Dermatology Paper - Oxybenzone Review 1 of 4

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To:IEM Committee <IEM.Committee@mauicounty.us>; County Clerk <County.Clerk@mauicounty.us>;

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11 attachments (3 MB)

Final Manuscript jocd_12449_Rev.pdf; 1 Microbead Law PLAW-114publ114.pdf; 2 FDA Sunscreen Monograph 2012.pdf; 3 Diffey et al 2000 In Vitro Broad Spectrum .pdf; 4 Guy et al Prevelance and Cost of Skin Cancer.pdf; 5 NCI Cancer Trends Progress Report Sun Protective Behavior.docx; 6 Warshaw et al NACDG Oxybenzone Dermatitis.docx; 7 SCCP COLIPA Opinion on Benzophenone 3 2006.pdf; 8 Verhulst and Goossens Contact Urticaria Benzophenone 3.pdf; 9 Dermatitis 25(1)3-10[2014] Benzophenones.pdf; 10 Bos_et_al-2000- 500 Dalton ruleExperimental_Dermatology.pdf;

Aloha Chair and Maui County Council,

Mahalo Nui Loa for inviting us to provide testimony before your committee. It was an honor and privilege.

We are submitting the attached studies to you, as requested by the Infrastructure and Environmental Management committee after our presentations on Oxybenzone and Octinoxate's effects on marine life and human health. After reviewing the attached studies, we are confident that you will all feel comfortable with your vote to support the legislation to ban the sale of SPF Sunscreen products containing Oxybenzone and/or Octinoxate.

Mahalo,

Craig Downs – Executive Director – Haereticus Environmental Laboratory

Joe DiNardo – Retired Personal Care Industry Toxicologist & Formulator

Notes:

- Because of the size of the files there will be several Emails sent per topic; all will be numbered appropriately.

- The first Email on the topic will contain the main article (Dermatology Paper – Oxybenzone Review, Oxybenzone HEL Monograph or Octinoxate HEL Monograph) and the references used to support the main article will be included.

- Chemical names used in the attached research papers may vary – please feel free to ask us to clarify any concerns you may have associated with terminology:

1) Oxybenzone = Benzophenone-3 (BP-3) and metabolites maybe noted as Benzophenone-1 and 4-Methylbenzophenone

2) Octinoxate = Ethylhexyl Methoxycinnamate (EHMC) = Octyl Methoxycinnamate (OMC)

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REVIEW ARTICLE

WILEY JCD Journal of Cosmetic Dermatology

Dermatological and environmental toxicological impact of the sunscreen ingredient oxybenzone/benzophenone-3

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Summary

Oxybenzone (Benzophenone-3) is an emerging human and environmental contaminant used in sunscreens and personal care products to help minimize the damaging effects of ultraviolet radiation. The Center for Disease Control fourth national report on human exposure to environmental chemicals demonstrated that approximately 97% of the people tested have oxybenzone present in their urine, and independent scientists have reported various concentrations in waterways and fish worldwide. Oxybenzone can also react with chlorine, producing hazardous by-products that can concentrate in swimming pools and wastewater treatment plants. Moreover, adverse reactions could very well be increased by the closed loop of ingesting fish contaminated with oxybenzone and/or washing the ingredient off our bodies and having it return in drinking water as treatment plants do not effectively remove the chemical as part of their processing protocols. In humans, oxybenzone has been reported to produce contact and photocontact allergy reactions, implemented as a possible endocrine disruptor and has been linked to Hirschsprung's disease. Environmentally, oxybenzone has been shown to produce a variety of toxic reactions in coral and fish ranging from reef bleaching to mortality. Lastly, with the rise in skin cancer rates and the availability of more effective sunscreen actives such as micronized zinc oxide and titanium dioxide, serious doubts about the relative prevention benefit of personal care products containing oxybenzone must be raised and compared with the potential negative health and environmental effects caused by the accumulation of this and other chemicals in the ecosystem.

KEYWORDS
contact dermatitis, environmental contaminant, toxicity

1 | INTRODUCTION

Consumer awareness about human health and environmental concerns associated with various ingredients used in personal care products is increasing markedly. Several state and Federal laws banning the use of polyethylene microbeads in cleansing scrubs, tooth pastes, and other consumer products were instituted in 2016 as a result of their presence in numerous fish species found in the food supply and the associated potential adverse health effects to humans.[1] In 2017, several bills have been introduced in the Hawaiian legislature that are designed to ban the use of oxybenzone in any consumer product—particularly if the intended use is near beaches—or, at a minimum, requiring a warning label stating that the chemical is harmful to coral and the aquatic environment. Oxybenzone is an aromatic hydrocarbon that acts as an ultraviolet (UV) light filter in sunscreen formulations. As would be expected, there has been significant debate regarding these proposed actions, with environmentalist calling for a ban on the chemical, industry voices questioning the scientific validity of the negative human/environmental toxicity data based on limited safety data conducted 20 to 40 years ago, and the

medical profession expressing concerns related to increasing the rate of skin cancer should UV blockers, like oxybenzone, be removed from sunscreen formulations. The present review examines the scientific evidence related to oxybenzone and posits that alternative formulation strategies using micronized zinc oxide and/or titanium dioxide are available which avoid the toxic effects. It is hoped that this examination will be useful to the dermatology community as it considers how to best respond to patient questions related to human health and environmental concerns associated with the use of oxybenzone.

2 | GENERAL INFORMATION

Common Name used on Drug Labels (Active Ingredient): Oxybenzone.

Common Name used on Non-Drug Labels (INCI Name): Benzophenone-3.

Common Technical/Chemical Name: 2-Hydroxy-4-Methoxyphenyl Phenylmethanone.

Common Trade Names: Eusolex 4360 and Escalol 567.

Chemical Abstract Service (CAS) Number: 131-57-7.

Molecular Weight (MW): 228.26 Daltons (g/mol).

3 | USES

Oxybenzone is commonly used as a short-wave (290 to 320 nm) ultraviolet light (UVB) and mainly short-wave UVA light (320 to 340 nm) absorber at concentrations up to 6% in sunscreen preparations and up to 0.5% in personal care products as a photo-stabilizer minimizing color and odor changes. It has been reported to be used in over 2000 personal care formulations spanning numerous product categories from skin and hair care to color cosmetics and fragrances. Additionally, it is used in plastics as an ultraviolet light absorber and stabilizer. In 1990, oxybenzone was added to the Environmental Protection Agency High Production Volume Challenge Program which identifies ingredients manufactured or imported into the United States in amounts equal to greater than one million pounds per year.

4 | UV ABSORPTION SPECTRUM, SUNSCREEN EFFICACY TESTING, AND SKIN CANCER RATES

With the recent attempts in Hawaii to ban the use of oxybenzone in sun protection factor (SPF) products, some have expressed concern over losing an effective UV absorber and possibly causing an increase in the number of skin cancers observed annually. The ability of a sunscreen product to protect against UV rays is not based on an individual ingredient contained in a formula, however, but rather how the formula performs, as a whole, when tested according to the Food & Drug Administration (FDA) guidelines for labeling and effectiveness testing; sunscreen drug products for over-the-counter human use.[2] For example, a product could contain the most effective UV-absorbing ingredients allowed (avobenzone, titanium dioxide, or zinc oxide), but if it is formulated in an inappropriate way that product would deliver little to no protection from the damaging effects of UV rays. This reality underlies why FDA has established these guidelines and requires as a matter of law that all formulas be tested for efficacy and stability prior to being sold in the marketplace. Therefore, any product sold in the United States that claims a SPF and, further, makes a broad spectrum claim—regardless of the ingredient(s) used in the product—can be trusted to perform according to the package labeling and protect against the carcinogenic effects of the sun.

