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Photoprotection in changing times – UV filter efficacy and safety, sensitization processes and regulatory aspects

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Synopsis

Since excessive sun exposure is tightly associated with different pathological changes of the skin, e.g. premature skin aging or the development of skin cancer, an appropriate protection of the skin against UV radiation is of particular importance. Sun protection products and UV filter substances have evolved continuously in the past few decades. New developments and improved technical conditions of production have led to increasingly effective and efficient products with broadband protection ability. Accordingly, legal requirements have also changed and expanded. Although certain trends exist to harmonize the regulation of sunscreens at a global level, there are still large differences how

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UV absorbers are approved, which testing methods are prescribed, and which general requirements sun protection products must meet.

Modern UV filters provide efficient protection against UVA and UVB radiation, are heat and photostable, user-friendly, cost-effective, water resistant and nontoxic. Since inorganic and organic UV filters are topically applied to the skin in relative high concentrations (up to 25 %), especially the assessment of their (photo)sensitization potential is of particular importance. Accordingly, skin sensitization is a key endpoint for the legally required safety assessment of cosmetic ingredients in Europe and many other countries.

This review will summarize the current regulatory status of different approved UV filters, will describe their beneficial and adverse properties, and will give an overview of how the efficacy of sunscreens can be evaluated. Finally, an insight into the basic mechanism of (photo)allergic reactions and existing skin sensitization test methods will be provided.

General introduction

In addition to the desired effects of sun radiation, such as the increase of general well-being [1], vitamin D3 synthesis [2], and positive therapeutic effects on some skin diseases, e.g. psoriasis [3], excessive UV radiation is mainly responsible for several types of severe skin damage. In addition to the direct visible acute damages such as sunburn or polymorphic light eruption, the undesirable long-term effects, most notably skin cancer, are a special cause for concern [5,6]. Today, it is generally accepted that a main environmental risk factor for skin cancer is the exposure to sunlight or UV radiation [7,8]. This finding, however, prevailed only gradually during the 20th century.

Until the end of the 19th century, there was no real market for sun protection products. A pale skin color was considered elegant and begat prosperity. With industrialization, the centuries-old ideal of beauty changed fundamentally. An improved living standard with more leisure time, a less-restricted

style of dress, and medical health advice to spend more time outdoors led to increased sun exposure and the initial need for skin protection [9,10].

Still, at the beginning of the 20th century, it was believed that solar heat was the actual cause of sunburn. Only in 1922 did Karl Eilham Hausser und Wilhelm Vahle observe that not heat but ultraviolet radiation between 280 nm and 315 nm was responsible for the formation of sunburn [11]. This finding was the cornerstone of sunscreen development.

Already in the middle of the 1930s, Franz Greiter and Eugene Schueller, the later founders of Piz Buin and L'Oreal, brought the first, simple sunscreens on the market. These products were mainly focused on the prevention of sunburn, i.e. ultraviolet (UVB) protection. Due to the success of the first sunscreens, there was a decreasing fear of sunburn, and sunbathing became increasingly popular [12,13]. However, with the extended exposure to the sun, the incidence of skin cancer also increased [14,15].

In Germany alone, the cases of cutaneous melanoma has nearly tripled between 1976 and 2003 [16] with approximately 140,000 new cases of skin cancer every year [17]. Recent increases in skin cancer incidence may directly reflect the change in leisure behavior over the last decades associated with short-term excessive tanning, especially during the summer holidays or due to the usage of tanning devices [18-20]. Here, it must be mentioned, that sunscreens with higher sun protection factor (SPF) may offer better protection ability, but conversely also entice the user to stay longer in the sun [21,22].

Furthermore, there has been a gradual depletion of the stratospheric ozone layer ("ozone hole") since the 1970s [23], which has led to increased UVB intensity at the surface associated with a rising number of skin cancer cases [24,25].

To avoid damage from UV radiation, the human body has developed different protection mechanisms, e.g. a thickening of the stratum corneum (hyperkeratosis) or skin pigmentation [26,27]. However, the body's protective mechanisms are not sufficient for long-term UV exposure, and additional protective measures are required.

The widespread sun protection campaigns since the 1980s have led to improved education of the population about the risks of excessive sun exposure and the importance of sunscreen usage [28-30]. This laid the foundation for a growing market of sun protection products, which have been continuously improved and adapted to consumer needs and the scientific progress. With increasing awareness of the harmful effects of sunlight, the demand for higher sun protection factors (SPFs) has continued to grow in recent decades. At the end of the 1970s, an SPF of about 20 was the maximum attainable SPF with existing technological capabilities and the limited number of available UV filters. Today, there is a variety of organic and inorganic UV filters, which are combined to reach a balanced UVA/UVB protection while maintaining a high SPF of greater than 50 due to an increase in the number of UV filters and their concentration in the products [31,32].

Furthermore, the usage of cosmetic UV filter substances has expanded to a large number of daily skin and hair care products [33,34]. It is therefore not surprising that more than 10,000 tons of UV filters are produced annually for the global market [35]. As a result, the skin is in constant contact with high quantities of UV filters throughout the entire year.

This increased usage must be studied critically, not only because of the increasing release of the UV filters into the environment [36,37] and their possible ecological impacts [38,39], but also due to their behavior on the skin.

Because of the particular application type and the typical chemical structure of organic UV filters, the assessment of their photostability is of great importance [40]. Photodegradation can lead to a loss of UV protection and the formation of photo products [41-43]. Additionally, due to the chemical

structure of the most common organic UV filters and their known photodegradation products, various reactions, e.g. with protein structures of the skin, are conceivable but not yet sufficiently investigated [44].

Structure of the human skin

The largest human organ is the skin with a surface area of about 2 m² and a weight of about 3 kg for an average adult, calculated without subcutaneous adipose tissue. The skin consists of several layers of epithelial tissue with an average thickness of between approximately 0.5 and 2 mm (palms and soles up to 4 mm) [45]. The skin fulfills several important vital functions. As an outer barrier, it protects the inner body against pathogens and mechanical, chemical, and physical impacts. With various sensory receptors, which are responsive against mechanical stimuli and temperature, the skin is an important sense organ. In addition, the skin prevents the uncontrolled loss of water and minerals and protects the human body against hypothermia. Finally, the skin is responsible for the production of vitamin D [46,47].

The human skin consists of three main definable layers, the epidermis, the dermis, and the subcutis (Fig. 1). The epidermis can be further divided into five sublayers: the stratum basale, stratum spinosum, stratum granulosum, stratum lucidum, and stratum corneum [48]. The basal/germinal layer (stratum basale) shows the highest mitotic activity of the skin. It is composed mainly of non-proliferating and proliferating keratinocytes (KC; “stem cells”); proliferating KCs undergo constant cell division and are the origin of the corneocytes, which are finally exfoliated at skin surface [49]. Each basal cell has a large nucleus, numerous free ribosomes, a few mitochondria, a small Golgi complex, and a rough endoplasmic reticulum (rER). Additionally, several Merkel cells (touch receptors) and the melanocytes are located in this layer.

In the multilayered spinous layer (stratum spinosum), a gradual transformation of KCs took place, they become larger, polygonal, and flattened in the upper part of the layer. The cells of the stratum spinosum synthesize intermediate filaments (cytokeratins), which are grouped into bundles, called

tonofilaments. KCs are connected to each other by multiple, prickle-like intercellular bridges (desmosomes) where the tonofilaments are anchored to the cell membrane. This reticulated structure gives the layer its typical name. In the stratum spinosum, the immune cells of the lymphatic system (Langerhans cells) are located.

The increased synthesis of keratohyalin in KCs of the granular layer (stratum granulosum) initiates the cornification (keratinization) of the cells. This is accompanied by a simultaneous loss of other cell organelles (e.g. the nucleus). Lipids contained within lamellar bodies of KCs are released into the extracellular space and form the lipid barrier of the skin.

What follows is the stratum lucidum. The clear and thin layer of dead skin cells is only present in the palms and soles.

The outer layer is the cornified/horny layer (stratum corneum) consisting of 10 to 30 layers of polyhedral, enucleated corneocytes. Corneocytes are surrounded by an envelope of cornified proteins (e.g. loricrin, involucrin, and filaggrins) filled with water-retaining keratin proteins. The corneocytes stick together through corneodesmosomes and are surrounded by hydrophobic lipids [50]. The intercellular organization of the lipids plays an important role in the barrier function of the skin [51,52]. They form two lamellar phases, with periodicities of approximately 6 and 13 nm [53]. The lipids inhibit both an inside-out water loss and the permeation of hydrophilic substances with a molecular weight of more than about 500 Dalton (DA) [54]. The main barrier functions of the epidermis are attributed to the stratum corneum, which prevents water loss from the body and provides mechanical protection, although the cell-cell junctions and the associated cytoskeletal proteins in the deeper layers also provide important protection ability [55-57].

In adult epidermis, there is a balance of cell proliferation and cell desquamation with a complete renewal approximately every 28 days [58]. The cell proliferation in the stratum basale is followed by

the differentiation of the cells in the stratum spinosum and granulosum, which ends with the transition in the horny layer.

The dermis is tightly connected to the epidermis through the basal layer. The main structural components of the dermis are collagen, elastic and extrafibrillar matrix fibers (connective tissue), which give the skin its mechanical stability. The hair follicles, sweat glands, sebaceous glands, and apocrine glands are located in the dermis. The dermis also contains numerous lymphatic vessels and blood vessels, which nourish both dermal and epidermal cells and are important for waste removal. Various immune cells, such as macrophages, lymphocytes and mast cells, are also located in the dermis. The dermis can be divided in the stratum papillare and the stratum reticulare [47].

With age, the human skin becomes thinner, more and more wrinkled, and loses some of its elasticity. The main reasons for this are the dehydration of the stratum corneum, an extensive cross-linking of collagen and the degeneration of the elastic fibers [59,60].

The subcutis or hypodermis lies below the dermis. It attaches the skin to underlying bones and muscles and contains the bigger blood vessels and nerves. It consists of loose connective tissue and elastin. The main cell types are fibroblasts, macrophages and adipocytes. Adipose tissue serves as thermal isolation, provides energy storage, and offers mechanical protection [61].

