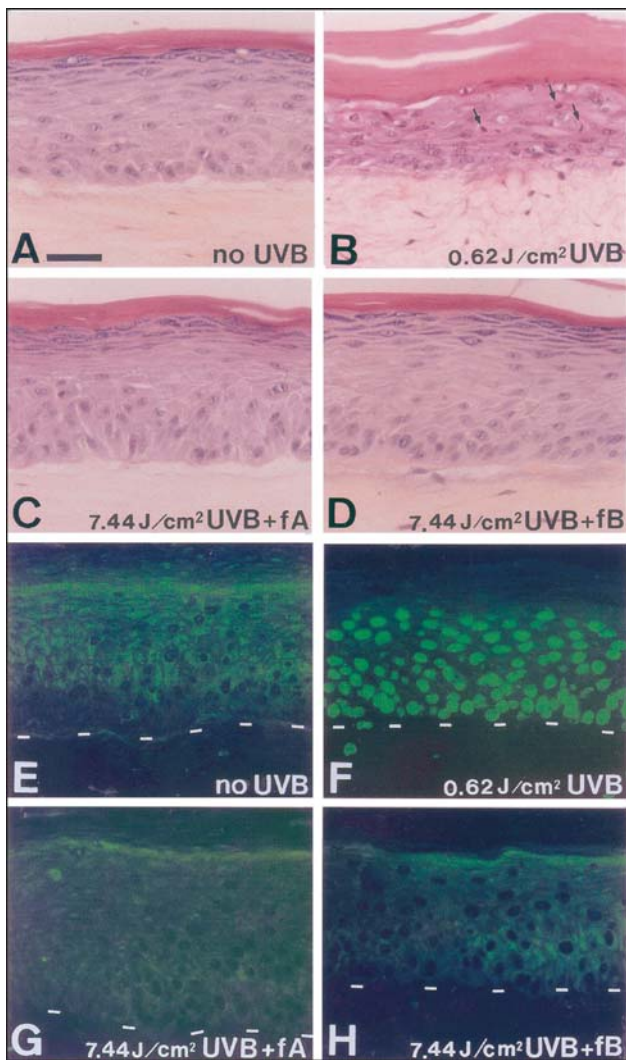


Figure 5. Visualization of SBCs and pyrimidine dimers after UVB exposure and treatment with formulations A or B. A-D: histological analysis of samples exposed to UVB and taken 24 hours after for SBC analysis. A: control sham irradiated sample. B: skin reconstructed *in vitro* exposed to  $0.854 \text{ J/cm}^2$  UV ( $0.62 \text{ J/cm}^2$  UVB). C-D: samples exposed to  $10.26 \text{ J/cm}^2$  UV ( $7.44 \text{ J/cm}^2$  UVB) and treated with formulation A (C) or B (D). E-H: samples taken immediately after UVB exposure and immunostained with the antibody directed against thymine dimers. E: sham irradiated sample. F: skin reconstructed *in vitro* exposed to  $0.854 \text{ J/cm}^2$  UV ( $0.62 \text{ J/cm}^2$  UVB). G: sample treated with formulation A and exposed to  $10.26 \text{ J/cm}^2$  UV ( $7.44 \text{ J/cm}^2$  UVB). H: sample treated with formulation B and exposed to  $10.26 \text{ J/cm}^2$  UV ( $7.44 \text{ J/cm}^2$  UVB). Note the formation of SBC (arrows) and DNA lesions in untreated skin reconstructed *in vitro* exposed to UVB and the absence of these damages in both sunscreen formulation treated samples. Bar:  $50 \mu\text{m}$ .

attributed to the UVB part of the spectrum [21, 40-42]. The dermal alterations seemed to be due to the UVA range and could be related to penetration of UVA radiation through the dermis [5, 6, 22, 43]. In the UV-SSR conditions the formulation having the higher PF-UVA provided better skin protection. Because high UV-SSR doses (including high level of UVA) led to the destruction of the

tissue protected with formulation B, the evaluation of SBC formation was not possible. The assumption that the low protection afforded by formulation B was due to poor UVA absorption was evaluated after UVB exposure. This study is the first one using such a "pure" UVB source which is not monochromatic. UVB radiation is generally obtained from fluorescent tubes. These tubes deliver a huge amount of short UVB wavelengths not present in the solar spectrum [44]. They are therefore often considered as physiologically irrelevant for photobiology experiments [45]. The present means of obtaining UVB radiation thus accurately reflects a more realistic UVB spectrum compared to outdoor conditions. SBCs as well as CPDs were indeed induced. However the doses required were higher than those using UVB fluorescent tubes [21]. This is probably due to the lack of highly energetic short UVB photons in the UVB-xenon spectrum. The two sunscreens were equally efficient with regard to SBC and CPD prevention, even at the highest UVB dose tested. These results are in agreement with those of Young *et al*, [46] showing that two sunscreens having similar SPF but a different spectral profile are equally efficient in preventing the formation of DNA dipyrimidine photolesions in human skin. Such a result is also in agreement with the correlation between erythema, thymidine dimers and SBC formation [47, 48].

Taken together, our results clearly point out that the value of SPF does not reflect the efficiency of sunscreens over the entire solar UV spectrum, and against major biological damage induced by sun exposure. In addition, they also confirmed human skin reconstructed *in vitro* as a powerful model for photobiology experiments and photoprotection evaluation. ■

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