It is important to note that SPF testing (UVB) is conducted in 10 human subjects, as outlined in the FDA testing guidelines. However, broad spectrum (UVA) testing is an analytical method (in vitro) that measures if a product has a critical wavelength of at least 370 nm, which represents 90 percent of the total area under the curve in the UV region. Based on the FDA definition, only zinc oxide, titanium dioxide, avobenzone, menthyl anthranilate, oxybenzone, and octocrylene would qualify out of all the approved actives noted in the sunscreen monograph (Table 1). It is important to note that based on these classification criteria, oxybenzone just makes the critical wavelength cutoff of at least 370 nm for UVA claims and would be tied for last place with octocrylene in terms of broad spectrum performance.

The average annual number of adults treated for skin cancer in the United States increased from 3.4 million in the 2002-2006 time period to 4.9 million annually between 2007 and 2011.[4] Correspondingly, the average annual total cost for managing skin cancer increased 126.2% from $3.6 billion to $8.1 billion. Importantly, the National Cancer Institute [5] consumer use data for adults aged 18 years or older between the years 2005 and 2015 report that 70.8% of all adults practice one of the three sun protective behaviors identified: (i) seeking shade and avoiding sun during peak hours (ii) wearing protective clothing; and (iii) using sunscreen. Of the three methods, only 33.7% reported applying sunscreens, while 38.4% relied on clothing and 39.1% usually sought shade.

Taking into account how products are tested for UV efficacy, the absorption spectrum of the currently approved FDA actives, and how

TABLE 1 Critical wavelength for commonly used UV filters with an attenuation of 370 nm and above[3]

FDA monograph sunscreen ingredients drug label name (INCI/Common Name)	Attenuation in NM	Peak absorption
Octocrylene	290-370	305-325
Oxybenzone (Benzophenone-3)	290-370	290-300 & 325-340
Menthyl anthranilate	290-380	340-350
Avobenzone (Butyl Methoxydibenzoylmethane)	290-390	355-370
Titanium dioxide	290-400	290-320
Zinc oxide	290-400	290-385

consumers actually deal with protecting against sun exposure, it is unclear that products containing oxybenzone offer any distinct benefits over other available options when it comes to reducing elevated epidemiological trends in skin cancer. Indeed, for those sunscreen users who have concerns and want the strongest UV protective against the sun, zinc oxide has the best UV attenuation (290-400 nm) and peak absorption (290-385 nm) of all actives, covering 100% of the UVB and 95% of the UVA spectrum. Zinc oxide can be used individually or with other actives, if UV protection above an SPF 30 is required for very sun sensitive individuals, and with the advent of micronized particles (100 nm or larger) product, esthetics are excellent and promote patience compliance. Lastly, it is more likely that patients would receive better protection from frequent application of sunscreens rather than solely relying upon higher SPF factors. For example, a product with a SPF 30 protects against 97% of UVB whereas a product with a SPF 50 protects against 98%; however, to gain that additional 1%, a SPF 50 product may contain almost twice the concentration of sunscreen actives, potentially increasing the chance of adverse reactions, particularly in patients with sensitive skin.

5 | SKIN REACTIVITY

In a study designed to describe allergens associated with a sunscreen source, the North American Contact Dermatitis Group evaluated both active and inactive ingredients in sunscreen products that may cause contact dermatitis. Standard patch testing in 23 908 patients was conducted between 2001 and 2010 and identified 219 (0.9%) positive reactions. The top three most frequent allergens in sunscreens were as follows: oxybenzone (70.2% for 10% concentration, 64.4% for 3% concentration), DL-alpha-tocopherol (4.8%), and fragrance mix I (4.0%).[6]

Similarly, the European Scientific Committee on Consumer Safety (SCCP) published an opinion paper[7] based on a review of 20 publications involving 6378 patients that were photo-patch tested for oxybenzone and other sunscreen actives between the 1981 and 2003. A total of 159 positive reactions were noted, leading to the conclusion that oxybenzone is a photoallergen. By way of comparison, only 19 photoallergic reactions were noted in these studies to p-aminobenzoic acid (PABA) and 34 photoallergic reactions to the various PABA esters.

Verhulst and Goossens[8] recently published a review and update of cosmetic products that have been reported to produce contact urticaria. Causative agents cited included phenoxyethanol, polyaminopropyl biguanide, oxybenzone, menthol, and a number of plant-derived ingredients including wheat and wheat protein hydrolyzates. Evidence of contact urticaria and, to a lesser degree, contact-mediated anaphylaxis was reported to be caused by oxybenzone.

The American Contact Dermatitis Society listed benzophenones as the 2014 Allergen of the Year, covering both allergy and photoallergy reactivity based on research reported by Heurung et al[9] They sighted oxybenzone (benzophenone-3) as the most frequent reactor in the class as well as the most prominent agent found in 68% of the 201 sunscreen products assessed. The authors also noted that oxybenzone showed high rates of cross-reactivity with the sunscreen active octocrylene, as well as ketoprofen, a topical nonsteroidal anti-inflammatory.

Additionally, oxybenzone has a molecular weight (MW) of 228.26 daltons, which raises concerns historically as a MW below 500 daltons has been associated with most of the common contact allergens.[10]

Cumulatively, there appears to be sufficient research demonstrating that oxybenzone possesses the potential to induce/elicit contact allergy, photocontact allergy, and contact urticaria reactions in humans. To put this into perspective, the sunscreen active p-aminobenzoic acid (PABA) and its esters have also been reported to produce allergic contact and photocontact dermatitis reactions[7,11] at somewhat lower reactivity rates than oxybenzone; however, PABA was forced into obscurity in the United States as a result of concerns from the medical community about sensitivity and subsequent competitive pressures on industry.

6 | ENVIRONMENTAL CONCERNS

In order to be effective, SPF products must be formulated to stay on the surface of the skin where they can reduce the penetration of UV energy to the underlying tissue. Therefore, when formulated in an effective SPF vehicle, oxybenzone demonstrates little absorption through the skin despite having a low MW. Gonzalez et al[12] observed an average excretion rate of 3.7% of the dose of a commercial sunscreen containing 4% oxybenzone when applied morning and night for 5 days. Accordingly, it is possible to estimate that if approximately 4% of oxybenzone in a sunscreen formulation is absorbed into the skin, 96% of the remaining dose is available to be washed off and enter various waterways. Corroborating this point, a 2008 study estimated that 4000 to 6000 tons of sunscreens were washed off in tourist reef areas annually[13]; as of 2017, scientists are currently estimating that 8000 to 16000 tons of sunscreen enter coral reefs each year.[14] The increase is the result of the continued growth of the global sunscreen market, which is projecting to reach sales of $11 billion by the year 2020. To better understand the implications of these figures, Tsui et al[15] sampled the waters of eight cities across four countries (China, United States, Japan, and Thailand) and the North American Arctic identifying twelve widely used aromatic hydrocarbon UV chemical filters. In general, concentrations of the chemicals increased with population density. Oxybenzone concentrations ranging from as high as 33 parts per trillion (ppt) in the Arctic to 5 parts per billion (ppb) in Hong Kong were identified. It should be noted that the surface waters sampled came largely from metropolitan areas featuring both commercial and industrial development, as opposed to beach or resort communities that see high levels of recreational water use by humans. Moreover, the concentrations in the Arctic waters suggest significant migration of toxic chemicals is occurring as current and tidal forces lead to water migration. The authors concluded that the findings represent various ecological risks to marine ecosystems, including promoting coral bleaching and adversely affecting reproduction in fish.