Natural sunlight

The spectrum of natural sunlight covers most of the electromagnetic spectrum with the highest intensity in the range of visible light. At the top of the earth's atmosphere, sunlight has a power of 1366 watts/m² and is comprised of about 50% infrared (IR) light, 40% visible light (VIS), and 10% ultraviolet (UV) light [62] (Fig. 2).

While humans sense IR radiation as heat and VIS radiation optically, UV radiation is not directly perceivable. Depending on the wavelength, UV radiation can be divided into UVC (200–280 nm),

UVB (280–315 nm), and UVA radiation (315–400 nm) [63]. Only part of UV radiation reaches the surface and depends on the location, the season, the clouds, the air pollution, and the humidity [64]. The majority of UVC and UVB radiation is absorbed by oxygen and ozone in the atmosphere. On average, approximately 20 times more UVA rays than UVB rays reach the earth's surface [65]. Due to their different wavelengths, UV rays can penetrate the skin to different depths and can cause cellular changes (Fig. 3) [66,67].

In addition to natural sources, artificial sources must also be mentioned. Except for occupational exposure, sun lamps and tanning beds are the most common source of artificial UV light in everyday life [68,69]. Commercial tanning beds emit high UVA levels and variable amounts of UVB (1–5 %) [70].

Because children and adolescents in particular show a higher vulnerability to UV radiation [71,72], some EU countries, including Germany, the United Kingdom, and Austria, have adopted legal provisions that prohibit adolescents under 18 years from indoor tanning [73].

Positive effects

Limited quantities of UV rays have different positive effects on human well-being and are described as relaxing and enjoyable. Most positive effects of solar radiation are seen in the context of UVB-induced production of vitamin D₃ in the skin [2,74]. By incident sunlight, 7-dehydrocholesterol, present in the plasma membranes of epidermal KCs and dermal fibroblasts is converted into previtamin D₃. By the rearrangement of double bonds (thermal isomerization), stable vitamin D₃ is formed and ejected into the extracellular space where it binds to the vitamin D-binding protein and thus enters the circulatory system. With protein binding, vitamin D₃ is converted into 25-hydroxyvitamin D₃ (25(OH)D). After being transported to the kidney, 25(OH)D is metabolized to 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D), its biologically active form [75].

During prolonged UV radiation, previtamin D₃ is photoisomerized to the two biologically inactive isomers, lumisterol and tachysterol, and vitamin D₃ is converted to suprasterol I and II and 5,6-transvitamin D₃, to prevent vitamin D intoxication [75,76].

Vitamin D₃ is required for the intestinal absorption of calcium and phosphorus and is therefore essential for healthy bone growth. Without vitamin D₃, only 10 to 15% of dietary calcium and about 60% of phosphorus are absorbed [77,78]. In childhood, severe vitamin D insufficiency can lead to growth retardation and skeletal deformities, such as rickets [79]. In adults, vitamin D deficiency leads to reduced bone mineral density and ultimately to osteoporosis [80,81]. In addition, as skeletal muscles have receptors for 1,25(OH)₂D, the hormonally active form of vitamin D, some studies suggest that vitamin D deficiency may also be related to muscle weakness [82,83].

For most people, approximately 90% of the human vitamin D requirement is covered by exposure to sunlight. Therefore, elderly people who remain indoors most of the time, heavily veiled women, and strong pigmented persons are especially affected by deficiencies [84-86]. The application of sunscreens with high SPF can also decrease vitamin D production [87].

In addition to its positive effects on the bone health, vitamin D can have many other positive effects on the human body. These include the stimulation of insulin production, effects on myocardial contractility, modulation of T and B lymphocyte function, prevention of inflammatory disease, promotion of hormone secretion, and decreased risk of developing colon cancer and rheumatoid arthritis [74,87,88].

In addition, UV radiation is also successfully used to treat several skin diseases, e.g. psoriasis and eczema [89,90]. Such treatment occurs under medical supervision and the benefits of the treatment and the risk of extensive UV radiation are weighed against each other at the beginning [91].

In general, balanced exposure to sunlight is essential to make optimal use of positive health effects without unnecessarily burdening the skin.

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Negative effects

UVB radiation

The basis of the biological effects of UV rays is their absorption by endogenous molecules and an associated excitation or even ionization of specific amino acids or nucleic acids. With decreasing wavelengths, the energy of the radiation and the damaging effects strongly increases.

Energy-rich, shortwave UVB rays mainly act in the epidermis and are the main cause of probably the best-known and most obvious acute negative effect of extensive sun exposure, the sunburn (erythema). In comparison to skin reddening that occurs immediately and is mainly caused by a temporary widening of blood vessels and an associated increased in circulation, UVB-induced erythema occurs some hours after UV exposure. Sunburn is seen as an inflammatory skin reaction, which can be associated with swelling, itching and even blistering [5,92].

With strong damage to skin cells or genetic material, the cells are subject to programmed cell death known as apoptosis. The consequence of this process is skin peeling and renewal of the skin after strong sunburn. This renewal is a kind of protection mechanism that prevents the replication of malignant cells and the formation of skin cancer [93-95].

UVB radiation has a strong carcinogenic effect. It causes direct damage to the DNA and RNA and leads to the generation of thymine-thymine cyclobutane pyrimidine dimers (TT-CPDs) (Fig. 4) and pyrimidine-pyrimidine (6-4) adducts (6-4 PPs) (Fig. 5) [6,96,97].

DNA photoproducts can be excised and replaced by different endogenous repair enzymes (specific endonucleases), which restore the original structure. However, if the repair mechanisms fail or the damage is too extensive, apoptosis is initiated by the tumor suppressor protein p53 (TP53). However, if the suppressor protein has also been damaged due to UV radiation, apoptosis does not occur. As consequence, it can come to an accumulation of mutations in the DNA during cell division, particularly to C-to-T or CC- to-TT transitions (“UV signature mutations”) [98,99]. In squamous-cell

carcinoma and partly basal-cell carcinoma, these mutations are mainly found in the TP53 gene [100-102].

Furthermore, there is also a direct association between exposure to UVB radiation and the formation of eye disease. Photokeratitis and photoconjunctivitis (inflammation of the cornea and conjunctiva) are known reversible acute effects [103,104], whether pathological changes such as cataracts, pterygia conjunctiva or macular degeneration develop only gradually [105,106]. Therefore, appropriate protection of the skin and also the eyes is important in bright sunlight.

UVA radiation

UVA radiation plays a major role in photoaging of the skin. Today, it is generally assumed that UVA radiation leads to a balance shift toward the collagen-degrading matrix metalloproteinases (MMPs) and a simultaneous down-regulation of their tissue-specific inhibitors [107,108]. The consequences are an accelerated and increasing degradation of collagen fibers with the concurrent inhibition of the formation of collagen and hyaluronic acid. This leads to an increased formation of deep skin folds, wrinkles and a loss of turgor [109,110].

Photoaging has also been shown to be correlated with the increased formation of mutations of the mitochondrial DNA, triggered by the formation of reactive oxygen species (ROS), e.g. superoxide radical, hydrogen peroxide, or hydroxyl radical [111,112]. This mutagenesis mainly affects the dermis and therefore the non-proliferating part of the skin, so that the UV-damaged skin cells cannot be eliminated by the endogenous repair mechanisms. This results in the dose-dependent accumulation of the damages of the mitochondrial genome [113,114]. However, the aging process is not limited to the dermis, but also affects the epidermis. The proliferation of healthy KCs is strongly disturbed in the presence of fibroblasts with a high content of mitochondrial DNA deletions, which show up in a weakening of the barrier functions of the skin [115].

Naturally, the skin has endogenous enzymatic antioxidants (e.g. superoxide dismutase or catalase) and non-enzymatic antioxidants (e.g. coenzyme Q10, glutathione or vitamin E), which provide protection against ROS [116,117]. However, UV radiation leads to a decrease in antioxidant enzymatic activity in cultured fibroblasts [118] and repeated UV exposure before enzyme activity fully returns can lead to additional damage to the skin tissue [119].

In addition, UVA radiation and the formation of ROS not only influence skin aging but also play a central role in the formation of cancer [120,121]. ROS are formed via absorption of the UV rays by cellular chromophores such as NADH or porphyrins. They can cause punctual DNA mutations, chromatid exchange, chromosome aberrations, or single-strand breaks, which explains their cytotoxic and carcinogenic potential [6,112,122].

Singlet oxygen is considered to be the most important ROS that oxidizes the guanine nucleotide to 8-hydroxy-2'-deoxyguanosine (8-oxodG), which is the main marker of oxidative DNA damages [123,124]. As a result, G-to-T or T-to-G transitions occur, which can be seen as typical UVA fingerprint mutations in keratinocyte-derived tumors and especially in melanoma [125-127].

Squamous cell and basal cell carcinoma

The occurrence of squamous cell and basal cell carcinoma provides evidence for the influence of long-term UV radiation on their formation. Both skin cancer types extend from the epidermis and are mainly found in skin areas exposed to extensive sun light. Accordingly, more than 80% of basal cell carcinomas and more than 75% of squamous-cell carcinomas are found on the head, neck or the hands [128]. They mostly grow there continuously at the same position, and a delocalization is rather rare, more often observed in cases of squamous-cell carcinoma (approximately 5%). Actinic keratosis is often seen as a precursor of squamous-cell carcinoma and is associated with the first skin changes of sun-exposed areas with scaly, thickened or crusty skin patches [129].

Depending to the different penetration depths of UVA and UVB rays, UVA fingerprint mutations are preferentially found in the basal layer, whereas UVB fingerprint mutations concentrate particularly on superficial layers [126]. Although UVB radiation is believed to play a greater role in the formation of non-melanoma skin cancer, also UVA radiation seem to be an important risk factor [121,130-132].

Another carcinogenic effect of both UVA and UVB radiation is its suppressive effect on the immune system of the skin [133]. In the skin different cell types are responsible for immune defense: KCs, monocytes, epidermal T cells, dermal macrophages, and Langerhans cells (LCs). These cells interact through a complex network of mediators such as prostaglandins and cytokines that coordinate a balanced immune response [134]. This complex organization is deeply altered by UV irradiation, which leads to the increased release of immune suppressive cytokines, e.g. interleukin 4 and 10 (IL-4, IL-10), hepatocyte growth factor (HGF), tumor necrosis factor-alpha (TNF- α), or transforming growth factor-beta1 (TGF- β -1), and the development of suppressive T regulatory cells [135,136].

