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PERSPECTIVES

Sunscreens and their usefulness: have we made any progress in the last two decades?

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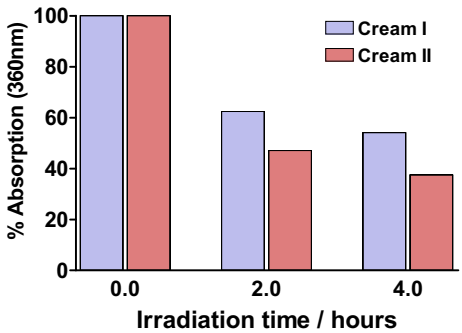
Abstract

Sunscreens have now been around for decades to mitigate the Sun's damaging ultraviolet (UV) radiation which, although essential for the existence of life, is a recognized prime carcinogen. Accordingly, have suncreams achieved their intended purposes towards protection against sunburns, skin photo-ageing and the like? Most importantly, however, have they provided the expected protection against skin cancers that current sunscreen products claim to do? In the last two decades, there have been tens, if not hundreds of studies on sunscreens with respect to skin protection against UVB (280−320 nm)—traditionally sunscreens with rather low sun protection factors (SPF) were intended to protect against this type of radiation—and UVA (320−400 nm) radiation; a distinction between SPF and UVA protection factor (UVA-PF) is made. Many of the studies of the last two decades have focused on protection against the more skin-penetrating UVA radiation. This non-exhaustive article reviews some of the important facets of what is currently known about sunscreens with regard (i) to the physical UV filters titanium dioxide (TiO_2) and zinc oxide (ZnO) and the mostly photo-unstable chemical UVB/UVA filters (e.g., octinoxate (OMC) and avobenzone (AVO), among others), (ii) to novel chemical sunscreen agents, (iii) to means that minimize the breakdown of chemical filters and improve their stability when exposed to UV sunlight, (iv) to SPF factors, and (v) to a short discussion on non-melanoma skin cancers and melanoma. Importantly, throughout the article we allude to the safety aspects of sunscreens and at the end ask the question: do active ingredients in sunscreen products pose a risk to human health, and what else can be done to enhance protection?

Graphic abstract

Significant loss of skin protection from two well-known commercial suncreams when exposed to simulated UV sunlight. Cream I: titanium dioxide, ethylhexyl triazone, avobenzone, and octinoxate; Cream II: octyl salicylate, oxybenzone, avobenzone, and octinoxate.



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1 Introduction

Sunlight UV radiation is classified as a complete carcinogen because it acts as both a mutagen and a non-specific damaging agent causing harmful effects to human health owing

to its properties as a tumor initiator and promoter of skin cancers (e.g., basal cell carcinoma, squamous cell carcinoma and malignant melanoma); it also causes several other skin disorders such as sunburns (erythema: redness of the skin), cataracts, skin photo-ageing, and suppression of the immune system. More than a million Americans are affected annually [1]. A 2010 analysis by Rogers and coworkers [2] estimated that the number of non-melanoma skin cancers (NMSCs) in Medicare beneficiaries increased dramatically, nearly 77% over the years 1992 to 2006 (1,158,298 in 1992 to 2,048,517 in 2006) at an annual age-adjusted rate per 100,000 beneficiaries of 3514 in 1992 to 6075 in 2006—the number of procedures for NMSCs in the Medicare population increased by about 16% in the period 2002–2006. In the last year of the survey (2006), the total number of reported NMSCs was about 3,507,693 while the total number of people treated for NMSCs was 2,152,500, which the authors [2] described as an under-recognized epidemic of skin cancers.

Educational programs designed to slow down the incidence of skin cancers by emphasizing sun protection have been rather disappointing at best [3]. It is clear that the connection between peoples' exposure to ultraviolet (UV) radiation and skin cancers craves for added photoprotection, which commercially available sunscreens (creams, lotions and others) were intended to provide.

Undeniably, skin disorders have become an ever increasing concern worldwide as society changed from a predominantly rural society (pre-WWII) to a mostly urban-based society in post-WWII. To avoid, or at least minimize such disorders, consumers have come to rely on the use of sunscreens (also referred by some as sun blockers) in the form of lotions, creams, sticks, gels, oils, butters, pastes, and sprays as a means of skin protection against the harmful effects of UVA and UVB radiations. Sunscreen formulations are intended to avoid skin damage, while allowing gradual tanning, both of which are attained using protective organic-based chemical UV filters and/or physical UV filters that absorb or otherwise, as the latter filters have also been said to scatter and reflect sunlight UV radiation. Regardless, natural UV sunlight also imparts beneficial effects on human health as it mediates the natural synthesis of vitamin D, together with formation of endorphins in the skin. Without sunlight, there would be no life!

As a constituent of the electromagnetic spectrum, the UV wavelengths fall between those of visible light and gamma radiation. They are partitioned into UVA, UVB and UVC segments. UVC photons comprise the wavelengths between 100 and 280 nm, while UVB comprises the wavelength range 280–320 nm; the UVA light is the least energetic falling between 320 and 400 nm. Sometimes UVA is further partitioned into UVA1 (320–340 nm) and UVA2 (340–400 nm)—Fig. 1. Each of the UV components exerts various effects on cells, tissues and molecules [1]. However,

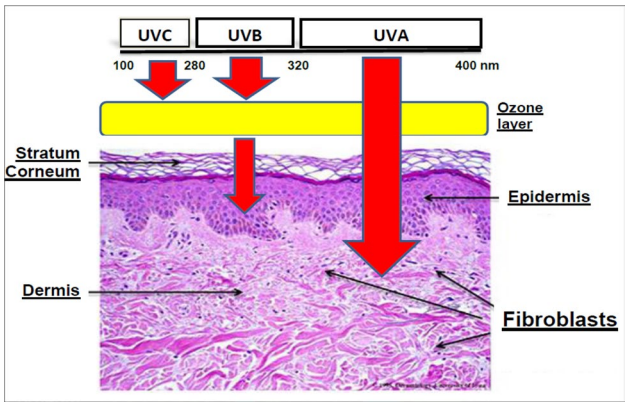


Fig. 1 UVA, UVB and UVC components of the electromagnetic spectrum between the visible range and the gamma radiation; also shown are the UV radiation and biologic effects on the skin. The arrows are meant to convey the extent of penetration of the UV radiation components into the epidermis and dermis. Adapted from D'Orazio and coworkers [1] through an open access article under the terms and conditions of the Creative Commons Attribution license; Copyright 2013 by the authors

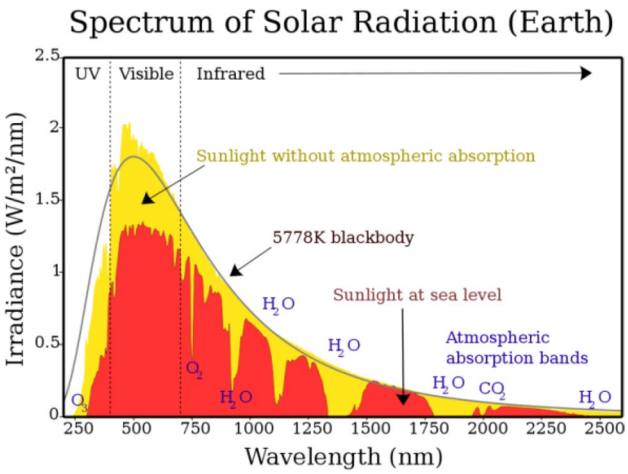


Fig. 2 Solar irradiance spectrum above the atmosphere and at the surface. Reproduced from https://en.wikipedia.org/wiki/Sunlight#/media/File:Solar_spectrum_en.svg (accessed August 13, 2020)

because atmospheric ozone absorbs the UVC component, ambient sunlight consists predominantly of 90–95% UVA and 5–10% UVB radiation (Fig. 2). Solar UV radiation penetrates the skin to different depths: the longer wavelength UVA penetrates deeply into the dermis (symbolized by the lengths of the arrows in Fig. 1), contrary to the UVB that is nearly completely absorbed by the epidermis. UVA radiation generates reactive oxygen species (ROS) that can damage DNA indirectly through photosensitizing reactions.

By contrast, UVB radiation is directly absorbed by the DNA leading to molecular rearrangements toward specific photoproducts such as, for example, cyclobutane dimers

Table 1 Skin photo-type, skin responses on exposure to UVB/UVA radiation, minimal erythema dose (MED) and a measure of cancer risk (adapted from Ref. [1])

Skin type	Properties	Skin response to UV	MED mJ/cm^2	Cancer risk
I	Bright white skin; Blue/green eyes; Freckles; Northern Europe; UK	Burns always; Peels; Never suntans	15–30	++++
II	White skin; Blue/hazel/ brown eyes; Red/blonde/brown hair; Europe/Scandinavia	Easily burns; Peels; Minimal suntans	25–40	+++/++++
III	Fair skin; Brown eyes; Dark hair; South/Central Europe	Moderate sunburns; Average suntans	30–50	+++
IV	Light brown skin; Dark eyes; Dark hair; Mediterranean, Asian, Latino	Minimal sunburns; Easily suntans	40–60	++
V	Brown skin; Dark eyes; Dark hair; East Indian, Native American, Latino, African	Rare sunburns; Easily suntans	60–90	+
VI	Black skin; Dark eyes; Dark hair; African, Aboriginal ancestry	No sunburns; Plentiful suntans	90–150	±

and other photoproducts that result in DNA modifications to DNA mutations and cancers [1]. Regarding the protective role of melanin on human skin from UV radiation, in order to cause the same level of damage to the skin as UVB radiation would necessitate more UVA radiation by nearly three orders of magnitude [4]. The latter is viewed to be less carcinogenic than UVB radiation since it does not interact as extensively with DNA as UVB does [5–8], which when directly absorbed by DNA [6] leads to significant mutagenic photolesions, (e.g., cyclobutane pyrimidine dimers and pyrimidine 6–4 photoproducts [5, 9]).

Important factors that make the skin sensitive to UV radiation and to skin cancer depend on the type of skin, which is subdivided into six photo-types (Table 1), all of which are based on various elements: skin color and melanin level. Thus, the fairer the skin is, the easier it is for the UV radiation to cause sunburns. That is, the minimal erythema dose (MED) of UV (predominantly UVB) needed to induce sunburns within a day or two of exposure to sunlight is rather low in developing erythema and swelling of the skin (edema).

Fair-skin individuals possess low melanin levels in the epidermis and thus are impacted considerably by UVB/UVA radiation. Consequently, they tend to develop sunburns rather than suntans on exposure to UV. Mutations that impair such individuals is likely due to less efficient DNA repair in melanocytes, as the skin is not efficient in blocking UV sunlight. Such individuals may also accumulate additional mutations from UV exposure because of defective DNA repair [1]—see Fig. 3.

One of the greatest risk factors in developing cutaneous melanoma pertain to individuals with fair skin complexion, as they possess low levels of the epidermal UV-blocking dark pigment Eumelanin (Fig. 4) [1]. Consequently, these individuals suffer much greater skin damage on exposure to UVB/UVA as such rays penetrate deeper into the epidermis and damage both keratinocytes and melanocytes. Accordingly, fair-skinned people are exposed to higher doses of

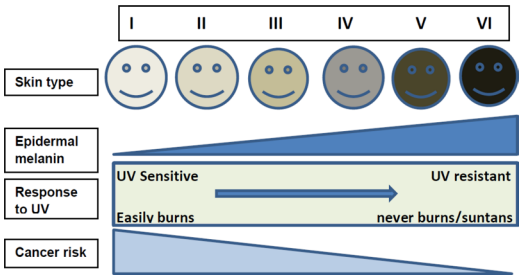


Fig. 3 Cartoon illustrating the influence of pigmentation on UV risks of skin cancer for individuals possessing various types of skins from I to VI. Adapted from Ref. [1]. Copyright 2013 by the authors and distributed under the Creative Commons Attribution license (http://creativecommons.org/licenses/by/3.0/)

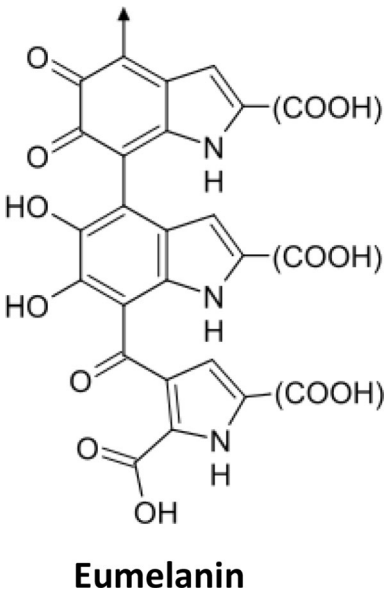


Fig. 4 Structural formula of Eumelanin (arrow denotes where the polymer continues)

UV radiation and thus to greater UV-induced mutations that (over time) contribute non-insignificantly to melanoma and to basal and squamous cell carcinomas, all of which can be circumvented to a certain extent by limiting exposure to UV sunlight. To minimize such exposure, consumers have come to rely on sunscreen products of various types and formulations. Most sunscreen formulations typically contain at least two chemical filters that display high SPF (sun protection factor) numbers, one of which predominantly screens the UVB radiation while the other screens much of the UVA radiation (Table 2) [10]—see sub-Sect. 3.2 for a description of the differences between the SPF and the UVA-protection factor UVA-PF. Often, these formulations may also contain physical filters such as nanosized/micronized titanium dioxide (TiO_2) or zinc oxide (ZnO).

The toxicological nature of sunscreen active agents found in increasing levels in the environment to which both wildlife and humans will ultimately be exposed to, albeit indirectly, has been described in the extensive review article of Ruszkiewicza and coworkers [11]. Specific emphasis was on the neurotoxicity of several organic filters such as, for example, octinoxate, oxybenzone, 4-methylbenzylidene camphor, 3-benzylidene camphor, and octocrylene (OCR), as well as the metal oxides ZnO and TiO_2, from which the authors concluded the need to revisit current safety and regulations vis-à-vis sunscreen agents. Means of alternative UV photoprotection must, therefore, be sought to minimize the spread of non-melanoma skin cancers (NMSCs) that could subsequently turn into malignant melanomas.

Consequently, the present perspective examines first the available FDA's approved sunscreen active agents (Table 2) [10] and subsequently examines what has been achieved in the last two decades with regard to novel sunscreen agents and their properties (where available) that relate to photostability, dermal absorption (i.e., skin penetration), and toxicity.

In practice, sunscreen formulations are tested for their photo-protective ability by measuring the time delay for erythema to appear on the skin that has been previously treated with topically applied sunscreens in comparison to untreated skin. Not least, sunscreen products are also tested for their dermatological compatibility (allergies and photoinduced allergies). However, from a photochemical and photophysical viewpoint, it is important to consider what events might occur to the sunscreen ingredients when they screen/block the UV radiation. Typically, as with any other molecule, absorption of the UV (and/or visible) radiation causes the sunscreen ingredients to be photoexcited from their ground electronic states to higher energy, potentially reactive excited state(s), subsequent to which they relax back to their respective ground electronic and vibrational states through a variety of photochemical and photophysical processes: for instance, prompt intramolecular vibrational redistribution, internal conversion, intersystem crossing, and luminescence (fluorescence and/or phosphorescence). The latter is undesirable in a sunscreen product and should, at the very least, be quenched by other ingredients within the sunscreen formulation.

Table 2 Sunscreen active ingredients included in the "stayed" 1999 final monograph (adapted from the data in Ref. [10])

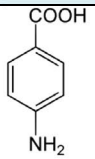
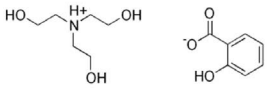
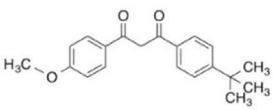
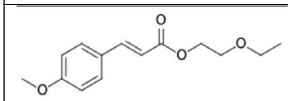
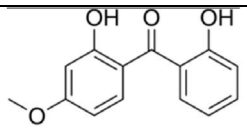
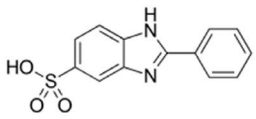
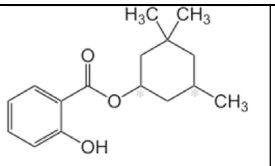
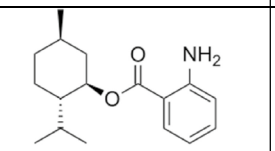
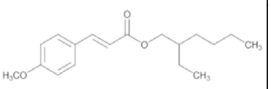
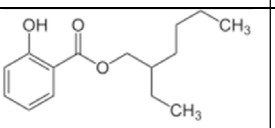
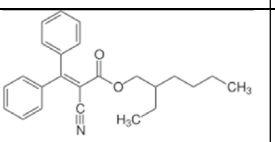
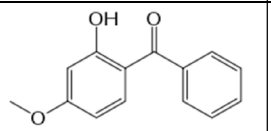
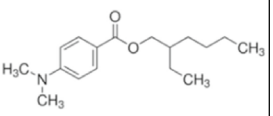
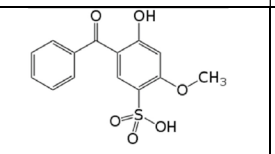
Active ingredients	Names	Type	Allowed max. % concn.
Category I			
ZnO	**Zinc oxide**	UVB/UVA absorber	**25**
TiO_2	**Titanium dioxide**	UVB/UVA absorber	**25**
Category II			
	**PABA** * *p*-Amino benzoic Acid	UVB absorber	**15**
	**Trolamine salicylate** **	UVB absorber	**12**
Category III			
	**Avobenzone; Parsol 1789**; Milestab 1789; Eusolex 9020; Escalol 517; Neo Heliopan 357; Butyl methoxy-dibenzoylmethane	UVA absorber	**3**
	**Cinoxate**; 2-ethoxyethyl *p*-methoxy-cinnamate	UVB/UVA absorber	**3**
	**Dioxybenzone**; benzophenone-8	UVB/UVA absorber	**3**
	**Ensulizole**; phenylbenzimidazole sulfonic acid	UVB absorber and minimal UVA absorber	**4**

Table 2 (continued)

	**Homosalate**, 3,3,5-Trimethylcyclohexyl 2-hydroxybenzoate	UVB absorber	**15**
	**Meradimate**; Menthyl anthranilate	UVA absorber	**5**
	**Octinoxate (OMC)**; Octyl methoxycinnamate; Eusolex 2292; Ethylhexyl methoxycinnamate; Uvinul MC80; Parsol MCX	UVB absorber	**7.5**
	**Octisalate;** 2-ethylhexyl salicylate; octyl salycilate	UVB absorber	**5**
	**Octocrylene**; Octocrilene; Uvinul N-539	UVB/UVA absorber	**10**
	**Oxybenzone**; Benzophenone-3; 2-Hydroxy-4-methoxybenzophenone	UVA absorber	**6**
	**Padimate O**; 2-ethyl hexyl dimethyl PABA; Escalol 507; octyl dimethyl PABA; OD-PABA; Sundown	UVB absorber	**8**
	**Sulisobenzone**; Benzophenone-4	UVB/UVA absorber	**10**

1.1 Physical UVB/UVA filters: category I

The mineral compounds ZnO and TiO_2 are found extensively in cosmetics, powders, eye shadows and pencils. Note that TiO_2 has been used as a sunscreen active agent since 1952 [12] as it was thought to block solar UV light from penetrating the skin over all UVB/UVA radiation wavelengths through mostly reflecting and scattering the UV light [13]. Factors that impinge on the latter two processes are the intrinsic refractive index, particle size, film thickness, and the nature of the dispersion in some appropriate medium. Regardless, Chiang and coworkers [14] concluded that although sunscreens may indeed prevent skin redness through absorption of the UVB/UVA sunlight and to some extent through inhibiting the skin's inflammatory response, sunscreens could in fact also be promoting, rather than protecting the skin from malignant melanomas.

A principal reason to use inorganic UV filters is that they not only afford a greater degree of UV protection, but as physical filters they have a further advantage that skin penetration is limited and they do not sensitize the skin [15]. Of the two approved physical filters, some believe that TiO_2 is very effective in absorbing mostly UVB, while ZnO absorbs mainly the UVA radiation, so that the combination of both TiO_2 and ZnO nanoparticles would provide broad UVB/UVA skin protection. Metal-oxide pigments with sizes greater than 100 nm tend to be poorly dispersed and reflect and scatter light to a greater extent than nanoparticles (NPs, size less than 100 nm); the former may result in undesirable visible white films on the skin, whereas the nanoparticles are easier to apply and are transparent on the skin [16]. However, micronization of these two pigments could make the nanoparticles more bioreactive and could thus facilitate their penetration into the skin and other tissues, thereby giving pause to their safe use. In addition, such nanoparticles dispersed in aqueous media absorb UV radiation and generate free radicals on the particle surface. As such, TiO_2 and ZnO nanoparticles have been associated with NP-induced cytotoxicity and genotoxicity. [17].

Contrary to some organic-based UV filters, inorganic-based sunscreens exhibit a wider UV absorption spectrum and combine absorption and scattering of UVA and UVB radiations. Nonetheless, a combination of organic and inorganic UV filters in topical formulations can display synergistic effects [18]. Although recognized as active long-wavelength UVA ingredients in sunscreen formulations, Diffey et al. [19] have claimed that TiO_2 and ZnO do not meet the requirement to be classified as broadspectrum products for critical wavelengths longer than 370 nm.

According to Beasley and Meyer [20], however, model sunscreen formulations that contained 3% avobenzone or 5% ZnO provided superior attenuation of UVA wavelengths longer than 360 nm, compared to formulations that contained 5% TiO_2. Sunscreen products of similar SPF values containing avobenzone or ZnO exhibited a significantly greater protection factor for UVA radiation (UVA-PF)— nearly threefold greater—than those containing TiO_2,

while addition of TiO_2 provided but modest UVA-PF values. Consequently, relative to formulations that contained photostabilized avobenzone or ZnO, TiO_2 was thought to provide neither the same level of UVA attenuation nor the same degree of UVA protection of human skin. In fact, TiO_2 was considered a poor substitute for avobenzone or ZnO in providing high levels of UVA protection to human skin [20]. Note that this would be true if the TiO_2 used by these authors were anatase TiO_2, whose absorption edge is 387 nm (bandgap = 3.2 eV) contrary to rutile TiO_2 whose absorption edge is around 405−410 nm (bandgap = 3.0 eV). Hence, anatase TiO_2 would be more effective against the shorter UVA wavelengths (less than 360 nm), and would consequently confer partial broad spectrum protection. Titanium dioxide has often been substituted for zinc oxide, especially as the latter is dermally absorbed and imparts some toxicity to the human body (see below). Related to the study of Beasley and Meyer [14], some social media forums claim that ZnO displays a broader relative attenuation of UVA/UVB radiation, while TiO_2 provides, so it seems, better UVB protection as illustrated in Fig. 5 [21, 22]. Also displayed in Fig. 5a are the relative attenuations of UVB/UVA radiation by the chemical filters oxybenzone, octinoxate, and avobenzone (Parsol 1789).

However, if one examined the absorptance spectra of rutile and anatase TiO_2 nanoparticles (as we have), rutile TiO_2 covers the entire UVA spectral region (Fig. 6; see also Ref. [23]). Evidently, there exist controversies in the literature [22, 23] that need to be addressed before the frequently used physical filter titanium dioxide (mostly the lesser photoactive rutile phase vis-à-vis the active anatase phase) in sunscreen formulations be thought to cover less of the UVA radiation relative to zinc oxide.

Even though the inorganic UV filters TiO_2 and ZnO do not absorb all of the UV radiation—for example, TiO_2 absorbs ca. 70% of the incident UV light—they do offer reasonably good UV shielding characteristics. Unfortunately, they also display high photocatalytic activity when exposed to UV radiation, which leads to formation of a significant number of reactive oxygen species (ROS; e.g., •OH radicals) that are harmful to human cells. These metal oxides also impact significantly on the photostability of cosmetics, an undesirable outcome of suncare products. More importantly, TiO_2 and ZnO have been shown to be important photocatalysts in the photodegradation of organic pollutants in both aqueous media and gas phase owing to the generation of hydroxyl radicals (•OH) and, to a lesser extent, hydroperoxyl radicals ($HO_2^{\bullet}$) and singlet oxygen ($^1O_2$), responsible for initiating photooxidations.

The failure of the U.S. FDA [10] to approve new broad-spectrum UVB/UVA filters has resulted in the approved inorganic filters ZnO and TiO_2 nanoparticles to play a more prominent role in photoprotection when used in sunscreen

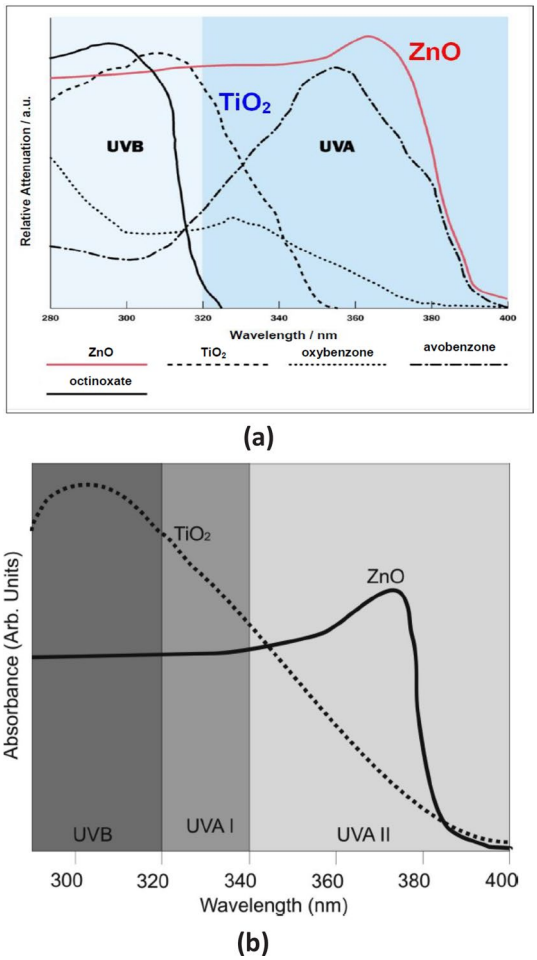


Fig. 5 **a** Spectra illustrating the relative attenuation spectra of the UVB/UVA radiation by oxybenzone, octinoxate, avobenzone (Parsol 1789), zinc oxide and (anatase) titanium dioxide. Reproduced from a social media forum [21]. **b** Spectra of TiO_2 and ZnO together with the partitioned UV regions. Reproduced from Ref. [22]. Copyright 2013 by the authors licensed under a Creative Commons License by Attribution 4.0

formulations. Nanosized metal-oxide particles increase their cosmetic acceptability as they are much less visible when applied to the skin [24]. Early formulations of mineral-based sunscreens often left a white chalky presence on the skin, which was most noticeable on darker skins. Consumer satisfaction has stimulated the commercialization of new sunscreen formulations with inorganic-based nanoparticles, so much so that the year 2018 witnessed a large increase in the number of available sunscreen products in the United States alone that contained solely ZnO and/or TiO_2 physical filters: ca. 17% in 2007 to about 41% in 2018 [24].

Risks to human health posed by ZnO and TiO_2 filters are relatively low given a lack of percutaneous absorption, as evidenced by a lack of absorption across both intact and damaged (tape-stripped) skin, except when humans are exposed to such mineral filters via inhalation. The latter has

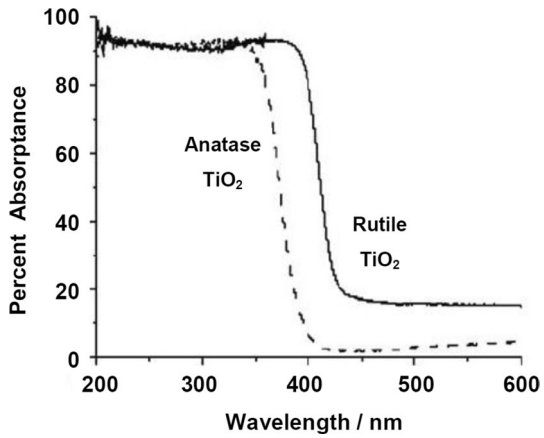


Fig. 6 Percent absorptance spectra of anatase TiO_2 versus rutile TiO_2 from our own work. Note the significant differences with the TiO_2 spectra in Fig. 5

prompted consumers to avoid sunscreen sprays that contain these nanoparticles. The risk of these metal oxides to the environment is also considered low [24]. Inhaled nanoparticles can induce inflammatory diseases or even exacerbate respiratory allergies and asthma, and they may also be involved in cardiovascular diseases and promote certain lung cancers [25]. Borase and coworkers [26] showed that incorporating latex-synthesized gold nanoparticles to such inorganic filters could provide a potent alternative to traditionally used harmful TiO_2 and ZnO nanoparticles in sunscreen products.

1.1.1 Zinc oxide

Nanosized ZnO has proven to be an attractive UVA filter for use in sunscreen formulations owing to its transparency across the visible light wavelength region, and its relatively high absorption in the UV spectral region, so essential in formulating efficient novel sunscreen products to attain broadspectrum protection [20]. Regardless, generation of ROS species under UV light by nano-ZnO remains a major issue, together with its decomposition into zinc ions (Zn^{2+}) when absorbed through the skin.