In 2008, Danovaro et al[13] were one of the first to report that oxybenzone had a negative impact on coral causing bleaching and death at concentrations of 33 and 50 parts per million (ppm). Additional research published in 2015 by Downs et al[16] identified oxybenzone as a phototoxicant, genotoxicant, and a skeletal endocrine disruptor in coral. They determined a lethal concentration 50 (LC50) for coral larvae that ranged from 139 to 3100 ppb depending on the specific test conditions. Coral cell LC50s for seven different coral species ranged from 8 to 340 ppb. The authors went on to measure the amount of oxybenzone at various locations (bays and open waters) at two different locations: Concentrations in the sampled waters from the U.S. Virgin Islands ranged from 75 to 1400 ppb and the Hawaiian Islands 0.8 to 19.2 ppb. Based on these findings, the water concentration of oxybenzone currently in the Virgin Islands overlaps the LC50 calculated for coral larvae and coral cells, while the waters in Hawaii are starting to reach levels that are within the range of the LC50 for coral.

The identification and accumulation of oxybenzone in waters cause concerns not just to coral, but to many other aquatic species as well. Braush and Rand [17] reviewed oxybenzone toxicity in Daphnia magna (invertebrate) and Oncorhynchus mykiss and Oryzias latipes (fish) and found LC50s of 1.9 ppm, 749 ppb, and 620 ppb, respectively. The authors further identified that UV filters have been shown to have bioaccumulation factors greater in fish than in water. For example, Gago-Ferrero et al[18] evaluated the accumulation of UV absorbers in a variety of fish in Spain and were able to extract oxybenzone from the tissue of white fish, rainbow trout, barb, chub, perch, and mussels. Taken together, these studies suggest the potential for increasing concentrations in species higher up in the trophic level, with humans poised to ingest the highest concentrations from the larger species that are regularly fished for human consumption.

UV filters enter the environment in two primary ways, directly from sloughing off while swimming around reefs or other waterways and indirectly via wastewater treatment plant (WWTP) effluent. In fact, even swimming in chlorinated pools and people washing sunscreens off their bodies while bathing raises several concerns. Researchers have observed that chlorine can react with oxybenzone producing chlorinated oxybenzone, which results in significantly more cell death than unchlorinated controls.[19] Another study evaluated oxybenzone transformation and kinetics after chlorination.[20] These results indicated that more genotoxic transformation products were produced in spite of the elimination of oxybenzone, posing potential threats to drinking water safety. Similarly, six water treatment plants in southeast Brazil evaluated WWTP levels of oxybenzone and observed (0.18 to 1.15 ppb) in both raw treated and chlorinated water, indicating that the compound was not removed by the water treatment process.[21] Additionally, Braush and Rand[17] reported that Switzerland estimated the input of 69 g of oxybenzone per 10,000 people per day into their WWTP.

The Centers for Disease Control and Prevention evaluated urinary samples obtained from 2517 participants aged 6 years and older between 2003 and 2010 and identified oxybenzone levels ranging from 15 ppb up to 3 ppm.[22] Meeker et al[23] recruited 105 pregnant women in northern Puerto Rico to provide urine samples and complete questionnaire data at three times during gestation. Urinary concentrations of oxybenzone ranged from 41.0 to 66.4 ppb and a positive association between biomarker concentrations, and self-reported use of personal care products was reported. An intraclass correlation coefficient (ICC) of 0.62 was determined for oxybenzone which was the highest among all the chemicals identified in the study. In contrast, urine samples were collected from 33 young Danish men over a 3-month period [24] with ICCs ranging from 0.69 to 0.80 and with more than 70% of the urine samples having detectable levels of oxybenzone. These data suggest that while most oxybenzone in personal care products is not significantly absorbed, sufficient quantities do enter the body such that meaningful levels can be measured in urine that finds its way to WWTP. Oxybenzone and/or its metabolite 4-methylbenzophenone may be more ubiquitous than generally thought (e.g., not just in sunscreens, cosmetics, and fragrances). The International Agency for Research on Cancer[27] has identified several sources of dietary exposure to these molecules in food or addition to food as a flavoring agent, its presence in drinking water as a contaminant, and through its migration from food packaging, printing inks, or recycled paperboard.

Kim and Choi[25] observed that oxybenzone has been detected in water, soil, sediments, sludge, and biota. Based on their review, the maximum detected level in ambient freshwater and seawater was 0.13 ppb and 0.58 ppb, respectively, and in wastewater, influent was 10.4 ppb. They also noted that in humans, oxybenzone has been detected in urine, serum, and breast milk samples worldwide with receptor binding assays showing strong adverse endocrine effects, including anti-androgenic and anti-estrogenic activity. Predicted no-effect concentration (PNEC) for oxybenzone was derived at 1.32 ppb; the levels observed in ambient water are generally an order of magnitude lower than the PNEC, but in wastewater influents, hazard quotients greater than 1 were noted. Lastly, Huo et al[26] looked at the relationship between maternal oxybenzone exposure and Hirschsprung's disease (HSCR) as well as its potential mechanism. HSCR is a neonatal intestinal abnormality that is derived from the failure of enteric neural crest cells migration to hindgut during embryogenesis from 5 to 12 weeks. The results showed that maternal oxybenzone exposure was associated with offspring developing HSCR, likely due to the chemical's inhibiting migration of highly specific cells.

7 | CONCLUSION

Based on the data reviewed, oxybenzone can be found globally in water, soil, sediments, sludge, and biota as well as in human urine, serum, and breast milk. As a sunscreen active, it is not as effective at protecting against UVA exposure as avobenzone, titanium dioxide, and/or zinc oxide. In humans, the chemical has been linked to Hirschsprung's disease is a confirmed contact allergen and photocontact allergen with some potential to induce contact urticaria and, to

a lesser degree, contact-mediated anaphylaxis. Environmentally, oxybenzone inhibits reproduction of coral and fish via embryo toxicity and/or causing male fish to be feminized, coral bleaching, and/or death. In summary, the potential negative health and environmental effects caused by the accumulation of this and other chemicals in the ecosystem needs to be taken into consideration by industry and regulatory agencies prior to the development and release of new and effective personal care products.

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Public Law 114–114
114th Congress

An Act

To amend the Federal Food, Drug, and Cosmetic Act to prohibit the manufacture and introduction or delivery for introduction into interstate commerce of rinse-off cosmetics containing intentionally-added plastic microbeads.

Dec. 28, 2015
[H.R. 1321]

Be it enacted by the Senate and House of Representatives of the United States of America in Congress assembled,

SECTION 1. SHORT TITLE.

Microbead-Free Waters Act of 2015.
21 USC 301 note.

This Act may be cited as the "Microbead-Free Waters Act of 2015".

SEC. 2. PROHIBITION AGAINST SALE OR DISTRIBUTION OF RINSE-OFF COSMETICS CONTAINING PLASTIC MICROBEADS.