In addition, UV light can alter and damage the LCs embedded between KCs in the epidermis. LCs normally identify exogenous substances through their surface profile, activate resting T lymphocytes and therefore initiate a specific immune response against the exogenous substances. With increasing radiation time and intensity, the number and functionality of LCs decreases. LCs from irradiated skin show a reduced expression of major histocompatibility complex (MHC) proteins of class II and have reduced antigen-presenting ability, probably due to a UV-induced reduction of co-stimulatory molecules like cluster of differentiation antigens (CD 80 and CD 86) [137,138]. Thus, malignant, degenerated cells cannot be detected and aren't rejected by the immune system [139].

Cis-Urocanic acid (UCA), the photoisomere of *trans*-UCA, can also promote systemic suppressive effects [140,141]. In mice with a histidine-rich diet, the total UCA concentration increased significantly. Under subsequent UVB irradiation, these test animals showed significantly higher suppression of contact hypersensitivity compared to normally fed mice [142]. The actual underlying mechanisms of UCA action are still unclear, but it is thought that UCA acts via different pathways.

For example, *cis*-UCA both has an effect on granulocytes and natural killer cells (NK) and reduces the number of LCs by at least 50% [143]. In addition, it can also modulate the action of different cytokines such as TNF- α , IL-6 or IL-8, and may initiate the formation of intracellular ROS [144-146].

Thus, UV radiation has a dual role: first, the induction of the carcinogenicity through direct and indirect DNA damage; and second, the additional suppression of the immunological tumor defense.

Malignant melanoma

Melanoma is a highly malignant tumor of the skin arising from the melanocytes. In 2012 there were more than 3,500,000 new cases of skin cancer across Europe, including more than 100,000 cases of melanoma [147]. Particularly alarming are especially the fast growth rates. In Europe and the USA, the incidence rates for cutaneous melanoma (CM) have tripled or quadrupled since the 1970s. With 40 to 60 new cases per 100,000 inhabitants every year, Australia and New Zealand have the highest incidence rates worldwide [148,149].

CM tends to metastasize in an early stage and is the world's most lethal skin disease [150]. The formation of CM in genetically susceptible individuals is directly associated with different risk factors include family history, nevi and the exposure to UV radiation from sunlight or artificial sources [151]. In contrast to the basal cell carcinoma and the squamous cell carcinoma, where mainly sun-exposed skin areas are affected and chronic cumulative sun exposure leads to the pathological skin changes, intermittent sun exposure of the skin seems to play a crucial role in the formation of acute malignant melanoma. Higher, e.g. job-related, moderate sun exposure seems to have an inverse association with the occurrence of melanoma [152]. It seems that skin's own protective mechanisms, such as skin pigmentation or the thickening of the horny layer, can partially prevent the formation of melanoma. In contrast, the skin of persons who are abruptly exposed to extensive sunlight is caught unprepared and is therefore more vulnerable. Accordingly, skin areas that are only intermittently exposed to extensive sunlight (e.g. the back or the inner thighs) and persons who work mostly inside are most frequently affected of melanoma [153].

Unprotected UV exposure and sunburns in childhood and adolescence increase the risk of skin cancer formation in adulthood. Anamneses of five or more sunburns in childhood can double the risk of subsequent melanoma formation [154,155]. The significance of premature sunburn was also confirmed by different animal studies, e.g. the HGF transgenic mouse model [156]. Here, neonatal test animals were exposed to erythematous doses of UV radiation, whereupon they developed melanocytic tumors in the form of early lesions up to metastases similar to human cutaneous malignant melanoma [157]. These results emphasize the critical role of early sunlight exposure for the formation of human melanoma.

Although UV radiation is seen as one of the most important risk factors in the formation of melanoma, the role of UVA and UVB radiation is still controversial [158,159]. Nevertheless, today it is generally accepted that both wavelength areas contribute in different ways to the development of the tumor [160,161].

A recent published study in 2012 found that there is a pigment-independent pathway initiated by UVB radiation through direct DNA damages and a second, pigment-dependent pathway under the influence of UVA radiation that causes indirect oxidative DNA damages in the melanocytes via ROS [162]. While after UVB radiation the typical markers TT-CPD and 6-4 PP are found in epidermal cells of both pigmented and unpigmented test animals, after UVA radiation only a low level of TT-CPD lesions is detectable. Instead, in pigmented test animals, 8-Oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) was found in the nuclei of extra-follicular melanocytes at the dermal/epidermal junction. This indicates a strong photooxidative interaction between UVA light and the melanin in the melanocytes and confirms that the presence of pigmented nevi is a strong risk factor for melanoma.

Because the uncontrolled proliferation of melanocytes is seen as a first critical step in the formation of melanoma, the expressions of different growth factors seem to have an important influence on the etiology of melanoma [163]. Melanocytes carry different growth factor receptors whereby the corresponding ligands are produced by the surrounding KCs in the epidermis and the fibroblasts in the

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dermis. The growth factors regulate survival, growth, and pigment production of the melanocytes. Mitogen-activated protein kinases (MAPK) are responsible for the homeostatic balance between melanocytes, KCs and fibroblasts and regulate cell growth and cell survival via the cascaded protein kinases, namely the rat sarcoma (RAS) kinase, the rapidly accelerated fibrosarcoma (RAF) kinase, the extracellular signal-regulated protein kinase (ERK), and the MAPK/ERK kinase (MEK) [164]. In over 60 % of malignant melanoma, somatic mutations of the B-RAF protein (an isoform of the RAF kinase) or the BRAF gene can be found [165,166]. The mutated B-RAF protein has an up to 800-fold increased kinase activity, resulting in an over-activation of the MAPK signaling pathway and a misdirected expression of different growth factors, e.g. increased production of basic fibroblast growth factor (bFGF), endothelin-1 (ET-1), and stem cell factor (SCF) in KCs and of bFGF, HGF and TGF- β in the fibroblasts [167,168]. This results in the activation of the melanocytes via paracrine ways and the uncontrolled proliferation of melanocytes.

For advanced melanoma cells, which increasingly produce a variety of cytokines and growth factors endogenously (e.g. bFGF) and thus ensure their own survival, growth, and spread via autocrine ways, increased independence from exogenous growth factors could be observed [169].

Finally, it must be noted, that the molecular processes that are responsible for the formation of the different types of skin cancer are very complex and multifaceted, and even after numerous studies, the detailed processes are still not fully understood and require further research to advance possible medical treatment options.

Natural photoprotection of the skin

The human skin has developed different natural protection mechanisms against UV radiation. The most important protection is the pigmentation of the skin by formation of melanin (Fig. 6), which acts as radical scavenger and ensures light absorption up to the visible range [170].

Melanogenesis starts tyrosinase-catalyzed with the formation of tyrosine to the catechol L-DOPA. By subsequent oxidation, ortho-quinone is formed. The non-enzymatic cycling step to leucodopachrome follows the tyrosinase-dependent transformation to dopachrome. Decarboxylation of dopachrome leads to 5,6-dihydroxyindole (DHI) and its oxidation to the 5,6-indolequinone or by direct oxidation of dopachrome to 5,6-dihydroxyindole-2-carbonic acid (DHICA). Polymerization of these units finally leads to eumelanin. In the presence of cysteine or glutathione, dopaquinone is converted to cysteinyl-dopa. Subsequently, the yellow-red pigment pheomelanin is formed through oxidative polymerization of cysteinyl-dopa via benzothiazinylalanine [171].

UVA radiation is mainly responsible for the immediate and persistent pigment darkening (IPD and PPD) by photooxidation of melanin precursors, which are already present in the skin. These reactions take place within a few minutes or hours but are mostly reversible, so that the tanning of the skin diminishes within a few days. UVB radiation results in delayed tanning reaction (DTR). Pigment formation takes place in the basal layer and is based on the proliferation of specific enzymes, especially tyrosinase. The melanocytes are stimulated to form melanin, which is transported to KCs to protect the cell nuclei in particular. With the movement of the epidermis cells, the pigment also moves toward the skin surface. The formation of the suntan takes several days and lasts for a few weeks [172].

Under excessive sun exposure, also the enzymatic activity of histidinase, in addition to that of tyrosinase, increases. As a result, KCs of the horny layer increasingly form UCA out of histidine. UCA absorbs in the long-wave UVA range under *cis/trans*-isomerization [140]. In the past, UCA has been used as a UV filter in various sun protection products. However, when it turned out that its *cis*-form shows a strong immunosuppressive effect, the substance was banned in the EU and other countries. However, the question whether the beneficial or detrimental effects of UCA predominate is still cause of discussion [146,173].

A further protection mechanism is the formation of the UV-induced hyperkeratosis. Under UV radiation (especially UVB light), the basal cells are stimulated to proliferate what causes a thickening of the horny layer. Without further exposure to UV radiation, the hyperkeratosis disappears [174].

Furthermore, there are the body's endogenous defense mechanisms, i.e. repair enzymes that are able to identify, cut, and replace faulty DNA sequences. For strongly damaged cells – so-called sunburn cells – apoptosis can be initiated as a protection mechanism [175,176]. And finally, there are endogenous redox systems, such as ubiquinone, glutathione, and α -lipoic acids, which have an antioxidative effect and react efficiently with free radicals before they can damage other cell constituents, such as lipid membranes, proteins, and nucleic acid. However, the quantities of these substances produced by the body itself are rapidly depleted under UV radiation by the formed ROS [177].

UV filter substances

Since natural skin protection becomes ineffective after a short time (depending on the skin type, between 10–40 min) and a lasting tan develops only slowly, other protection measures, e.g. avoidance of direct midday sun, wearing of protective clothes and sunglasses, and usage of appropriate sun protection products, are needed for longer stays in the sun. To prevent sunburns and protect the skin from serious damage, sunscreens must meet certain criteria. They should be photostable, dissipate the absorbed light energy through photophysical and photochemical pathways without the formation of harmful reactive intermediates, be water resistant, and well tolerated; in addition, they should not penetrate the skin [178,179]. Normally, sunscreens contain a combination of organic filters and inorganic UV filters, which ensure effective protection across the whole UVA and UVB range [31].