Because of its insolubility in aqueous media (at neutral pH of 7) and in biological fluids, the systemic absorption of ZnO is precluded when topically applied, even if it were used at its maximum eligible concentration of 25% (Table 2), irrespective of the formulation of the sunscreen product. According to the Federal Register [10], nanosized ZnO does not penetrate into or through human skin to any large extent, and thus appears to be of no consequence to any possible adverse health issues. The FDA's review of available data from animal and human studies, coupled to the data on the physical properties of ZnO, concluded that the transdermal absorption of zinc oxide from any topically applied sunscreen formulation would be extremely unlikely, and that any minimal absorption that may occur would not result in any adverse health effect [10]. Available studies on the dermal penetration of ZnO suggest that, regardless of particle size, penetration would be limited primarily to the upper layers of the non-living stratum corneum, with most penetration occurring only into skin folds and furrows or hair follicles; any ZnO particles that achieve transdermal absorption would dissociate into Zn^{2+} and O^{2-} ions [27].

Although dermal absorption is a major route when exposed to ZnO nanoparticles (NPs) in sunscreen applications, a few studies reported that ZnO NPs do not penetrate into the deeper layers of the skin [28–32]. By contrast, the 2010 study by Gulson et al. [33] demonstrated that ZnO NPs do penetrate the skin, albeit to a limited extent, as evidenced by a small increase of zinc ions (Zn^{2+}) in blood and urine samples following a 5-day exposure of humans with healthy skin to sunscreen products that contained ZnO NPs. The earlier (1996) in vitro study by Pirot and coworkers [34] also showed that human skin absorbed about 0.34% of ZnO NPs after a 3-day exposure. Nonetheless, the risk of dermal absorption on exposure to ZnO NPs appears to be rather low when considering solely the common human behavior when applying sunscreens, as other sources also need to be considered—for example, eating and drinking with sunscreen-laden hands and lips, and gastrointestinal or pulmonary exposure to ZnO, together with occupational exposure that might be of concern to some [16]. Toxic effects are likely caused by the presence of Zn^{2+} ions from the decomposition of ZnO NPs in the medium or in cells [11].

The dissociation of ZnO into zinc ions (Zn^{2+}) from commercial sunscreens exposed to UVB light irradiation was investigated by Martorano et al. [35] to assess the cytotoxicity of accumulated Zn^{2+} in human epidermal keratinocytes (HEK). The significant increase in the quantity of Zn^{2+} ions was irradiation intensity dependent and ZnO concentration dependent. Cytotoxic assays revealed a reduction in cell viability as a function of ZnO concentration. Real-time cytotoxicity assays with propidium iodide also showed that treatment with UVB-irradiated ZnO sunscreen caused a late- or delayed-type cytotoxicity in HEK. Moreover, Zn^{2+} ions induced the production of reactive oxygen species in HEK, which led the authors [35] to conclude that UVB irradiation produced an increase in Zn^{2+} through dissociation of sunscreen ZnO, a consequence of which was the accumulation of free or labile Zn^{2+} that caused the cytotoxic effects and oxidative stress.

Recently, Mueen and coworkers [36] successfully synthesized zinc oxide/ceria (ZnO/CeO_2) nanoparticle composites via a simple route that involved precipitating CeO_2 nanodots onto a commercial ZnO nanopowder at pH 9 and at various Ce/Zn ratios (2.5 at%, 5 at%, and 10 at%). The

nanocomposites achieved efficient UV filtering applications. In addition, the small amount of CeO_2 that decorated the commercial ZnO surface led to a stronger, more selective absorption of light within the UV range. Loads of 10 at% ceria on ZnO effectively reduced the photocatalytic degradation of a dye (crystal violet) by nearly 97% within a 30 min exposure to UV light, and about 99% under simulated sunlight for 30 min [36].

1.1.2 Titanium dioxide

Under dark conditions, titanium dioxide is an innocuous/inactive product, in as much as it has been used safely in dental pastes, oral capsules, suspensions, tablets, dermal preparations and non-parenteral medicines; in addition, its toxicity is rather low and thus widely used in biomedical applications because of its biocompatibility. Under UV irradiation, however, titanium dioxide is anything other than a photo-inactive ingredient.

The lack of solubility of titanium dioxide in aqueous media and in biological fluids precludes any transdermal absorption of more than a minimal amount, regardless of concentration and formulation of the sunscreen product. Given that TiO_2 is insoluble and unreactive under physiologic conditions, and does not penetrate into skin or enter into a systemic circulation to any meaningful extent, the FDA's Federal Register [10] considered the available data on TiO_2 adequate enough to approve it and allow it to be used in sunscreen formulations. Moreover, a study by FDA's researchers [37] showed that TiO_2 particles in sunscreens maintain their original sizes and shapes as observed in powdered samples.

Our extensive experience of over ca. 40 years in heterogeneous photocatalysis [38–43] led us to question the suitability of TiO_2 and other metal-oxide pigments in sunscreen formulations. Through the use of chemical methods, we showed that all sunscreen TiO_2 samples extracted from commercial sunscreen lotions/creams catalyzed the photooxidation of phenol (a representative phenolic organic substrate, as well as tens of other organic pollutants). We further demonstrated that simulated sunlight illuminated TiO_2 also catalyzed the damage to DNA both in vitro and in human cells (cultured human fibroblasts) [44]. As Fig. 7 demonstrates, supercoiled (S) DNA plasmids were first converted to the relaxed form (R), and then to the linear form (L). DNA strand breakages were established.

Simulated sunlight alone had little effect on the plasmids. However, the TiO_2 samples extracted from commercial sunscreens were photoactive toward plasmid damage, which could be suppressed by such quenchers as dimethyl sulphoxide (DMSO) and mannitol. This showed that the damage to DNA was caused by photogenerated ·OH radicals [44]. Comparison of pure anatase TiO_2 versus rutile TiO_2 showed

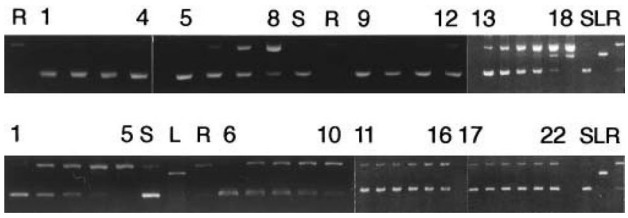


Fig. 7 Relaxation of plasmids caused by illuminated TiO_2 and ZnO and suppression by DMSO and mannitol. In both panels, S, L and R show the migration of supercoiled, linear and relaxed plasmids, respectively. Top panel: plasmid relaxation found after illumination with sunlight alone for 0, 20, 40 and 60 min (lanes 1–4) and with 1% anatase (lanes 5–8) or 1% rutile (lanes 9–12) TiO_2 for the same times. Lanes 13–18: illumination with TiO_2 from sunscreen SN8 for 0, 5, 10, 20, 40 and 60 min. The results are typical of those found with various samples. Bottom panel: illumination with 0.2% ZnO for 0, 10, 20, 40 and 60 min before (lanes 1–5) or after (lanes 6–10) adding DMSO; and with 0.0125% sunscreen TiO_2 for 0, 5, 10, 20, 40 and 60 min after adding 200 mM DMSO (lanes 11–16) or 340 mM mannitol (lanes 17–22). Reproduced from Ref. [44] with permission under an Elsevier User License

the latter to have no impact on the plasmids. A later study by Buchalska and coworkers [45] revealed that TiO_2 (bare or otherwise coated with an inert metal oxide) extracted from several commercially available sunscreen products caused the photodegradation of the Azur-B dye and the oxidation of α-terpinene, both attributed to the presence of photogenerated singlet oxygen ($^1O_2$). The study by Rampaul et al. [46] revealed that the inorganic component in sunscreens caused significant cellular damage of cultured human skin cells, either by the presence of uncoated TiO_2 in the sunscreen formulations, or else the integrity of the coating on TiO_2 may have been compromised [47].

Comet assays (Fig. 8) confirmed the damage to DNA in human cells caused by UV-illuminated TiO_2. Damage suppression by DMSO again implied the activity of hydroxyl radicals on DNA.

Consequently, to the extent that photoactive TiO_2 specimens extracted from commercial sunscreen lotions caused damage to both DNA plasmids in vitro and to whole human skin cells in cultures by photogenerated ·OH radicals, [44] it was imperative that this sunscreen agent TiO_2 be modified so as to minimize/suppress the formation of the ·OH radicals and thus considerably reduce its photoactivity [48]. Such reduction of photocatalytic activity of TiO_2 significantly decreased, if not totally suppressed, damage caused to DNA plasmids (Fig. 8), to human cells, and to yeast cells (see below) compared to non-modified specimens when exposed to UVB/UVA simulated solar radiation.

UV light alone appeared to have had little, if any, consequence as the plasmids remained in their supercoiled form (S). Addition of TiO_2, however, caused a number of supercoiled plasmids of the circular, double-stranded DNA to be

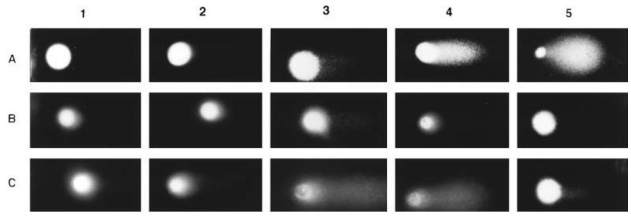


Fig. 8 Damage inflicted on human cells as revealed by comet assays. Row A: cells were exposed on ice for 0, 15, 30 and 60 s, giving comets falling into five classes: 1, class 0; 2, class I; 3, class II; 4, class III; 5, class IV. Rows B and C: examples of comets obtained using simulated sunlight, MRC-5 fibroblasts and sunscreen TiO_2 (0.0125%). For each exposure, 100 cells were scored, and comets were classified by comparison with the standards in Row A. Row B: no treatment (1); sunlight alone for 20, 40 and 60 min (2–4); and effect of TiO_2 in the dark for 60 min (5). Row C: sunlight with TiO_2 for 0, 20, 40 and 60 min (1–4); and for 60 min with TiO_2 and 200 mM DMSO (5). Reproduced from Ref. [44] with permission under an Elsevier User License

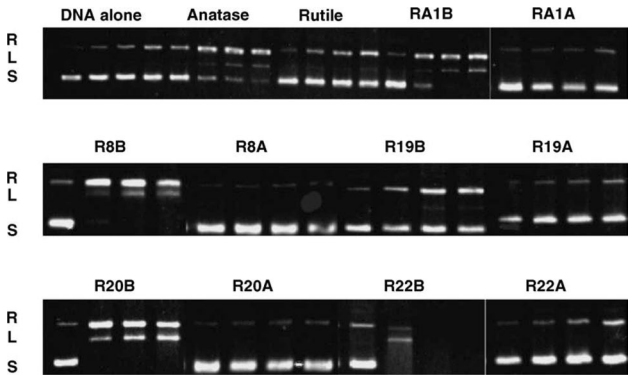


Fig. 9 Relaxation and migration of supercoiled (S), relaxed (R) and linear (L) forms of DNA plasmids caused by (top panel) UVA/UVB irradiation of DNA alone, irradiation in the presence of anatase, rutile and the selected TiO_2 specimens RA1B and RA1A specimens; (middle panel) irradiation in the presence of R8B, R8A, R19B and R19A samples; and (lower panel) with R20B, R20A, R22B and R22A titanium dioxide specimens (Note B means before modification, and A means after modification). Irradiation times were 0, 10, 20 and 30 min. Reproduced from Ref. [48] with permission (license No. 4954810511975). Copyright 2006 by Elsevier B.V

converted to the relaxed (R) and linear forms (L) (Fig. 9). Relative to untreated TiO_2, whatever damage (if any) was caused to DNA strands by the modified titanium dioxide specimens was considerably diminished, and was comparable to whatever damage was inflicted by UV light alone acting on plasmid DNA (control experiment). Specifically, as evidenced by the single- and double-strand breaks, a certain change in DNA plasmids was displayed by the RA1B and R8B TiO_2 specimens (B: before modification). Interestingly, the RA1B TiO_2 specimen caused complete disappearance of supercoiled plasmids after only 20 min of illumination, while only 15% of the supercoiled plasmids were damaged after 30 min of irradiation in the presence of the modified RA1A (A: after modification) specimen. By comparison, the R8B and R20B specimens cleaved plasmid DNA after only 10 min. In the presence of treated R8A and R20A TiO_2s, the survival rate of plasmid DNA was greater than ~ 90% (30 min). By contrast, the R22B sample completely destroyed all forms of the plasmids after 20 min of UVA/UVB irradiation; the survival rate in the presence of the modified R22A specimen was ca. 80% after the 30-min irradiation period.

Synergistic effects of TiO_2 specimens were also demonstrated in the presence of the sunscreen active agent padimate-O (Table 2) on DNA plasmids. The survival rate of yeast cells in the presence of TiO_2 as well as in the presence of the chemical UV filters padimate-O and avobenzone under UV irradiation were examined by Serpone coworkers [48]. DNA plasmids were irradiated by UV light alone, as well as in the presence of untreated and modified TiO_2 specimens for a 30-min period under otherwise identical conditions of irradiation. As clearly shown in Fig. 10, modified RNA TiO_2 specimens were not toxic to yeast cells as exemplified by the results of the droplet test for the R9A and R9B specimens, in comparison to the case when the yeast cells were subjected to UV illumination alone and in the presence of padimate-O and Parsol 1789. Under dark conditions, TiO_2 had no effect on the survival rate of yeast cells, while the effect of UV-irradiated TiO_2 was greater than that caused by UV light alone. Control experiments showed that, in the absence of TiO_2, the yeast cells survived after 40 min of irradiation with UVA/UVB simulated sunlight (Fig. 10a). The R9B TiO_2 specimen caused a significantly greater number of kills of the yeast cells than did the modified R9A sample: cell death was complete after 10 min of illumination with R9B (Fig. 10b), while all the yeast cells survived even after 40 min of irradiation in the presence of the R9A titania specimen (Fig. 10c). For comparison, the UVA filter avobenzone (Fig. 10) and the UVB padimate-O sunscreen agent (Fig. 10e) were highly toxic to yeast cells causing cell death almost immediately upon UV irradiation.

Clearly, passivation of TiO_2 particles had a significant influence in decreasing the extent of damage inflicted on DNA plasmids, on whole human skin cells, and on yeast cells relative to non-modified TiO_2 specimens when exposed to UVA/UVB simulated sunlight. Passivated TiO_2 specimens caused no damage to these in vitro skin models and protected DNA from the harmful UVA/UVB radiation. By contrast, Parsol 1789 (avobenzone) and padimate-O caused considerable damage to yeast cells, which confirmed the earlier work on DNA plasmids by Damiani and coworkers [49] and by McHugh and Knowland [50]. Several of the TiO_2 specimens were also tested by the comet assay technique, which further confirmed damage to DNA caused by illuminated TiO_2 on whole human skin cells in vitro [48]. Several

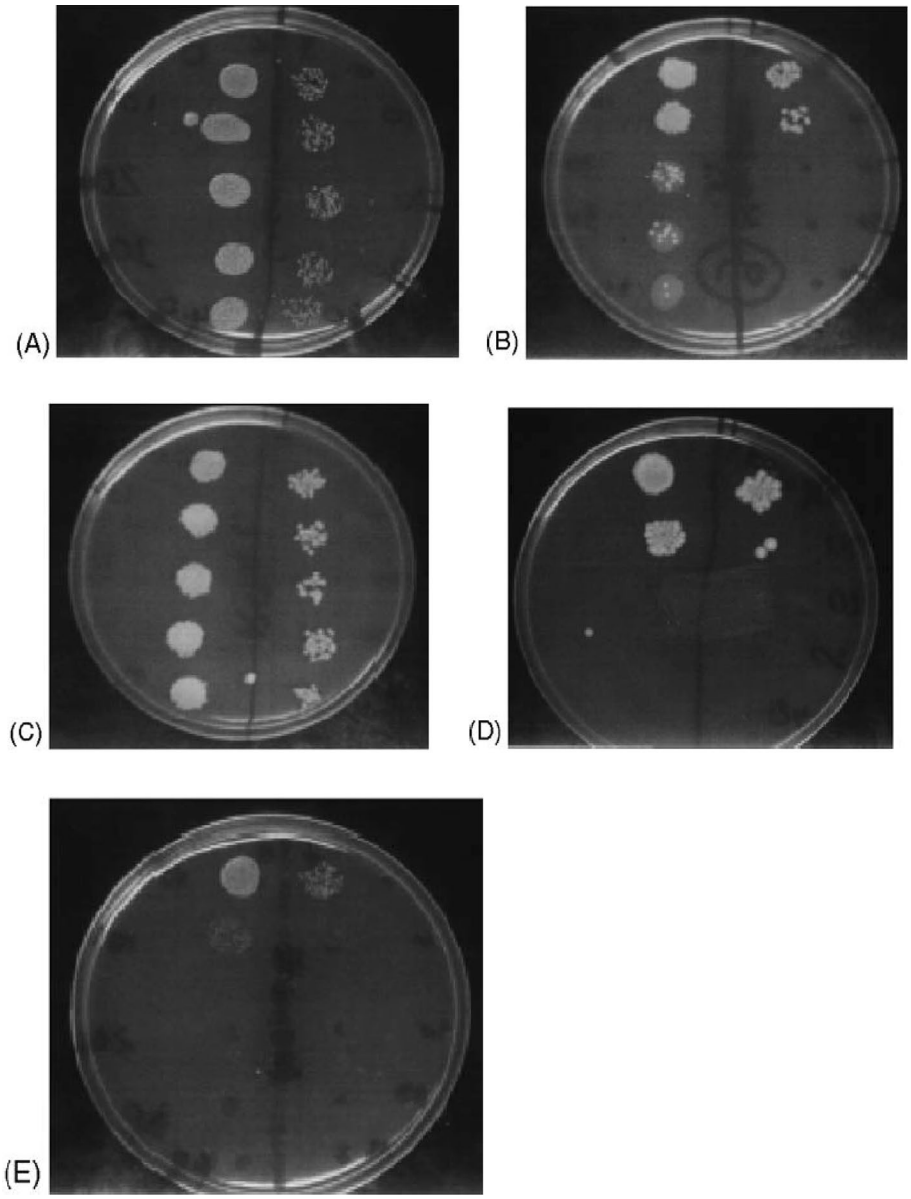


Fig. 10 Survival of yeast cells on UV illumination for 0, 10, 20, 30 and 40 min (from top to bottom) in each Petri dish: **a** yeast cells alone; **b** R9B titania; **c** R9A titania; **d** Parsol 1789; and **e** Padimate-O. The Petri dish was divided into two parts to repeat the experiments. Note that the number of yeast cells on the left was twofold greater than the cells on the right. Reproduced from Ref. [48] with permission (license No. 4954810511975. Copyright 2006 by Elsevier B.V

studies into the penetration of TiO_2 into the skin demonstrated that related nanoparticles do not permeate into intact and damaged skin, but remain in the stratum corneum and epidermis without reaching the brain or peripheral organs [50–54]. Low level cytotoxicity in human HaCaT keratinocytes have inferred a low toxic potential of nanomaterials at the skin level, owing to their great photostability and low ionizing capacity [55–57].

No titanium dioxide species were detected in both intact and damaged skin subsequent to exposing needle-abraded human skin to TiO_2 nanoparticle suspensions for a 24-h period. However, titanium dioxide was found in the epidermal layer (0.47 ± 0.33 μg/cm^2) of intact skin, although its concentration was below the limit of detection in the dermal layer. The concentration of titanium dioxide found in damaged skin was nearly identical (0.53 ± 0.26 μg/cm^2) to that found in the epidermal layer of intact skin [57].

Cytotoxicity studies on HaCaT cells by Crosera and coworkers [57] demonstrated that TiO_2 NPs displayed cytotoxic effects after a 7-day exposure, albeit only at very high concentrations that reduced cell viability. Their study also showed that TiO_2 NPs did not permeate intact and damaged skin but was detected in the stratum corneum and epidermis, indicating that these nanocompounds are potentially toxic at the skin level only after a long-term exposure. Nonetheless, no safety evaluations have been carried out on TiO_2 NPs in sunscreens in real-world sunburned skin from long-term chronic UV exposure.

Using electron paramagnetic resonance (EPR) spectroscopy, Rancan and coworkers [58] examined the possible

formation of intracellular free radicals in nanoparticle-based formulations used in cosmetics and dermatology that were exposed to UVB radiation—e.g., TiO_2 and amorphous SiO_2 nanoparticles. They evaluated the influence of nanoparticle surface chemistry on particle cytotoxicity toward HaCaT cells (uncoated TiO_2 was the positive control). Contrary to SiO_2 nanoparticles, which showed no formation of intracellular free radicals both in the dark and under UVB irradiation, photoactivated TiO_2 nanoparticles generated a large number of intracellular free radicals. However, non-toxic concentrations of silica particles did enhance the toxicity of UVB radiation.

Although TiO_2 nanoparticles do not appear to permeate through human skin when TiO_2-containing sunscreens are topically applied, the fact remains that to the extent that such nanoparticulates are used in such diverse fields as foodstuffs, cosmetics and medical industries, among others, there is a clear necessity to examine their potential toxicity on biological systems, irrespective of the entry mechanisms. In this regard, Yu and coworkers [59] exposed human bronchial epithelial cells (16HBE14o-) to 50 and 100 μg/mL of TiO_2-NPs for 24 and 48 h; the TiO_2-NPs induced (i) endoplasmic reticulum stress in the cells, (ii) disrupted the mitochondria-associated endoplasmic reticulum membranes and calcium ion balance, and (iii) caused an increase in autophagy.

Similarly, Shukla et al. [60] exposed mice orally to TiO_2 nanoparticles over a 14-day period. This resulted in a significant alteration in the level of hepatic enzymes and liver histopathology (dose, 100 mg/kg of body weight), as well as a significant oxidative DNA damage to liver cells that was attributed to oxidative stress.

A related study by Weir and coworkers [61] quantified the amount of titanium from TiO_2 in common food products, derived estimates of human exposure to dietary nano-TiO_2, and discussed the impact of the nanoscale fraction of TiO_2 that enters the environment. They concluded that because of the millions of tons of titanium-based white pigment used annually, testing ought to focus more on food-grade TiO_2 than that used in many environmental health and safety tests, in which much lower amounts are used in products that are less likely to enter the environment.

1.2 Chemical UVB/UVA filters

Mechanistically, to minimize, if not preclude sunburns and some of the more severe skin damage, the chemical filters present in sunscreen formulations reported in Table 2 should dissipate the absorbed UV radiation via photophysical and/or photochemical pathways that preclude the formation of the cytotoxic singlet oxygen ($^1O_2$), together with other reactive oxygen species (ROS) and harmful reactive intermediates. A propos, a recent 2018 study by Uco and coworkers [62] reported an innovative non-invasive method that uses in vitro three-dimensional human skin models to appraise the cytotoxicity and phototoxicity of sunscreen formulations without exposure to UV radiation, and subsequent to UVB/UVA exposure, which caused the sunscreens to photodegrade. This calls attention to the photostability of sunscreens, a topic we discuss later. All formulations were found to be toxic under all conditions, including the control formulation [62]. Prior to the phototoxicity radiation process, cell viability of photodegraded formulations was higher, inferring that some of the formulation components degraded into products of reduced toxicity. The widely used UVA sunscreen agent avobenzone (Parsol 1789) was more unstable/toxic than the UVB octinoxate sunscreen agent under otherwise identical test conditions. More importantly, the sunscreens and their formulations were, to some extent, toxic to skin model cells, even when not exposed to UV radiation. [62].

Most of the more widely used UV sunscreen chemical filters have tended to be members of the cinnamate (UVB) and dibenzoylmethane (UVA) classes: e.g., octinoxate and avobenzone. Within this context, the US Federal Register on OTC drugs [13] discourages the simultaneous presence of these two representatives in sunscreen formulations because of their inherent photo-instability that could produce some undesirable photoadducts formed between octinoxate and photogenerated fragments of avobenzone [63]. Such photoadducts could potentially be toxic to DNA and, not least, possible photoisomerization reactions occurring (in some cases) might yield species less absorbing of UV radiation and thus less useful as sunscreen agents. Moreover, several sunscreen-active ingredients are very good triplet sensitizers that convert harmless triplet oxygen ($^3O_2$) into the well-known cytotoxic singlet oxygen ($^1O_2$) species [64–68].

2 FDA's categories of chemical UVB/UVA sunscreen filters

2.1 Category II filters

PABA (4-aminobenzoic acid) was one of the first active ingredients used in sunscreen formulations [69] as it absorbed UVB radiation. The beneficial effects were demonstrated in experiments carried out in vivo on mice that revealed PABA could reduce UV damage and protect against skin tumors in rodents [70]. However, in vitro studies on animals showed that PABA increased the risk of cellular UV damage [71, 72]. Accordingly, PABA was taken off the market and no longer used in sunscreen formulations because of its adverse effects on skin (allergies), and its discolouring and staining effect on clothing [73]. Clinical information that includes a number of studies on allergic and photoallergic skin reactions to PABA have shown rates of PABA-induced skin reactions to be 8% or greater and thus its discontinued

use [10, 74–77]. Additionally, analyses of urine samples from human subjects who were exposed to topical PABA application revealed that PABA also penetrated the skin and entered systemic circulation [71, 72] ; it was unclear to what degree such transdermal absorption took place [10]. Yet another study found an association between autoimmune disorder and use of PABA [73]. Nonetheless, a derivative a PABA (i.e., padimate-O) continues to be used in sunscreen formulations (Table 2).

Trolamine salicylate, an ingredient found in Aspercreme and Aspergel, comprises both trolamine and salicylic acid, the latter acting as a non-steroidal anti-inflammatory (NSAI) drug used widely as an analgesic and as an anti-pyretic agent [10]. Although it was included in FDA's Stayed 1999 Final Monograph for sunscreens at a concentration of up to 12%, and proposed as a Category III active ingredient in the tentative final monograph for OTC external analgesic drug products in the 1983 Federal Register [78], it was evident that, in order to act as an external analgesic, trolamine salicylate had to penetrate the skin (transdermal absorption) to reach the relevant sites [79, 80]. To be an effective sunscreen agent, however, trolamine salicylate must remain on the skin surface so that it absorbs the UV radiation. Accordingly, as a result of some non-insignificant safety concerns associated with the use of trolamine salicylate as an active ingredient in sunscreens, the FDA revised its classification of trolamine salicylate and currently considers it a Category II ingredient [10]. In addition, a review by the FDA of hundreds of commercially available sunscreen products indicates that trolamine salicylate is no longer being used in sunscreen formulations [78].

2.2 Category III sunscreens

Of the twelve sunscreen agents listed in Category III of Table 2: (i) cinoxate, (ii) dioxybenzone, (iii) ensulizole, (iv) homosalate, (v) meradimate, (vi) octinoxate, (vii) octisalate, (viii) octocrylene, (ix) padimate-O, and (x) sulisobenzone lack general recognition with respect to safety and effectiveness as sunscreen ingredients. [10] To FDA's knowledge, only homosalate and octisalate [81], and octinoxate [81–84] have been evaluated for human absorption, albeit with significant limitations [10]. Rates of transdermal delivery of some active ingredients in approved drug products has shown that those containing active ingredients with physical properties (melting points and molecular weights) similar to many of the sunscreen active ingredients (i) through (x) are supplied transdermally with success, and thus are systemically available [85]. This underpins the potential for systemic exposure of humans to these sunscreen ingredients and to their transdermal absorption. In addition, mammalian assays on homosalate [86–92] and padimate-O [10] have demonstrated hormonal effects. Homosalate, [86–92] octinoxate [93, 94], and octocrylene [95] have shown similar hormonal effects in both in vitro and in vivo assays.

Dermal penetration studies have been carried out on homosalate [81, 84], octinoxate [81, 96–101], octisalate [81, 84, 101–105], octocrylene [95, 106], padimate-O [100], and sulisobenzone [107, 108]. Results have shown that, except for homosalate, these ingredients do permeate into the epidermis and/or dermis. Several factors potentially influence and increase the permeation and/or penetration of these ingredients, among which are vehicle composition and the presence of other active ingredients.

Another no less important harmful aspect of using sunscreen-based products is that some people may develop photocontact allergies to the active agents, which may be photoallergenics. For instance, Karlsson et al. [109] noted that photodegraded dibenzoylmethane based sunscreens may be an important causal source in the development of photocontact allergies to sunscreens. In this regard, one of the most frequently observed photoallergens in current use is avobenzone, which photodegrades into arylglyoxals and benzyls; the latter two photoproducts are cytotoxic rather than allergenic as evidenced by local lymph node and cell proliferation assays. There is strong evidence that the arylglyoxals may be the principal source of photocontact allergies experienced by some people to the dibenzoylmethane class of sunscreens. [109].

2.3 The peculiar cases of oxybenzone and avobenzone

2.3.1 Oxybenzone

Oxybenzone (OB) is one of the organic-based chemical filters and a representative of the class of benzophenones found extensively in sunscreen formulations but, unlike the inorganic-based metal oxides ZnO and TiO_2, absorbs a non-insignificant amount of the UVB/UVA radiation. Spectra in Fig. 11 display intense broad absorption bands in each of the UVA, UVB and UVC spectral regions, thereby making it a suitable substrate to absorb incident broadband UV solar radiation [110]. Oxybenzone is fairly photostable for several hours following exposure to UV radiation [111], although some studies have questioned its suitability as a sunscreen ingredient because of some adverse dermatological effects and endocrine disruption [112–115].