(a) IN GENERAL.—Section 301 of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 331) is amended by adding at the end the following:

"(ddd)(1) The manufacture or the introduction or delivery for introduction into interstate commerce of a rinse-off cosmetic that contains intentionally-added plastic microbeads.

"(2) In this paragraph— Definition.

"(A) the term 'plastic microbead' means any solid plastic particle that is less than five millimeters in size and is intended to be used to exfoliate or cleanse the human body or any part thereof; and

"(B) the term 'rinse-off cosmetic' includes toothpaste.".

(b) APPLICABILITY. Effective dates. 21 USC 331 note.

(1) IN GENERAL.—The amendment made by subsection (a) applies—

(A) with respect to manufacturing, beginning on July 1, 2017, and with respect to introduction or delivery for introduction into interstate commerce, beginning on July 1, 2018; and

(B) notwithstanding subparagraph (A), in the case of a rinse-off cosmetic that is a nonprescription drug, with respect to manufacturing, beginning on July 1, 2018, and with respect to the introduction or delivery for introduction into interstate commerce, beginning on July 1, 2019.

(2) NONPRESCRIPTION DRUG.—For purposes of this subsection, the term "nonprescription drug" means a drug not subject to section 503(b)(1) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 353(b)(1)).

(c) PREEMPTION OF STATE LAWS.—No State or political subdivision of a State may directly or indirectly establish under any authority or continue in effect restrictions with respect to the manufacture or introduction or delivery for introduction into interstate 21 USC 331 note.

commerce of rinse-off cosmetics containing plastic microbeads (as defined in section 301(ddd) of the Federal Food, Drug, and Cosmetic Act, as added by subsection (a)) that are not identical to the restrictions under such section 301(ddd) that have begun to apply under subsection (b).

21 USC 331 note.

(d) RULE OF CONSTRUCTION.—Nothing in this Act (or the amendments made by this Act) shall be construed to apply with respect to drugs that are not also cosmetics (as such terms are defined in section 201 of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 321)).

Approved December 28, 2015.

LEGISLATIVE HISTORY—H.R. 1321:

HOUSE REPORTS: No. 114–371 (Comm. on Energy and Commerce).
CONGRESSIONAL RECORD, Vol. 161 (2015):
Dec. 7, considered and passed House.
Dec. 18, considered and passed Senate.

○

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration

21 CFR Parts 201 and 310

[Docket No. FDA–1978–N–0018] (Formerly Docket No. 1978N–0038)

RIN 0910–AF43

Labeling and Effectiveness Testing; Sunscreen Drug Products for Over-the-Counter Human Use

AGENCY: Food and Drug Administration, HHS.

ACTION: Final rule.

SUMMARY: The Food and Drug Administration (FDA) is issuing this document to address labeling and effectiveness testing for certain over-the counter (OTC) sunscreen products containing specified active ingredients and marketed without approved applications. This document addresses labeling and effectiveness testing issues raised by the nearly 2,900 submissions that we received in response to the sunscreen proposed rule of August 27, 2007 (2007 proposed rule). The document also identifies specific claims that render a product that is subject to this rule misbranded or would not be allowed on any OTC sunscreen product marketed without an approved application. The document does not address issues related to sunscreen active ingredients or certain other issues regarding the GRASE determination for sunscreen products. The document requires OTC sunscreen products to comply with the content and format requirements for OTC drug labeling contained in the 1999 Drug Facts final rule (published in the **Federal Register** of March 17, 1999, by lifting the delay of implementation date for that rule that we published on September 3, 2004).

DATES: *Effective Date:* This final rule is effective June 18, 2012. For additional information concerning this effective date, see section X in the preamble of this document. The incorporation by reference of a certain publication listed in this rule is approved by the Director of the Federal Register as of June 18, 2012.

Compliance Date: The compliance date for all products subject to this final rule with annual sales less than $25,000 is June 17, 2013. The compliance date for all other products subject to this final rule is June 18, 2012.

Implementation date: FDA is lifting the delay of implementation date for § 201.66 as published at 69 FR 53801, September 3, 2004.

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SUPPLEMENTARY INFORMATION:

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I. Overview of Document

A. Rulemaking History

This section of the document does not discuss every regulatory action associated with OTC sunscreen products. It highlights the major regulatory actions that are related to the regulatory actions being taken in this document. For a complete list of all regulatory actions associated with OTC sunscreen products, please refer to our Web site: *http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/Over-the-CounterOTCDrugs/StatusofOTCRulemakings/ucm072134.htm.*

In the **Federal Register** of May 12, 1993 (58 FR 28194), we published a proposed rule for OTC sunscreen products that identified active ingredients we tentatively considered to be generally recognized as safe and effective (GRASE), as well as associated labeling and sun protection factor (SPF) testing to be required for these OTC sunscreen products (the 1993 proposed rule). The SPF test and corresponding labeling reflect the level of protection against sunburn, which is caused primarily by UVB radiation. The 1993 proposed rule also explained the importance of protection against UVA radiation (58 FR 28194 at 28232 and 28233). The proposed rule referenced published UVA test methods but did not propose a specific method (58 FR 28194 at 28248 to 28250). Rather, the proposed rule stated that a sunscreen product could be labeled as "broad spectrum," or labeled with a similar statement, if it protected against UVA radiation as demonstrated by one of the published UVA tests or a similar test.

In April 1994, we reopened the administrative record to allow additional submissions concerning UVA-related issues. We also announced a public meeting to be held in May 1994 to discuss UVA testing procedures (59 FR 16042, April 5, 1994). We held the public meeting to gather more information to help us determine the most appropriate UVA test method and labeling.

In November 1997, Congress enacted the Food and Drug Administration Modernization Act of 1997 (FDAMA), which addressed OTC sunscreen products among other FDA issues. Section 129 of FDAMA stated that "not later than 18 months after the date of enactment of this Act, the Secretary of Health and Human Services shall issue regulations for over-the-counter sunscreen products for the prevention or treatment of sunburn." We then determined that the GRASE active ingredients, SPF testing requirements, and related labeling were issues that we could finalize within the timeframe set by FDAMA. Because we had not previously proposed specific UVA testing and labeling requirements, we did not have sufficient time to finalize these UVA requirements within the FDAMA timeframe.

In the **Federal Register** of May 21, 1999, we published a final rule for OTC

sunscreen products (64 FR 27666). The 1999 sunscreen final rule added the sunscreen monograph (regulations) in part 352 (21 CFR part 352) and included an effective date of May 2001. The 1999 sunscreen final rule stated that we would publish a proposed rule outlining UVA testing and labeling requirements at a future date. In 2000, we extended the effective date for the 1999 sunscreen final rule to December 2002 (65 FR 36319, June 8, 2000).

In December 2001, we stayed the December 2002 effective date of the 1999 sunscreen final rule indefinitely. We took this action because we planned to revise part 352 to add UVA testing and labeling requirements so that OTC sunscreen products would be tested and labeled for both UVB and UVA radiation protection. We included these revisions in a proposed rule that published in the **Federal Register** of August 27, 2007 (72 FR 49070). The 2007 proposed rule identified UVA testing and labeling that we proposed should be required for all OTC sunscreen products. The proposed rule also revised SPF testing and corresponding labeling from the 1999 final rule. The proposed rule did not lift the existing stay of the effective date for part 352.