As sun protection products are used in relatively high concentrations over the whole body surface, their tolerability is of particular importance in addition to good performance. Accordingly, appropriate dermatological tests are required as part of the safety assessment according to the European Cosmetics Regulation No. 1223/2009 [180]. Although all UV filter substances are tested for their

(photo)irritative and (photo)sensitization potential, their actual allergic potential may only turn up after years of widespread usage in various products.

Legal requirements and recommendations

The legal status of sunscreen products differs from country to country, sometimes substantially. There is no overarching binding definition for sunscreens or UV filters worldwide, but many countries use the definition of the EU Cosmetics Regulation No. 1223/2009. Thereafter, UV filters are substances that are exclusively or mainly intended to protect the skin against UV radiation by absorbing, reflecting, or scattering UV light. In some countries such as the European Union, China, India, South Africa, Japan, and the countries of the Association of Southeast Asian Nations (ASEAN) and Mercosur in South America, sunscreens are regulated as cosmetics. In contrast, in the USA, Australia, and New Zealand, sunscreens are regulated as drugs. The same applies for Canada, except for sunscreens that solely use titanium dioxide (TiO₂), zinc oxide (ZnO), or *p*-aminobenzoic acid as UV filters. They fall under the category “Natural Health Products” (NHP) and are therefore less regulated. Which UV filters can be used for cosmetic products differs between countries (Table I). In Europe, Annex VI of the EU cosmetics regulation lists all UV filters allowed for cosmetic products in the EU with their respective maximum use concentrations. At the moment, the list contains 27 substances if considering the recent inclusion of the UV filter tris-biphenyl triazine [181] and the deletion of PABA according to directive 2008/123/EC [182]. ZnO is currently not yet listed as an approved UV filter, but due to a positive assessment of the Scientific Committee on Consumer Safety (SCCS), it is expected that ZnO will also be included during one of the next revisions of the Annexes. The lists of approved UV filters for China (numbering 28), India (numbering 27), and the ASEAN countries (numbering 28) are mainly oriented toward the European cosmetics regulation. In the USA, only 16 UV filters are approved for sunscreens, of which only 10 are also approved for Europe, but with partially different maximum use concentrations [183]. Although the Food and Drug Administration (FDA) further accepted eight UV filters already approved in Europe for possible future submission in the time and extent application (TEA) process, there is no prospect of an imminent approval. For Australia and New Zealand, 29 UV filters have been approved, of which 23 substances are also part of

the EU cosmetics regulation plus 5 UV filters also approved in the USA. The Canadian Sunscreen Monograph is strongly oriented toward FDA requirements. The Monograph lists currently 20 UV filters, which are approved drug medical ingredients or natural health products medicinal ingredients for sunscreens. In Japan, the usage of 34 UV filters is permitted. Some of them, such as benzophenone-9 or pentyl dimethyl PABA, are solely approved in Japan, whereas several UV filters that are approved within the EU or other Asian countries are forbidden. In the Mercosur countries, 33 UV filters are approved for usage in cosmetic products. A special case is South Africa (SA), which adopts nearly all UV filters approved by any reliable major organization. Therefore, in SA there are currently 48 approved UV filter substances. For all of the above-mentioned countries, there are only 9 UV filters, octocrylene (OCR), homosalate, benzophenone-3 (BP-3), phenylbenzimidazole sulfonic acid (PBSA), ethylhexyl methoxycinnamate (EHMC), butyl methoxydibenzoylmethane (BMDDBM), and ethylhexyl salicylate (EHS) that are approved worldwide but have different maximum limits, so it is nearly impossible for a manufacturer to use one uniform formulation in all markets.

Sun protection products have an important protective function. Therefore, their efficacy and the basis on which the efficacy is claimed are important public health issues that must be controlled. The efficacy of sun protection products is first given by the sun protection factor (SPF), which is the quotient between the minimal erythema dose (MED) with applied sunscreen and the MED without sunscreen. Therefore, the SPF is an indicator of protection against UVB radiation. However, considering the erythema action spectrum, also UVA-II (315-340 nm) seems to contribute to the formation of erythema to some extent [184].

In 1956, the radiation physicist Rudolf Schulze first introduced the term “protection factor,” which was initially only used among dermatologists [185]. In 1962, the chemist Franz Greiter picked up the term and defined it precisely in the way we know “SPF” today [186]. The SPF should allow the consumer a direct and easily understandable comparison. However, as *in vivo* SPF determinations depends on different parameters such as number and selection of the test persons, kind of application, and irradiation source or the exact waiting time to evaluate the skin reddening, the need for a uniform

methodology soon arose. In 1976, the US Food and Drug Administration (FDA) proposed the first standardized method to determine the SPF of sunscreens [187]. Further standards similar to the FDA method followed, e.g. by the Standards Association of Australia (SAA) in 1986 [188] and by the Japan Cosmetic Industry Association (JCIA) in 1991 [189]. The first standardized method in Europe was the COLIPA SPF Test Method, developed in collaboration of major European manufacturers of sun protection products and contract testing laboratories in 1994 [190]. In 2003, COLIPA (since 2012: Cosmetics Europe), JCIA and the South African Cosmetic, Toiletry & Fragrance Association (CTFA-SA) published the jointly improved International SPF Test Method (ISPF) [191], which was revised in 2006 with the additional participation of the Cosmetic, Toiletry and Fragrance Association of the United States (CTFA-USA) [192]. In December 2010, the International Organization for Standardization (ISO) published the ISO Standard “Cosmetics - Sun protection test methods - *In vivo* determination of the sun protection factor (SPF),” which replaced the earlier test method [193].

Today, Korea, Columbia, the Mercosur Countries (Argentina, Brazil, Paraguay, and Uruguay), Australia, New Zealand, Canada, and the ASEAN countries (Indonesia, Malaysia, the Philippines, Singapore, Thailand, Brunei, Myanmar, Cambodia, Laos, and Vietnam) have adopted methods referring to FDA or ISO standards. China is also considering an adoption of a SPF standard.

The standardized amount of sunscreen applied in the *in vivo* test situation is 2 mg/cm^2 . However, different studies have shown that the actual “in-use” levels of applied sunscreens are often significantly lower, which decreases their protection ability [194,195] so that the SPF claimed on the product cannot be reached [196]. In general, the protection capability of a sunscreen depends on other factors in addition to the SPF and the actual amount of sunscreen applied. These include the respective skin type of the user, the frequency of re-application, the subsequent activities (swimming, drying of the skin, contact with sand, etc.), and the total formulation of the product [197].

For a long time, the efficacy of sun protection products was mainly concentrated on a high SPF, giving consumers a false sense of security and encouraging them to stay in the sun longer. Although a

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sunscreen of SPF 30 offers twice the protection of a sunscreen with SPF 15 (according to a halved UVB transmittance of 3.3 % against 6.7 %) and therefore allows to stay twice as long in the sun, a high SPF says almost nothing about UVA protection and during the prolonged exposure time, the skin is increasingly defenseless against UVA radiation, without any directly visible signs but an increased risk of melanoma [198,199]. In addition, it is known that the determination of especially high SPF is difficult due to the necessarily long irradiation time and the decreasing reproducibility [200,201]

As it became clear throughout the years that UVA radiation is not only responsible for premature skin aging but shows also mutagenic and carcinogenic properties, the development of sunscreen products with the best possible broadband protection was pushed forward [31,199,202]. In 2006, the European Commission (EC) issued a recommendation on the efficacy of sunscreen products including a UVA-PF/SPF ratio of at least 1:3 [203]. By doing so, the EC and the industry seek to further ensure that sunscreens protect consumers against both UVB and UVA radiation, and that there are easy-to-understand efficacy levels based on standard criteria. Although not legally binding, European manufacturers consequently reformulated their products according to the recommendations. To indicate that a sunscreen product offers the recommended minimum UVA protection, COLIPA has issued a standardized UVA-label, the “UVA-seal.”

Depending on the respective country, different established methods are used to determine the UVA protection ability of a product (Table II). One possibility to determine the UVA protection performance of a sunscreen is the *in vivo* measuring of PPD or IPD after long-term or short-term UVA radiation [204,205]. The PPD was originally developed in Japan where it is still the prescribed method to measure UVA protection [206]. Today there is also an ISO standard, which is based on the principles recommended by JCIA [207].

Another way to test the UVA or broad-spectrum protection ability of a product is by the usage of appropriate *in vitro* methods. In Europe, this development started with the determination of the UVA/UVB ratio, which compares the area under the curve in the UVB and in the UVA ranges, as

well as the critical wavelength (CW) [208]. Both tests are based on the assessment of the UV transmittance of a thin sunscreen film spread over a defined area of a roughened substrate before and after controlled UV exposure. The CW describes the range of protection over the “whole” UV spectrum (290–400 nm) and is the wavelength at which 90% of the cumulative area under the total absorbance curve from 290 to 400 nm occurs. The longer the critical wavelength is, the broader the UVA protection of a product. According to the FDA 2011 Final Rule, the determination of the CW is the prescribed standard method to define whether a product offers broad spectrum protection ($CW \geq 370$ nm) or not [209].

The internationally standardized ISO method “Determination of sunscreen UVA photoprotection *in vitro*” [210] is also based on absorbance measurements. The ISO replaced the earlier reference method of COLIPA, first published in 2007 [211] and last updated 2011 [212] due to the results of a collaborative study concerning the roughness of the plate material used [213]. The aim is to determine the CW and the UVA protection factor (UVA-PF) by the convolution of the transmittance results with the PPD action spectrum and the spectrum of the UVA-filtered solar simulator. Due to the European recommendation, the UVA-PF/SPF ratio of an effective sunscreen is ≥ 0.33 .

In Australia and New Zealand, for a long time the “Australian Standard” was the established *in vitro* method to determine UVA protection [214]. Once a sunscreen reduces the transmission in the range of 320–360 nm by at least 90%, a product could claim broad-spectrum UV protection. For products with low or moderate SPF, the methods offers adequate protection, but with a higher SPF the ratio between UVA and UVB protection increases disproportionately. In addition, the Australian Standard method excludes a large portion of the UVA-I spectrum (340–400 nm). Therefore, in the recent Australian and New Zealand Sunscreen Standard [215], the previous standard method was replaced by the ISO 24443:2012 [210].