Ab initio electronic structure calculations on oxybenzone by Karsili and coworkers [112] have inferred that ultrafast dynamics are mostly responsible for the efficacy of oxybenzone as a sunscreen, as a result of internal conversion (IC) via a barrierless electron-driven excited state hydrogen atom transfer, a likely mechanism of energy deposition along the enol RO–H reaction coordinate (Fig. 12) [116, 117].

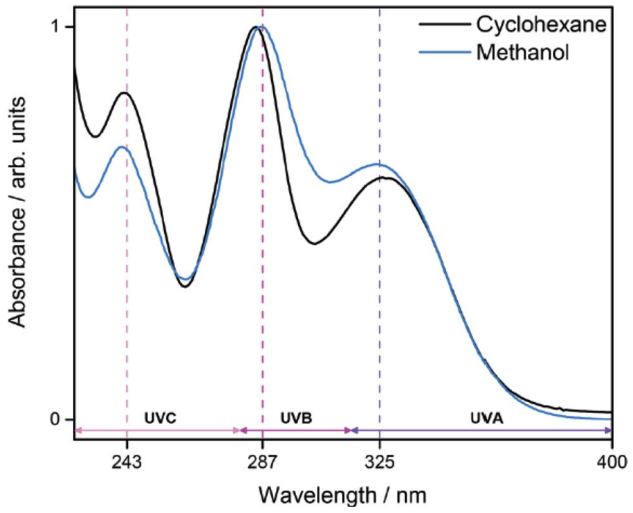


Fig. 11 UV–visible spectrum for enol-OB in solution in cyclohexane (black) and methanol (blue) displays three absorption maxima at ca. 325 nm, 287 nm and 243 nm, each highlighted by a vertical dashed line. Reproduced from Ref. [110] with permission from the European Society for Photobiology, the European Photochemistry Association, and The Royal Society of Chemistry

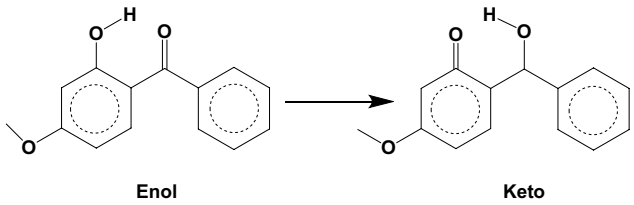


Fig. 12 Enol and keto tautomers of oxybenzone undergoing rapid tautomerization into the keto tautomer on exposure to UVA irradiation [111, 117]

In addition to being used as a sunscreen agents in no less than 588 products, oxybenzone is also found in 81 different types of lipsticks, in 111 lip balms, in 172 facial moisturizers, hair sprays, perfumes, hair conditioners, and not least as a fragrance enhancer and as a photostabilizer of other sunscreen ingredients [118–120]. Available data indicate that transdermal absorption and the systemic availability of oxybenzone are not non-insignificant [82, 121, 122]. Transdermal absorption of oxybenzone in 16 women and 9 men, who were treated with a topically applied sunscreen product containing 4% oxybenzone, showed prolonged systemic availability of oxybenzone following UV exposure [122]. The authors established that although UV exposure was somewhat insignificant to affect the urinary excretion of oxybenzone, renal excretion continued for 5 days following the last application of the sunscreen.

Topical applications of oxybenzone on human skin caused it to penetrate the skin directly via intercellular laminae of the stratum corneum, or otherwise by the passive diffusion under high-concentration gradient conditions, to ultimately reach the blood stream [123]. As well, Gonzalez and coworkers [124] examined the topical application of a commercially available sunscreen that contained 4% oxybenzone over a 5-day period on 25 volunteers. They discovered that ca. 4% of oxybenzone was absorbed into the system [124].

Likewise, Frederiksen et al. [125] detected oxybenzone in more than 80% of urine samples of healthy Danish children and adolescents at a median concentration of 0.92 ng/mL, while Janjua and coworkers [82] detected oxybenzone levels of up to 81 ng/mL in urine samples and up to 238 ng/mL in plasma upon repeated whole-body topical applications of 2 mg/cm^2 of a sunscreen formulation over a 4-day period. The insect repellent *N*,*N*-diethyl-*m*-toluamide (DEET) enhanced skin penetration of oxybenzone as did DEET itself when both repellent and sunscreens were applied concurrently [126]. This led to a systemic circulation of the highly lipophilic oxybenzone that is subsequently transported to different organs, as evidenced by detection in rats' livers [119, 127, 128] and in the brain at levels of 15.5−34.1 ng/g [126].

In their 2008 study, Janjua and coworkers [82] sampled plasma and urine from 15 males (average age, 26) and 17 post-menopausal females (average age, 65) subsequent to a 4-day whole-body topical application of a sunscreen product (dose, 2 mg/cm^2) containing 10% oxybenzone. Figure 13 illustrates the non-insignificant absorption and systemic availability of oxybenzone; octinoxate (10%) and 3-(4-methylbenzylidene) camphor (4-MBC; 10%) were also detected in plasma and urine, albeit to a lesser extent. Confirming the results of Janjua et al. [82], Ye and coworkers [129] also detected oxybenzone in the urine of 30 adults.

Related to the above finding, a 2008 national survey by the United States Center for Disease Control (CDC) [118] of 2,500 individuals aged from 6 years and upwards revealed that oxybenzone was readily absorbed into the body in 97% of those surveyed. Higher levels of oxybenzone were found in young girls and women than in young boys and men, likely due to differences in the use of sunscreens and, where relevant, the use of other body-care products. Most disturbing, however, is the study by Wolff and coworkers who found that 96% of 6–8 year old girls showed detectable amounts of oxybenzone in their urine [130]. Hence, the effects of oxybenzone on children can be nefarious as the surface area of a child's skin relative to body weight is far greater than that of adults, so that the potential dose of a chemical subsequent to being dermally exposed is ca. 1.4 times greater in children [89]. Moreover, children are less likely than adults to detoxify and excrete chemicals; their developing organs are more vulnerable to damage from chemical exposures,

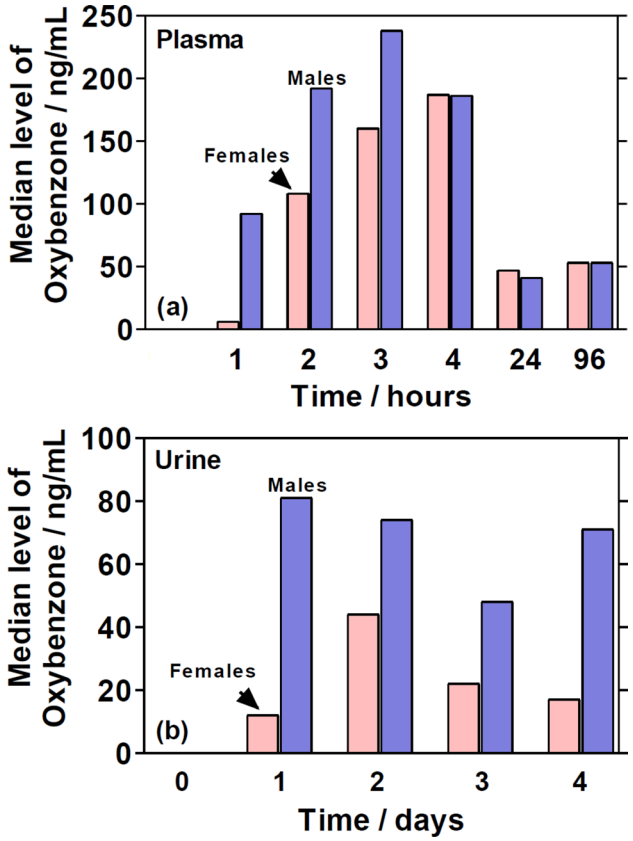


Fig. 13 **a** Concentration of oxybenzone in plasma with daily topical application; **b** concentration of oxybenzone in urine as a function of daily application. Plotted from the data reported in Table 2 of the study by Janjua and coworkers [82]

and not least children tend to be more sensitive to low levels of hormonally active compounds [83, 131].

This notwithstanding, oxybenzone has also been associated with allergic reactions triggered upon exposure to UV radiation. In their study of photoallergic contact dermatitis in 82 patients, Rodriguez and coworkers [132] observed that more than 25% of the patients displayed photoallergic reactions to oxybenzone, while the study of Bryden et al. [133] reported that 20% of those examined in photo-patch tests displayed allergic reactions on exposure to oxybenzone. Besides being able to penetrate the skin, oxybenzone also causes skin damage as it forms free radicals on exposure to UV sunlight [111, 134, 135]. Oxybenzone also aids other chemicals such as the herbicide 2,4-dichlorophenoxyacetic acid to penetrate the skin [136].

Whatever fraction of oxybenzone not absorbed by the body, ultimately it finds its way to contaminate the aqueous ecosystems through washing from the skin when bathing, water playing, and swimming [137, 138]. Wastewater treatment plants remove but a fraction of oxybenzone [139] so that it eventually finds its way into lakes, rivers, and sea waters.

A recent 2020 review article by Suh and coworkers [140] observed that because of the high systemic absorption of oxybenzone and octinoxate by the skin, these agents have been banned in Key West, Florida, and in Hawaii owing to their toxic effects to marine ecosystems. Of the 29 studies examined by the authors, which addressed the impact of these active agents to human health, it seems that even though there have been reported adverse effects through association of oxybenzone levels on the thyroid hormone, on testosterone level, on kidney function and on pubertal timing, elevated systemic levels of oxybenzone appeared to have had no adverse effects on male and female fertility, on female reproductive hormone levels, on adiposity, on fetal growth, on a child's neuro-development, and on sexual maturation.

2.3.2 Avobenzone

The 2019 Federal Register [10] expressed some concerns regarding the fact that no in vivo studies have been carried out to examine the transdermal absorption of avobenzone, even though in vitro studies [85] were carried out, albeit with several weaknesses that limited their usefulness in assessing the potential absorption of avobenzone from formulated sunscreens. Nonetheless, certain chemical properties of avobenzone point to the potential for transdermal absorption when present in sunscreen products. The available data on avobenzone also indicate that it may permeate into at least the dermis and epidermis, which would, therefore, suggest the possibility for avobenzone to impact the development of skin tumors. [10].

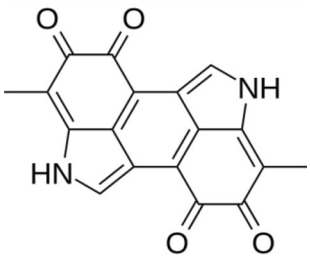
A comprehensive profile on avobenzone in topical sunscreen products was reported by Kockler and coworkers [141] with regard to toxicity, photostability as it may be susceptible to photodegradation, and to its being photostabilized by other agents within the sunscreen products. Avobenzone is highly photo-unstable with respect to other UV absorbers as it photodegrades rapidly on exposure to UV radiation, with its efficacy decreasing from 50 to 90% after 1 h of exposure to sunlight [142, 143]. Accordingly, avobenzone is often combined with a photostabilizer in sunscreen formulations to minimize or altogether suppress such rapid photodegradation.

2.3.3 Other sunscreens

In vitro measurements have revealed that octinoxate penetrates into the dermis to about 0.2–4.5% of applied dose, although systemic absorption is significantly lower. For instance, whole-body topical application of a sunscreen (in cream format) containing 10% octinoxate penetrated through the skin as detected in plasma to an extent of 10 ng/mL in females and 20 ng/mL in males (maximal concentrations),

as well as up to 5 ng/mL in urine of females and 8 ng/mL in males [83]. Octinoxate is also an endocrine disruptor as it interferes, to various extents, with the endocrine system [144–146]. Moreover, exposure to octinoxate has had a statistically non-insignificant effect on testosterone and estradiol levels [83].

The highly lipophilic 4-methylbenzylidine camphor (4-MBC) sunscreen is also absorbed through the skin as evidenced by its occurrence in human tissues and in placenta [147]. It has also been found in urine (4 ng/mL) and in plasma (18 ng/mL) subsequent to repeated topical applications of 2 mg/cm^2 of a sunscreen formulation over a 4-day period [82]. Furthermore, it exhibits toxic activity as an estrogenic endocrine disruptor [87, 88, 145].



Melanin

Fig. 14 Structural formula of melanin

3 Sunscreens and SPF factors

3.1 Sunscreen ingredients

Although UV radiation is an important prerequisite to human health by producing the indispensable vitamin D, there is nonetheless a public health debate regarding the potential risk of vitamin D deficiency from sunscreen use, as sunscreens could impair the synthesis of vitamin D if used in the suggested amount of 2 mg/cm^2; unlikely, however, at quantities below 1.5 mg/cm^2 that are more likely what users would in effect ultimately apply upon rubbing sunscreens onto the skin under real life conditions [148]. As emphasized earlier, sunlight UV radiation exhibits nefarious consequences on overexposure because of its leading role in such health issues as sunburns, photo-ageing and formation of skin cancers, most important of which are the malignant melanomas [110]. Thus, the principal motive to provide broadband photoprotection is to develop novel sunscreens that comprise a combination of UV chromophores.

A primary natural absorber of UV radiation that provides protection to underlying epidermal and dermal elements is melanin (Fig. 14) whose absorption spectrum illustrates why it provides adequate protection from UV radiation. [149] The melanogenesis process causes the skin to darken on exposure to UV radiation, which causes melanin skin pigmentation to absorb light effectively, following which it dissipates over 99.9% of the absorbed UV radiation. [150]. Consequently, melanin protects skin cells from UVB radiation damage, reduces the risk of folate depletion and dermal degradation. Nonetheless, exposure to UV radiation increases the risk of malignant melanoma, a cancer of the melanocytes (melanin-producing neural crest-derived cells located in the bottom layer (the stratum basale) of the skin's epidermis) [149].

Individuals with more concentrated melanin—that is, people with a dark skin tone—show a lower incidence to skin cancer, although a relationship between skin pigmentation and photoprotection remains a debatable and elusive issue [4]. Absorption spectra of epidermal melanin pigmentation in vivo, together with the reactivity of melanin precursors and metabolites to UVA and visible radiation (400−700 nm), have shown that epidermal melanin is not a simple passive absorber that acts as a neutral density filter in the skin as it absorbs UV/visible light strongly at all wavelengths. A study of the action spectra for erythema and pigment reactions to UV radiation in subjects possessing different levels of pigmentation (i.e., different colored skins) found, however, that epidermal melanin does not act as a neutral density filter at all wavelengths, in that while it provides no or little protection toward induced erythema at 295 and 315 nm, it does provide some protection at 305 and 365 nm [149]. Thus, the exact role of epidermal melanin pigmentation in humans needs to be reconsidered.

Absorption of UVB and/or UVA radiation by UV chromophores minimizes the effects of overexposure and thus avoids inevitable skin photodamage. To attain such protection against the nefarious UV rays, however, the chromophores in sunscreens must absorb the radiation energy and then must dissipate that energy rapidly as heat before they photodegrade and before they undergo undesirable reactions with other sunscreen components that would substantially decrease their efficacy. Interestingly, some sunscreen ingredients have been retired from the market: for example, PABA [65], octocrylene [151, 152], and apparently oxybenzone may also have been retired [153].

In addition to the two inorganic UV physical filters ZnO and TiO_2, sunscreen formulations often also comprise organic-based classes of products such as the *p*-aminobenzoates, salicylates, cinnamates, dibenzoylmethanes, camphor derivatives, benzophenones, and benzimidazoles. Of these, only the benzophenones and dibenzoylmethanes cover the UVA spectral region with a representative of the latter class (the enol form of avobenzone) being the most widely used UVA filter. Unfortunately, this filter is highly unstable under UV light as it undergoes an enol → keto phototautomeric reaction followed by intersystem crossing to the triplet state and ultimately C–C bond breaking [154, 155]. Accordingly,

addition of a triplet–triplet (T–T) quencher in the sunscreen formulation ought to improve the photostability of avobenzone [156, 157]. One such T–T quencher, and thus a stabilizer, often added to sunscreen formulations is the (*E*)-isomer of 4-methylbenzylidene camphor (4-MBC; Fig. 15) used especially in the cosmetic industry for its ability to protect the skin against UVB radiation, and which turns out to be rather photostable; it is currently authorized for use in formulations in Europe and in Australia [158]. Based on this rather photostable quencher/stabilizer compound, Pinto da Silva and coworkers [158] examined theoretically the UV absorption of 4-MBC with the main objective being the design of novel UVA filters so that photodegradation could be avoided without the use of other molecules.

To achieve that objective, the authors [158] investigated a series of camphor derivatives based on the (E)-isomer of the benzylidene camphor (BC; Fig. 15) structure. Figure 16a illustrates the eight camphor derivatives with various R_1 and R_2 substituent groups, while Fig. 16b displays the excitation spectra of two of these derivatives (DSBC1 and DSBC2) in comparison with the spectrum of the parent 4-MBC UV filter. Both DSBC1 and DSBC2 derivatives present an absorption band that is red-shifted by 0.35–0.69 eV relative to 4-MBC, and thus cover a more substantive UVA spectral region.

Along similar lines, Losantos and coworkers [159] synthesized a series of compounds suitable for sunscreen formulations owing to their accompanying enhanced stability, rapid dissipation of light energy, and reduced toxicity. The compounds were based on mycosporine-like amino acids (MAAs) found in many marine and freshwater organisms that absorb UV radiation, and thus of interest for human skin protection. Some 20 different compounds were prepared by modifying, for example, some of the critical features of the MAAs cyclohexenimine basic structure: namely, cycle size, substituents, and counterions (Fig. 17) [159].

In particular, these MAAs should prove very useful in photoprotection of skin from the damaging UV rays because they absorb light in both the UVA and UVB range without generating free radicals, and also because they can scavenge

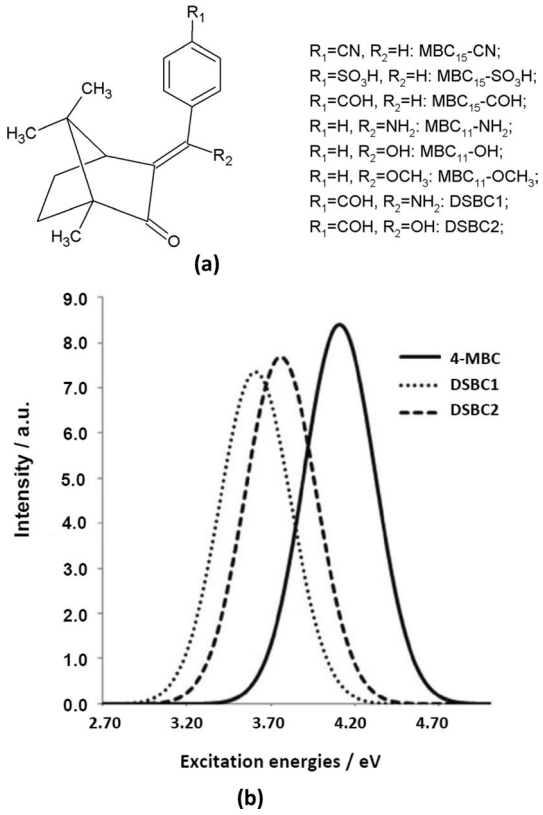


Fig. 16 **a** Schematic representation of the newly designed BC derivatives. **b** Calculated excitation spectra, in ethanol, of 4-MBC, DSBC1 and DSBC2. Reproduced from Ref. [158] with permission from the European Society for Photobiology, the European Photochemistry Association, and The Royal Society of Chemistry

reactive oxygen species (ROS), and are thus excellent antioxidants by suppressing $^1O_2$-induced damage [160]. The MAAs are low-weight, are water-soluble, are thermally and photochemically stable, and are non-fluorescent compounds that absorb light strongly between 310 and 360 nm [161]. Representative systems illustrated in Fig. 18 were evaluated for their absorption of UV radiation (Fig. 19), as well as for

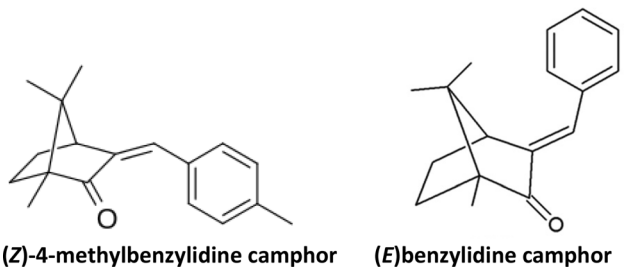


Fig. 15 Structural formulae of (*Z*)-4-methylbenzylidine camphor (4-MBC; known also as enzacamene, eusolex 6300 and parsol 5000) and (*E*)benzylidene camphor

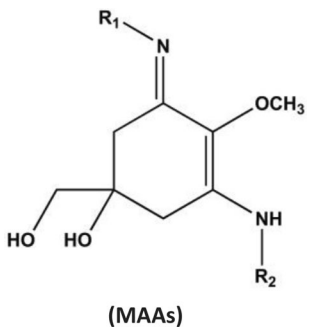


Fig. 17 Critical features of the mycosporine-like amino acids (MAAs) based on the cyclohexenimine structure, where R_2 is a carboxylic acid function

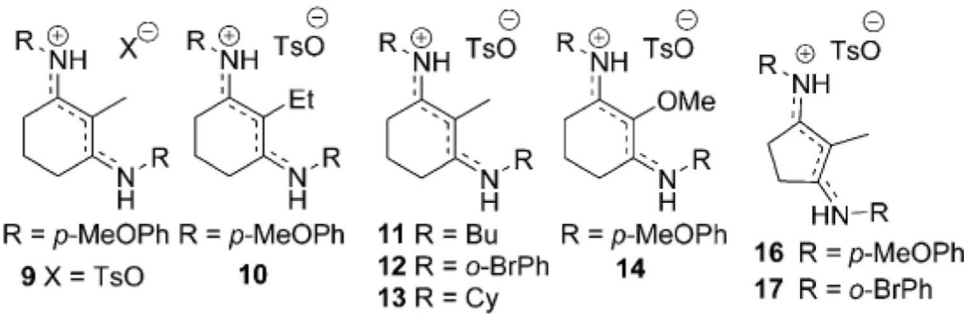


Fig. 18 Selected synthesized compounds 9 through 14 plus 16 and 17 used to investigate their respective properties as potentially novel sunscreen agents. Adapted from Losantos and coworkers [159] with permission (License No. 4954831248661). Copyright 2017 by Wiley–VCH Verlag GmbH & Co. KGaA, Weinheim

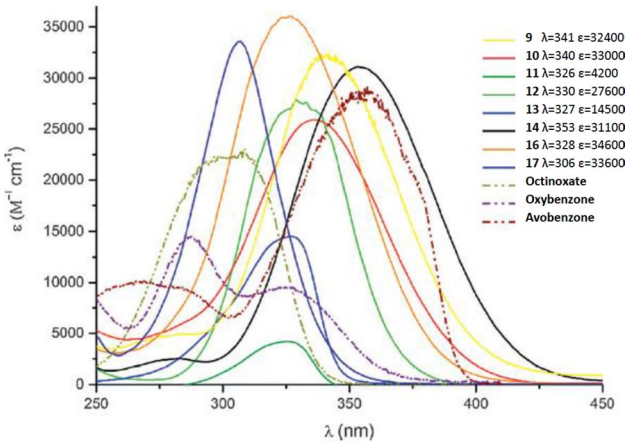


Fig. 19 Absorption spectra (displayed as extinction coefficients, ε) of representative compounds 9–14, 16, and 17 in comparison to the commercial sunscreen ingredients octinoxate, oxybenzone, and avobenzone. Adapted from Losantos and coworkers [159] with permission (License No. 4954831248661). Copyright 2017 by Wiley–VCH Verlag GmbH & Co. KGaA, Weinheim

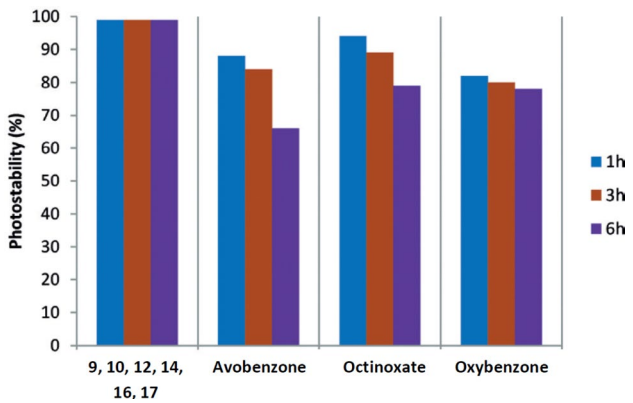


Fig. 20 Histograms illustrating the relative photostability of the synthesized systems 9, 10, 12, 14, 16, and 17 for 1, 3 and 6 h of exposure to UV radiation in comparison to avobenzone, octinoxate and oxybenzone. Adapted from Losantos and coworkers [159] with permission (License No. 4954831248661). Copyright 2017 by Wiley–VCH Verlag GmbH & Co. KGaA, Weinheim

their photostability (Fig. 20) in comparison to some currently used sunscreen ingredients (avobenzone, octinoxate, and oxybenzone).

The absorption spectra (Fig. 19) revealed a tunable wavelength (λ_{max} = 306–360 nm) with very high absorption coefficients (even higher than commercial ingredients). Moreover, no photodegradation occurred even after 16 h of UV irradiation, in contrast to commercial sunscreens—one compound (9, see Fig. 18) remained photostable over a whole summer of being exposed to natural sunlight [159]. These MAAs outclassed most of the commercially available UV filters. Addition of two of these compounds (10% of 16 and 17) in a real formulation containing 10% octinoxate and 5% avobenzone boosted its SPF number from about 29 to 73. Accordingly, these MAAs are rather promising in developing new sunscreen formulations that exhibit significantly greater efficacy [159].

An interesting strategy to limit, if not altogether avoid skin penetration by sunscreen agents, while retaining UV protection and achieve a synergistic effect on UV protection, is to load them onto suitable nanoparticles that would, therefore, not only absorb the UV radiation, but would also scatter the UV light. To the extent that the water-resistant colorless UVB filter Parsol®MCX (i.e., octinoxate) is often incorporated into sunscreen formulations in combination with others in the lipid phase of cosmetic products, it would be a suitable candidate for loading into hybrid nanoparticles composed of lipids and silica. Toward this purpose, Andreani and coworkers [18] developed a new sunscreen formulation that consisted of hybrid Solid Lipid Nanoparticles (SLN)/Silica particulates loaded with Parsol®MCX, and subsequently incorporated into a hydrogel for skin applications as it induced no irritation effects in the HET-CAM test (hen's egg-chorioallantoic membrane). Loading of the Parsol into SLN increased the SPF factor; as well, storage of the Parsol®MCX/ SLN/SiO_2 over a period of 30 days at 25 °C showed the particulates to remain physicochemically stable.

The system hydroxypropyl-β-cyclodextrin (HP-β-CD)/avobenzone loaded onto lipid microparticles was investigated in vivo by Scalia and coworkers [162] to assess the

percutaneous penetration of avobenzone by the tape stripping technique, a minimal invasive procedure that removes the upper cutaneous layers (stratum corneum) with adhesive tape strips. Compared to the suncream with the non-encapsulated sunscreen agent in which ca. 9.7% of the applied dose penetrated, the amount of avobenzone diffusing into the stratum corneum increased with the formulations that contained the avobenzone/HP-β-CD complex (ca. 17% of applied dose), or with the microparticles loaded only with avobenzone (ca. 15% of applied dose). By contrast, the level of avobenzone penetrating the stratum corneum was significantly lower with the micro-encapsulated avobenzone/HP-β-CD complex (ca. 6% of applied dose). The reduced percutaneous penetration of avobenzone attained by the complex enhanced not only the UV filter efficacy but also limited any potential toxicological risks [162].

A preliminary randomized trial with 24 healthy participants (mean age, 35 years old; 12 women; 14 black or African-Americans) was carried out by Matta and coworkers [163] to examine the effect of topical application (quantity: 2 mg/cm^2 over 75% of the body) of four commercially available sunscreen formulations maximally used with regard to the concentration of sunscreens found in plasma. The first sunscreen spray contained 3% avobenzone, 6% oxybenzone, and 2.35% octocrylene; the second spray contained 3% avobenzone, 5% oxybenzone, and 10% octocrylene; the sunscreen lotion contained 3% avobenzone, 4% oxybenzone, and 6% octocrylene; the suncream contained 2% avobenzone, 10% octocrylene, and 2% ecamsule. The maximal concentrations of avobenzone found in plasma were, respectively, about 61%, 77%, 46%, and 32% for the two different sprays, the lotion, and the cream. The corresponding quantities of oxybenzone were ca. 67% for one of the sprays and ca. 52% for the other spray, while the amount for the sunscreen lotion was 44.5%. For octocrylene, the quantities found in the plasma were 102% for the first sunscreen spray, 113% for the second sunscreen spray, about 66% for the sunscreen lotion, and 47% for the suncream. By comparison, the quantity of ecamsule (Mexoryl SX; Fig. 21) was 166% for the suncream. Systemic concentrations greater than 0.5 ng/mL (the limit recommended by the FDA for safety) were reached on the first day subsequent to four applications of all four products [163]. Note that the 0.5-ng/mL threshold reflects the highest plasma level below which the carcinogenic risk of any unknown compound would be less than 1 in 100,000 after a single dose [10, 163].