On September 3, 2004 (69 FR 53801), we delayed until further notice the implementation date for the Drug Facts final rule (64 FR 13254, March 17, 1999) (21 CFR 201.66) for OTC sunscreen products. The Drug Facts final rule (21 CFR 201.66) establishes general labeling format and content requirements for all OTC drugs. We explained that we postponed the implementation date for general Drug Facts labeling requirements for sunscreens because we did not expect to issue the sunscreen final rule containing UVA testing and product-specific labeling requirements (*i.e.*, this document) by the Drug Facts implementation date of May 2005. Therefore, we delayed the implementation date until further notice to prevent sunscreen product manufacturers from having to relabel their products at two closely related time intervals, as initially required by the 1999 Drug Facts final rule and the 1999 sunscreen final rule.

B. Scope of This Document

This final rule establishes the labeling and testing requirements for OTC sunscreen products containing specific ingredients or combinations of ingredients and marketed without an approved application under section 505 of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 355) (the FD&C Act). The requirements in this final rule will help ensure that these currently marketed sunscreen products are appropriately labeled and tested for both UVA and UVB protection. In addition, the requirements in this final rule will help ensure the proper use of these sunscreens and greater consumer protection from the damaging effects of UV radiation. This final rule also identifies claims that render a product that is subject to this rule misbranded or are not allowed on any OTC sunscreen drug product marketed without an approved application.

As described in the previous section of this document, we issued the 2007 proposed rule as a proposed amendment to the sunscreen monograph requirements in 21 CFR part 352 primarily to establish UVA testing and labeling requirements so that all OTC sunscreen products marketed under the sunscreen monograph would be tested and labeled for both UVB and UVA radiation protection. Sunscreen active ingredients, UVB testing, UVB labeling, and other conditions under which sunscreens would be considered GRASE and not misbranded had been addressed in the 1999 (stayed) final rule. In response to the 2007 proposed rule, however, we received submissions from the public concerning all aspects of the sunscreen monograph (*i.e.*, the conditions specified in the 1999 final rule and the 2007 proposed rule). As discussed further in this section, some of the issues regarding the monograph conditions raised in the public submissions will require further evaluation by us. Therefore, we are not issuing a final monograph with GRASE conditions for sunscreens in this document. Instead, we are publishing this final rule establishing labeling and the effectiveness testing upon which it relies, which applies to the same sunscreens that were the subject of the 2007 proposed rule to amend the monograph, because it is in the best interest of public health to publish this final rule while we work on remaining issues that need to be addressed in order to publish a final monograph. This labeling will help ensure that these products are not misbranded by providing specific indications, directions, warnings, and other important information to help consumers select and use them appropriately.

In this final rule, then, we are codifying in 21 CFR part 201 requirements for OTC sunscreen products containing specified active ingredients and marketed without approved applications under section 505 of the FD&C Act (21 U.S.C. 355) (hereafter referred to as "covered" products). With respect to these covered products, this new section 21 CFR 201.327 includes requirements for labeling and the effectiveness testing upon which it relies. Because we have not yet resolved all of the issues regarding conditions under which sunscreens are GRASE and not misbranded, the stay of 21 CFR part 352 remains in effect. Although we are not yet codifying these labeling and related effectiveness testing provisions in the monograph regulation, they do embody the agency's current determination on appropriate regulation of these aspects of sunscreens that were previously identified as falling within the monograph in part 352, and supersede the prior approach embodied in the never-effective provisions of 21 CFR part 352 subparts C and D. While this rule does not lift the stay of part 352, we are lifting the delay of implementation date for the Drug Facts labeling requirements of 21 CFR 201.66. In addition, this rule codifies certain specific claims that render a covered product misbranded or are not allowed on any OTC sunscreen drug product marketed in the United States without an approved application.

We note that all provisions of new 21 CFR 201.327 and the amendments to 310.545 included in this rule apply only to the aforementioned covered products, and references in this document to "covered" products recognize this limitation. Manufacturers of sunscreen products that are already being marketed pursuant to an approved application can contact FDA's Center for Drug Evaluation and Research to discuss supplemental submissions that would enable them to include labeling on their products like that specified in this final rule.

C. Issues Outside the Scope of This Document

There are a number of issues that were raised in public submissions responding to the 2007 proposed rule that are outside the scope of this document. The issues fall into two categories:

- GRASE determination for sunscreen products and active ingredients
- Issues affecting multiple OTC drug monographs

As explained below, in this document, we are not addressing these issues related to determining the GRASE status of sunscreen products or sunscreen active ingredients and are not addressing the issues described below affecting multiple OTC drug monographs.

1. Issues Regarding GRASE Determination for Sunscreen Products and Active Ingredients

A large number of submissions on the 2007 proposed rule raised issues related to the conditions that define what constitutes a GRASE finished OTC sunscreen product, irrespective of its active ingredients. These included over 1000 submissions requesting that we limit the monograph to sunscreens that offer broad spectrum protection and have SPF values of 15 or higher. Because this final rule is a labeling rule, and not a monograph, we do not address these issues here but plan to address them in future rulemakings regarding the monograph and conditions for general recognition of safety and effectiveness.

This rule also does not address issues related to the GRASE status of sunscreen active ingredients that are included in the 2007 proposed rule (proposed 21 CFR 352.10 and 352.20). We received 20 submissions raising questions about the safety of ingredients in sunscreens (Ref. 1). Ten of the submissions specifically asked that we ensure that none of the ingredients are carcinogenic. Others asked that we ensure that all ingredients in sunscreens are safe without citing a specific concern. We intend to address carcinogenicity and other safety considerations related to sunscreen active ingredients in a future rulemaking.

We also received submissions requesting that we increase the GRASE concentration of avobenzone from 3 percent to 5 percent (Ref. 1). Another submission points out that there are two USP [1] monographs for zinc oxide:

- Zinc oxide (Ref. 2)
- Zinc oxide neutral (Ref. 3)

The submission would like us to clarify that zinc oxide in OTC sunscreen products can meet the specifications of either USP monograph (Ref. 1). We intend to address all of these issues regarding GRASE determination for sunscreen active ingredients in future rulemakings.

In addition, we received two submissions requesting that we classify three new ingredients not previously marketed in the United States as GRASE: bemotrizinol, bisoctrizole, and octyl triazone (Ref. 1). We found these active ingredients eligible for review under the OTC drug monograph system in 2003 (octyl triazone) and 2005 (bemotrizinol and bisoctrizole) (68 FR 41386, July 11, 2003, and 70 FR 72449, December 5, 2005). We are currently reviewing the safety and effectiveness data submitted for these and other sunscreen active ingredients found eligible for potential addition to the monograph. When we complete our review, we will issue proposed rules stating our tentative conclusions on the safety and effectiveness of all of these ingredients.

2. Issues Affecting Multiple OTC Drug Monographs

This final rule also does not address three issues raised in response to the 2007 sunscreen proposed rule that are not specific to sunscreen products. Because these issues apply more generally to multiple categories of OTC drug products, we are not addressing these issues in this final rule, which is limited to OTC sunscreen products.

The first issue concerns the inclusion of expiration dates on sunscreen labels. We received 12 submissions requesting that we require OTC sunscreen products to be labeled with an expiration date (Ref. 1). Currently, regulations in 21 CFR 211.137(h) do not require that an expiration date be included in labeling if an OTC drug product does not have any dosage limitations and is stable for at least 3 years. This regulation applies to many OTC drug products, including sunscreen products. Any modification of the existing regulations would require publication of a proposed rule addressing all OTC drug products affected by the expiration date regulations.