All of the countries previously mentioned allow at least one of these UVA determination methods (Table II). However, a possible agreement on one common standard seems unlikely, at least for the near future.

Water resistance also has an influence on the efficacy of a sun protection product and can be reached by the usage of UV filters with only minimal water solubility, polymers, and an appropriate emulsifier system. Since 2005, there is a standardized COLIPA testing method to determine the water resistance of a product [216]. It is based on the comparison of the SPF of a tested sunscreen product after a defined period of immersion in water with the original SPF. The term “(very) water resistant” can be used if the remaining SPF after 2 (4) baths of 20 minutes is $\geq 50\%$ of the original SPF (IC 90%). The FDA method is similar in the methodology, but the big difference is that no decrease in the SPF level is allowed after the respective testing time.

Since possible photodegradation may have a big influence on the efficacy of a sun protection product, a test of the photostability of a sunscreen over the whole UVA and UVB range is also required. One possibility to determine the photostability is the usage of an *in vivo* method in which a fixed amount of sunscreen is applied on the skin of a test subject followed by irradiation. The UV filters are recovered by tape stripping and extracted from the tapes, and their residual presence is quantified by high-performance liquid chromatography (HPLC) and spectrophotometry. The difference in the determined UV filter amount of an irradiated and non-irradiated sample allows a direct assessment of the photostability of a sun protection product. In 2004, COLIPA has issued a comparable *in vitro* testing guideline, which was also based on the HPLC determination of the UV filter amount of a tested sun protection product before and after UV irradiation [217].

The above-mentioned *in vitro* test methods for the determination of SPF, UVA protection, and photo degradation are based on results obtained *in vitro* with the help of poly(methyl methacrylate) (PMMA) or roughened quartz plates. Whether these results can be transferred directly to the real

conditions of the skin is questionable, as reactions of the skin under UV exposure or interactions of the products with the skin are not covered by the test conditions [218-221].

Besides the testing of the performance of UV filters and sunscreens, the issue of patent freedom has become a major concern for UV filter and sunscreen manufacturers worldwide. At the moment, all newly developed UV filters are patented before their introduction to the sunscreen market. For example, BASF holds several patents for the use of ZnO in sun care formulations in the USA or L'Oreal for the effective UVA filters Mexoryl SX and Mexoryl XL. Furthermore, the patents do not extend solely to the UV filters themselves, but also to the combinations of different UV filters (e.g. octocrylene and 4-MBC) or the underlying technologies. This strongly constrains the cosmetic usage of such ingredients for competitors and can even make usage impossible [222].

Inorganic UV filters

For these UV filters, the term “physical UV filters” was initially used, attributed to their first known mechanism of sun blocking through the physical manner of reflection and scattering. However, small inorganic UV filter particles also absorb part of the incident light. The combination of absorption, reflection, and scattering leads to a protection across both the UVA and UVB spectrum [223].

The best-known inorganic UV filters are TiO₂ and ZnO. TiO₂ is listed in Annex VI of the Cosmetics Regulation as a permitted UV filter up to a concentration of 25% in the finished product. TiO₂ is known to exist in the three crystal forms, anatase (tetragonal), brookite (orthorhombic), and rutile (tetragonal) with different UV-attenuating properties. ZnO is currently not listed in Annex VI as a permitted UV filter. So far, however, there were different national approvals, e.g. in the German Cosmetics Ordinance [224], which allowed the usage of ZnO as a UV filter. Currently, the EC is about to implement microfine and nanofine ZnO as UV filter in Annex VI of the Cosmetics Regulation based on the previous positive opinions of the Scientific Committee of Consumer Safety [225,226].

In the past, mineral UV filters formed an opaque film on the skin that limited their cosmetic use. Due to technological advantages, micronized oxides are now obtained on a nanometric scale (<100 nm) and show other properties of light refraction and diffraction, resulting in a thin transparent layer that is easy to spread with even better protection abilities [227]. For both nano TiO₂ and nano ZnO, the SCCS concluded that their usage in sun protection products can be considered as safe [226,228]. The only exception is made for the usage of ZnO and TiO₂ nanoparticles in spray products, because there is evidence that these particles can have severe effects in the lungs upon inhalation. Although very effective and positively evaluated by the SCCS, the usage of nanomaterials is still questioned critically. The main points of criticism are the possible altered toxicological potential of extremely small particles, their conceivable penetration, especially through (sun)damaged skin, and their toxicological effects in the body [229,230].

Toxic effects of ZnO and TiO₂ nanoparticles are seen in their ability to evade immunological defence mechanisms, to form complexes with proteins and in their photochemical activity [231,232]. When exposed to UV radiation *in vitro*, nanoparticles emit electrons responsible for the formation of highly reactive oxygen species, e.g. hydroxyl radicals or hydrogen peroxide [233-235]. In general, they are able to damage cell compounds such as cell membranes, proteins, and DNA and are therefore cytotoxic [236-238]. When comparing the three crystal forms of TiO₂, the anatase and the amorphous form show higher photoreactivity and cytotoxicity than the rutile form [239-241]. This finding is partly reassuring insofar as, in sunscreens, the rutile form is mainly used due to its higher UV absorption [242].

Of course, the results of those *in vitro* cell tests are not directly transferable to *in vivo* conditions, primarily because the increased formation of ROS at a viable cell level requires the penetration of the particles into the skin.

To reduce the undesirable photoreactivity of TiO₂ and ZnO nanoparticles, the surface activity of the particles can be reduced by an appropriate coating of the material with both inorganic (aluminum

oxide, silica, zirconia) or organic (silanes, silicones, dimethicones) substances [243,244]. An appropriate coating is stable over the whole production process, can help to ensure that the particles can be readily incorporated in the formulation, and protects the particles against agglomeration (1–100 μm) and deposition [245].

Permeation of small particles through the stratum corneum may occur via different pathways: intracellular, intercellular, or through the appendage route (i.e. along hair follicles, sweat pores, or skin glands) [231]. Due to their low solubility and the impermeable character of corneocytes, intracellular penetration of TiO_2 or ZnO is rather unlikely [230]. Although aggregation of the small particles in hair follicles was observed [246,247], the follicles must be seen as more similar to accumulation sites from which particles are gradually transported out with the sebum flow without further penetration in the living skin [248,249]. What remains is the intercellular pathway, which is also limited due to the densely packed stratum corneum (SC) structure. Intercellular spaces between the SC corneocytes are likely small and measure between 0.5–7 nm and 20–30 nm [250]. However, the spaces can be expanded by the application of topical products [251] or after stress, e.g. caused by UV radiation [252,253].

The penetration of nanoparticles in the skin has been subject of numerous studies, some of which have had different results. Most *in vitro* and *in vivo* studies using both animal and human skin verify that TiO_2 and ZnO nanoparticles do not penetrate the underlying layers of either intact or UV-damaged skin [254–260]. The penetration is mostly limited to the SC, and hence systemic absorption is unlikely. One reason for this could be that nanoparticles tend to aggregate into larger structures (30–150 nm), which may reduce their ability to penetrate the skin [256,261–264]

Nevertheless, as sunscreens are also applied especially to sun-stressed or damaged skin with weakened barrier functions, there is a further need for substance-specific research.

Organic UV filters

The group of organic UV filters includes different substance classes, which can be divided into UVA and UVB filters due to their specific absorption characteristics. Most organic UV filters contain aromatic compounds conjugated with carbonyl groups. The excitation of electrons in the respective benzene ring leads to absorption in the UV range. The absorption range and strength of the different UV filter classes (Fig. 7) is additionally affected by further substituents [265].

The frequency of use of the different organic UV filters has changed significantly in the past 20 years due to scientific evidence and the development of new UV filter substances. To get an overview of the most commonly used UV filters in Germany in 2013 and 2014, we compared 178 sun protection products, 137 skin care products (e.g. face cream, hand cream, make-up), and 47 lip care products with UV protection, due to their ingredients declaration (Fig. 8).

Dibenzoylmethane derivatives

Butyl methoxydibenzoylmethane (BMDBM) is the most common UVA filter in cosmetic products and is included in approximately 80% of the sun protection products in Germany (Fig. 8). With the worldwide recommendation of a balanced UVA/UVB protection, the usage of BMDBM has gained further importance. BMDBM has replaced the former 4-isopropyl dibenzoylmethane (I-DBM), the production of which was discontinued in 1993 due to its high photoallergic potential [266]. However, BMDBM is also a known contact allergen among the UV filters [267,268].

Another critical point is the photoinstability of BMDBM, especially when it is used in combination with ethylhexyl methoxycinnamate (EHMC) [43,269,270]. The resulting photodegradation products also have a specific reaction potential and may be seen as further triggers for contact allergies [271]. However, with an appropriate UV filter combination, e.g. with octocrylene (OCR) or by encapsulation, photodegradation can be decreased to a minimum [272-275].

Benzophenone derivatives

The two UV filters benzophenone-3 (BP-3) and benzophenone-4 (BP-4), both approved in the EU, show very good photostability and broad-spectrum protection over the whole UVB and UVA ranges [276,277]. Therefore, they are not only used in sunscreens and various skin care products, especially in non-European countries [278], but also as typical light stabilizers in plastics and coatings [279]. However, used alone they do not provide complete UVA protection. Several studies have demonstrated that BP-3 shows a clear tendency to penetrate the human skin and was found in urine and even mother's milk [280-282]. This is particularly critical because BP-3 is considered a substance with endocrine potential [283-285]. In addition, BP-3 and BP-4 are seen as the most common photoallergens among the UV filters [286-289]. Therefore, the usage of BP-3 and BP-4 in sun protection products in Germany decreased to a minimum (Fig. 8).

p-Aminobenzoate derivatives

The UVB filter 4-aminobenzoic acid (PABA) was one of the first commercially available and one of the most popular UV filters worldwide [290]. However, it soon became apparent that both PABA and its derivatives were known triggers of photoallergic reactions [291,292].