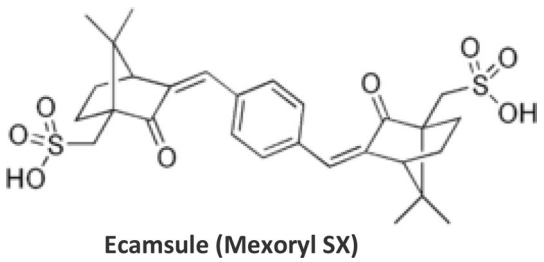


Fig. 21 Structural formula of ecamsule, also known as mexoryl SX

In a later randomized clinical trial study that involved six sunscreen active ingredients (avobenzone, oxybenzone, octocrylene, homosalate, octisalate, and octinoxate) contained in four commercially available sunscreen products (lotion, aerosol spray, non-aerosol spray, and pump spray) and 48 random participants with a mean age of 39 years (24 women an 24 men of whom 23 were white, 23 were African-Americans, 1 was Asian and one was of unknown ethnicity), Matta et al. [164] found that the maximal concentrations of all 6 active ingredients in plasma were greater than 0.5 ng/mL, which was surpassed on the first day after a single topical application of all sunscreen ingredients. Nearly one third of the participants developed a rash. The study further showed the following:

(a) Avobenzone: the maximal concentrations in plasma were: 7.1 ng/mL (lotion), 3.5 ng/mL (aerosol spray), 3.5 ng/mL (non-aerosol spray), and 3.3 ng/mL (pump spray).
(b) Oxybenzone: the concentrations were: about 258 ng/mL (lotion) and 180 ng/mL (aerosol spray).
(c) Octocrylene: concentrations were: 7.8 ng/mL (lotion), 6.6 ng/mL (aerosol spray), and 6.6 ng/mL (non-aerosol spray).
(d) Homosalate: concentrations were: 23.1 ng/mL (aerosol spray), 17.9 ng/mL (non-aerosol spray), and 13.9 ng/mL (pump spray).
(e) Octisalate: concentrations were: 5.1 ng/mL (aerosol spray), 5.8 ng/mL (non-aerosol spray), and 4.6 ng/mL (pump spray).
(f) Octinoxate: concentrations were: 7.9 ng/mL (non-aerosol spray), and 5.2 ng/mL (pump spray).

Clearly, all six of the tested active ingredients administered in four different sunscreen formulations were systemically absorbed. The plasma concentrations surpassed, in some cases, the FDA's threshold of 0.5 ng/mL by several orders of magnitude [10, 164]. As pointed out by the authors [164], however, their study was not without some limitations:

1. A change from indoor to an outdoor setting would have affected the results because the inherent heat and natural sunlight would have better represented real-life sunscreen application, so that the data would have displayed greater variations.
2. The study was meant neither to assess the absorption difference by types of formulation nor by skin types.

3. Because of the different amounts of sunscreen ingredients between products, the data from tape stripping were qualitative rather than quantitative across all four products.

3.2 Sun protection factors (SPF for UVB) and UVA protection factors (UVA-PF)

The SPF value typically reported on the labels of sunscreen products refers to the ratio of the energy required to produce a minimal erythema dose through the sunscreen compared with the energy required to produce the same reaction in the absence of the sunscreen [165]. Consequently, SPF gives one an idea of how long one can remain exposed to the sun's UV radiation before being sunburned. For instance, if one were to sunburn normally in 10 min without a sunscreen, a topical application of a sunscreen with an SPF of 15 signifies that it would then take about 150 min (2.5 h) before observing reddening of the skin—that is, the sunscreen would provide 15 times the protection if it were properly applied at the dose of 2 mg/cm^2 used to determine the SPF factor; in this case, the sunscreen would filter out only ca. 92% of the UVB radiation, while an SPF 30 sunscreen would filter out about 97%. Protection against UVA radiation was estimated to be ca. 10% of the SPF rating for UVB radiation. [165] Consequently, a topically applied sunscreen with an SPF 30 would protect against the UVA radiation with an equivalent UVA-PF of about 3 and probably even less than 3.

SPF assessments are often established under indoor controlled conditions such as humidity, artificial UVB radiation with light irradiances similar to outdoor natural sunlight, but with no wind, no light reflection, no sweating, no toweling, and topical applications repeated hourly using 2 mg/cm^2 of the sunscreen lotion or cream being tested [165]. Accordingly, actual use conditions under UVB/UVA sunlight are likely to decrease the SPF value on the label of the sunscreen product. In this regard, our personal experience (and that of a former collaborator at Oxford University) suggests that when people apply a sunscreen (lotion or cream) they do so by rubbing and rubbing to the point that the amount of sunscreen actually applied may be far less than the 2 mg/cm^2 standard, thereby lessening the SPF value. In addition, most people apply the sunscreen at the beach under the hot Sun—totally against recommendations made by several Cancer Societies or Government Agencies. It cannot be overemphasized that sunscreen protection against UVA radiation is not quantified with regard to both exposure and protection, although in recent years the "+" symbol is being used on some labels to infer a certain level of protection afforded by a sunscreen product against UVA: the greater the number of "+", the greater is the implied protection against UVA radiation; in other words, "++++" means double the protection vis-à-vis "++".

Australia is by far the best laboratory to test various sunscreen products commercially available and thus provides a base with which to regulate sunscreens and their use. Other countries may regulate differently. For example, the United States FDA's Federal Register of 1999 mandated the classification scale for sunscreens to reflect the following:

- Sunscreens with $2 < \text{SPF} < 12$ →→ Minimal sunburn protection.
- Sunscreens with $12 < \text{SPF} < 30$ →→ Moderate sunburn protection.
- Sunscreens with $\text{SPF} \geq 30$ →→→→ High sunburn protection.

In addition, the FDA no longer permitted the use of labels that claimed the sunscreens to act as sunblocks of UVB/UVA radiation or the claim of extended wear, taken to mean a greater number of hours for which one is protected, or the absolute claim all-day protection; the newer allowed labels can only suggest: helps prevent sunburn or higher SPF gives more sunburn protection [165].

By contrast, through its Therapeutic Goods Administration, the Australian Government [166] has placed the upper SPF limit for marketing a sunscreen product to be restricted to "SPF 50+" with possible additional labels:

1. A broad spectrum sunscreen with an SPF of 30 or higher is permitted to carry the following indications:
   a. May assist in preventing some skin cancers.
   b. May reduce the risk of some skin cancers.
   c. Can aid in the prevention of solar keratoses.
   d. Can aid in the prevention of sunspots.

2. A broad spectrum sunscreen with an SPF of 4 or higher is permitted to carry the following indication:
   a. Can aid in the prevention of premature skin ageing.

Factors that typically impinge on the damage to skin caused by UV radiation are: (i) race, (ii) skin type, and (iii) climate with, it seems, Australia being the region that presents the highest rate of developing cutaneous malignant melanoma relative to other geographical regions [167]. It is disturbing to see that ca. 40% of all cancers in the general public results from skin cancers that comprise 80% of basal skin carcinomas, 16% of squamous cell carcinomas, and 4% melanomas [168, 169]. Consequently, it should be fairly evident to beachgoers and suntan aficionados that the use of sunscreens alone will not prevent all the potential risks from one's overexposure to the Sun's radiation. As such, the FDA [10] has urged manufacturers of sunscreen products to include voluntarily a Sun alert statement to the effect that limiting sun exposure, wearing protective clothing, and

using sunscreens may reduce the risks of skin photo-ageing, skin cancers, and other harmful effects of the sun.

A 2013 analysis by Wang and coworkers [170] on trends in the level of photoprotection, afforded by commercially available sunscreen products against excessive exposure to UV radiation for the period 1997 to 2009 in the United States, revealed a noteworthy increase in the number of available sunscreens displaying higher SPF values. Specifically, the number of low SPF products (SPF 4–14) decreased from 27% in 1997 to 6% in 2009, while the number of products containing the UVA filters avobenzone and zinc oxide increased from 5% in 1997 to 70% in 2009. There were significant improvements into sunscreen products, the most significant of which was the extent of increased protection against the shorter UVA1 wavelengths (320−340 nm) [170].

Using an in vitro method, Diffey and coworkers [19] were one of the first groups to assess the broadspectrum UV protection afforded by sunscreen products. They recognized that although the SPF value on sunscreens' labels provided an effective index of protection against erythemally solar UV radiation, largely confined to the UVB wavelengths and to the shorter UVA1 wavelengths (320−340 nm), there was a void as to how broadspectrum protection could be assessed against the longer UVA2 wavelengths (340−400 nm). Accordingly, they investigated 59 commercially available sunscreen products and multiple experimental formulations with one or more UV filters using absorption spectroscopic techniques; a quantity of 1 mg/cm^2 was applied to a hydrated synthetic collagen substrate irradiated with a solar simulator to estimate the critical wavelength at which the integral of the spectral absorbance curve reached 90% from 290 to 400 nm. In a later study [171], they examined the effect of UVB absorbing sunscreens on the reflectance and consequent protection of Caucasian skin in vivo using olive oil as the vehicle for the octinoxate and avobenzone. All the formulations reduced the reflectance of the skin throughout the UVA spectral range (320−400 nm), with the formulations containing UVB and (UVB + UVA) absorbers reducing the reflectance to a greater extent than the sunscreen vehicle alone, thereby facilitating greater penetration of the UV radiation to the skin's lower epidermis and dermal layers, and thus lessening the sunscreen's efficacy.

The efficacy of avobenzone, zinc oxide, and titanium dioxide in broadspectrum sunscreen products to provide UVA protection was characterized by Beasley and Meyer [20] by measuring in vitro the absorbance profiles using an inert substrate (Vitro Skin®) and by determining the UVA protection factors (UVA-PF) on human skin. Loss of avobenzone had a non-insignificant impact on SPF and UVA-PF values. The authors also appraised the photostabilizing influence of specific ingredients in formulations that precluded, or otherwise minimized the UV-induced photodegradation of avobenzone, while the photostability of commercial sunscreen products was quantified by the extent to which the absorbance within the UVB and UVA spectral regions changed subsequent to irradiation of thin films of product on inert substrates [20]. Apparently, TiO_2 provided neither the same level of UVA attenuation nor the same degree of UVA protection on human skin as did products containing photostabilized avobenzone or ZnO, which led the authors to infer that TiO_2 was not a suitable substitute for avobenzone or ZnO in providing high levels of UVA protection to human skin. This inference is rather curious as our nearly 40-year experience in studying metal-oxide photocatalysts suggests that both TiO_2 (bandgap of anatase TiO_2: 3.2 eV; absorption edge: 387 nm; bandgap of rutile TiO_2: 3.0 eV; absorption edge: 413 nm) and ZnO (bandgap, 3.3 eV; absorption edge, 376 nm) display similar spectra (see, for example, Figs. 5, 6).

The above notwithstanding, however, the efficacy of sunscreen products is most relevant when determined on the skin surface it is intended to protect, namely human skin in vivo. In this regard, Gillies et al. [172] used a non-invasive in vivo protocol to assess the UVA efficacy of topically applied sunscreens using diffuse reflectance spectroscopy (DRS) to measure changes in optical properties of the skin decoupled from biological responses. The assessment of UVA efficacy of oxybenzone and avobenzone at different concentrations (0–5%) was carried out with 20 volunteers enrolled for each product measured by DRS and 10 different individuals for each product measured by the human phototest (quantity of sunscreen, 2 mg/cm^2). The UVA efficacy at each concentration of product was estimated from the measured transmission spectra of the products convoluted with the spectrum of the Xenon light source, adequately filtered to acquire the UVA spectrum from 320 to 400 nm as well as the action spectrum of erythema. Results of the UVA efficacy from the DRS technique closely correlated with those from human phototesting [172]. Even so, however, they encouraged individuals to seek shade when outdoors, use photoprotective clothing (including hats) and sunglasses, and apply sunscreens on exposed areas by hand, preferably indoors about 20−30 min before exposure to natural solar radiation.

A newer non-invasive approach to assess SPF values in vivo using DRS spectroscopy and in vitro transmission was reported by Ruvolo and coworkers [173]. The method required neither extensive UV irradiation procedures, nor biological responses typically used to establish sunscreen efficacy. It combined an evaluation of the absolute UVA absorption spectrum (measured by DRS) with the spectral shape of the UVB absorbance in the spectral region 290–320 nm of the test material determined by in vitro thin film spectroscopy. Measurement of the in vivo UVA absorption spectrum involved the assessment of the remitted

intensity of monochromatic UVA radiation (320–400 nm) before and after a sunscreen product was applied on the skin; the method was limited to skin types I, II and III (see Table 1).

Sunscreen formulators always claim that their products provide good protection against UVB/UVA radiation. To ascertain such claims, Garoli and coworkers [174] investigated whether in vitro SPF data correlated with the SPF values given on the products labels; also examined were the photostabilities of commercially available products. The method used to assess in vivo SPF values is the internationally accepted COLIPA standard test for sunscreen products [175], a rather expensive and time consuming method that does not facilitate routine quality control, in addition to which results tend to be controversial and often not reproducible, so that in vitro techniques have been developed based on absorbance/reflectance measurements of sunscreens applied on suitable UV transparent substrates [174]; a revised COLIPA in vitro method has been reported [176]. The study by Garoli et al. [174] revealed a good correlation between the in vitro measured SPF values and the SPF reported on the labels of sunscreen formulations; the correlation was especially good at the lower quantities of product applied (e.g., 0.65 mg/cm^2) on the substrate, as demonstrated in Fig. 22a, b. To the extent that the standard quantity used for SPF determinations is 2 mg/cm^2 calls attention to a lesser reliable correspondence than claimed. Photostability tests

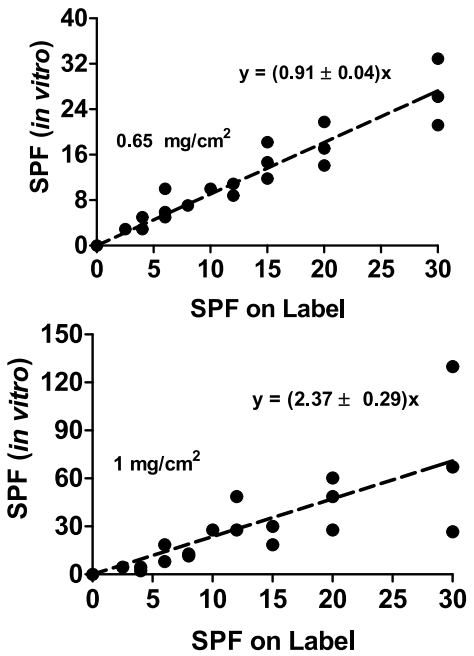


Fig. 22 Correlation between in vitro SPF values and SPF values reported by sunscreen formulators for quantities of 0.65 mg/cm^2 and 1 mg/cm^2 of sunscreen product applied. Adapted from the data reported by Garoli and coworkers [174] with permission (License No. 4954810064863). Copyright 2008 by Elsevier B.V

indicated that infrared radiation (heat) also played a role in the photodegradation of sunscreens.

Inasmuch as in vitro SPF screening methods of oleosome-based SPF products show significant inconsistencies and low reliability in SPF values, Yang et al. [177] developed a more reliable spectrophotometric method to screen oil body-based SPF formulations that contained two broadly used organic UV sunscreen active agents: octinoxate and avobenzone loaded into safflower oil bodies and formulated into oil-in-water emulsion-based finished products. The reliability between the in vivo protocol carried out in a clinical laboratory and the spectrophotometric method used in the authors' laboratory showed a significantly higher level of reproducibility and reliability compared to the U.S. FDA-directed in vivo SPF testing method.

It must be recognized that the in vitro star system (*) or the "+" methodologies used to suggest a level of UVA protection afforded by sunscreens are not absolute in deciding UVA skin protection as they represent but a ratio of the total integrated UVA/UVB absorption. It is possible, therefore, that usage of traditional broadspectrum sunscreens may well fail to fully protect against skin damage by radical species generated either directly by UVA, when skin is exposed to natural sunlight, or indirectly from the photo-degradation of the UVA filters as evidenced by anisotropic spectra of DMPO-trapped oxygen-centred radicals from lipid oxidation detected in irradiated sunscreens with added DMPO [178]. To this end, Haywood et al. [178] developed an ex vivo ESR/spin trapping protocol to measure sunscreen protection against solar-simulated radiation-induced structural radical damage to skin. Results showed that sunscreen formulations with SPF 20+ and UVA protection reduced the quantity of DMPO-trapped protein radical adducts by 40–65% when applied at a dose of 2 mg/cm^2. Accordingly, the authors suggested that sunscreen protection could be improved through the design of broadspectrum filters that will minimize radical leakage and lipid oxidation. In an analogous study, Wang and coworkers [179] proposed a novel strategy to minimize the extent of UVA-radiation damage caused by formation of radical oxygen species. It involved addition of antioxidants to sunscreen formulations, as available commercial sunscreen products provided free radical protection only through the presence of UV filters rather than through antioxidants.

A question that needs to be asked is whether a sunscreen with a higher SPF provides greater protection against UV radiation relative to a product with a lower SPF. In this regard, Ghiasvand et al. [180] performed a thorough evaluation of data collected in a Norwegian study conducted between 1991 and 2007 that implicated over 147,900 people. The sunscreen with the higher SPF rating (SPF greater than 15) showed superior beneficial effects with respect to melanoma risk than a sunscreen product with a lower SPF

rating (≤ 15). This was corroborated by Williams and coworkers [181] who carried out a randomized, double-blind, split-face, clinical trial under natural sunlight exposure for a sunscreen product with an SPF 100+ rating against one with an SPF 50+ rating. The study involved 199 healthy men and women participants of 18 years or older, each of whom sported both sunscreens simultaneously during activities, with no restrictions imposed other than the treatment area being identified. Reddening of the skin was clinically assessed the day following exposure, while the efficacy of each sunscreen was evaluated from a comparison of sunburns between treatment areas assessed separately for each treated area. After approximately 5 to 7 h of sun exposure, about 55% of the participants (110 of 199) were more sunburned in the area that was treated with the SPF 50+ sunscreen, whereas only about 5% (10 out of 199) showed similar results with the SPF 100+ [181]. Clearly, the SPF 100+ sunscreen was significantly more effective in protecting against sunburn than was the SPF 50+ sunscreen under actual use conditions.

Chou and coworkers [182] used UV–Vis spectroscopy to compare rapidly the efficacy of various sunscreen formulations toward UVB absorption in two stages: the first stage examined formulations from a single brand (brand S) with SPF values of 8, 15, 30, and 50 under identical conditions (0.02 g of a sunscreen sample in 70 mL of a 70/30 *iso*-propanol/water solution), while the second stage compared several other commercial sunscreen formulations of different brands all displaying SPF 30 with the formulation of brand S also with SPF 30. Figure 23 reports the UV–Vis absorption spectra of the four sunscreens from the same brand X indicating that all formulations absorbed the UVB radiation, albeit to a different extent: SPF 50 > SPF 30 > SPF 15 > SPF 8; that is, maximal absorbance correlated linearly with the SPF value for the same brand's sunscreens. No less

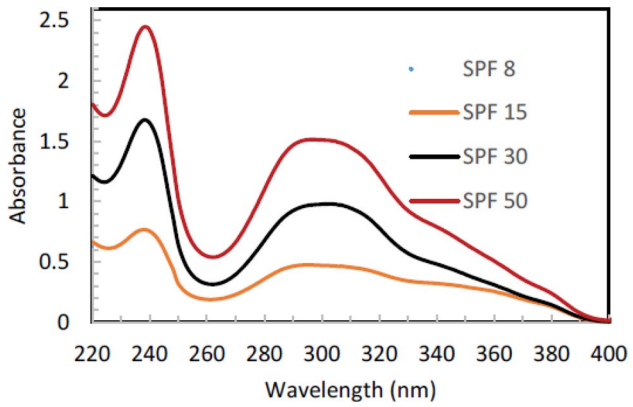


Fig. 23 UV–Vis absorption spectra for the SPF 8, 15, 30 and 50 sunscreen formulations of Brand S. Reproduced with permission from Ref. [182] through the Creative Commons Attribution 4.0 License and Scholars Open Access (Open Access Publisher)

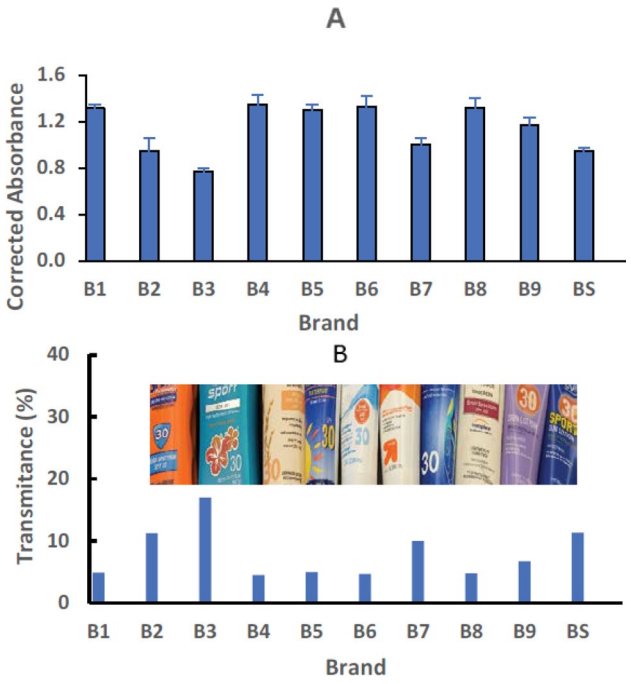


Fig. 24 Corrected absorbance (**a**) and transmittance (**b**) at λ_{max} of different sunscreen formylations from different brands with an SPF 30. Reproduced with permission from Ref. [182] through the Creative Commons Attribution 4.0 License and Scholars Open Access (Open Access Publisher). Copyright by the authors

than 9 different brands of sunscreen products (B1 to B9) were examined subsequently and compared with the Brand S (BS) formulation. Figure 24a [182] shows that although they have the same SPF 30, they display some variations as they absorb different quantities of UVB radiation; six brands (B1, B4–B6, B8, B9) absorbed UVB light more than Brand S, while brands B2 and B7 displayed comparable absorption with Brand S. Figure 24b illustrates the transmittance of all 10 sunscreens that varied from 4 to 17% indicating that most of them would provide a satisfactory protection against UVB as they absorbed 89% or more of the UVB rays.

On a more practical aspect, Vergou et al. [183] used non-invasive optical and spectroscopic techniques to investigate the La Roche-Posay's sunscreen Anthelios XL Fluide Extreme with SPF 50+ that consisted of 7% octocrylene, 5% bemotrizinol, 5% octisalate, 4% avobenzone, 3% drometrizole trisiloxane (Mexoryl XL), 2.5% titanium dioxide, and 0.5% Mexoryl SX (ecamsule). Optical and spectroscopic results revealed a highly homogeneous distribution of this sunscreen on both the skin's surface and in the deep furrows. According to the authors [183], it provided a high protective efficacy of the sunscreen in both the UVA and UVB spectral ranges.

For comparison, Im and coworkers [184] investigated the lipophilicity, chemical stability, and enzymatic hydrolysis of fatty ester prodrugs of salicylic acid, together with

an in vitro evaluation for UV protection. Of the several systems examined (octanoyl, nonanoyl, decanoyl, lauroyl, myristoyl, and palmitoyl oxysalicylate), the palmitoyl oxysalicylate showed significantly higher dermal accumulation in all types of skins relative to its parent salicylic acid, thus making it a potential candidate for UV protection because of the lack of skin permeation, smaller uptake in the lipid phase, and relatively lower skin accumulation [184].

The natural sunscreen soybean oil (contains vitamin E) absorbs UVB radiation and possesses antioxidant properties, and thus can lessen the photooxidative damage caused by the presence of UV-generated reactive oxygen species; many other natural oils provide not enough UV protection. A combination of soybean oil (2.73%) avobenzone (3%), octinoxate (7.5%), and paraffin oil (0.27%) yielded a nanoemulsion formulation with SPF ca. 22 (assessed in vitro by UV spectrophotometry), higher than a sunscreen nanoemulsion without the soybean oil (SPF ≈ 17), and also higher than a sunscreen emulsion composed of avobenzone and octinoxate (SPF = 15) [185]. The nanoemulsion sunscreen combination proved more stable over a 12-week period at ambient temperature than was the sunscreen emulsion.

To enhance the sun protection factor of commercial sunscreens, Borase and coworkers [26] incorporated latex-synthesized gold nanoparticles (2 and 4 wt%) that increased the SPF from 2.4 to 24.1% (UV-Vis absorption spectroscopy) than the parent sunscreen devoid of gold nanoparticles.

An unanswered question is whether an individual's pretreatment with cosmetic creams/lotions impacts the distribution and adhesiveness of sunscreens on skin, and thus on the degree of UV protection. The homogeneity of sunscreen distribution, its resistance to water, and its effective UV protection were evaluated by Kluschke et al. [186] who enrolled 18 volunteers using a combination of tape stripping and UV–Vis spectroscopy. Was there a gain or loss of efficacy of the sunscreen before and after swimming? It appears that cosmetic skin pretreatment affected neither sunscreen homogeneity, nor its effective UV protection prior to contact with water. However, compared to a non-pretreated skin, there was a considerable loss of water resistance and loss of sunscreen, and thus loss of UV protection. Accordingly, it would be wise for the consumer to avoid applying cosmetic creams/lotions prior to the topical application of sunscreens [186].

Using an innovative approach for UV skin protection, Andreani and coworkers [18] developed a novel sunscreen formulation that consisted of a hybrid system comprising solid lipid nanoparticles and silica particles (SLN-Si; mean size, 210 nm) loaded with octinoxate (Parsol®MCX), and subsequently incorporated into a hydrogel for skin administration. Determination of SPF was carried out using the Mansur equation (Eq. 1) [187] for UVB wavelengths (290–320 nm) at 5 nm increments that yielded an estimated SPF of 10.6 for the Parsol-SLN-Si combination.

$$\mathrm{SPF}_{\mathrm{spectrophotometric}} = \mathrm{CF} \times \sum_{290}^{320} \mathrm{EE}_{\lambda} \times I_{\lambda} \times \mathrm{Abs}_{\lambda}, \tag{1}$$

where CF is a correction factor, EE_{λ} refers to the spectrum of the erythemal effect, I_{λ} represents the spectrum of the solar intensity, and Abs_{λ} is the sunscreen absorbance. By contrast, Diffey et al. [188] estimated the SPF of sunscreens from transmission measurement data using Eq. 2,

$$\mathrm{SPF} = \frac{\sum_{290}^{400} E_{\lambda}\varepsilon_{\lambda}}{\mathrm{PF}_{\lambda}}, \tag{2}$$

where E_{λ} is the spectral irradiance of terrestrial sunlight under some defined conditions (for example: mid-day, mid-summer sunlight for Southern Europe) and ε_{λ} is the relative efficacy of UV radiation at wavelength λ (nm) that produced delayed erythema on human skin (the so-called erythema action spectrum); both sets of data were available from published literature. The monochromatic protection factor at wavelength λ (nm) $\{\mathrm{PF}_{\lambda}\}$ is the ratio of the transmittance data recorded at wavelength λ with no sunscreen applied to a section of the 3 M Transpore™ tape to the transmittance data recorded with sunscreen applied onto another section of the tape.