The second issue concerns the term "final monograph." One submission argued that we should not use this term because it is inaccurate (Ref. 1). As the submission states, "FDA is to continually evaluate products, so nothing is ever finalized." This issue applies to monographs representing all categories of OTC drug products. Therefore, we are not addressing the issue in this document.

The third issue concerns the country of origin listing for all ingredients (*i.e.*, both active and inactive ingredients) on a sunscreen drug product. We received a submission requesting that we provide the country of origin for each ingredient. The submission also requested that manufacturers be required to provide specific details about what each ingredient does in the product. This issue applies to all OTC drug products and, therefore, we are not addressing it in this document.

D. Enforcement Policy

As noted, no final monograph is currently in effect for OTC sunscreen drug products, and in its absence, questions may arise regarding FDA's enforcement policy for OTC sunscreen products marketed without approved applications. To clarify expectations for industry, elsewhere in this issue of the **Federal Register**, we are announcing the availability of a draft guidance document, explaining the agency's intended enforcement policy for these products until a final sunscreen monograph becomes effective.

E. Summary of Major Revisions to the Labeling and Effectiveness Testing Included in the 2007 Proposed Rule

In response to the 2007 proposed rule, we received almost 2,900 submissions from the public. Of these submissions, over 2,500 expressed general support for the proposed rule and urged us to finalize and implement the new rule quickly. Three hundred twenty-five of the submissions raised approximately 90 specific issues related to the proposed rule. We have addressed the issues specifically relating to labeling and effectiveness testing in this final rule. Based on the submissions received, and the information and data included in those submissions or otherwise available to us, we have re-evaluated our position on several issues in the 2007 proposed rule and made several changes to our proposed labeling and testing requirements. Tables 1, 2, 4, and 5 in this document summarize the labeling and effectiveness testing requirements included in the 2007 proposed rule as well as the labeling and effectiveness testing required by this final rule:

- Table 1: PDP Labeling (discussed in section III)
- Table 2: Drug Facts Labeling (discussed in section IV)
- Table 4: SPF Test (discussed in section VI)
- Table 5: Broad Spectrum Test (discussed in section VIII)

Rather than summarizing all of the revisions to the labeling and testing included in the 2007 proposed rule, we are highlighting what we consider to be the most important revisions in this section of the document.

We made the following changes to the proposed labeling:

1. The proposed UVA "star rating" is not required on the PDP.

2. A combined "Broad Spectrum SPF" statement is required on the PDP for sunscreen products that pass the broad spectrum test established in new 21 CFR 201.327(j). To pass the broad spectrum test, the amount of UVA protection must increase as the SPF value increases.

3. For sunscreen products that pass the broad spectrum test established in new 21 CFR 201.327(j) and have SPF

[1] United States Pharmacopeia.

values of 15 or higher in accordance with the SPF test in 21 CFR 201.327(i):

a. The "Sun Alert" warning proposed as the first warning in 2007 is not required (Warning proposed located in 21 CFR 352.52(c)(1)).

b. A new indication statement may be included to inform consumers that using the product "as directed with other sun protection measures (see Directions [in bold italic font]) decreases the risk of skin cancer and early skin aging caused by the sun."

c. A new direction statement has been added informing consumers that exposure to the sun increases the risk of skin cancer and early skin aging and providing a list of specific sun protection measures that can decrease this risk.

4. For any OTC sunscreen product that does not pass the broad spectrum test in 21 CFR 201.327(j), or that are broad spectrum with an SPF value less than 15, this final rule, like the 2007 proposed rule, requires that the first warning indicate the adverse consequences of spending time in the sun. The wording of this warning has been revised to state, "Skin Cancer/Skin Aging Alert [in bold font]: Spending time in the sun increases your risk of skin cancer and early skin aging. This product has been shown only to help prevent sunburn, not [in bold font] skin cancer or early skin aging."

We also made the following changes to the effectiveness testing proposed in 2007:

1. The number of subjects required in the SPF test has been reduced from 20 subjects to 10 subjects.

2. One in vitro test is required to demonstrate broad spectrum protection rather than the two previously proposed tests (an in vitro test and an in vivo test).

3. The broad spectrum test is a pass/fail test based on the critical wavelength value of 370 nm [2].

II. Administrative and Other Issues

Some of the submissions that we received following publication of the 2007 proposed rule made the following requests involving administrative issues (Ref. 1):

- Extend the comment period of the 2007 proposed rule.
- Lift the stay on 21 CFR part 352, imposed in 2001 (66 FR 67485).
- Allow interim marketing of products containing avobenzone with ensulizole and avobenzone with zinc oxide.
- Set an effective date for this final rule other than the 18 months proposed in the 2007 proposed rule.
- Revise the preemption language included in the 2007 proposed rule by deleting any references regarding the rule's potential preemption of State tort law.

Our positions on these issues are discussed in the remainder of this section of the document.

All of the requests to extend the comment period were submitted before the November 28, 2007 **Federal Register** notice in which we extended the comment period of the 2007 proposed rule (72 FR 67264). In that notice, we extended the close of the comment period from November 26, 2007, to December 26, 2007. We have not received any more requests to extend the comment period since December 2007.

With regard to requests to lift the stay of 21 CFR part 352 (the OTC sunscreen monograph), as already discussed, our 2007 proposed rule anticipated amending the testing and labeling provisions of that monograph and subsequently lifting the stay. However, comments received on the 2007 proposed rule not only addressed labeling and effectiveness testing for final sunscreen formulations, but also raised other issues about the monograph conditions for OTC sunscreen products that require further consideration. As a result, we are not finalizing amendments to part 352 at this time nor lifting the stay placed on that section as enacted in 1999 (66 FR 67485). Rather, this final rule establishes in 21 CFR 201.327 labeling requirements and the effectiveness testing upon which it relies for covered OTC sunscreen drug products. We intend to lift the stay on part 352 when we reach our final conclusions on the conditions under which sunscreen products are GRASE and not misbranded, including a determination regarding sunscreen active ingredients, and publish a revised final monograph. In the interim, the labeling and effectiveness testing provisions of this rule apply to covered OTC sunscreen products.

We received a request that we allow interim marketing of avobenzone combinations in proposed § 352.20(a)(2) prior to issuing a final rule for part 352. Subject to our enforcement discretion, we will continue to allow the marketing of avobenzone combinations provided for in the 1999 sunscreen final rule. However, we are not allowing marketing of the additional avobenzone combinations discussed in the 2007 proposed rule until we reach a final conclusion on the GRASE determination for sunscreen active ingredients and combinations of those ingredients.

We are requiring that this final rule become effective in 1 year, even though we considered 18 months in the 2007 proposed rule (72 FR 49070 at 49110). We are allowing products with annual sales less than $25,000 to comply with this rule in 2 years, as stated in the 2007 proposed rule. In response to the proposed rule, we received one submission arguing that we should require this final rule to become effective in 1 year (Ref. 1). The submission stated that a later effective date would have a negative public health impact. We received eight submissions arguing that we should extend the effective date from the proposed 18 months to 3 years (Ref. 1). The submissions listed the following reasons for allowing more than 18 months:

- Repackaging
- Relabeling
- Testing/retesting
- Removing products from market
- Impact on small businesses

The most common argument was that more time would be needed to test/retest OTC sunscreen products for broad spectrum protection in accordance with both the in vitro and in vivo UVA test methods included in the proposed rule.