In 2008, PABA was banned as a cosmetic UV filter in the EU. The usage of ethylhexyl *p*-aminobenzoic acid (OD-PABA) has also significantly decreased in the past 20 years, and nowadays it has been almost completely replaced by other UV filters.

Salicylate derivatives

The UVB filters ethylhexyl salicylate (EHS) and homosalate are typical representatives of this group. Salicylate derivatives are rather weak UVB absorbers but are often used to augment other UVB filters. Due to their insolubility in water, they show good water resistance. Cosmetically used salicylates are rarely associated with allergic or photoallergic reactions [268,293,294] and do not have notable estrogenic activity [295,296]. In addition, EHS shows only a slight tendency to penetrate the

skin [297]. Therefore, especially EHS with its good photostability and solubility is a common ingredient in sunscreens (Fig. 8).

The UV absorber triethanolamine salicylate is water-soluble and is a typical photoprotective agent in hair care products.

Camphor derivatives

Due to their excellent photostability, the camphor derivatives 3-benzylidene camphor (3-BC) and 4-methylbenzylidene camphor (4-MBC) were popular UVB filters for a long time [298]. In 1994, 4-MBC was found in about 30% of the sun protection products [299]. Even in the period from 2004 to 2006, 4-MBC was still a typical sunscreen ingredient [300]. In the past several years, the camphor derivatives have been under more and more criticism due to their possible endocrine potential [283-285]. The increasing pressure of the public and different nongovernmental organizations, like e.g. Women in Europe for a Common Future (WECF) or the German Friends of the Earth (BUND), resulted in the near-cessation of use of 3-BC and 4-MBC in sun protection products in Germany (Fig. 8).

Terephthalylidene dicamphor sulfonic acid (TDSA, Mexoryl SX), a further camphor derivative, is an effective UVA filter, which was developed and patented by L'Oréal in 1982 and was approved 1991 as cosmetic ingredient in Europe. In 2006, Mexoryl SX was also approved by the FDA as part of the sunscreen "Anthelios SX". This was the first approval of a product with a new UV filter since 1988. TDSA shows good protection ability against the negative effects of UVA radiation, like skin pigmentation, epidermal hyperplasia, or the decrease of skin hydration and elasticity [301-303]. In addition, TDSA is photostable and shows no tendency for percutaneous absorption [304].

Cinnamate derivatives

Ethylhexyl methoxycinnamate (EHMC, octinoxate) is one of the most common UV filters worldwide and is often used in combination with other UVB filters to achieve high SPF values. EHMC is

generally well tolerated but shows a certain photodegradation potential, especially in combination with BM-DBM, associated with a decrease of its protection ability [268,305,306]. However, encapsulation of EHMC can significantly improve its photostability [307,308].

For EHMC, which is seen as another UVB filter with possible estrogenic activity [283-285,309] and a tendency to permeate the skin [297,310], the frequency of use in Germany strongly decreased. In 1994, EHMC was an ingredient of about 65% of all sunscreens on the market [299]. We found it in about 15% of the observed sun protection products currently sold in Germany. However, EHMC is still one of the most important UVB filters in daily care products (Fig. 8).

The UVB filter OCR also belongs to the group of cinnamates. Due to its good photostability and its photo-stabilizing effect, especially toward BMDBM [274,311], OCR can be found in approximately 80% of all observed sunscreens in Germany (Fig. 8). It is assumed that under irradiation an energy transfer can occur between OCR and BMDBM in an excited state (triplet), which results in the very efficient deactivation of BMDBM from the triplet to the ground state via heat release and therefore stabilization [178].

However, due to its widespread usage, there is more and more evidence that OCR has developed into one of the most common photoallergens [312-315].

Triazones

Recently, the development of new UV filters is based on the so-called 500 Da rule [54]. With an increased molecular weight of more than 500 Da, skin penetration is reduced. This reduction is associated with increased safety and efficiency of such substances. Due to the extension and multiplication of their chromophoric groups, the UVB filter ethylhexyl triazone (EHT), the UVB filter diethylhexyl butamido triazone (DEBT, iscotrizinol, Uvasorb HEB), and the broad-spectrum UV filter bis-ethylhexyloxyphenol methoxyphenyl triazine (BEMT, bemotrizinol, Tinosorb S) have a molecular weight well above 500 Da. These UV filters show comparatively high absorption coefficients,

significant anti-inflammatory effects and are highly efficient and very photostable [222,316-318]. In addition, especially Tinosorb S can improve the photostability other UV filter substances in a sunscreen [316]. Due to their various positive properties, triazones are incorporated in more and more sun protection and skin care products (Fig. 8). Recently, tris-biphenyl triazine (Tinosorb A2B) has been added to Annex VI of the EU Cosmetics Regulation [181]. It is the first UV filter with a particle size below 100 nm that is explicitly approved for the use in European cosmetics. It is a water-dispersible, broad spectrum, micronized UV filter that protects the skin especially against UV radiation between 290 nm and 340 nm, thus closing the gap between “classical”, pure UVA or UVB filters. It is the first UV filter, which was newly added to Annex VI since 2005.

Benzotriazoles

Also the broad-spectrum UV filters drometrizole trisiloxane (DTS, Mexoryl XL) and methylene bis-benzotriazolyl tetramethylbutylphenol (MBBT, Bisotrizole, Tinosorb M) fall under the 500 Da rule. Accordingly, their skin penetration is minimal and (photo) allergic reactions to these UV filters also appear to be further rare [319,320]. Mexoryl XL was the first photostable UV filter, which offers protection over the whole UVB and UVA ranges. The combination of Mexoryl SX and XL has an additional synergistic effect on their protection abilities [321].

Tinosorb M is manufactured in the form of organic microfine particles and can be dispersed in the water phase of a sunscreen. Accordingly, it combines the properties of organic and inorganic UV filters and scatters, reflects and absorbs UV radiation. Tinosorb M shows a good photostability and broad-spectrum ability over the whole UVB, UVA-I and UVA-II range [322,323].

Development of allergic and photoallergic reactions

As described previously, several organic UV filters are known triggers of allergic and photoallergic reactions responsible for approximately 55–80% of the positive photopatch test results [287,292,324]. Their mostly lipophilic character and their small molecular size allow sufficient skin penetration, which is the basic requirement to initiate an allergic response.

Mechanism of sensitization

Allergic contact dermatitis (ACD) is a type IV hypersensitivity response (delayed type) and is the result of a T-lymphocyte-mediated immune response to an allergenic substance that comes into contact with the skin. Hence, in contrast to other allergic reactions, ACD is not antibody mediated but is cell mediated [325]. ACD consists of two phases: the induction (sensitization) phase and the elicitation (expression) phase (Fig. 9). The first phase may take from a few days to a few years, depending on the allergic potential of the substance. After sensitization, the second phase takes approximately 1–2 days to fully develop, hence the name “delayed type.” In the first phase, small molecules with allergenic potential (haptens) penetrate through the skin. Some substances are initially non-immunogenic but become potent haptens due to enzymatic processes in or on the skin (prohaptens) or due to non-enzymatic processes, e.g. oxidation or photoactivation (prehaptens) [326,327]. In the skin, the haptens bind covalently to nucleophilic amino acid side chains (mainly lysine and/or cysteine residues) of MHC proteins, which are carrier proteins, and form an antigenic complex, which is internalized and processed by dendritic LCs [328].

At the same time, an uptake of haptens by KCs activates the release of different cytokines, e.g. the interleukins IL-1 β , IL-1 α , and IL-18, and different growth factors, e.g. tumor necrosis factor- α (TNF- α) and granulocyte-macrophage colony-stimulating factor (GM-CSF) [329]. In addition, the contact of KCs with haptens induces different protective pathways such as the oxidative stress response pathway [330-332]. This pathway is mediated by the antioxidant response element (ARE), which is an enhancer sequence that transcriptionally regulates different genes that encode phase 2 enzymes to maintain the cellular redox status and to protect against oxidative damage. Activation of ARE is regulated by the specific transcription factor, nuclear factor (erythroid-derived 2)-like 2 (Nrf2), which in turn is negatively controlled by the Kelch-like ECH-associated protein (Keap1), a protein that binds Nrf2. Keap1 is redox sensitive, and the cysteine groups in its structure can easily be oxidized, which decreases its affinity for Nrf2. As a result, Nrf2 is released from Keap1, translocates to the nucleus where it binds to ARE, and induces the transcription of different antioxidative genes

[333]. An allergenic substance can also induce the release of Nrf2 due to its binding to the cysteine side chains of the Keap1 protein [334].

Additionally, KCs show metabolic potential and supply enzymes (e.g. cytochrome P450), which are involved in the activation of prohaptens to immunologically effective haptens [335].

Under the influence of the released cytokines and chemokines, the loaded LCs migrate toward the regional lymph nodes. There, LCs present the hapten-protein complex to naive T cells, which recognize the antigen and start the cascade of antigen-specific commitment. The proliferation of memory and effector T-lymphocytes is initiated, which migrate in the peripheral tissue. Upon re-exposure to the same or a very similar hapten, the LCs become haptenized once more and are rapidly intercepted by the already-existing antigen-specific T cells in the epidermis (lower activation threshold). Proinflammatory cytokines and chemokines are released locally, which promote an inflammatory reaction with typical ACD symptoms such as redness, itching, swelling of the skin, formation of blisters, and an oozing rash. Due to the development process, an ADC can be divided into skin penetration, possible metabolic activation, protein binding, activation and migration of LHCs, and proliferation of T cells [325,336-338].

Skin sensitization test methods

Allergic contact reactions have become frequent skin complaints with increasing incidence rates during recent years. It is estimated that the prevalence of contact allergies is about 15-20% in the general population [339,340]. Therefore, the evaluation of the sensitization potential of chemical substances, especially cosmetic ingredients that are intentionally applied directly on the skin, is of great importance. Accordingly, manufacturers of cosmetics are legally obliged to secure the safe usage of their products before they are placed on the market. In addition, many chemical substances, including cosmetic ingredients, must be assessed under the European chemicals regulation REACH (1907/2006/EC) [341]. The evaluation of skin sensitization is one mandatory endpoint when producing or importing a ton or more of a substance in or to Europe.