So far, the above discussion has focused on the notion that SPF values point to efficacies of sunscreen products to protect people from the damaging UV radiation. Studies that have estimated SPF indices of various sunscreen formulations have made no less than two critical assumptions: (a) SPF is independent of light irradiance, and (b) SPF is independent of the UV radiation dose. Bacardit and Cartoixà [189] revisited theoretically the role of irradiance and the UV dose in determining the magnitude of SPF, especially when sunscreen ingredients undergo photodegradation on exposure to UV radiation. The authors estimated that the two assumptions may be valid if the relaxation times of the sunscreen active ingredients were less than ca. 10 ns (relaxation rate $> 10^8$ s) as relaxation of sunscreen molecules would normally occur within picoseconds. As noted by the authors, however, where relaxation dynamics are far greater than 10 ns—i.e., in the microsecond timeframe—the performance of the sunscreens may be compromised as the SPF would be different under natural sun irradiance from SPF obtained under solar simulated conditions in a laboratory setup. Figure 25 [189] illustrates a simulation for a sunscreen with an SPF 50 except that the relaxation time was set at $\tau_r = 10$ µs. On irradiating at an erythematic effective dose up to 400 mJ/cm^2, the SPF would be 34 under natural solar conditions (280 – 400 nm range; irradiance, 61 W/m^2 [197]), while SPF would be 13 if it were assessed in a solar simulator

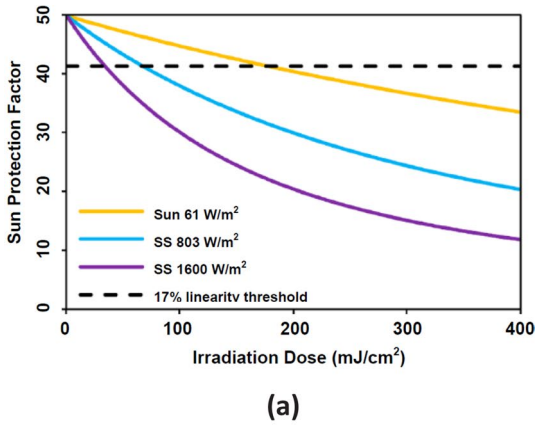


Fig. 25 **a** Plots illustrating the SPF versus irradiation dose at different UV light irradiances E_0 under natural sunlight (Sun) and simulated sunlight (SS); **b** cartoon illustrating the SPF behavior under solar conditions and under low and high UV light irradiances in a laboratory setup. Adapted with permission from Ref. [189]. Copyright 2020 by the American Chemical Society

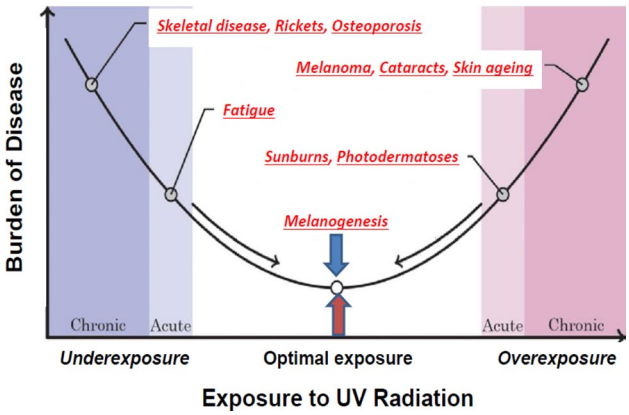


Fig. 26 Plot illustrating the burden of disease exhibited by humans (and plants) in response to exposure to UV radiation that might lead to a number of ailments as a result of acute and chronic underexposure or overexposure. Arrows indicate the gene-regulated pathways to respond to changing exposure to UV radiation so as to minimize the incidence of disease. Adapted from Baker and Stavros [192] as a pre-approved permission request (https://us.sagepub.com/en-us/nam/pre-approved-permission-requests-journals). Copyright 2016 by SAGE Publishing

(irradiance, 1600 W/m^2). Moreover, a sunscreen that may be photostable for 5 h under natural solar conditions would behave differently if it received the same irradiation dose under accelerated solar simulator conditions. [189].

In their eloquent and extensive article, Baker and coworkers [191] summarized some of the natural protective tools displayed by individuals in response to changing levels of UV radiation and further emphasized the time when such tools may be inadequate and thus cause the burden of disease to increase (Fig. 26) [192]. The article by Mancuso et al. [193] discussed various UV filters, the benefits of sunscreens, the regulations and controversies pertaining specifically to sunscreens, the importance of broadspectrum protection, the concerns regarding sunscreen formulations and their photostability, together with education and compliance of people vis-à-vis the use of sunscreens. Within this context, and bearing in mind the effects originating from being exposed to UV radiation relative to physiological responses in individuals, it is relevant to note that more than 90% of the body's vitamin D requirements are satisfied by the UVB-mediated conversion of 7-dehydrocholesterol to previtamin D_3, a precursor of vitamin D [194]. The potential neurotoxicity of sunscreen ingredients was described by Ruszkiewicza and coworkers [11] in their review on the question of cost–benefit and large scale use of sunscreens. The authors further emphasized that those adverse neurotoxic effects of UV filters at concentrations significantly greater than those observed in the environment and in human tissues should not be ignored, as they may point to potential pathological mechanisms that could occur under other conditions and/or in sensitive populations [11].

We have already alluded to the consequences of being overexposed to the Sun's UVB radiation with regard to the development of skin cancers (basal and squamous cell carcinomas, and malignant melanomas) that, according to the study by Lucas and coworkers [195], were responsible for 55,000 fatalities worldwide, not to mention the worldwide 3 million annual cases of cataracts and the possible link to some mental illnesses. By contrast, underexposure to the Sun's UVB light can cause a vitamin-D deficiency, which can subsequently cause the development of skeletal disease and osteoporosis [194, 196].

3.3 Photostability of sunscreens

Photostability of a sunscreen formulation is a most crucial property for an effective photoprotection of skin under sunlight UV. To the extent that most sunscreen UV filters absorb sunlight energy, they may undergo some sort of photoreaction to generate some photoproducts that can absorb radiation in other spectral regions, in which case their photoprotective efficacy would be reduced. In view of their structural nature, UV filters are also good photosensitizers, so that when exposed to UV radiation, their photochemical intermediates or stable photoproducts could easily interact with skin components that would cause phototoxic and/or photoallergenic responses. Most important, however, photoproducts could themselves be toxic, which would then necessitate their overall safety to be considered carefully when using sunscreens.

Health risks originating from sunscreen products, whether or not they are photostable, comprise phototoxicity, photoirritation, and/or photoallergic responses, although in the longer-term responses are also associated with photoageing of skin and increased danger of skin cancers [143]. Contrary to others' viewpoints, Nash and Tanner [143] indicated that the prevalence of photoirritation and photoallergic responses to sunscreen formulations is seemingly rare vis-à-vis other adverse effects: for example, skin irritation or sensitization produced either by cosmetics or by topically applied drugs, and do not necessarily directly implicate photoproducts from UV-irradiated sunscreen filters. Moreover, for the photo-unstable combination of octinoxate and avobenzone, the long-term reduction in skin cancer benefiting humans likely outweighs adverse consequences ascribed to photodegradation. The authors defined photostability and photo-instability and have objectively assessed the acute and chronic toxicological consequences that might result from human exposure to UV-irradiated sunscreen filters and to their plausible photodegradation products.

A major focus of formulators of sunscreen products is attaining maximal efficacy of their products and so, whether by necessity or design, they integrate measures to achieve and enhance photostability as all chemical UV filters will sooner or later undergo some degree of photodegradation. Nash and Tanner [143] also emphasized that human exposure to potentially photo-unstable UV filters as well as sunscreen products that may or may not cause health risks under conditions of sunscreen uses. The view that the potential formation of photoproducts from photo-unstable sunscreens may be hazardous and could potentially lead to unacceptable human health risks appears not to be supported by an historical understanding of adverse events associated with exposure to photostable or photo-unstable sunscreen products [143]. Their view notwithstanding, however, formulators may be more inclined on convincing people that their sunscreen products are safe through various marketing strategies than focus on the actual safety of their products to human health.

In the present context, personal observations in the late 1990s noted that manufacturers of sunscreen lotions/creams used a variety of marketing strategies to lure consumers to purchase their products. For instance, in 1996 their strategy popularized the presence of micronized TiO_2 particles in sunscreen products (purchased by the author in Italy) in which the label stated:

> "…protects the skin against sunburns and harmful effects induced by the sun because its water-resistant anti-UVA + UVB protective system contains Micro-reflectors™ that act as small mirrors to protect the skin from the sun's harmful rays".

A year later, the strategy emphasized the presence of Mexoryl SX as one of the active agents, whereas in 1998 the marketing strategy—at least in one of the sunscreen products sold in the UK—focused on the photostability of the sunscreens, while in 2000 the marketing emphasized the presence of Parsol 1789 (i.e., avobenzone) as the sunscreen active ingredient. Given these observations and in collaboration with a colleague from Oxford University (UK), we began an extensive study in 1997 of sunscreen products and their active ingredients with a focus on the photostability of the organic chemical UV filters and the effect of inorganic physical UV filters on supercoiled DNA plasmids, inasmuch as some of the sunscreens also contained TiO_2 (and/or ZnO), which at the time was being investigated extensively by us [41–43] and others as the pillar highly-photoactive catalyst to eliminate organic pollutants from various aqueous and atmospheric environments. [197] Table 3 summarizes the 10 or so commercially available sunscreen (SN_x; $x = 1$ to 10) lotions investigated—also given are the SPF on the labels and the active ingredients destined for UVB/UVA protection.

Currently, it is fairly evident that, for an appropriate protection against the Sun's UV radiation, the applied sunscreen lotions must be photostable so as to afford the consumer the kind of protection implicit by the value of the SPF factor, a value that applies solely and specifically to UVB radiation, not to UVA radiation. Consequently, the question is whether or not the sunscreen's ingredients are photostable on exposure to natural sunlight.

Despite earlier claims, our studies demonstrated that they were unstable toward UV radiation [111, 197], since any molecule that absorbs UV energy must somehow dissipate that energy. This can proceed either via (i) radiative decay (emission), and/or (ii) non-radiative decay as heat, and/or (iii) the photoactivated molecule undergoes some sort of photoreaction that leads to its breakdown and generates photoproducts. As a consequence, they no longer provide

Table 3 Summary of active ingredients in the sunscreen lotions together with their corresponding SPF numbers

Sunscreen lotion	SPF	Active ingredients noted in sunscreen formulations
NP1[a]	15	Octinoxate; aloe vera gel (natural product)
SN1	19	Octinoxate; oxybenzone (OB); phenyl benzimidazole sulfonic acid (PBSA); titanium dioxide
SN2	25	4-Methylbenzylidine camphor (MBC); avobenzone (AVO); terephthalylidene dicamphor sulfonic acid (TDSA); titanium dioxide
SN3[b]	25	4-Methylbenzylidine camphor; terephthalylidene dicamphor sulfonic acid; avobenzone; titanium dioxide
SN4[b]	25	4-Methylbenzylidine camphor; terephthalylidene dicamphor sulfonic acid; avobenzone; titanium dioxide
SN5	15+	Octinoxate; octyl salicylate (OS); titanium dioxide
SN6	20+	Octinoxate; 4-methylbenzylidene camphor; titanium dioxide
SN7	30	Titanium dioxide; avobenzone; triethanolamine salicylate (TES); terephthalylidene dicamphor sulfonic acid
SN8	–	Titanium dioxide
SN9	15	Titanium dioxide
SN10	25	Titanium dioxide; zinc oxide

[a]Cream made up in a pharmacy in Torino, Italy

[b]Different brands but contained the same active ingredients

the expected UV protection and may even become an indirect cause of allergies and/or photoinduced allergies. This deficiency of photostability of chemical UV filters is now a well-recognized issue, which could be alleviated if two or more active ingredients were mutually present in a commercial formulation, or else other molecules might be present that would quench the photoreactions. Moreover, it is important to note that the efficacy of a sunscreen is more often tested for its ability to prevent skin erythema than it is at the molecular and cellular level to assess its efficacy toward prevention of skin cancers.

Accordingly, apart from having a large absorption cross section in the UVA and UVB regions of the electromagnetic spectrum, the active agents in these photoprotective sunscreen products must safely dissipate the excess energy—preferably as heat—without the release of photoproducts or initiation of subsequent harmful photochemistry.

3.4 Behavior of a UVB filter under irradiation: octinoxate

Of the UVB filters noted in Table 3, octinoxate appears to be the most widely used UVB absorber, and together with the UVA filter avobenzone is, to a large extent, a significant component of commercial sunscreen formulations marketed in most countries [198]. Consequently, both these UV filters were examined for their photostabilities in natural product gels (Aloe Vera) and in various polar (water, methanol) and nonpolar (hexane) solvents. The photostability of oxybenzone was also investigated as it is yet another UVA filter often used in formulations. It cannot be overemphasized, however, that photoproducts produced from the photodegradation of UV filters may absorb lower wavelength UV light relative to their parent compounds and may cause allergic skin reactions, among other toxic effects, as noted by Butt and Christensen [64] who demonstrated that exposure of octinoxate to UV light increased its toxicity to mouse cells.

Figure 27a illustrates the photo-instability of octinoxate in Aloe Vera gel as the sunscreen vehicle dissolved in aqueous methanolic media subjected to simulated sunlight UV illumination (sunscreen formulation NP1; see Table 3); the attenuance of NP1 was reduced by ~65% after 2 h (see Fig. 27c) [111]. Figure 27b displays the highly photostable Aloe Vera gel, an original Brazilian plant gel used in cosmetics to heal sunburn-damaged skin. The Aloe leaf gel extract NP2 contained a natural UVB/UVA sunscreen which photodegraded by less than ca. 5% after 2 h of exposure to simulated sunlight in aqueous media.

In a related study of various UVB filters, Ricci et al. [199] investigated the photostability of octinoxate when exposed to UVA radiation in the absence and presence of TiO_2, often used in combination with organic filters in sunscreen formulations (see e.g., Table 3). They found that, in the absence of TiO_2, octinoxate undergoes the expected *E*/*Z* isomerization, a process that describes the absolute stereochemistry of double bonds. By contrast, the presence of TiO_2 caused the photoinduced mineralization of octinoxate with the process being markedly faster in the presence of a surfactant than it was in a sunscreen formulation, as well as in aqueous suspensions.

By comparison, our own work [111] demonstrated that the extent of photodegradation of octinoxate was significantly greater in aqueous media (90%; Fig. 28a) than in n-hexane media (40%; Fig. 28b) subsequent to its exposure to UV for 30 min. However, nearly complete degradation (ca. 95%) of octinoxate occurred in the latter non-polar solvent after 2 h of illumination; quantum yields of degradation were $\Phi_{320nm} \geq 0.04$ (in water) and $\Phi_{290nm} \geq 0.02$ (in

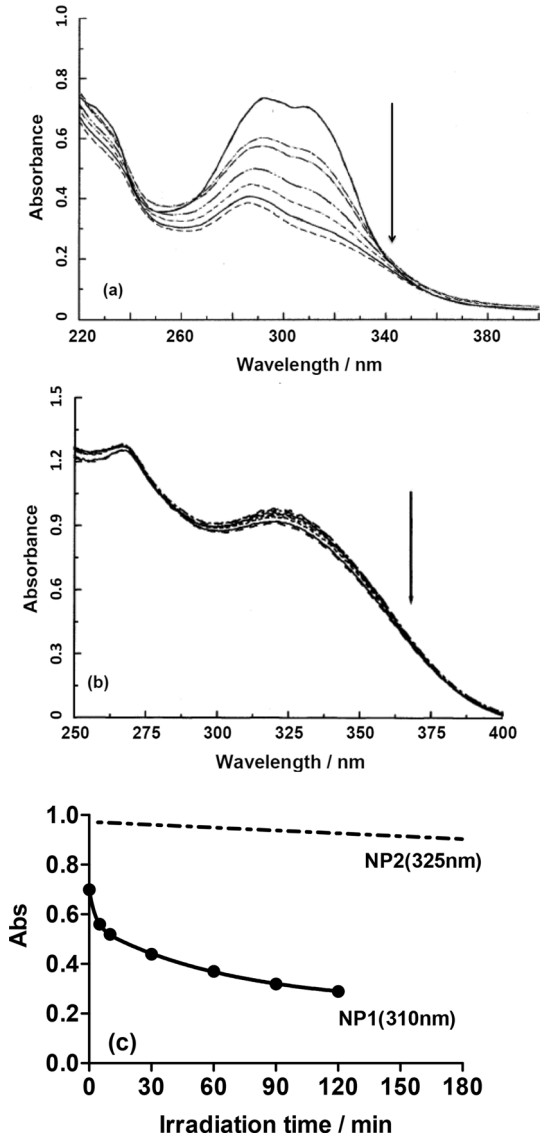


Fig. 27 **a** Absorbance changes during the simulated sunlight UV illumination of the NP1 sunscreen formulation containing octinoxate and Aloe Vera gel as the vehicle (see Table 3) dissolved in 20/80 v/v% water/methanol; **b** absorbance changes during the simulated sunlight illumination of the pure Aloe Vera gel (NP2) in aqueous media; **c** plot displaying the photodegradation of the octinoxate filter (NP1 formulation) versus the relatively photostable Aloe Vera gel (NP2). Reproduced and adapted from Ref. [111] with permission from the European Society for Photobiology, the European Photochemistry Association, and The Royal Society of Chemistry

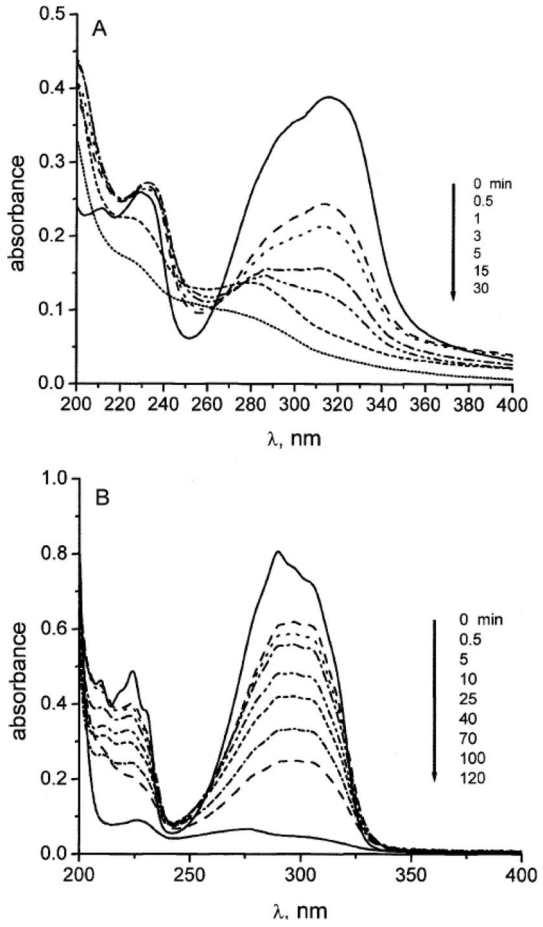


Fig. 28 Time course of the spectral changes occurring during the UVB/UVA irradiation of octyl methoxycinnamate (octinoxate) under aerobic conditions in (**a**) aqueous media, and in (**b**) *n*-hexane. Reproduced from Ref. [111] with permission from the European Society for Photobiology, the European Photochemistry Association, and The Royal Society of Chemistry

n-hexane). When exposed to simulated or natural sunlight, octinoxate undergoes change from the primary *trans*-form to the *cis*-form causing the UVB filtering efficacy to decrease because the *cis*-form has a significantly lower extinction coefficient (ε_{cis} = 12 600 at 291 nm; ε_{trans} = 24 000 at 310 nm) [200]. The degradation was due, in part, to some E/Z isomerization, with the *cis*-isomer absorbing less UV radiation, and in part due to the formation of photodegradation products [111].

Along similar lines, the influence of nanoparticle-based systems on the photoinduced degradation of *trans*-2-ethylhexyl-*p*-methoxycinnamate (octinoxate) was investigated by Perugini and coworkers [201] who discovered that the photodegradation of this UV filter in emulsion vehicles could be diminished by loading octinoxate onto ethyl cellulose (EC) or onto poly-D,L-lactide-co-glycolide (PLGA) nanoparticles. The extent of degradation was about 52% for free octinoxate versus ca. 35% for the sunscreen-loaded PLGA nanoparticles. Loading octinoxate onto EC nanoparticles was of little consequence. Evidently, the PLGA nanoparticles system enhanced the photostability of this UVB filter [201], which can also be enhanced upon loading it onto polymethyl methacrylate (PMMA) microspheres. Incorporating these octinoxate-loaded PMMA microspheres into a cream base

led to a fourfold increase in the efficacy of the sunscreen formulation [202].

The photostability of octinoxate could be enhanced further by reducing the extent of photoisomerization through encapsulation or otherwise loading this UVB filter onto nanoparticles in sunscreen formulations [203]. Insofar as octinoxate responds to UV light causing it to be photo-unstable and as it can potentially permeate through the skin, Ambrogi and coworkers [204] improved both safety and photostability of octinoxate through inclusion into the pores of mesoporous silicate MVM-41 and then entrapping it by plugging the pore openings.

Likewise, Trotta et al. [205] demonstrated that irradiation of octinoxate encapsulated in lipid microparticles in a suncream with simulated UV light led to a decrease of its photodecomposition from 56% when free to 46% when encapsulated, which improved its in vitro efficacy, its photostability and its water resistance; similar observations were reported for avobenzone. On their part, Wu and coworkers [206] encapsulated various organic UV filters (e.g., oxybenzone, avobenzone, octinoxate, and diethylamino hydroxybenzoylhexyl benzoate) in polymethyl methacrylate. These PMMA-encapsulated UV filters showed significant improvements of their safety, photoprotective ability, and photostability.

Although octinoxate is a UVB filter with significant absorption in the wavelength range of 290−320 nm, its spectrum also shows a lower-energy tail that extends well into the UVA spectral region of 310−400 nm. In this regard, Hanson and coworkers [207] revealed that the environment played a critical role in determining the photoresponse of octinoxate to UVB and UVA radiation. The dominant photochemical response was photoisomerization for a dilute solution of octinoxate in an organic solvent, attaining a solvent-dependent photostationary state between *trans*-octinoxate and *cis*-octinoxate isomers from which some additional photodegradation could occur, although the subsequent decrease in absorption was less than ca. 2%. Nonetheless, once the photostationary state was reached, the overall absorption of octinoxate in solution typically remained around 60–70% of its original absorbance. Moreover, they demonstrated something rather interesting. Compared to monomeric octinoxate, aggregation of octinoxate led to both photoisomerization and irreversible photodegradation that yielded a complex mixture of photoproducts which, besides the rapid loss of UV protection, was a matter of some concerns as some of the photoproducts could sensitize the formation of singlet oxygen ($^1O_2$) under UVA irradiation. Accordingly, the authors [207] recommended that sunscreen formulators consider strategies to avoid, or otherwise reduce such aggregation from occurring in commercial sunscreen products.

Paralleling the study of Hanson et al. [207], Stein and coworkers [208] found that exposure of octinoxate to UV radiation led to formation of numerous photoproducts—they isolated and characterized the major products from the photolysis of this UV filter that included two major stable octinoxate cyclodimers, one of which was δ-truxinate resulting from head-to-head dimerization of two octinoxate molecules, and the other was the cyclodimer α-truxillate produced from head-to-tail dimerization of two octinoxate molecules (see Fig. 29). In addition, the authors [208] found that octinoxate, 4-methoxybenzaldehyde, and the two cyclodimers were significantly toxic to cells. The photoproduct 2-ethylhexanol was not cytotoxic, meaning that different photoproducts from octinoxate photolysis contributed differently to the overall cellular toxicity. Echoing these observations, Piard et al. [209] noted that although cinnamates such as octinoxate (290−320 nm) and octocrylene (250−360 nm) may be efficient toward UVB protection, their photostabilities left much to be desired.

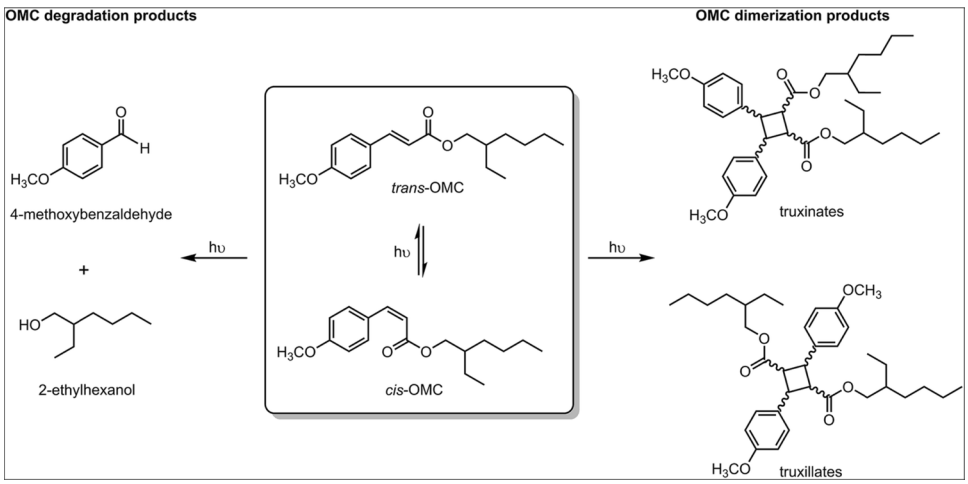


Fig. 29 Photodegradation of the UVB filter octyl methoxycinnamate and formation of the photoproducts 4-methoxybenzaldehyde and 2-ethylhexanol, together with the two cyclodimers δ-truxinate and α-truxillate. Reproduced from Ref. [208] with permission from The Royal Society of Chemistry

3.5 Behavior of a UVA filter under irradiation: avobenzone (Parsol 1789)

Avobenzone is sensitive to the solvent environment with respect to polarity, protic/nonprotic, as well as to the presence of other solutes, all of which have an impact on its photostability when present in various sunscreen formulations. Because of the various tautomers, the chelated enol form CE is in equilibrium with two nonchelated enol forms NCE1 and NCE2 (see Fig. 30a), as well as with the isomers of avobenzone; the chelated enol form CE (the Z isomer) is also in equilibrium with the keto form K and both display different photochemistry which makes the photochemical behavior of avobenzone rather complex [210]. As a consequence, Cantrell and McGarvey [210] decided to examine the photochemical properties of avobenzone by 355-nm laser flash photolysis to assess the decay kinetics of the nonchelated enol (NCE) forms and, together with results from temperature dependence studies, subsequently proposed a mechanistic sequence for the decay of NCE.

Figure 30 illustrates the decay of NCE1 that implicates a pre-equilibrium stage involving formation of NCE2, which yields the CE form through a facile single-bond rotation. However, a process involving the direct conversion of NCE1 to CE was not precluded by the authors [210]. Subjecting solutions of avobenzone to 266-nm laser photolysis yielded the triplet state of the keto (K) form (lifetime τ = ca. 500 ns) that was subsequently quenched when exposed to oxygen.

The same year (2001), Chatelain and Gabard [211] introduced the new UV filter Tinosorb S (Fig. 31) into oil-in-water sunscreen formulations to examine the photostability of avobenzone on exposure to UV radiation. Tinosorb S prevented the concentration-dependent photodegradation of this otherwise popular UVA filter, which led to a sustained SPF even after the formulation had been irradiated for up to 30 MED doses (MED, minimal erythema dose). To the extent that avobenzone destabilizes octinoxate, the authors also tested the effect of tinosorb S in sunscreens containing the (avobenzone + octinoxate) combination. The presence of tinosorb S protected both UV filters from being photodegraded and thus led to an improved photostability and efficacy of sunscreens containing both UV filters.

The well-known photo-instability of avobenzone in sunscreen formulations, especially in the presence of another UV filter such as titanium dioxide, led Wakefield and coworkers [212] to enhance the photostability of avobenzone by doping titanium dioxide with Mn^{2+} cations; the attenuation of UVA increased by up to 3 times the amount attainable by comparable commercial undoped titanium dioxide.

The photochemistry of avobenzone was also examined in solutions and in sunscreen films by Schwack and Rudolph [213] who suggested that UV-irradiated avobenzone

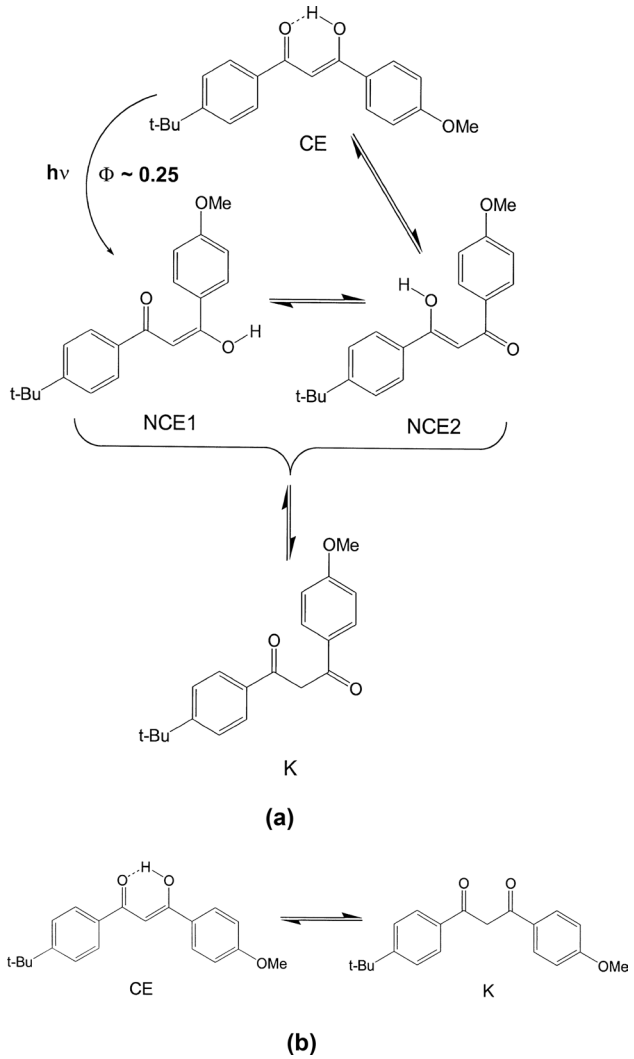


Fig. 30 **a** Proposed mechanism for the decay of NCE1 that involves a pre-equilibrium with the NCE2 tautomer; facile single-bond rotation within NCE2 yields the CE form. The K form may also be in direct equilibrium with the CE form, which accounts for ca. 90% of the avobenzone in solution. Reproduced from Ref. [210] with permission (License No. 4954801070359) Copyright 2001 by Elsevier Science B.V

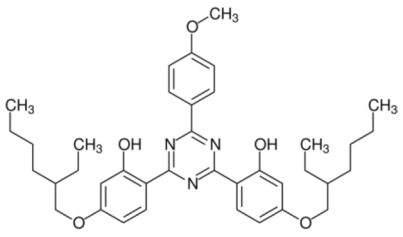


Fig. 31 Structural features of the UV filter tinosorb S, also known as escalol S and bemotrizinol

decomposes via formation of two different free radical fragments, which Sayre et al. [214] identified (ESR techniques) as carbon-centered free radicals that persisted for several minutes after UV irradiation had been terminated. The latter authors proposed that the sunscreen matrix stabilized and/or trapped these free radicals, thus permitting the decay through a solid-state-like bimolecular recombination to predominate. The photochemistry of this UVA filter was also investigated by Schrader et al. [215] who demonstrated the formation of a series of cyclobutane dimers from irradiated solutions of structurally analogous iso-amyl methoxycinnamates, which provided further evidence that processes other than *E/Z* photoisomerization occurs; these photodimers further decomposed thermally.