We agree with the submission which stated that it would be beneficial for consumers to have this rule become effective within 1 year. As explained in section VIII.A of this document, we are not requiring manufacturers to demonstrate broad spectrum protection by conducting in vivo and in vitro tests. This final rule requires that manufacturers conduct only the simpler and less expensive nonclinical in vitro test to demonstrate broad spectrum protection. In vitro tests are substantially shorter than in vivo tests. Therefore, we are setting an effective date for this rule 1 year from the date of publication in the **Federal Register**. However, we are providing two years for all products with annual sales less than $25,000 to comply with this rule. In addition, in order to ensure that limited testing laboratory capacity does not result in sunscreen shortages during the transition to the new rule, we intend to exercise enforcement discretion for a period of time with regard to the SPF test for certain OTC sunscreen products on the market by June 17, 2011 (see our draft guidance entitled "Guidance for Industry: Enforcement Policy—OTC Sunscreen Drug Products Marketed Without An Approved Application" announced elsewhere in this issue of the **Federal Register**).

The submissions stating that additional time is necessary for

[2] Nanometers.

repackaging and relabeling did not submit any information or data to support these arguments (Ref. 1). The argument that more than 18 months is needed to remove non-compliant products from the market is not valid. In the 2007 proposed rule, we indicated that sunscreen products which are already distributed by the effective date of the final rule would not be expected to be relabeled or retested in conformity with the final rule conditions unless these products were subsequently relabeled or repackaged after the effective date (72 FR 49070 at 49109). Consistent with this statement, we do not expect non-compliant products introduced or delivered for introduction into interstate commerce prior to the compliance dates specified for this final rule to be removed from the market.

We received a submission that expressed concern about the agency's preemption discussion in the 2007 proposed rule (72 FR 49070 at 49109 and 49110) and requested that we delete any discussion regarding the rule's potential preemption of State tort law (Ref. 1). The submission claimed that we exceeded our authority when we stated that section 751(a) of the FD&C Act displaces both State legislative requirements and State common law duties. The submission argued that Congress intended to preserve State common law claims by including section 51(e), which exempts State product liability claims from express preemption under section 751(a) of the FD&C Act. The commenter appears to have construed our statement in a way that would nullify section 751(e) of the FD&C Act. We did not intend to suggest that section 751(a) of the FD&C Act preempts State product liability claims, whether based on State legislative enactments or common law, because section 751(e) exempts such actions from the express preemption provision in section 751(a). However, it is important to note that section 751(e) of the FD&C Act exempts only those common law claims that are based on State product liability law. Our revised preemption discussion in section XII remains consistent with applicable law.

The submission also requested that we delete any references to implied preemption. In this final rule, we have omitted any statement regarding implied preemption because, although implied preemption may arise, such scenarios are necessarily case-specific. Section XII of this document makes clear that the sole statutory provision giving preemptive effect to the final rule is section 751 of the FD&C Act.

III. Principal Display Panel (PDP) Labeling

In response to the 2007 sunscreen proposed rule, we received 45 submissions requesting that we revise the proposed principal display panel (PDP) labeling (Ref. 1). We are revising the PDP labeling based, in part, on these submissions (see table 1 of this document). We have decided that the PDP labeling included in this document will simplify the purchase decision for consumers by allowing them to more easily find important information included on the PDP.

TABLE 1—SUMMARY OF PDP LABELING IN THE 2007 PROPOSED RULE AND THIS FINAL RULE USING A BROAD SPECTRUM SPF 30 WATER RESISTANT SUNSCREEN PRODUCT AS EXAMPLE A AND AN SPF 6 SUNSCREEN THAT IS NOT BROAD SPECTRUM AND NOT WATER RESISTANT AS EXAMPLE B

Labeled information	2007 Proposed rule	This final rule
Effectiveness Rating [1]	Example A: "UVB SPF 30 High" "UVA ★★★☆ High" Example B: "UVB SPF 6 Low" "No UVA Protection"	Example A: "Broad Spectrum SPF 30" Example B: "SPF 6"
Water Resistance	Example A: "Water Resistant" Example B: No statement on water resistance	Example A: "Water Resistant (40 minutes)" Example B: No statement on water resistance
Educational Statement	Examples A & B: "UV rays from the sun are made of UVB and UVA. It is important to protect against both UVA and UVB rays."	Examples A & B: No educational statement

[1] The UVA rating in the 2007 proposed rule is a four-tier rating (low, medium, high, highest). The UVA testing in this final rule is pass/fail—a product is either allowed or not allowed to include a broad spectrum statement depending on results of the test described in new 21 CFR 201.327(j) (see section VIII of this document).

A. SPF Statement

In the 2007 sunscreen proposed rule, we proposed redefining the acronym "SPF" as the "sunburn protection factor." We also proposed that the term "UVB SPF" would be required on the PDP of all OTC sunscreen products (proposed 21 CFR 352.50(a)). This term would be followed by the numerical value determined from SPF testing and one of the following descriptors: "low," "medium," "high," or "highest." For example, a sunscreen product could have contained the statement "UVB SPF 40 High" on the PDP.

We received 12 submissions regarding the SPF statement in response to the 2007 sunscreen proposed rule (Ref. 1). Collectively, the submissions made the following requests:

1. Do not change the definition of SPF to "sunburn protection factor"
2. Remove UVB from "UVB SPF"
3. Redefine the "highest" product category descriptor to include SPF 50
4. Require SPF values expressed in multiples of 5
5. Label SPF as the percent of UVB radiation screened

As discussed in the remainder of this section, we agree with the first and second requests, but are not granting the other three requests.

In this final rule, unlike the 2007 proposed rule, we have no express definitional section. However, we identify "SPF" as an abbreviation for "sun protection factor" in new 21 CFR 201.327(a)(1), and use it consistently in this way throughout the rule. This use of the term SPF is identical to the definition in the 1999 stayed sunscreen final rule (64 FR 27666). For products that are not broad spectrum, the term "SPF" will appear on the PDP with the numerical SPF value calculated according to the test method in new 21

CFR 201.327(i). For broad spectrum sunscreen products, the term "Broad Spectrum SPF" will appear on the PDP along with the numerical SPF value calculated according to the test method in new 21 CFR 201.327(i).

The term "UVB" will not be required as part of the SPF statement. We are also not requiring the descriptor (*e.g.*, "high" or "low"). We included these two requirements in the 2007 proposed rule because we had concluded that the requirements would help consumers understand the side-by-side SPF numerical rating in conjunction with the UVA star rating, which included the term "UVA" and the same descriptors (72 FR 49070 at 49084). As discussed in section III.B of this document, the UVA star rating is not being included in this final rule, and as discussed below, we have concluded that neither the term "UVB" nor the descriptor is necessary for consumers to understand the effectiveness statement.

Neither the term "UVB" nor a descriptor (*e.g.* "low" or "high") had been included on sunscreen labels prior to our 2007 proposal, and consumers had been able to make purchase and use decisions based on SPF values alone. Under this final rule, the SPF value will be expressed on the PDP by including the term "SPF," followed by the numerical value determined from the SPF test, similar to how it has appeared on the labels of OTC sunscreen products for more than 30 years. As described in section III.B of this document, for products passing the critical wavelength test in new 21 CFR 201.327(j), the SPF value statement will be expressed as "Broad Spectrum SPF" followed by the numerical SPF value calculated according to the test method in 21 CFR 201.327(i).