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For a long time, sensitization was tested using different animal methods, e.g. guinea pig-based tests like the Guinea pig maximization test (GPMT), the Buehler Test, or the local lymph node assay (LLNA). Even today, these methods are the only generally recognized test systems currently authorized by the Organization for Economic Cooperation and Development (OECD) [342-345]. For the original LLNA, radiolabeled thymidine is used as a marker to determine the cellular proliferation in the auricular lymph node of the test animals after repeated topical application of the test chemical [346]. Today, several variants of the LLNA have been developed in which the radioactive materials are replaced, e.g. by 5-bromo-2-deoxyuridine in the LLNA:BrdU-ELISA [347]. By testing a minimum of three different concentrations of the test substance, it is not only possible to differentiate between sensitizers and non-sensitizers, but also to assess the respective sensitizing potential via the EC3 value, the effective concentration of a test chemical to provoke a 3-fold increase in lymph node cell proliferation. Guinea pig tests are based on a visual assessment of a skin reaction induced after topical application (Buehler test) [348] or intradermal and topical application of the test substance (GPMT) [349]. The latter two can only provide information as to whether a substance is a sensitizer or not and have limitations when the sensitization potency of a substance must be assessed.

Even today, the above-mentioned OECD methods are the only validated tests that are currently implemented in the REACH regulation or the corresponding Test Methods Regulation (EC) No. 440/2008 [350]. Nevertheless, REACH expressly requires and promotes the usage of appropriate alternative test methods. Moreover, since July 11, 2013, the new Cosmetics Regulation completely bans animal testing for both finished cosmetic products and cosmetic raw materials. Therefore, it is not surprising that in the last 15 years great efforts have been made to develop promising animal-free testing methods. According to the five accepted mechanistic key events, i.e. skin penetration/bioavailability (1), peptide reactivity/haptenation (2), epidermal inflammation (3), dendritic cell activation and migration (4), and T cell proliferation (5), different approaches were followed. It is likely that a combination of different analysis methods using different endpoints may offer an overall high accuracy for prediction [351].

Bioavailability: In the case of sensitization, bioavailability describes the ability of a substance to penetrate the skin and the possible activation of a weak or non-sensitizing substance into a sensitizer. The chemical structure and the physical properties of a substance allow an initial assessment. For example, a low molecular weight (<500 Da) and melting point (<200°C), and an octanol/water partition coefficient (log K_{ow}) between 1 and 5 suggest a certain penetration potential [352]. Therefore, the consideration of such data can be useful for *in silico* structure activity relationship (SAR) tools.

Most of the data on the skin penetration have been obtained by *in vitro* or *ex vivo* studies using Franz diffusion cells and human or animal skin but also artificial skin constructs (ASC) [353]. Human skin models can be divided into skin equivalents, having both KCs and dermal substitutes (collagen and fibroblasts), and reconstituted human epidermis (RHE) models, consisting of multiple layers of KCs to mimic the epidermis. Several commercial RHE are available, but these model systems lack dendritic cells or T lymphocytes, which are crucial for the sensitization process [354,355]. Recently, a Japanese research group led by Uchino developed skin with an additional collagen/vitrigel layer, which contains KCs, fibroblasts, and dendritic cells (DCs) (VG-KDF-Skin) [356]. However, although different RHE models such as EpiSkin, EpiDerm or SkinEthic have already been implemented under the OECD guidelines as proven test systems for the endpoints of skin corrosion and skin irritation [357,358], there is currently no approved method for the area of sensitization with these test systems. Although some studies show promising results, it turns out that the permeability of the available reconstructed skin models is much higher compared with human skin, especially for lipophilic substances [359-361]. Additionally, the test systems cannot currently achieve ASC that are comparable complex as human skin, especially considering skin-specific metabolic activation processes [362,363]. Hence, also the European Union Reference Laboratory for alternatives to animal testing (EURL ECVAM), which promotes the scientific and regulatory acceptance of non-animal tests, concluded that the currently available models of human skin equivalents used to determine sensitization require further optimizations before they can be considered for evaluation by ECVAM.

Haptenation: For a majority of organic substances, the covalent binding to skin proteins is a crucial step during the sensitization process [364,365]. Such reactions can be described as nucleophilic-electrophilic processes. Therefore, the assessment of the molecular structure of chemical substances allows a first estimation of their reactivity and is the basis of structure-activity relationship approaches [366-368]. To draw conclusions about the toxicity of a substance due to its structure, it is useful to group the substances into different reaction mechanistic domains such as Michael acceptors, Schiff base formers, SN2 and SNAr electrophiles, and acylating agents, which were proposed by Aptula and Roberts [369]. Inclusion of quantitative assessments has led to different *in silico* quantitative structure–activity relationship (QSAR) models, e.g. the Toxtree and the DEREK NEXUS software, or the OECD (Q)SAR Application Toolbox to name only a few. These models use structural alerts and defined exclusion rules to assess the skin sensitization potential of a substance by performing a trend analysis and read-across [370-372]. The additional inclusion of further physical-chemical parameters such as the Log K_{OW} can further improve the accuracy of the assessment.

Another approach is the usage of *in chemico* methods such as the direct peptide reactivity assay (DPRA), the peroxidase peptide reactivity assay (PPRA), or the Electrophilic Allergen Screening Assay (EASA) to generate data for the reactivity of chemicals toward model nucleophiles [373-376]. The DPRA uses two model peptides with lysine and cysteine as reaction targets. The depletion of the peptides, measured with HPLC-UV, is seen as an indicator for the sensitization potential of a substance. Another possibility to determine the reactivity of chemicals towards model peptides, is to detect unoccupied thiol or lysine binding sides after incubation with 5,5'-dithiobis-2-nitrobenzoic acid or fluorescamine [377].

In a new generation of DPRA, the peroxidase peptide reactivity assay (PPRA), horseradish peroxidase, and hydrogen peroxides are additionally used to follow some metabolism and oxidation processes and to incorporate pro- or prehaptenes that require some kind of activation [375,378]. The DPRA has completed validation by EURL ECVAM, which supports the development of an appropriate OECD Test Guideline (TG) [379]. Recently, a draft TG on DPRA is under discussion at

OECD. To further extend the outcome of such peptide reactivity assays, an LC-MS/MS determination can be linked, which allows not only quantitative but also qualitative assessment due to the determination of possible adducts, dimerization processes, etc. [380,381].

Epidermal inflammation: KCs are the dominating cell types in the epidermis and are the first skin cells, which come into contact with substances that penetrated through the stratum corneum. KCs show metabolic activity and are able to express various (pro)inflammatory cytokines (e.g. IL-1 α or IL-18) and different adhesion and growth factors upon contact with sensitizers and photosensitizers [382,383]. This is the basis of a whole series of proposed alternative methods, e.g. the cell-based AREn32 or NCTC 2544 IL-18 assays and the SenCeeTox, or SENS-IS assays [384-390]. With SENS-IS, for which human 3D reconstructed epidermis (EpiSkin) is used, a quantification of 62 biomarkers, which are split into three groups (irritation, ARE, and SENS-IS) is possible [391]. SenCeeTox measures changes in gene transcription of up to 6 genes using different concentrations of the test substances and may not only identify sensitizers but also accurately predict their potency category [392,393].

The contact of KCs with skin sensitizers can also induce cyto-protective cellular pathways, e.g. Nrf2-Keap1-ARE regulatory pathway, which plays an important role in the protection of cells against oxidative and xenobiotic damage [394]. Keap1, with its hapten-reactive cysteine residues, plays a crucial role here. Based on this approach, the KeratinoSens and the LuSens assays were developed to measure the keratinocyte activity by the induction of a luciferase gene under the control of the antioxidant response element (ARE) [395-397]. In February 2014, ECVAM published its recommendation on the KeratinoSens. It is expected that the recommendation will facilitate scientific discussions at OECD in view of developing further OECD Test Guidelines for skin sensitization [398].

Dendritic cell activation: During contact between an allergen and the skin, dermal dendritic cells and the immature dendritic LCs serve as antigen-presenting cells able to internalize and process the

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hapten-protein complex and present the allergen-MHC-complex to naive T cells [399,400]. As a key issue in the sensitization process, many investigators see potential for the use of dendritic cell lines for the development of animal-free alternative methods. As LCs are only present in the skin in small numbers and tend to mature during extraction and cultivation processes, other dendritic cell lines such as the human myeloid leukemia cell lines THP-1, U-937, MUTZ-3, or KG-1 are isolated, cultivated, and used under standardized conditions. Dendritic cell activation can be measured by chemokine (e.g. DC-CK1, CCCL5, CCL19, and MIP-1 α) and cytokine secretion (e.g. IL-1 β , IL-1 α , IL-8, and TNF- α) and through the expression of cell surface markers (e.g. CD40, CD54, CD86, and C-C chemokine receptor type 7 (CCR7)) [401]. Under the available test systems, the myeloid U937 skin sensitization test (MUSST) and the human cell line activation test (h-CLAT) are the most advanced test systems in terms of both the number of tested substances and the standardization procedure. MUSST is based on changes in CD86 expression of the U937 cell line measured by flow cytometry [402,403]. During h-CLAT, CD86, and CD54 expression of a THP-1 cell line is also measured using flow cytometry [404,405]. Both methods are currently part of an ECVAM coordinated prevalidation study. Another approach is the analysis of gene expression changes after the contact of DCs with allergens. The VITASENS assay measures changes in gene expression of 13 genes, the cAMP-responsive element modulator (CREM), and the monocyte chemotactic protein-1 receptor (CCR2), determined as predictive biomarkers of CD34+ progenitor cells (CD34-DC) after contact with sensitizing chemicals [406,407]. The Genomic Allergen Rapid Detection (GARD) assay utilizes genomic biomarker signatures based on the cellular responses of the human myeloid cell-line MUTZ-3 toward sensitizers. The biomarker signatures provide measurements of numerous genes that are involved in immune response and cytoprotective mechanisms [408].

In addition, the increased cytokine/chemokine secretion upon contact with sensitizers is the basis of various other dendritic cell assays. For example, IL-8, as a sensitive biomarker, seems to be promising for distinguishing between sensitizers and non-sensitizers [409-411]. Quantification can be performed by enzyme-linked immunosorbent assay (ELISA), which allows high sample throughput for possible

screening. However, only a limited number of chemicals have been tested so far, so that the results still do not allow conclusive assessment of the validity of these methods.