In their study on sunscreen filters, Sayre and coworkers [214] irradiated films of a commercial sunscreen formulation that consisted of the UVA filters avobenzone and oxybenzone and the UVB filter octinoxate. The UV-induced changes in UV transmittance of sunscreen films correlated with changes in the concentrations of the sunscreen active agents. Exposure of the formulation to 2.0 MEDs caused only about 25% of the original protection to remain, while significant protection was afforded by the photostable oxybenzone (nearly 100% remained). [214] Although only 20% of the avobenzone remained in the sunscreen films, about 40% of the octinoxate survived (Fig. 32).

Evidently, octinoxate was also photolysed to a non-insignificant extent in the thin films of the sunscreen formulation, a process that dominated over photoisomerization. This observation strongly supported the notion that the photoinstability of one sunscreen agent caused the degradation of another sunscreen agent. In their earlier investigation of filter-filter interactions in sunscreen formulations, Johncock [63] discovered that fragments of avobenzone interacted with octinoxate to form yet undefined molecular structures, while Butt and Christensen [64] noted that related photoproducts from the photoinduced breakdown of octinoxate could be phototoxic, thus further questioning the safe use of such sunscreens.

The ability of UV filters to absorb the sunlight UV energy must be sustained for long periods to attain the proper photoprotection that commercial sunscreen products claim to provide. In most cases, assessing whether a sunscreen UV filter is photostable or photo-unstable when exposed to natural or simulated sunlight is often carried out in such solvents as methanol, ethanol, acetonitrile, hexane, and cyclohexane (among others), when in fact commercial sunscreen formulators do not use these solvents in the manufacturing of suncare products. This was highlighted by Vallejo et al. [216] who investigated the photostability of avobenzone in such solvents as well as in solvents intended for sunscreen and cosmetic lotions and creams: for example, in such vehicles as mineral oil and isopropyl myristate that provide a more complex media for the photochemistry to occur.

Figure 33 illustrates the absorbance decrease at 358 nm of avobenzone dissolved in ethanol, methanol and hexane, followed by a 14-h irradiation period with simulated sunlight. The decrease in absorbance was significantly less and much slower in ethanol and methanol than it was in hexane, clearly indicating that avobenzone is more photostable in polar/protic solvents. [216].

By contrast, the photodegradation of avobenzone in cosmetic solvents (e.g., mineral oil, isopropyl myristate, octyldodecanol, cyclomethicone, decyl-oleate, hydrogenated polyisobutene, and isopropyl palmitate) was much faster under simulated sunlight, requiring irradiation for only a

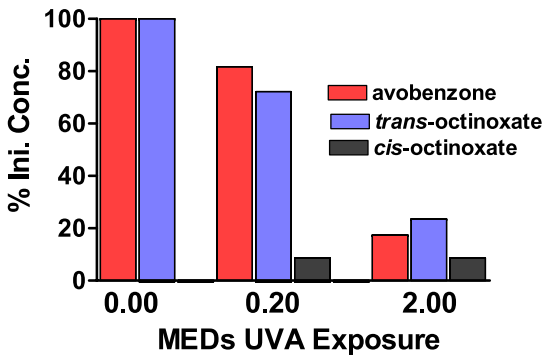


Fig. 32 Histograms showing the percent of the sunscreen avobenzone, *trans*-octinoxate, and *cis*-octinoxate remaining subsequent to being exposed to 0.20 and 2.0 MEDs. Oxybenzone (not shown) was used as an internal standard and was assumed to have been photostable throughout the photolysis. Initially, the octinoxate was all in the *trans*-isomeric structure, although subsequent to UV exposure was converted into a limited quantity of the *cis*-isomer. Octinoxate proved to be photo-unstable as 60% of it was lost. Adapted from Ref. [214] with permission (License No. 4954841256806). Copyright 2005 by the American Society for Photobiology

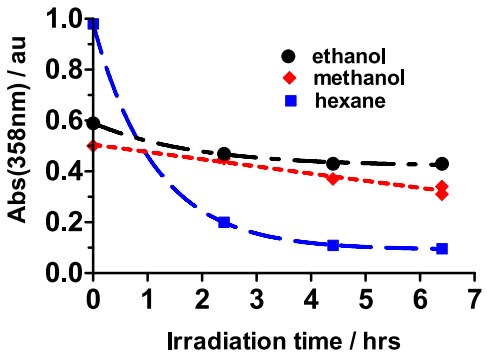


Fig. 33 Plot showing the 358-nm absorbance decrease of avobenzone dissolved in ethanol (black circle), methanol (red rhombus) and hexane (blue square). Adapted from Ref. [216]. Plataforma Open Access de Revistas Electronicas Espanolas y Latino-americanas. The Universidad de Antioquia. Medellín, Colombia

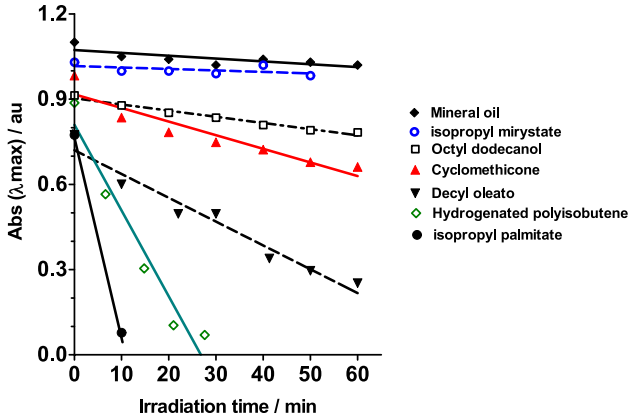


Fig. 34 Comparative decrease in absorbance of avobenzone in (from top to bottom): mineral oil, isopropyl myristate, octyldodecanol, cyclomethicone, decyl-oleate, hydrogenated polyisobutene, and isopropyl palmitate. Adapted from Ref. [216]. Plataforma Open Access de Revistas Electronicas Espanolas y Latinoamericanas. The Universidad de Antioquia. Medellín, Colombia

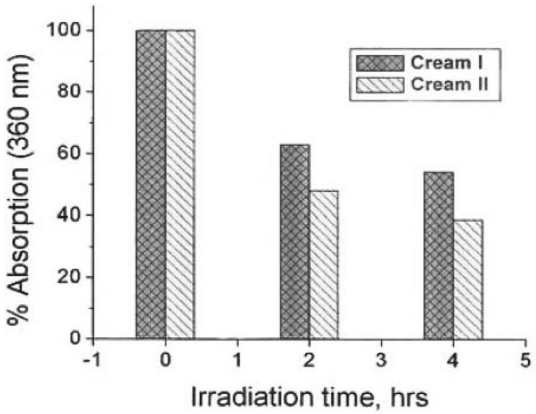


Fig. 35 Photochemical fate of two well-known commercial sunscreen emulsions (labelled cream I and cream II) under exposure to UV radiation in a solar simulator; SPF = 30 for both suncreams. Reproduced from Ref. [217] with permission from the European Society for Photobiology, the European Photochemistry Association, and The Royal Society of Chemistry

1-h period (see Fig. 34). Although these cosmetic solvents have similar dielectric constants and a similar oleic nature, it is evident that avobenzone experienced a significantly different solvent-dependent photochemical behavior.

Photodegradation of avobenzone was expected to be faster in hydrogenated poly-isobutene owing to its non-polar character. However, as this solvent consists of a long hydrocarbon chain (versus hexane for instance) and is highly viscous, it may have minimized the photodegradation of this UV filter. In addition, because of the low molar absorptivity of avobenzone, it was less likely to undergo photodegradation by α-cleavage in hydrogenated poly-isobutene. [216] Nonetheless, isopropyl myristate and mineral oil offered the best solvent media for attaining a very photostable avobenzone, even when these two solvents were combined as the absorbance decay did not depend on the solvent ratio.

3.6 Photochemistry occurring in sunscreen formulations that contain UVB and UVA filters

Owing to the aforementioned issues, a systematic investigation of two well-known and popular commercial suncreams was undertaken in one of our laboratories [217] (see also Refs. [218, 219]) to assess the potential interaction(s) that may occur between different solar UVB/UVA filters contained in two sunscreen formulations. To this end, we examined a widely used commercial sunscreen (cream I) that also contained titanium dioxide and ethylhexyl triazone, whereas the widely used and more expensive commercial sunscreen (cream II) contained, in addition, octyl salicylate and oxybenzone (benzophenone-3); see Fig. 35 and Table 4. To unravel the degradation events, also examined was the photochemistry of octinoxate and avobenzone (Parsol 1789) in pure solvents separately and in combination (in solution), as well as in neat form, together with their photochemistry when present in the actual sunscreen emulsion, as their combination typically yields sunscreens with high SPF numbers.

Irradiation of these sunscreens as thin films (thickness, 10–50 μm in keeping with the quantity typically used by consumers) by simulated sunlight (290–400 nm; light irradiance, 27 W m^{-2}) led to a conspicuous loss of absorption at 360 nm within a few hours (up to 4 h)—see Fig. 35. Both sunscreens displayed significant degradation (about 40–50%). Accordingly, we also examined [217] the photochemical fate of the active agents used in these two sunscreen formulations. Our specific objective was to address the photochemistry/photostability of octinoxate and avobenzone; their absorption spectra in acetonitrile solutions are illustrated in Fig. 36. Also examined was the photochemistry of octinoxate and avobenzone in the presence of Degussa P25 TiO_2 in acetonitrile dispersions, which upon irradiation with simulated sunlight with continuous stirring led to their complete photodegradation. [217].

To increase the photostability of avobenzone in vivo and to reduce its transdermal penetration in skin, Yang and coworkers [220] proposed complexing this UVA filter with hydroxypropyl-β-cyclodextrin (HPCD; Fig. 37) at various concentrations (30, 20, 10, and 0%) of the cyclodextrin, followed by irradiating with UVA light at doses of 100, 250, and 500 kJ/m^2. With regard to concentrations of HPCD, the photostability decreased in the order 30% > 20% > 10% > 0% with the 30% HPCD formulation affording the best photoprotection, as evidenced by the lowest extent of sunburn cell formation and edema induction. The photostability of avobenzone could also be enhanced in the presence of the photostable antioxidant photostabilizer

Table 4 Active agents present in two commercially available sunscreen formulations

Cream I		Cream II	
Sunscreen active agent	Function	Sunscreen active agent	Function
Titanium dioxide	Broadband UVB/UVA absorber	Octyl salicylate	UVB absorber
Ethylhexyl triazone	UVB absorber	Oxybenzone	Broadband UVB/UVA absorber
Avobenzone (Parsol 1789)	UVA absorber	Avobenzone (Parsol 1789)	UVA absorber
Octinoxate	UVB absorber	Octinoxate	UVB absorber

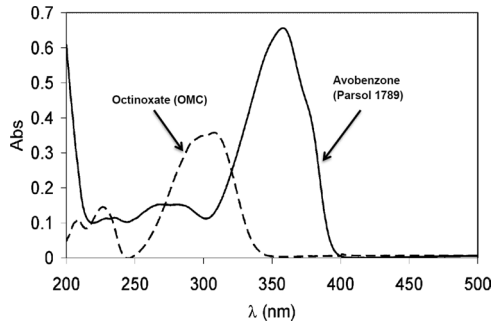
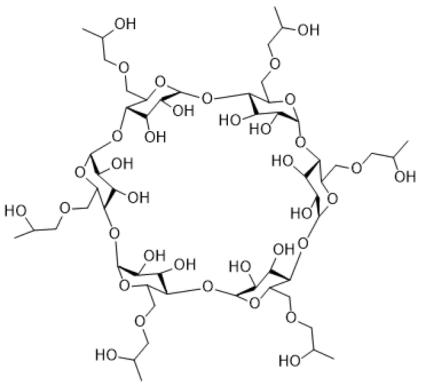


Fig. 36 Absorption spectra of acetonitrile solutions of octinoxate and avobenzone. Reproduced from Ref. [217] with permission from the European Society for Photobiology, the European Photochemistry Association, and The Royal Society of Chemistry



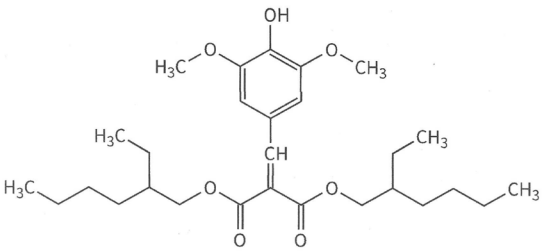


Fig. 37 Structural features of hydroxypropyl-β-cyclodextrin (HPCD) and di-2,2′-diethylhexyl-3,5-dimethoxy-4-hydroxybenzylidene malonate, also known as diethylhexyl syringylidene malonate (DESM)

di-2,2′-diethylhexyl-3,5-dimethoxy-4-hydroxybenzylidene malonate (also known as diethylhexyl syringylidene malonate; DESM; see Fig. 37) [221].

Using simple model systems, we discovered that irradiation of a mixed solution of a cinnamate and a diketone led to a [2 + 2] photocycloaddition process yielding cinnamate dimers and cyclobutyl-ketone photoadducts (see e.g., Fig. 38) that subsequently fragmented into substituted oxopentanoates and oxobutanoates. Similar findings were observed when examining the photochemistry occurring in the two commercial sunscreen formulations that simultaneously contained the two active agents exposed to a combined UVA/UVB output irradiance of 44 W m^{-2} [217]. Evidently, the simultaneous presence of dibenzoylmethanes (e.g., avobenzone) and other sunscreens (octinoxate) did not enhance their photostability (durability). Rather, it led to a non-insignificant loss of photoprotection when the sunscreens are used under actual applicative conditions; the toxic effects, if any, of the photoproducts certainly necessitate serious assessment.

With rare exceptions, the active ingredients in the sunscreens did undergo photochemical changes with, in some cases, the formation of free radicals in such a way that the sunscreen lotions lost considerable Sun protection efficacy, and this after only a relatively short time when exposed to simulated sunlight UVB/UVA radiation [217]. Our work confirmed the findings of Sayre et al. [214] who reported on the associated photolysis of avobenzone and octinoxate that predominated over the expected *E/Z* photoisomerization, and that irradiation of a film of this product produced free radicals (EPR) that persisted even after exposure to UV radiation had been terminated.

The observed stabilization of avobenzone is a result of (i) triplet-state energy transfer from avobenzone to DESM and (b) scavenging of reactive species such as singlet oxygen responsible for the photodegradation of avobenzone, although DESM did not improve the in vivo SPF but did

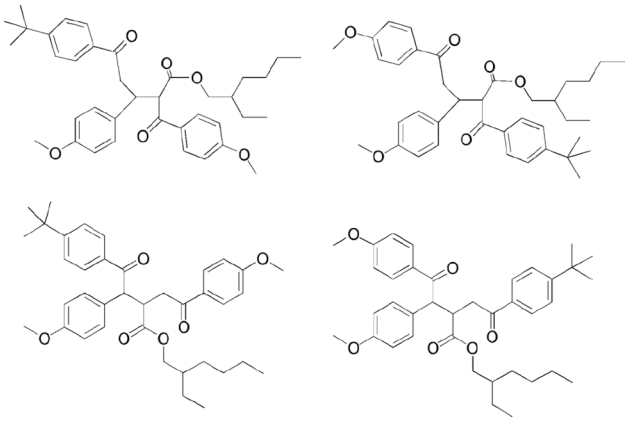


Fig. 38 Examples of cross photoadducts formed during the UV irradiation of such a cinnamate as octinoxate and a diketone such as avobenzone in acetonitrile solutions and in the commercial suncreams I and II. Reproduced from Ref. [217] with permission from the European Society for Photobiology, the European Photochemistry Association, and The Royal Society of Chemistry

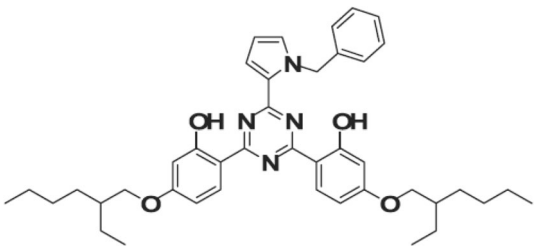


Fig. 39 Structural features of the photostabilizer 2-(1-benzyl-1H-pyrrol-2-yl)-4,6-bis[4-(2-ethylhexyloxy)-2-hydroxyphenyl]-1,3,5-triazine

boost the SPF by about 5 units in high-SPF sunscreens [221]. Indeed, photostable broadspectrum sunscreens with high SPF (> 30) have been obtained with avobenzone in combination with DESM and a UVB sunscreen agent such as homosalate and octisalate, among others. Regardless, the diketo form of this dibenzoylmethane derivative was responsible for its photodegradation. Undesirable effects of avobenzone—such as phototoxicity and photoallergy resulting from its photo-instability—could be diminished by the presence of the 1,3,5-triazine UVB filter {2-(1-benzyl-1H-pyrrol-2-yl)-4,6-bis[4-(2-ethylhexyl-oxy)-2-hydroxy-phenyl]-1,3,5-triazine} (Fig. 39) that can efficiently quench the triplet excited state of photoactivated avobenzone. [222] Although the photodegradation of avobenzone can be retarded when combined with other UV filters, such combination can also enhance its photodegradation whenever a photoreaction occurs between the different components in the sunscreens as we have seen between avobenzone and octinoxate, for example [217].

The photosensitization by avobenzone vis-à-vis biologically relevant targets was shown by Paris and coworkers [155] to cause (i) lipid peroxidation, (ii) cytotoxicity to human keratinocytes, (iii) a decreased survival of yeast cells, (iv) oxidative modification of albumin, and not least (v) strand breaks of supercoiled DNA plasmids. A later study by this group [222] on filter-filter interactions focused attention on the photostabilization of avobenzone, quenching of its triplet excited state, and on its reactivity with singlet oxygen. Specifically, the photoreactivity of avobenzone was examined in the presence of six commercial solar UV filters: (1) octinoxate, (2) bis-ethylhexyloxyphenol methoxyphenyl

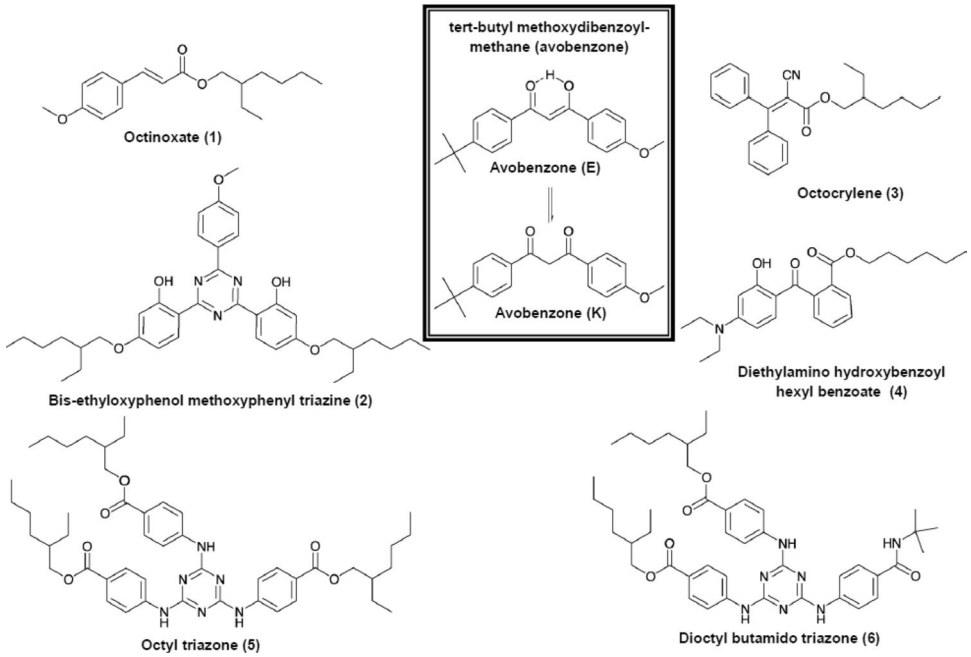


Fig. 40 Illustration of avobenzone and the structures of the other UV filters 1 through 6. Adapted from Ref. [222] with permission from the European Society for Photobiology, the European Photochemistry Association, and The Royal Society of Chemistry

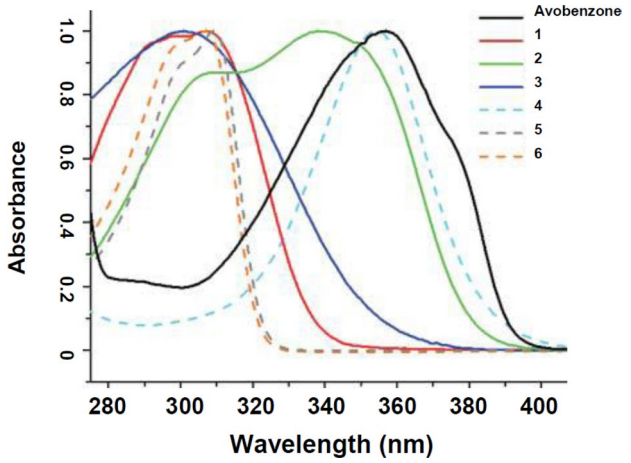


Fig. 41 Normalized UV spectra of avobenzone and the UV filters 1 through 6, namely (1) octyl methoxycinnamate, (2) bis-ethylhexyloxy phenol methoxyphenyl triazine, (3) octocrylene, (4) diethylamino hydroxybenzoyl hexyl benzoate, (5) octyl triazone, and (6) dioctyl butamido triazone. Adapted from Ref. [222] with permission from the European Society for Photobiology, the European Photochemistry Association, and The Royal Society of Chemistry

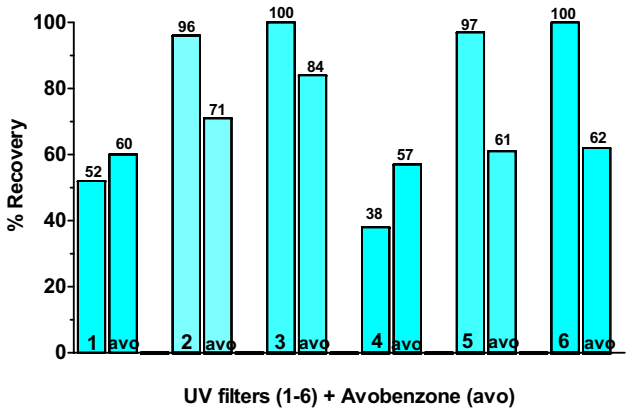


Fig. 42 Percent recovery of UV filters 1–6 as defined in Fig. 40 and of avobenzone after 4 h of irradiation with simulated sunlight; UV filters were irradiated in combination with avobenzone (avo). Adapted from Ref. [222] with permission from the European Society for Photobiology, the European Photochemistry Association, and The Royal Society of Chemistry

triazine, (3) octocrylene, (4) diethylaminohydroxybenzoylhexyl benzoate, (5) octyl triazone, and (6) dioctyl butamido triazone (Fig. 40). The photostability of the sunscreens took into account (a) the tautomerization of avobenzone, (b) the formation of its triplet excited state in its diketo form and its quenching in the presence of other (mostly) UVB filters, (c) the photoreactivity of UV filters under triplet photosensitization, (d) the quenching of singlet oxygen by UV filters, and (e) the degradation of $^1O_2$ under singlet oxygenation conditions. Amongst the UV filters investigated, avobenzone was the most significant representative for both its absorption capability (Fig. 41) and its commercial availability.

Following UV irradiation separately in miglyol solvent for up to 4 h in a solar simulator, the filters 1–6 of Fig. 40 displayed a reasonable tolerance to UV radiation with high recovery from 92 to 100%, except for octinoxate (1), which underwent a photoisomerization reaction with a 72% recovery, while avobenzone underwent an oxidative photofragmentation reaction with a 44% recovery. By contrast, irradiation of mixtures of avobenzone combined with filters 1 through 6 revealed that avobenzone underwent some filter-filter photoreaction with losses from 16% for the avobenzone/filter 3 combination to 43% for the avobenzone/filter 4 combination (Fig. 42) [222]. Overall, the rate of photodegradation of avobenzone decreased in the presence of other filters, particularly in the combination with filters 2 and 3, while the protective effect was more than counterbalanced by an augmented degradation of filters 1 and 4 (Fig. 42). In addition, the key processes that also accounted for the loss of avobenzone when combined with the other UVA and UVB filters was due to quenching of the triplet excited state of its diketo isomer and photodegradation of the added filters subsequent to triplet photosensitization [222].

Incorporating avobenzone into solid lipid microparticulates (SLMs), together with the photostabilizer UVB filter 4-methyl-benzylidene camphor (4-MBC; Fig. 15) also reduced the photodegradation of avobenzone to ca. 34% for the non-encapsulated avobenzone/4-MBC, to 25% for avobenzone-loaded microparticles in combination with free MBC, and to about 17% for the encapsulated avobenzone/4-MBC [223]. Clearly, encapsulation of both avobenzone and the methylbenzylidene camphor into SLMs was more effective in photostabilizing avobenzone than SLMs loaded solely with avobenzone. The relevance of the encapsulation protocol under the latter conditions afforded, however, but limited information regarding the photochemical behavior of avobenzone as sunscreen formulations typically implicate both a UVA and a UVB filter to attain broadband protection. And to the extent that the photoinduced decomposition of a sunscreen UV filter is affected by the presence of other UV absorbers, the resulting photo-instability of UV filter combinations is likely far different from what might be observed for a single sunscreen agent. Accordingly, Scalia and Mezzena [198] also encapsulated avobenzone with yet a different UVB filter in the lipid microparticles, octocrylene (Table 2) that also acted as a photostabilizer.

To mimic the conditions prevalent in commercial sunscreen formulations, Scalia and Mezzena [198] subsequently determined the photoprotection efficacy on adding the LM-encapsulated filters into an oil-in-water emulsion. Co-loading avobenzone and octocrylene in lipid microparticles reduced the light-induced decomposition of avobenzone to about 22% from 26% for avobenzone-loaded microparticles alone under simulated UV radiation. By comparison, the

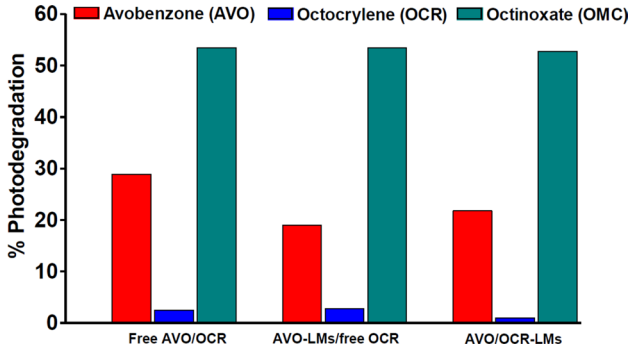


Fig. 43 Histograms displaying the percent photodegradation of avobenzone (AVO), octocrylene (OCR) and octinoxate (OMC) in their formulations after 1 h of UV irradiation with simulated sunlight. Adapted from Ref. [198]. Copyright 2009 by Springer Nature and the American Association of Pharmaceutical Scientists

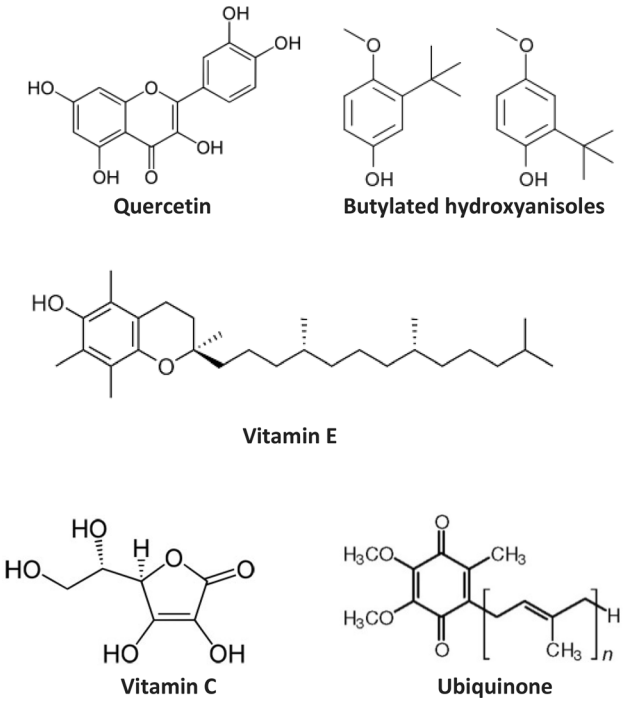


Fig. 44 Structural features of quercetin, butylated hydroxyanisoles, Vitamin E, Vitamin C, and ubiquinone

percent loss of avobenzone was 33% in the avobenzone/octinoxate filters combination applied onto a Transpore™ tape, while the loss of avobenzone was ca. 29% for the avobenzone/octinoxate/octocrylene formulation, with the difference being statistically significant [198]. For comparison, the loss of octinoxate in the avobenzone/octinoxate filters combination on lipid microparticles was reduced from 62 to 54% for the octinoxate-loaded microparticles.

By contrast, octocrylene appeared rather stable under simulated sunlight with a loss of less than 4%. Consequently, the co-loading of avobenzone with octocrylene in lipid microparticulates produced a clearly distinct enhancement of the stability of the UVA filter under simulated sunlight. Figure 43 illustrates a comparison in terms of the percent photodegradation for the formulations containing non-encapsulated avobenzone and octocrylene or lipid microparticles loaded only with avobenzone in conjunction with free OCR. The data of Fig. 43 also demonstrate that the co-loading of octocrylene enhanced significantly the photostability of avobenzone encapsulated in lipid microparticles compared with classical combinations of free avobenzone and octocrylene, or containing the microparticle/entrapped avobenzone with the non-encapsulated octocrylene. Thus, the sunscreen formulations developed by Scalia and Mezzena [198]—based on avobenzone-loaded lipid microparticles—might prove a useful alternative to conventional suncare products that contain free avobenzone as the UVA filter of choice.

In their later study, Scalia and Mezzena [157] reported on the influence that the natural antioxidant quercetin (Fig. 44) had on the photostability of model creams that contained 3 wt% avobenzone and 4 wt% octinoxate exposed to simulated sunlight at an irradiance similar to that which prevails in natural sunlight. Quercetin enhanced the concentration-dependent photostability of both UV filters. For instance, the photodegradation of avobenzone was reduced from 40 to 28%, while for the octinoxate it was reduced from 51 to 42%. In addition, quercetin was much more effective at the lower concentrations than the more commonly used octocrylene stabilizer and the antioxidants vitamin E and butylated hydroxyanisole (Fig. 44).

Along similar lines, Afonso and coworkers [224] studied the effect of such antioxidants as vitamin C, vitamin E, and ubiquinone (Fig. 44) in photostabilizing avobenzone dissolved in dimethyl sulfoxide, or otherwise incorporated in a sunscreen formulation irradiated with simulated solar radiation (irradiance, 750 W/m^2). The most effective photostabilizer of the sunscreen formulations was ubiquinone, which also led to an increased SPF factor. Evidently, antioxidants prove valuable ingredients for sunscreen formulations as they afford the photostabilization of the avobenzone filter, boost the SPF number, and not least prevent skin photo-ageing.

We have already seen that the SPF factor and the photostability of UVA filters can be improved by the presence of antioxidants (e.g., vitamin E) that can potentially scavenge reactive singlet oxygen, inasmuch as without such scavengers avobenzone would degrade under UV radiation from either simulated or natural sunlight, and would thus significantly reduce its efficacy in skin protection. Using an in vitro spectrophotometric method as a means to further mitigate the photodegradation of avobenzone, Abdassah and coworkers [225] investigated the efficacy of avobenzone toward skin

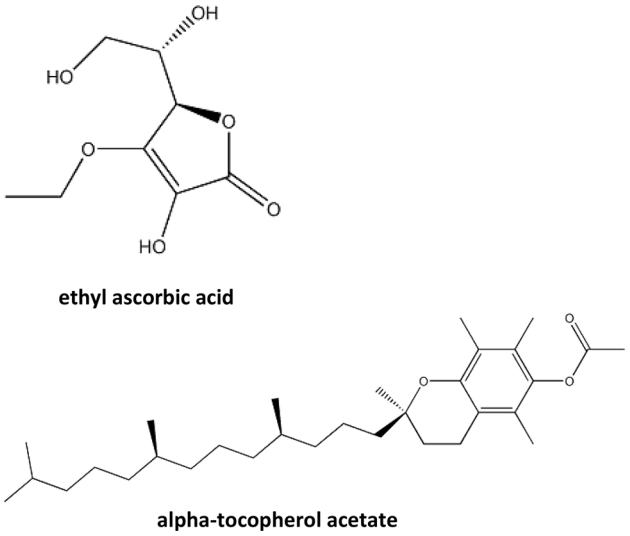


Fig. 45 Structural features of ethyl ascorbic acid and alpha-tocopherol acetate

protection (SPF) together with its photostability when combined with the antioxidants ethyl ascorbic acid and alpha-tocopherol acetate (Fig. 45) in cream formulations at various concentrations of these antioxidants (Table 5).

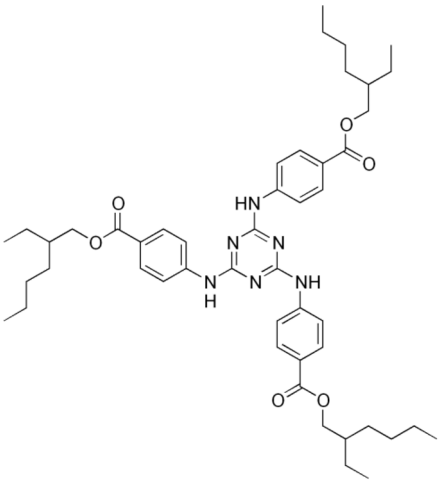
The cream formulations were irradiated with a UVA lamp (4.7 mW/cm^2) for a 15-h period that corresponded to the UVA dose that reaches the Earth's surface during the hours 14:00 to 17:00 on a sunny day [225]. Under these conditions, the best photostability of avobenzone was shown by the F4 formulation that contained 2% avobenzone and 2% ethyl ascorbic acid with the concentration of avobenzone decreasing by about 12% over that irradiation period (Table 5). However, although the addition of ethyl ascorbic acid and alpha-tocopherol acetate might have improved the SPF value, it may not have improved the effectiveness of protection against erythema/UVB radiation [226].

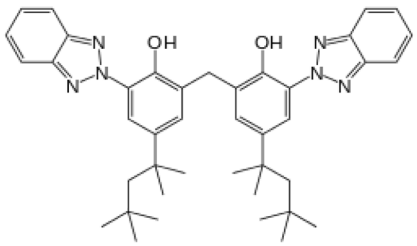
The photoprotective efficacy and photostability of 15 sunscreen products (marketed mostly in Europe) having the same label SPF subjected to natural sunlight were investigated by Hojerová and coworkers [226], who demonstrated how different were the photoprotection imparted by sunscreen formulations of identical SPF. Of the various combinations of UV filters, two were constant throughout 7 of the 15 formulations: octinoxate and avobenzone (Parsol 1789) to which were then added other UV filters. The sunscreens were loaded onto a polymethyl methacrylate plate (roughness, 2 μm) for a 0.75 mg/cm^2 layer that was subsequently exposed to sunlight radiation. Results showed 7 of the formulations were photo-unstable throughout the whole UVB/UVA spectral range, while the other 8 formulations were photo-unstable in the UVA2 range (340−400 nm). The most photo-unstable sunscreen formulations were those that contained the combinations (octinoxate + avobenzone + phenylbenzimidazole sulphonic acid) and (octinoxate + avobenzone + phenylbenzimidazole sulphonic acid + ethylhexyl triazone)—see Fig. 46. By contrast, under the conditions used, the most photostable formulation within the UVA2 range consisted of the combination (octinoxate + ethylhexyl triazone + methylene bis-benzotriazolyl tetramethyl butylphenol).

When buying a sunscreen product, the consumer is certainly not expected to assess whether or not the sunscreen product is photostable; however, the consumer recognizes something about the SPF factor in that the greater the SPF is, the greater is the expected photoprotection afforded by that product. Photostability is completely disconnected from the SPF; however, only in a few instances are consumers told that a sunscreen product is photostable because the photoactive ingredients may have been incorporated into a vehicle that stabilizes them. The claim that a given sunscreen or cosmetic product is photostable was questioned by Gonzalez and coworkers [227] because photostability tends to vary from brand to brand, as photostability also depends on such factors as the presence of (i) preservatives and (ii) oxygen radical scavengers, as well as the nature of the (iii) base formulation. Sunscreen labels are too often silent on this topic and, moreover, on reading the list of ingredients in such products, the consumer is not expected to know which of the active ingredients may or may not be photostable/photo-unstable when exposed to natural sunlight. Accordingly, the authors [227] investigated the photostability of

Table 5 Cream formulations containing avobenzone and other components in the base cream F1

Formulation	Contents	Quantity (wt%)
F1	Base cream	0.1 g
F2	Avobenzone	2
F3	Avobenzone; ethyl ascorbic acid	2 0.5
F4	Avobenzone; ethyl ascorbic acid	2 1
F5	Avobenzone; ethyl ascorbic acid	2 2
F6	Avobenzone; ethyl ascorbic acid; alpha-tocopherol acetate	2 0.5 1
F7	Avobenzone; ethyl ascorbic acid; alpha-tocopherol acetate	2 1 1
F8	Avobenzone; ethyl ascorbic acid; alpha-tocopherol acetate	2 2 1





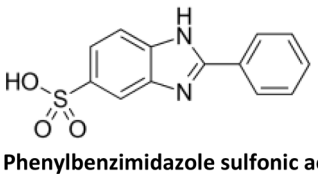


Fig. 46 Structural formula of ethylhexyl triazone, Methylene bis-benzotriazolyl tetramethylbutylphenol, and Phenylbenzimidazole sulfonic acid

no less than 7 commercial sunscreen products marketed in Sweden after exposure to natural sunlight (UV_{nat}) and to simulated sunlight UV radiation (UV_{art}). Three of the sunscreens consisted only of organic chemical filters (1, 2, 5), while three others were composed of inorganic (TiO_2) and organic chemical filters (3, 4, 6); the remaining sunscreen product (7) contained solely the inorganic physical filters titanium dioxide and zinc oxide.

In their study, Gonzalez et al. [227] placed 0.5 mg/cm^2 of the sunscreens between plates of silica, following which the transmittance was determined by absorption spectroscopy; key results are summarized in Table 6. Three of the sunscreens became unstable (1–3) after 90 min of being subjected to natural sunlight illumination (UV_{nat}), whereas three others (4–6) were photostable even after being exposed to UV_{nat} for 120 min; five of the sunscreens (2, 4−7) were photostable in the UVB region. Although sunscreen (5) was photostable under natural sunlight—it contained neither of the inorganic filters—its photostability was likely due to a vehicle that precluded degradation and/or due to the microstructures of the sunscreen emulsion [227]. Several of the commercially available sunscreens were not photostable, and although sunscreens (4) and (6) with TiO_2 particles seemed photostable, sunscreen (3) was the exception. In addition, avobenzone degraded during UV exposure in three out of the six sunscreens.

Following recognition that adequate skin protection by sunscreens from sun damage necessitated a photostable combination of UV filters that could also provide a suitable level of UVA protection, in 2012 researchers from L'Oréal Research and Innovation (Chevilly-Larue, France) and La Roche-Posay Pharmaceutical Laboratories [228] reported a new combination of UV absorbers in an oily emollient to increase both photostability and efficacy (SPF), as well as the UVA protection factor (UVA-PF). The total quantity of UV filters was to be as little as possible to avoid (a) antagonistic skin reactions, (b) potential impact to the environment,

Table 6 Active ingredients in the seven sunscreen products marketed in Sweden, together with their SPF factor, the UV irradiation dose after 90 min, and their photostability under natural sunlight UV (UV_{nat}) for 90 min (after the data given in Ref. [227])

No.	Active ingredients	SPF factor	UV dose after 90 min (kJ/m^2)	Photostability after 90 min under UV_{nat}
1	Octinoxate; Avobenzone	4	180	Unstable after 30 min; Unstable in UVB region
2	Octinoxate; Avobenzone; Oxybenzone	14	180	Unstable; Stable in UVB region
3	Octinoxate; Avobenzone; Oxybenzone; 4-methylbenzilidine camphor; TiO_2	10	210	Unstable after 30 min; Unstable in UVB region
4	Avobenzone; Ethylhexyl triazone; 4-methylbenzilidine camphor; TiO_2	10	210	Stable in UVB region
5	Avobenzone; 4-methylbenzilidine camphor	6	–	Stable throughout
6	Avobenzone; Octocrylene; TiO_2; Terephthalylidene dicamphor sulfonic acid	10	140	Stable throughout
7	TiO_2; ZnO	15	130	Stable throughout

and (c) to guarantee a texture acceptable for better application and usage.

The photostability of avobenzone was assessed by introducing this UVA filter in a simplex oil-in-water emulsion in the presence and absence of bis-ethylhexyloxyphenol methoxyphenyl triazine (BEMT) and isopropyl lauroyl sarcosinate (ILS) following exposure to simulated solar UV radiation for a duration equivalent to delivering 18 J/cm^2 of UVA (320–400 nm); the latter corresponded to a dose received during a 1-h exposure to zenithal sun [228]. With a total concentration of 8.11% of UV filters, sunscreen C consisting of 2.46% terephthalylidene dicamphor sulfonic acid (TDSA; Ecamsule; Mexoryl SX) and 5.65% bis-ethylhexyloxyphenol methoxyphenyl triazine (BEMT; Tinosorb S) in the simplex emulsion yielded an SPF of ca. 22 and a UVA-PF of 13 (Fig. 47), whereas the TDSA alone in the same emulsion (Sunscreen B) gave an SPF of 5 and a UVA-PF of 5. By comparison, the BEMT alone in the same emulsion (Sunscreen A) yielded an SPF about 9 and a UVA-PF ca. 5. [228].

L'Alloret and coworkers [228] concluded that it was possible to obtain a sunscreen product that was photostable for more than 4 h under natural sunlight by solubilizing the UVA filters terephthalylidene dicamphor sulfonic acid, bis-ethylhexyloxyphenol methoxyphenyl triazine, and avobenzone in the oily derivative of isopropyl lauroyl sarcosinate which, when combined with titanium dioxide and the UVB filter octocrylene, could produce a sunscreen with a UVA-PF of 38 and an SPF of 50. In addition this product would prevent UV-induced biological damage and skin reactions of polymorphous light eruption.

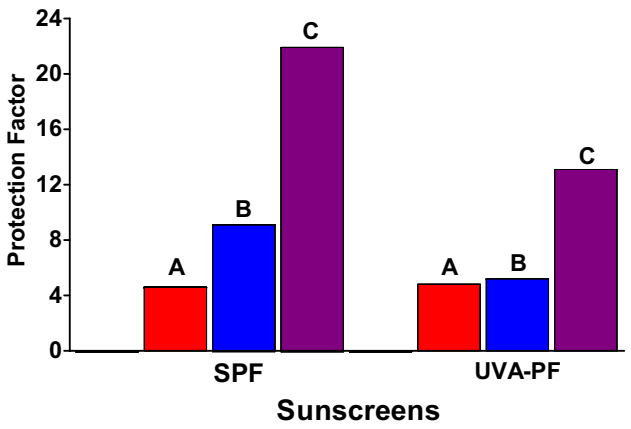


Fig. 47 Histograms comparing the SPF and UVA-PF values for sunscreens A, B, and C showing the synergistic effect of the association of terephthalylidene dicamphor sulfonic acid (TDSA) and bis-ethylhexyloxyphenol methoxyphenyl triazine (BEMT). Sunscreen A = bis-ethylhexyloxyphenol methoxyphenyl triazine; Sunscreen B = terephthalylidene dicamphor sulfonic acid, and Sunscreen C = (bis-ethylhexyloxyphenol methoxyphenyl triazine + terephthalylidene dicamphor sulfonic acid). Adapted from Ref. [228]; Open Access article distributed under the terms of the Creative Commons Attribution Non-commercial License which permits any non-commercial use, distribution, and reproduction in any medium, provided the original author(s) and source are credited

Additional studies on the photostability of UV filters were reported by Kockler and coworkers [47] who investigated the effect of particle size of TiO_2 on the photostability of avobenzone (1.0 wt%) and octocrylene (1.5 wt%) formulated in a microemulsion that consisted of Mygliol®812, glycerol oleate, oleth-20 and water phases, to which were also added phenoxyethanol, xanthan gum, and either nano-TiO_2 (size < 25 nm) or micro-TiO_2 (size ca. 0.6 µm). The oil-soluble avobenzone and octocrylene dissolved in the internal phase, while TiO_2 was evenly dispersed in the external phase. Exposing the microemulsion with the combination (avobenzone + octocrylene + nano-TiO_2) to simulated sunlight for about 15 h revealed a 12% greater photodegradation of avobenzone compared to the combination involving micro-TiO_2. This was attributed to the larger surface area of nano-TiO_2, to an increased generation of reactive oxygen species (e.g., ·OH radicals and $^1O_2$, among others), and to the formation of photoproducts that could cause allergic skin reactions and other toxic effects. Photoproducts from the photodegradation of avobenzone proved cytotoxic to the amino acid arginine; they also displayed photosensitive effects on skin as demonstrated by local lymphonode in vivo assays [109]. To avoid, or at least to minimize the photodegradative capability of TiO_2, it is often coated either with silica or with dimethicone or with aluminium hydroxide to reduce the formation of reactive oxygen species. Nonetheless, uncoated TiO_2 continues to be used in suncare products as a broadband UV filter.

To get a better handle on the effect of TiO_2 sizes in sunscreen emulsions, Kockler et al. [47] also examined, separately or in a combination, the UV filters avobenzone (AVO) and octocrylene (OCR) to determine their percent recovery after a nearly 15-h exposure to artificial sunlight. Results displayed in Fig. 48 show that irradiation of avobenzone alone resulted in less than 4% recovery in the absence of TiO_2, as well as in the presence of coated and micro-TiO_2. [47] Regardless, TiO_2 enhanced the photodecomposition of avobenzone despite light scattering effects by the metal-oxide particulates. No avobenzone was recovered after irradiation in the presence of nano-TiO_2. When combined with octocrylene, the photostability of avobenzone improved somewhat; recovery of avobenzone without TiO_2 and with coated TiO_2 and micro-TiO_2 increased to more than 12%, which failed vis-à-vis the acceptable range of 90–120% required for a UV-filter concentration in a sunscreen product [164]. By contrast, octocrylene remained largely photostable with recoveries of more than 96% subsequent to irradiation. Nonetheless, there was a non-insignificant decrease to ca. 88% in the presence of nano-TiO_2; a similar trend

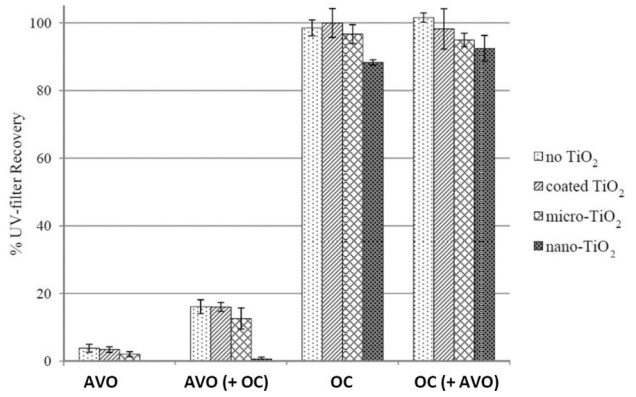


Fig. 48 Percent recovery of avobenzone (AVO) and octocrylene (here denoted OC) with and without the various TiO_2 materials after irradiation in a solar simulator. Reproduced from Ref. [47] through the Creative Commons Attribution license (http://creativecommons.org/licenses/by/3.0/). Copy-right 2014 by the authors

was witnessed in combination with avobenzone, although the effect of nano-TiO_2 was less pronounced. Along similar lines, Kim et al. [229] investigated the role of TiO_2 as an added inorganic physical UV filter in sunscreen products in combination with avobenzone and octinoxate to improve the various functions expected of a sunscreen product. TiO_2 nanocomposites of varying sizes present in three different types of oil-in-water sunscreen formulations mediated the photocatalyzed decomposition of avobenzone and octinoxate.

As further pointed out by Kockler et al. [47], caution must be clearly exercised whenever manufactured sunscreen formulations contain combinations of chemical UV filters and nano-TiO_2, as the latter nanoparticulates are not only becoming more commonly used in sunscreens, but are also being used in other cosmetic products. Figure 49a shows that 41% of avobenzone and 7% of octinoxate degraded following irradiation of the formulations in the absence of TiO_2 nanocomposites, whereas addition of the nano-composites into sunscreen formulations significantly increased the photocatalytic concentration-dependent degradation of both UV chemical filters [229]. As well, compared to the control experiment (no nanocomposites present), the photodegradation of octinoxate increased from 7 to 14%, 12%, and 16% in the presence of 1 wt%, 3 wt%, and 5 wt%, respectively, of 10-nm sized hydrophilic TiO_2 nanocomposites (WP-S; see caption of Fig. 49 for meaning). The presence of up to 3% WP-S caused a decrease in the degradation of avobenzone. By contrast, the presence of 5% WP-S in the sunscreen formulations increased the photodegradation of avobenzone, suggestive of a competitive photodegradation between octinoxate and avobenzone [229]. For comparison, the presence of 15-nm small-sized hydrophobic TiO_2 nanocomposites (OP-S) led to a significant increase in the photodegradation

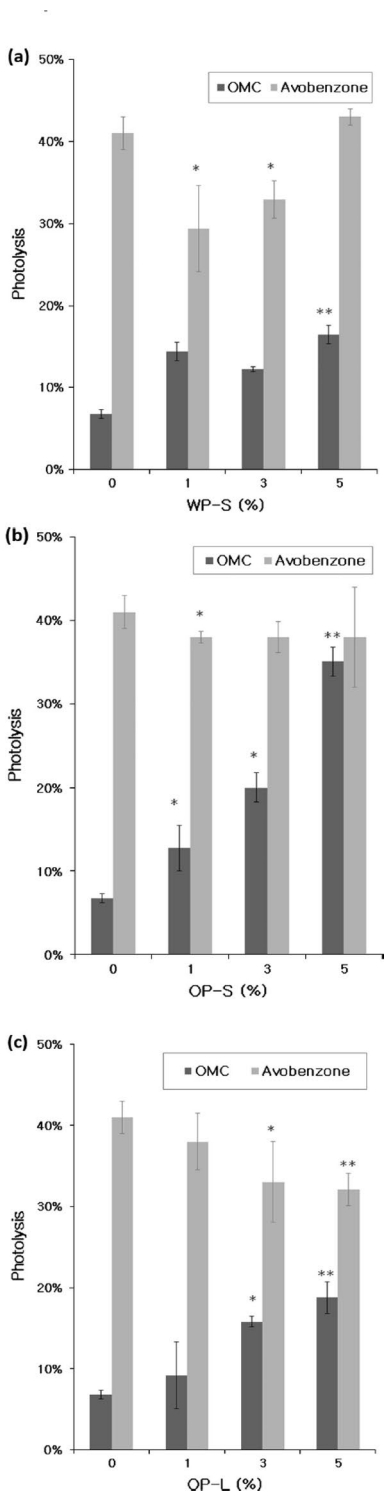


Fig. 49 Percent photodegradation of octinoxate (OMC) and avobenzone in suncreen formulations in the presence and absence of varying quantities of nano-TiO_2 after 1 h irradiation with simulated sunlight: **a** WP-S (~10-nm small-sized hydrophilic TiO_2 nanocomposites), **b** OP-S (~15-nm small-sized hydrophobic TiO_2 nanocomposites), and **c** OP-L (~200-nm large-sized hydrophobic TiO_2 nanocomposites). Reproduced from Ref. [229] with permission (License No. 4954800280237). Copyright 2015 by Elsevier B.V

of octinoxate, while the extent of photodegradation of avobenzone decreased somewhat (Fig. 49b). However, in the presence of 200-nm large-sized hydrophobic TiO_2 nanocomposites (OP-L), there was an increased photodegradation of octinoxate, while the extent of photodegradation of avobenzone decreased significantly (Fig. 49c).

As an effective scavenger of reactive oxygen species, quercetin was also used [229] as an inhibitor of the photodegradation of avobenzone and octinoxate that earlier had been shown to effectively inhibit their photolysis [157]. The inhibitory effects of quercetin on the photodegradation of these organic UV filters were even more apparent in the absence of TiO_2 nanocompsosites [229], though the photocatalytic effects of WP-S and OP-L on octinoxate and avobenzone appeared rather weak but nonetheless observable. By comparison, even though the OP-S nanocomposites demonstrated the lowest photocatalytic ability and the highest UV screening capability, nonetheless they did enhance significantly the photodegradation of octinoxate.

The photostability of *trans*-resveratrol (RES; Fig. 50) used in cosmetic formulations and that of the classical antioxidant beta-carotene (BCT; Fig. 50) also present in sunscreens, which correlated directly to their performance and safety in suncare products, was addressed by Freitas and coworkers [230] who assessed the influence of RES and/or BCT on the photostability of five UV-filters: octinoxate, avobenzone, octocrylene, bemotrizinol, and octyl triazone (also known as ethylhexyl triazone; Uvinul T 150) in three different combinations after being exposed to UVA radiation. Together with octinoxate and avobenzone, RES and BCT were significantly photodegraded after UV exposure producing no less than 11 identified degradation products, all of which originated from the four components. The combination RES + BCT in a sunscreen enhanced the photostability of avobenzone. In this regard, the combination of antioxidants in sunscreens revealed that using the (RES + BCT + the UV filters) combination led to more photostable formulations.

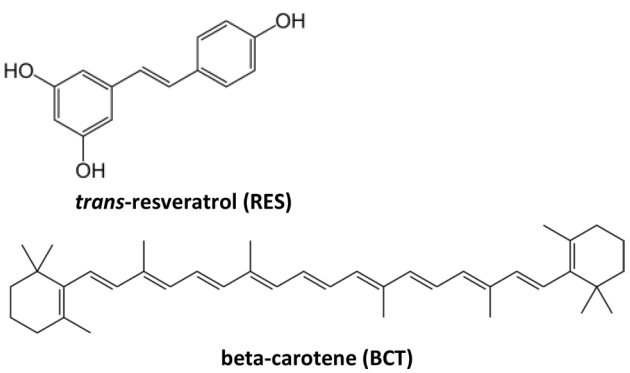


Fig. 50 Structural features of *trans*-resveratrol (RES) and beta-carotene (BCT)

Aside from any academic interests, the patent literature on work done with regard to enhancing the photostability of avobenzone and its various formulations for possible commercial implementation was reviewed in 2015 by Kumar and Deshpande [231].

4 Skin cancers: causes, developments, remedies

There is no such thing as a safe or healthy tan—tanned skin is damaged skin!

With such a strong statement, it is important to recognize that the incidence of skin cancer has been increasing in the last few decades, mostly because commercial sunscreen formulations protect largely against solar-induced erythema [148] (i.e., sunburns). Despite the many claims that sunscreens also protect against skin cancers [115], a direct cause-effect relationship has yet to be demonstrated for the most malignant of the skin cancers: the melanomas that have accounted for 287,723 deaths worldwide in 2018 [232] as a result of various factors, amongst which is an increase in sunlight exposure time that promotes skin cellular problems and exceeds the requirement for the light-mediated synthesis of vitamin D [233].

Regular applications of broadband UVB/UVA sunscreens are expected to prevent skin cancers and should minimize UV-induced immunosuppression and skin ageing. According to Bens [148], significant benefits from regular sunscreen uses have yet to be established toward the primary prevention of basal cell carcinomas and malignant melanomas, and although the effect of sunscreens may avoid actinic keratoses, their effect remains incomplete at best with regard to squamous cell carcinoma and skin ageing.

Nitric oxide (NO) is both a radical scavenger and a messenger (a natural warning system), which expresses itself by the appearance of redness (erythema) on those parts of skin overexposed to the sun's radiation. The use of sunscreens was meant to retard, if not suppress this natural warning and thus allow people to be exposed unreasonably to UV sunlight that sunscreens were not meant to block completely; a small percentage of sunlight—perhaps 1–2% depending on the SPF—always gets through and is absorbed deep into the epidermis and dermis. This leads to changes in the structure of skin cells and eventually to cancer-causing mutations in the DNA of these cells. Related to this, in the early 1990s, Garland et al. [234] noted that the widespread public health recommendations of the time were likely more harmful with regard to the use of sunscreens to prevent a variety of cutaneous malignancies (e.g., basal cell carcinoma, squamous cell carcinoma and melanoma) than the advice to control exposure to the sun's UV radiation by more traditional tested

means: (a) limiting the time spent in the sun and (b) using appropriate covering (hats, clothing). They further noted that countries, where chemical sunscreens had been recommended and adopted, experienced a non-insignificant increase in cutaneous malignant melanoma with its ultimate accompanying rise in death rates. As a case in point, the death rates in the United States between the 1950s and the 1990s caused by the melanomas doubled in women and tripled in men [235].