T cell proliferation: As seen in the LLNA, the proliferation of naive T cells after contact with a chemical substance is a reliable indicator that a substance is immunogenic. Therefore, several publications concentrate on the feasibility of in-vitro T-cell proliferation followed by a co-culture of sensitizer-treated dendritic cells (DC/T-cell co-culture protocols) and the expression of selected biomarkers [412-414]. While the first generation of classical human T cell priming assays (hTCPA) could only detect strong or extreme sensitizers, the usage of human peripheral blood with fewer regulatory cells increased the sensitivity of the methods and allows the determination of moderate and weak sensitizing properties [415,416]. Although T cell-based assays are complex and have limitations, they are seen as useful and valid future tools in the development of alternative methods [417].

It is generally accepted that no single approach of non-animal testing methods reflecting the key mechanisms of skin sensitization will be sufficient to predict the sensitization potential of a substance. However, the combination of different approaches in a combined test battery has the potential of a full replacement toolkit [418]. So far, one of the most promising approaches is a test system combined and evaluated under the leadership of BASF. Bauch et al. combined the DPRA, the LuSens, or KeratinoSens assay and the mMUSST and h-CLAT. They reached an accuracy of 94% for 54 test substances with no false positive or false negative events and thus exceeded even the results of the LLNA [390,419].

Conclusion

The compatibility and performance of sun protection products is essential for their safe use. The selection of (photo)stable UV filters, an appropriate combination of different UVA and UVB absorbers, and a cosmetically pleasant formulation provide the basis for effective protection against UV radiation. Unfortunately, a globally harmonized system for the evaluation, testing and approval of

sunscreens is still missing, making it almost impossible for a manufacturer to formulate sun protection products that can be approved globally.

The market for sun protection products is constantly changing based on the progress of scientific knowledge. Nevertheless, the approval of new UV absorbers is a major challenge, especially in certain countries, such as USA.

Although the role of sun protection products will further increase, the widespread use of UV filters in multiple cosmetic products should not be disregarded due to their impacts on compatibility and their fate in environment.

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Tables and Figures

Table I Approved UV filters worldwide with their maximum usage concentration

Table II Overview of prescribed UVA determination methods in different countries

Figure 1 Layers of Skin: The skin is composed of two main layers: the epidermis, made of closely packed epithelial cells, and the dermis, made of dense, irregular connective tissue that houses blood vessels, hair follicles, sweat glands, and other structures. Beneath the dermis lies the hypodermis, which is composed mainly of loose connective and fatty tissues. (This content is available for free at <http://cnx.org/content/col11496/1.6>) [420]

Figure 2 Spectrum of natural sunlight and the light-filtering effect of the atmosphere (according to data of [62,63,65])

Figure 3 Percent transmittance of UVA, UVB, and UVC radiation (according to data of [67])

Figure 4 Formation of thymine cyclobutane dimers (according to [421])

Figure 5 Formation of the pyrimidine (6-4) pyrimidone photoproduct (according to [421])

Figure 6 UV-induced transformation of tyrosine into pre-melanin metabolites and into melanin (according to [421,422])

Figure 7 Basic chemical structures of commonly used UV filters

Figure 8 Overview of the most commonly used cosmetic UV filters, available on the German market in 2013 and 2014, divided into sunscreens, skin care products, and lip care products

Figure 9 Schematic illustration of the sequence of an allergic response (mechanism according to [423])

	EU	CN	US	AU	CA	JP	IN	ZA	ASEAN	Mercosur
PABA (and its esters - JP)	5%	15%	-	15%	4%	5%	15%	-	15%	
Camphor benzalkonium methosulfate	6%	6%	-	6%	-	-	6%	6%	6%	6%
Homosalate	10%	10%	15%	15%	15%	10%	10%	10%	10%	15%
Benzophenone-3	10%	10%	8%	10%	6%	5%	10%	10%	10%	10%
Phenylbenzimidazole sulfonic acid	8%	8%	4%	4%	4%	3%	8%	8%	8%	8%
Terephthalylidene dicamphor sulfonic acid	10%	10%	-	10%	10%	10%	10%	10%	10%	10%
Butyl methoxydibenzoylmethane	5%	5%	3%	5%	3%	10%	5%	5%	5%	5%
Benzylidene camphor sulfonic acid	6%	6%	-	6%	-	-	6%	6%	6%	6%
Octocrylene	10%	10%	10%	10%	10%	10%	10%	10%	10%	10%
Polyacrylamidomethyl benzylidenecamphor	6%	6%	-	-	-	-	6%	6%	6%	6%
Ethylhexyl methoxycinnamate	10%	10%	7.5%	10%	7.5%	20%	10%	10%	10%	10%
PEG-25 PABA	10%	10%	-	10%	-	-	10%	10%	10%	10%
Isoamyl p-methoxycinnamate	10%	10%	-	10%	-	-	10%	10%	10%	10%
Ethylhexyl Triazone	5%	5%	-	5%	-	5%	5%	5%	5%	5%
Drometrizole Trisiloxane	15%	15%	-	15%	15%	15%	15%	15%	15%	15%
Diethylhexyl butamido triazone	10%	10%	-	-	-	-	10%	10%	10%	10%
4-Methylbenzylidene camphor	4%	4%	-	4%	6%	-	4%	4%	4%	4%
3-Benzylidene camphor	2%	2%	-	-	-	-	2%	2%	2%	2%
Ethylhexyl salicylate	5%	5%	5%	5%	5%	10%	5%	5%	5%	5%
Ethylhexyl dimethyl PABA	8%	8%	8%	8%	8%	10%	8%	8%	8%	8%
Benzophenone-4	5%	5%	10%	10%	10%	10%	5%	10%	5%	10%
Benzophenone-5	-	-	10%	-	10%	-	-	5%	-	5%
MBBT	10%	10%	-	10%	-	10%	10%	10%	10%	10%
DPDT	10%	10%	-	10%	-	-	10%	10%	10%	10%
BEMT	10%	10%	-	10%	-	3%	10%	10%	10%	10%
Polysilicone-15	10%	10%	-	10%	-	10%	10%	10%	10%	10%
Titanium dioxide	25%	25%	25%	25%	25%	no limit	25%	25%	25%	25%
DHMB	10%	10%	-	10%	-	10%	10%	10%	10%	10%
Tris-biphenyl triazine (nano)	10%	-	-	-	-	-	-	-	-	-
Zinc oxide	*	25%	25%	no limit	25%	no limit	-	25%	25%	25%
Menthyl anthranilate	-	-	5%	5%	5%	-	-	5%	5%	5%
Cinoxate	-	-	3%	6%	3%	5%	-	5%	-	3%
Benzophenone-8	-	-	3%	3%	3%	-	-	3%	-	3%
TEA salicylate	-	-	12%	12%	12%	-	-	12%	-	12%
Diethanolamine methoxycinnamate	-	-	-	-	10%	-	-	8%	-	-
Benzophenone-1	-	-	-	-	-	10%	-	10%	-	-
Benzophenone-2	-	-	-	-	-	10%	-	10%	-	-
Benzophenone-6	-	-	-	-	-	10%	-	5%	-	-
Benzophenone-9	-	-	-	-	-	10%	-	no limit	-	-
Methyl-2,5-disopropylcinnamate	-	-	-	-	-	10%	-	10%	-	-
1-(3,4-Dimethoxyphenyl)-4,4-Dimethyl-1,3-Pentadiene	-	-	-	-	-	7%	-	7%	-	-
Ethylhexyl Dimethoxybenzylidene Dioximidazoline Propionate	-	-	-	-	-	3%	-	3%	-	-
Fenilic Acid	-	-	-	-	-	10%	-	10%	-	-
4-(2-Beta-Glucopyranosiloxy)propoxy-2-Hydroxybenzophenone	-	-	-	-	-	5%	-	-	-	-
Glyceryl Ethylhexanoate Dimethoxycinnamate	-	-	-	-	-	10%	-	10%	-	-
Glyceryl PABA	-	-	-	-	-	4%	-	5%	-	-
Isopentyl trimethoxycinnamate trisiloxane	-	-	-	-	-	7.5%	-	7.5%	-	-
Mixture: Isopropyl p-methoxycinnamate + Ethyl Disopropylcinnamate + Methyl-2,4-disopropylcinnamate	-	-	-	-	-	10%	-	10%	-	-
Pentyl Dimethyl PABA	-	-	-	-	-	10%	-	-	-	-
Digalloyl Trioleate	-	-	-	-	-	-	-	5%	-	-
Ethyl Dihydroxypropyl PABA	-	-	-	-	-	-	-	5%	-	-

* Banned since 2008 (123/2008/EC)

PABA: para-4-Aminobenzoic acid

MBBT: Methylene Bis-Benzotriazyl Tetramethylbutylphenol

BEMT: Bis-Ethylhexyloxyphenyl methoxyphenyl triazine

DPDT: Dodecyl phenyl dibenzimidazole tetrasulfonate

DHMB: Diethylamino hydroxybenzyl hexyl benzoate

	UVA determination method
Europe	ISO 24442:2012 (PPD)
	ISO 24443:2011 (in vitro) + critical wavelength ≥ 370 nm
USA	FDA final rule 2011 – critical wavelength > 370 nm
Canada	FDA final rule 2011 – critical wavelength > 370 nm
	COLIPA in vitro method + critical wavelength
	ISO 24442:2012 (PPD)
	JCIA Standard (PPD)
China	JCIA Standard (PPD)
Japan	JCIA Standard (PPD)
ASEAN	ISO 24442:2012 (PPD)
	ISO 24443:2011 (in vitro) + critical wavelength ≥ 370 nm
Mercusor	FDA final rule 2011 – critical wavelength > 370 nm
	COLIPA in vitro method + critical wavelength
	ISO 24442:2012 (PPD)
	ISO 24443:2011 (in vitro) + critical wavelength ≥ 370 nm
	JCIA Standard (PPD)
South Africa	Documented test results of an acceptable authority, but broad spectrum ratio UVA/UVB 1:2:5