Currently, skin cancer is also the most commonly diagnosed cancer in Canada [236], and although melanoma accounts for only 4% of all skin cancers, it is responsible for 80% of skin cancer deaths. In the 20-year period between 1992 and 2012, the occurrence of skin cancers increased by 38.4%. Most disturbing, about 7300 new cases of melanoma were diagnosed in 2017 alone, causing 1250 deaths—by comparison, in 1989 there were 2400 new cases and 500 deaths. Melanoma is one of the few types of cancer that is increasing within the Canadian population. A current estimate of Canadians developing melanoma is 1 in 74 for women and 1 in 56 for men [237]. In a 2005 study on melanoma and skin cancer prevention, Gallagher [237] estimated the risk of melanoma and skin cancer in the white population of Canada to be ca. 1 in 76 for melanoma (incidence about 1.3% of which almost 15% of cases proved fatal), about 1 in 20 for squamous cell carcinoma, and approximately 1 in 4 for basal cell carcinoma. Apparently, the incidence of skin cancer could be reduced by 50–75% upon regular use of sunscreens [236], but with the caveat that (if and only if) the general public apply sunscreen effectively and use sufficient quantity of sunscreen (2 mg/cm^2 of skin surface) to reach the level of SPF of the sunscreen product. Our personal observations, however, indicate that people continue to fail to heed to this simple recommendation. In line with this, Diffey—one of UK's foremost expert on sunscreens—pointed out that consumers apply much less than this quantity [238], typically between 0.5 and 1.0 mg/cm^2, if not less, which amounts to ca. 20–50% of that expected from the sunscreen product, since quantity (i.e., thickness [239]) is an important factor in sun protection along with other factors: (i) uniformity of application, (ii) cosmetic 'feel' of sunscreen, (iii) resistance to water immersion and sand abrasion, and (iv) when, where and how often sunscreen is re-applied [238]. Not least, beachgoers fail to apply sunscreen prior to UV sunlight exposure and fail to apply sunscreen over all the exposed skin [240]. Recognizing that sunscreens used in the 1980s−1990s came with low SPF numbers and were mostly based on UVB filters, the resulting effective SPF was more likely around 2–3 and consequently the total UVB/UVA radiation dose impinging on the exposed skin was likely 70–90% of that received by unprotected skin for a similar time period [241]. By comparison, if a broadspectrum sunscreen with SPF 25 were applied at ca. 1.0 mg cm^{-2}, the effective SPF would be around 8–10 so that the total solar UV radiation (UVA/UVB) dose impacting the exposed skin would fall between 20 and 30%. Clearly, current commercially available sunscreen products with SPFs 50+ and 100+ are expected to reduce significantly the amount of UV radiation that penetrates the epidermis and dermis, and thus minimize its consequences, especially as sunscreens now also include broadband UVA filters.

Be that as it may, however, let us examine what skin cancers look like from a simplistic viewpoint, with the reminder that skin cancers begin in skin cells as a result of different causes and to varying degrees of malignancy [242].

4.1 Non-melanoma skin cancers

4.1.1 Basal cell carcinoma

Basal cell carcinoma is the most common type of skin cancer that occurs in the outermost layer of the skin—the epidermis (Fig. 1)—and makes up nearly 75% of all diagnosed non-melanoma skin cancers. Typically, this carcinoma appears as a pink or translucent nodule on a sun-exposed area of skin, most often the face and neck (Fig. 51) [242].

People most at risk in developing this carcinoma are those that overexpose themselves regularly to the sun's UV radiation and suffer severe sunburns. Particularly prone are people with skin type I, people with a compromised immune system, and people over 60 years of age. However, this type of skin cancer does not spare people in their teens or early twenties, as well as people with the genetic condition basal cell nevus syndrome. Nonetheless, when discovered early, basal cell carcinoma can be removed surgically, although the treatment depends on factors such as the patient's age, the medical history, and health status [242].

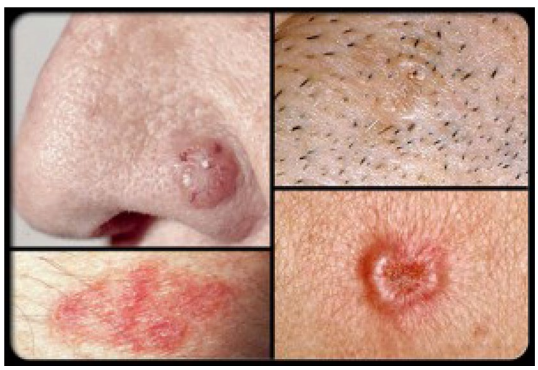


Fig. 51 Illustrations of how basal cell carcinoma presents itself on the face and neck. Reproduced from Ref. [242]: https://www.melanomanetworkca/skincancer

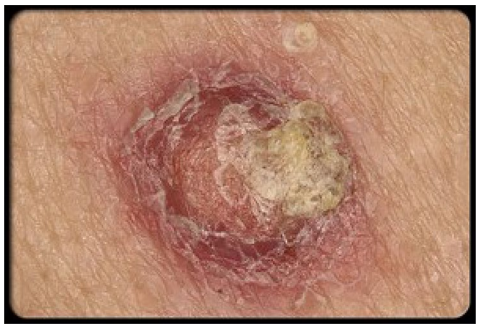


Fig. 52 Illustrations of how squamous cell carcinoma presents itself on skin exposed to the UV sunlight. Reproduced from Ref. [242]: https://www.melanomanetwork.ca/skincancer/

4.1.2 Squamous cell carcinoma

Squamous cell carcinoma (Fig. 52) is the second most common form of skin cancer, which typically occurs in areas of the body exposed to UV sunlight: face, neck, bald scalp, hands, shoulders, arms and back, although it can also spread to other parts of the body. A leading cause of this type of skin cancer is also frequent sun exposure. Healthy individuals with skin type I and certain patients are particularly at high risk [242].

4.2 Melanoma

Melanoma is one of the fastest growing skin cancers worldwide, a lethal form of skin cancer that affects cells (the melanocytes) that produce the pigment melanin, which is primarily responsible for imparting color to the skin. The greater the skin is exposed to the UV radiation, the greater is the production of melanin, and to the extent that most melanocytes are found in the deepest part of the epidermis and grow down into the dermis (Fig. 1), makes melanoma of the skin most common [243]. Nonetheless, unlike other cancers, melanoma can occur anywhere on the body: in men it occurs mostly on the head, neck and back, whereas in women melanoma occurs mostly on the back and lower legs.

Figure 53 displays three forms of melanoma: (i) the most common is the superficial spreading melanoma, which makes up nearly 70% of the melanomas and usually appears as a dark brown to black stain spreading from an existing or a new mole, particularly in the areas of the skin exposed to UV radiation; (ii) the nodular melanoma, which makes up nearly 10% of the melanomas and appears as a firm domed bump on the skin that can grow rapidly down through the epidermis into the dermis where it can metastasize or spread to other parts of the body; and (iii) the lentigo maligna melanoma, which grows slowly initially, appears as a dark stain that earlier may have looked like a large or irregular freckle and usually occurs on the face or arms of middle-aged or older people [243]. The likely prime risk factors in developing melanoma are: (i) family history, (ii) age, and (iii) exposure to UV sunlight during childhood years that ended in sunburns—note that the incubation period for melanoma to show its deleterious effects is around 20–40 years so that a direct relationship (cause-effect) between sunscreen use, even in early childhood, and development of melanoma is difficult to establish over such a long period of time [244].

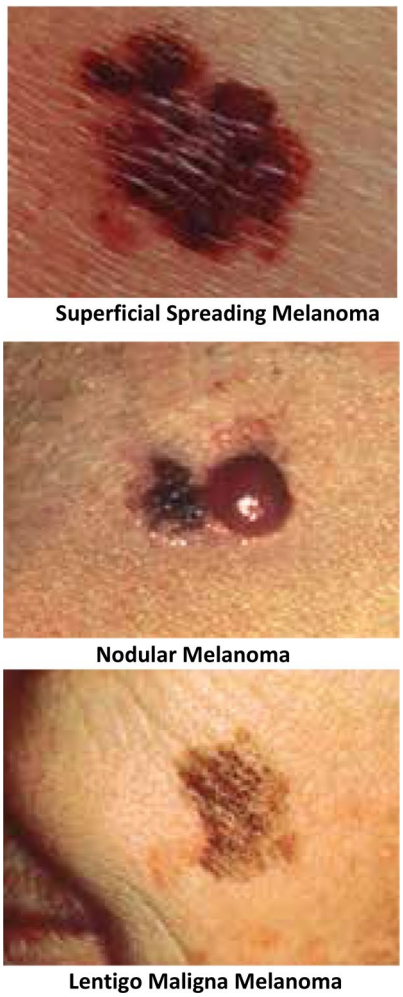


Fig. 53 Illustrations of three of the different types of melanomas. Reproduced from https://www.melanomanetwork.ca/types-of-melanoma/ (accessed, September 14, 2020)

4.3 Does the use of sunscreens prevent the development of skin cancers?

Indeed, what did pre-2005 evidence demonstrate on the efficacy of sunscreens with regard to protection against malignant skin cancers? [237]. Apparently, (1) there was no convincing evidence that sunscreen use reduced the risk of basal cell carcinoma; (2) there was good evidence that when properly applied, sunscreen use could reduce the risks of

actinic keratoses and squamous cell carcinoma; (3) however, there was no adequate confusion-free evidence on which one could definitely establish a direct relationship between the use of sunscreens and the development of melanoma. Thus, no firm pre-2005 evidence existed of any protective value by sunscreen products with regard to melanoma risk. The latter was also echoed by Diffey [241] who examined whether such observations [237] were to be expected given the period during which case–control studies were conducted up to about 2005 with sunscreens that were commercially available at the time, together with how sunscreens were used and applied in practice. There was much confusion in the 1990s and beyond subsequent to a report by the International Agency for Research on Cancer [245] which reviewed and analyzed no less than 15 previous studies on the evaluation of the potential preventative effect of sunscreens against cutaneous melanoma. Their analysis showed that:

1. four of the studies provided little evidence of an effect of sunscreen use on the risk of developing melanoma,
2. three studies showed significantly lower risks in developing melanoma in sunscreen users compared to non-users, while
3. the other eight studies showed significantly higher risks in developing melanoma by sunscreen users.

Although the latter eight studies suggested that development of melanoma was associated with sunscreen use, the later analyses by Huncharek and Kupelnick [246] and by Dennis et al. [247] showed no relationship between development of malignant melanoma and sunscreen use. Thus, the confusion continued to prevail, mostly because given the length of time for melanoma to appear, it is difficult, if not impossible, to associate melanoma directly with sunscreen use because people are not expected to remember whether long ago they used sunscreens regularly, and whether they also used clothing and shade for sun protection in their daily activities.

Sober [248] was somewhat puzzled that not all melanomas are caused by solar UV exposure (other possible influences included genetic factors and immune system deficiencies [249]) as the fraction of melanoma differed from nearly 90% for Australians with skin type I/II to ca. 0% for people with skin type VI (Fig. 3). He was also confounded by the dearth of strong data with regard to which UV wavelengths are responsible for the melanomas, although some studies had suggested that UVA radiation plays a role in the development of melanoma via oxidative stress, free radical generation, and the degradation of vitamin D, but not necessarily direct DNA damage [250–252]. Studies from Australia and elsewhere, which inferred sunscreen users were at higher risk to develop melanoma than non-users, added to the confusion. The work by Planta [253] noted that despite the decades' long availability and promotion of sunscreens, the frequency of cutaneous malignant melanoma (CMM) between 1973 and 2003 increased by 81% in the United States and continued to escalate at a rate of ca. 3% per year; there was also scant evidence that sunscreen uses protected against malignant melanoma [254]. As well, a number of studies suggested that sunscreen use did not significantly decrease the risk of developing melanoma, but in fact may actually have enhanced the risk of developing cutaneous malignant melanoma and sunburns [246, 255–258]. Other studies also reported that sunscreen users were actually more likely than non-users to develop sunburns, and consequently increase their risk of developing melanoma [258–260]. That cosmetics incorporating chemical UV filters fail to protect the skin against melanoma was also raised by Arct and Pytkowska [261] in that such failure likely stemmed from side effects of filter activity and/or from products of their photodegradation [262].

By contrast, a 2018 online statement, released by the University of Sidney's Cancer Epidemiology and Prevention Research group of the School of Public Health and Melanoma Institute Australia [263], indicated that Australians aged 18–40 years who used sunscreens regularly in childhood reduced their risk of developing melanoma by 35–40% vis-à-vis those who rarely used sunscreens. The objective of that research study by Watts and coworkers [264] was to assess whether there was a correlation between early-life use of sunscreens and the association between sunscreen use and risk of cutaneous melanoma before age 40. The study involved over a thousand participants aged from 28 to 38 years, many of whom were women (57–62%) with most participants being of British/Northern European ethnicity, and thus possessing lighter skin pigmentation and a stronger history of blistering sunburns. Subgroup analyses showed that the protective association of sunscreen with melanoma was stronger for people reporting blistering sunburns, receiving a diagnosis of melanoma at a younger age, or having some or many nevi [264]. Apparently, total lifetime sun exposure was unrelated to melanoma risk, although total sun exposure, inversely weighted by sunscreen use (a measure of sun exposure unprotected by sunscreens), was significantly associated with melanoma risk and appeared stronger for people having (a) lighter pigmentation, or (b) some or many nevi, or (c) using sunscreens to stay longer in the sun. The authors [264] concluded that regular sunscreen use during childhood was associated with significant reduced risk of developing cutaneous melanoma among young adults.

According to the United States Environmental Protection Agency (EPA), melanoma is currently (2020) one of the most common cancers amongst adolescents and young adults aged 15–29, as UV exposure and sunburns during childhood are significant risk factors in developing melanoma [249]. Although it accounts for only about 3% of

skin cancer cases, melanoma is responsible for more than 75% of skin cancer deaths. More new cases of skin cancer are diagnosed each year in the United States than are new cases of breast cancers, prostate cancers, lung cancers, and colon cancers combined [249].

Based on available studies then, the jury is still out as to whether or not sunscreen use provides protection against skin cancers, as a single blistering sunburn before the age of 20 can increase the risk of developing melanoma later in life [242]. In that case, how can one protect oneself from the carcinogenic UV radiation? The United States Center for Disease Control (CDC) recommends the following rules for appropriate protection for families with dynamic outdoor activities [265]:

1. Seek shade, especially during midday hours. This includes 10 am to 4 pm, March through October, and 9 am to 3 pm, November through February. Umbrellas, trees, or other shelters can provide relief from the sun.
2. Be extra careful around surfaces that reflect the sun's rays, like snow, sand, water, and concrete.
3. Wear sun protection gear like a hat with a wide brim and sunglasses to protect your face and eyes.
4. Sunglasses protect your eyes from UV rays and reduce the risk of cataracts and other eye problems. Wrap-around sunglasses that block both UVA and UVB rays offer the best protection by blocking UV rays from the side.
5. Wear a long-sleeved shirt and pants or a long skirt for additional protection when possible. If that's not practical, try wearing a T-shirt or a beach cover-up.
6. Apply a thick layer of broad spectrum sunscreen with an SPF of 15 or higher at least 15 min before going outside, even on cloudy or overcast days. Reapply sunscreen at least every 2 h and (especially) after swimming, sweating, or toweling off.

The Australian study quoted above [264] reported that 35–40% of participants saw a reduction in developing melanoma, which also means that 60–65% of participants did not – in essence only 1 out of 3 people, who regularly used sunscreens in childhood, had a negative response to developing melanoma. This calls attention to also use alternative means, in addition to sunscreens, to protect oneself against the solar radiation as recommended by the Center for Disease Control [265], by the American Academy of Dermatology Association [266], by the Mayo Clinic [267], and by the Johns Hopkins University School of Medicine [268], among several other agencies in different countries. For instance, the National Health Service of the UK also makes similar recommendations [269]:

(i) spend time in the shade between 11 am and 3 pm from March to October,
(ii) make sure you never (sun)burn,
(iii) cover up with suitable clothing and sunglasses,
(iv) take extra care with children, and
(v) use at least factor (SPF) 30 sunscreen.

Unfortunately, beachgoers on the French Cote D'Azur beaches and on Italy's Adriatic and Mediterranean beaches (1995–2005 personal observations), more often than not, tended to violate the CDC's rule (1) as the time they spend at the beach is exactly between 10 am and 4 pm, and sunscreens were topically applied at the beach under the hot sun [violation of CDC's rule (6)]. These rules notwithstanding, however, are the sunscreen active ingredients safe to use? Especially, the chemical (organic) UV filters?

4.4 Do active ingredients in sunscreen products pose a risk to human health?

Chemical UV filters in sunscreens, suncare products, and cosmetics raise some significant problems since, as noted earlier, they can penetrate into the skin and affect hormone activity and photochemical reactions in sunscreen-covered skin (see Ref. [261] and references therein). Not only may these products pose a direct health risk when in contact with the skin, but also when present in municipal sewage as they may not be destroyed completely, even in modern wastewater treatment plants.

At the 2014 International Conference on Cosmetics, Arct and Pytkowska [261] raised some important issues with regard to chemical UV filters that are worth noting:

- The majority of classical UV filters penetrate the skin, permeate it, reach the circulatory system and impact a systemic action on the body.
- Exposure of sunscreen-treated skin to UV generates reactive oxygen species that have serious consequences when penetrating the outermost skin layers.
- The presence of ca. 2% chemical UV filters in urine, plasma, and breast milk confirms the penetration of the filters oxybenzone, octisalate and octinoxate in the skin (Treffel's studies).
- In vitro studies confirm the ability of oxybenzone, 3-(4-mehtylbenzylidine) camphor, and octinoxate to penetrate the epidermis and permeate the skin.
- Oxybenzone, 3-benzylidine camphor, 3-(4-methylbenzylidine) camphor, octinoxate, homosalate, padimate-O, and PABA display estrogenic activity—i.e., these UV filters can bind to and activate estrogen receptors.
- In vitro tests reveal that oxybenzone, 3-benzylidene camphor, 3-(4-methylbenzylidene) camphor, octinoxate,

homosalate, and padimate-O show pronounced antagonistic activity against androgen receptors.
- Several UV filters undergo photochemical changes and photodegradation when exposed to UV radiation: e.g., avobenzone, octinoxate, padimate-O, and ethylhexyl triazone are photo-unstable, with some generating $^1O_2$ that reacts with sunscreen components and yields photoproducts that may be allergenic and thus irritate the skin.

Because of these issues, the FDA recommended that chemical UV filters be tested further for safety if present in plasma at concentrations greater than 0.5 ng/mL, because at higher concentrations they could be a leading cause of skin cancers, and/or otherwise could cause injury to developmental and reproductive systems.

Figure 54 illustrates the chemical UV filters that have been found in significant amounts in plasma, well above FDA's threshold. [10] Clearly, all six UV filters penetrated/permeated through the skin and ultimately got into the blood stream, even after a single topical application of the sunscreen product. After 4 days, the level of those six UV filters increased significantly, some more than others, and yet some still showed high levels after a 3-week period without re-applying the sunscreens. Particularly bothersome is oxybenzone whose concentration increased 180-fold after the first application, and subsequently soared to 500 times after a 4-day period [163, 164]. The authors [163, 164] and the FDA administration [10, 270] have recommended additional studies by sunscreen/cosmetic manufacturers to further ascertain the safety of chemical UV filters. However, they were silent as to whether or not consumers should change

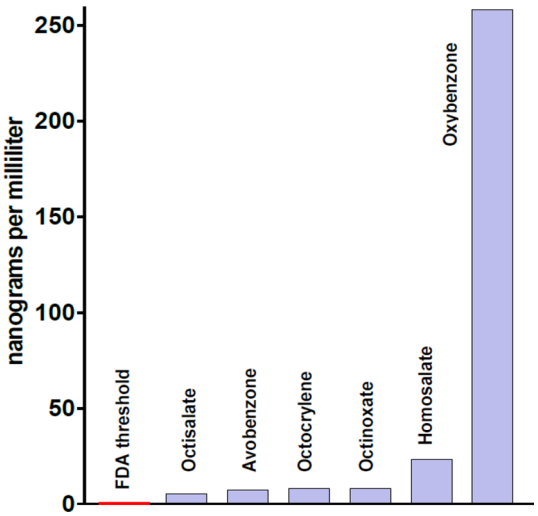


Fig. 54 Concentrations in ng/mL of some sunscreens active chemical UV filters found in human plasma well beyond the FDA's "safe" threshold of 0.5 ng/mL, subsequent to exposure of topically applied sunscreens to UV radiation over a 4-day period. Plotted from data reported by Matta and coworkers [163, 164]

their sunscreen practices, or whether they should avoid those sunscreen products altogether. They further noted that, although UV filters in chemical-based sunscreens cannot be considered safe, it does not mean they are unsafe—a rather unusual and confusing statement! So which is it—are they or are they not?

Oxybenzone, has been shown to be damaging to breast development, to infant birth weight, to sperm function, and not least to exhibit relatively high rates of skin allergies; it is also a weak estrogen disruptor and a moderate anti-androgen (see Ref. [271] and refs. therein). By comparison, octinoxate exhibits moderate rates of skin allergies and hormone-like activity. Animal studies showed that octinoxate also affects the reproductive system, as well as causing thyroid and behavioral alterations. Homosalate is known to disrupt estrogen, androgen and progesterone, and yields toxic photoproducts subsequent to its photoinduced breakdown [271]. Interestingly, the Environmental Working Group—to whom consumers might turn to for online information on sunscreen use, their safety and which sunscreen product may be best—reported that 84% of 831 sunscreens tested failed to pass the health and environmental muster, and that more than half the sunscreens made questionable claims regarding longevity, water resistance, and UV protection [272].

5 Concluding remarks

There is no question in anyone's mind that the Sun's UV radiation is a prime carcinogen responsible for the development of skin cancers and for other damaging effects. Other than exposing oneself for about 15–20 min to this radiation for the synthesis of vitamin D, an immediate defense against UV radiation might be complete avoidance. Obviously, this line of defense would be totally impracticable, and so the second line of defense is for people to use various commercially available sunscreens/cosmetics products applied topically on exposed skin to attain a level of protection against the nefarious consequences of UV radiation. However, as noted throughout this article, the use of sunscreens (and cosmetics) that contain either or both physical and chemical UV filters is not without its problems.

The 16 UV filters listed in Table 2 and some used in sunscreens and cosmetics are referred to as over-the-counter drugs (OTC) [10] in the United States as they may affect the health of the American consumers from the harmful consequences of solar UV radiation. Accordingly, they are regulated by the Food and Drug Administration. Other countries do the same, although these UV filters are often considered as "cosmetic components", not as drugs. The FDA partitioned the 16 filters into three categories: (a) Category I lists the physical filters TiO_2 and ZnO, both of which are considered by the FDA as being generally recognized as safe

and effective (thus, as being GRASE); (b) those in Category II are not GRASE owing to significant safety issues, and so are not recommended for use in sunscreens; and (c) for the chemical filters in Category III, the FDA lacks sufficient safety data to consider them as being in the GRASE classification [10, 270]. In their new proposed regulations [270], the FDA anticipates:

1. Raising the maximal SPF value on sunscreen labels from SPF 50+ to SPF 60+.

However, raising the SPF is typically done by increasing the concentrations of the UV filters and consequently will also raise the amount of these chemical filters to find their way in human plasma and perhaps elsewhere.

2. Requiring sunscreens with SPF 15 or higher to also include filters to provide consumers with broadspectrum protection, especially against UVA radiation.
3. Products that combine sunscreen uses with insect repellents will not be considered as being GRASE (note that the simultaneous use of both products enhances skin penetration of both).

Considering TiO_2 and ZnO as being two broadspectrum filters in GRASE is rather questionable [44], because even the least photoactive form of TiO_2 (viz., rutile) caused strand breaks in DNA plasmids as did ZnO (see Fig. 7). Nonetheless, nanosized TiO_2 samples synthesized and subsequently modified [48] showed that such TiO_2 specimens could be made so as to have no consequence on DNA plasmids (Fig. 9), or on the survival rate of yeast cells (Fig. 10) when exposed to simulated sunlight. Untreated TiO_2 samples together with avobenzone (Parsol 1789) and padimate-O, however, significantly enhanced the kill rates of yeast cells.

Two additional rules by the Center for Disease Control, by the FDA, and by other similar agencies regarding topical applications of sunscreens are important and should be adhered to:

Apply the sunscreen indoors about 15–20 min prior to sunbathing/swimming or for other daily activities.

Yet most people tend to apply, more often than not, sunscreens at the beach under the hot sun. Surely, they would not wax their car under a hot sun – so why would they apply sunscreens on their bodies under a hot sun?

Re-apply sunscreens every 2 h, or else immediately after swimming and towelling off.

As most sunscreen chemical filters tend to be photounstable, it is important to re-apply (refresh) sunscreens every 2 h (preferably in the shade). However, at the cost of some of the sunscreen brands (20 years ago, this author paid about 120 Euros for a 2 fl. oz. suncream, about 60 mL) for use in our examination of the photostability of chemical filters [111], and later to examine the photochemical consequences of a UVB filter with a UVA filter [217]. It is doubtful that people will re-apply sunscreens either because of the high cost (in some cases) or because they may simply forget to do so.

Our choice of a simple photostable sunscreen would consist of the natural product Aloe Vera gel (see Fig. 27b) as the base vehicle and our modified titanium dioxide particles such as the RA1A, R8A and R20A samples whose effects on DNA plasmids have been illustrated in Fig. 9. Are there other choices? Indeed there are!

Natural compounds such as the flavonoids [e.g., the polyphenolic systems Quercetin (Fig. 44) and Rutin (Fig. 55)] present in several fruits—for instance, in grape fruits, strawberries, apples, tea, vegetables and red wine—in combination with current sunscreen active agents could afford an

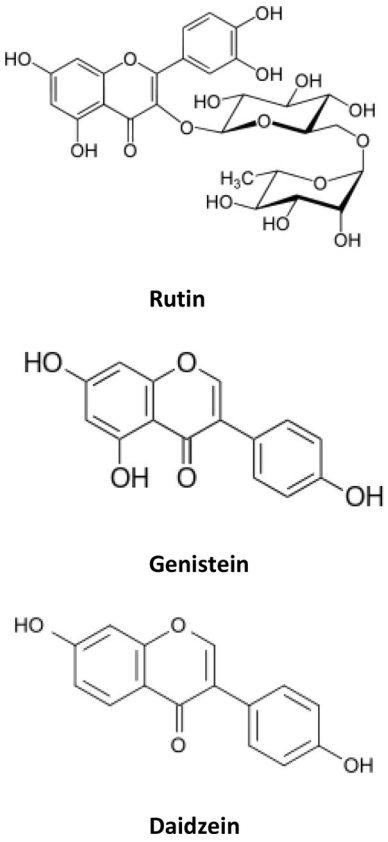


Fig. 55 Structural features of some natural compounds that could play a role in sunscreens: Rutin (2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-[α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyloxy]-4*H*-chromen-4-one), Genistein (5,7-dihydroxy-3-(4-hydroxyphenyl) chromen-4-one), and Daidzein (7-hydroxy-3-(4-hydroxyphenyl)-4H-chromen-4-one)

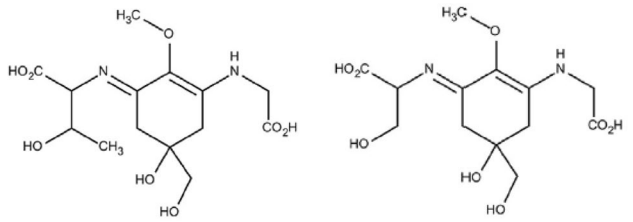


Fig. 56 Structural features of the two MAAs present in Helioguard 365: porphyra-334 and shinorine

interesting strategy for skin photoprotection against UV radiation [273]. Also, the isoflavones Genistein and Daidzein (Fig. 55) possess anti-photocarcinogenic and anti-photoageing properties, and thus could avert UVB-induced sunburns.

The role that the mycosporine-like amino acids (MAAs; Figs. 17, 18) could play as sunscreen agents to protect against damage by harmful levels of UV radiation was briefly described earlier. They can block specific consequences of oxidative damage preventing lipid peroxidation and formation of superoxide radicals.

Although earlier studies have proposed MAAs as possible sunscreen agents, they have yet to be exploited on a large scale. Only a few products are available, one of which is Helioguard 365 that contains porphyra-334 and shinorine (in a ratio of 11.5–1; Fig. 56) extracted from the red alga *Porphyra umbilicalis* [274] to protect against UVA radiation with, however, minimal protection from the more damaging UVB radiation. The concentration of MAAs in one commercial sunscreen is very small (0.0005% w/v) compared to the concentrations of UV filters typical in most sunscreen products (0.5–10% w/v) and consequently will have a minimal, if any, influence on the SPF of the sunscreen [274].

It must be further emphasized that MAAs are highly water-soluble and so are not suitable for incorporation into sunscreen formulations intended for UV protection on beaches, but they are more appropriate for use in daily suncare products as aqueous formulations possess better sensorial properties than oil-based formulations [274].

As good as sunscreens may appear to be, none block 100% of the UV radiation, as some of this radiation (however small) will still get through deep into the epidermis and dermis, so that the other recommendations regarding additional alternative means for sun protection merit thoughtful considerations.

So to come back to the question: have we made any progress in the last two decades? Evidently, much remains to be done on three fronts: first and foremost are (a) the safety issues of sunscreen ingredients, (b) the photostability of sunscreens, especially the photostability of the UVA filters remains an important issue, and (c) the direct cause-effect relationship between sunscreen usage and skin cancers remains to be demonstrated unambiguously.

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Compliance with ethical standards

Conflict of interest The author reports no conflicts of interest.

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