We received five submissions objecting to the definition of SPF as "sunburn protection factor" and only one submission supporting the definition (Ref. 1). The submissions objecting to the definition argued that, if the term "sunburn protection factor" is used, consumers may mistakenly assume that a higher SPF value means a higher probability of sunburn. Additionally, they argued that sunscreen products protect against various harmful effects of sun exposure, such as early skin aging and skin cancer, in addition to protecting against sunburn. Some submissions suggested that the term "sunburn protection factor" will lead consumers with darker skin to assume that they do not need sunscreen products because they do not burn easily (Ref. 1).

We agree with the arguments provided by the submissions suggesting that the term "sunburn protection factor" may be misleading. In the 2007 sunscreen proposed rule, we revised the definition of SPF from "sun protection factor" to "sunburn protection factor" because we thought that the new definition was more descriptive of what an SPF value represents (72 FR 49070 at 49077). The SPF value is determined from a clinical test with sunburn as the endpoint. However, for broad spectrum sunscreen products, the SPF statement also serves as a relative measure of the magnitude of broad spectrum protection (Ref. 4). In this final rule, while we do not codify a separate definitional section, we continue to use the term "SPF" to mean "sun protection factor," as we had done in the 1999 final rule (21 CFR 201.327(a)(1)).

In this final rule, we are also revising the effectiveness statement so that the term "UVB" is not required before the term "SPF," as proposed in the 2007 proposed rule (proposed 21 CFR 352.50(a)). We received six submissions requesting this revision (Ref. 1). These submissions argued that "UVB SPF" is an incorrect representation of the SPF value determined from a test using a solar stimulator that emits both UVA and UVB radiation. The submissions point out that sunburn is not caused solely by UVB radiation. It is well known that UVA radiation contributes up to 20 percent of the skin's sunburn response (Refs. 5 and 6). One submission points out that if a sunscreen product blocked 100 percent of the incident UVB radiation and none of the erythemally effective UVA radiation, the sunscreen product would have SPF values no higher than 11 (if only 9 percent or 1/11 of UV radiation reaches the skin) (Ref. 4).

We agree that UVA radiation contributes to the development of sunburn. Although the contribution of UVA to sunburn is less than UVB, it is still significant (Ref. 5). Further, as stated in the submissions, protection against UVA radiation is necessary to achieve higher SPF values (Ref. 5). We proposed including the term "UVB" in the SPF statement in the 2007 proposed rule to help consumers understand that the SPF effectiveness rating is different from the UVA effectiveness (star) rating being proposed (72 FR 49070 at 49084). However, as discussed elsewhere in this final rule we are not requiring a UVA effectiveness rating on the PDP (see section III.B.). Therefore, the term "UVB" is not necessary as part of the SPF statement. In this final rule, we are not requiring the term "UVB" be placed before the term "SPF."

In the 2007 sunscreen proposed rule, we stated that the SPF value should be followed by one of the descriptors "low," "medium," "high," or "highest" (proposed 21 CFR 352.50(a)). The proposed descriptors were included to help consumers understand the SPF value because the label would have included identical descriptors for the UVA star rating. As discussed in section III.B. of this document, we are not requiring a UVA effectiveness rating on the PDP. Therefore, descriptors are no longer required to distinguish the SPF value from the UVA rating on a sunscreen label. Because we are not requiring a descriptor after the SPF value on the PDP in this document, the request to include SPF 50 sunscreen products in the "highest" category is no longer relevant.

We received two other requests for revision to the SPF statement with which we do not agree. First, a submission stated that SPF values should only be labeled in multiples of five to be consistent with SPF labeling recommendations by the European Commission (Ref. 7). Second, one request from a submission suggested that SPF values should be expressed as the percent of UV absorption. The submission argued that the current SPF values are misleading because consumers believe an SPF 15 sunscreen product is not very protective even though it screens 93 percent of UV radiation.

We do not agree with either submission. Based on SPF test data we have reviewed, we find that SPF values for sunscreen products generally can be determined with a precision that allows the products to be labeled with SPF values in intervals of less than 5 units (Ref. 1). Therefore, there is no mathematical or statistical basis for restricting SPF values to multiples of five. Contrary to the second request, consumers have relied on SPF values for over 30 years and are familiar with this format. Therefore, expressing SPF values as percentages may be confusing. It would imply that the stated percentage of the entire UV spectrum is absorbed by a sunscreen. However, the SPF values only reflect protection against the portion of the UV spectrum that causes sunburn. Additionally, the percentages of UV radiation screened that the submission notes are theoretical. The percentages are determined in a laboratory setting and not under actual use conditions. For example, laboratory tests may show that an SPF 15 sunscreen absorbs 93 percent of UV rays, but, under actual use conditions, the level of protection provided by an SPF 15 sunscreen product may be significantly below 93 percent. There are a number of factors

that lead to this decreased protection, the most important being under-application of the sunscreen product (72 FR 49070 at 49092). Therefore, if SPF values were expressed as percentages, consumers might mistakenly believe that the sunscreen products they are using provide more protection than they really do provide under actual use conditions.

B. Broad Spectrum Statement

In response to the 2007 proposed rule, we received over 50 submissions collectively making the following four requests regarding the UVA effectiveness rating (Ref. 1):

1. Do not require UVA 4-star rating system.
2. Do not require "no UVA protection" statement if a product does not protect against UVA radiation.
3. Do not require the UVA statement to be the same size as the SPF statement.
4. Perform label comprehension studies prior to implementing proposed PDP labeling.

The submissions included arguments, but no data, to support these requests.

We agree with the first and second requests. However, we are not granting the third and fourth requests. Our reasons for these decisions are explained below, but we first summarize the related provisions of this final rule. We are not requiring a star rating or descriptors to indicate the level of UVA protection as proposed. Instead, to indicate the level of UVA and UVB protection, we are establishing a pass/fail broad spectrum test and a broad spectrum labeling statement. If a sunscreen product passes the broad spectrum test (see section VIII.B. of this document), under this final rule, the PDP of the product must include the statement "Broad Spectrum SPF [insert numerical SPF value resulting from testing under paragraph (i) of this section]," without any "UVA" reference (§ 201.327(a)(1)(i)). We are requiring the Broad Spectrum SPF statement to appear as continuous text with no intervening text or graphics. We are also requiring that the entire text be the same font style, size and color on the same background color. (§ 201.327(a)(1)(ii)).

With regard to the submissions received, nearly all of the 50+ submissions argued against requiring the 4-star rating system to display the level of UVA protection on the PDP of OTC sunscreen products (Ref. 1). Many submissions stated that the presence of stars and a number (SPF) on the PDP will lead to consumer confusion. Some submissions argued that consumers may be confused when determining whether a star is filled or empty, thereby not knowing the UVA protection level. Other submissions argued that consumers are familiar with star ratings, but that the star rating for items such as movies and hotels are based on recommendations and not rigorous data. They suggested several options for labeling UVA protection, such as a numerical rating or another symbol other than stars.

Some submissions suggested that the UVA rating should be proportional to the SPF value but requested that there not be two ratings on the PDP. The submissions cited the European Commission's recommendation that UVA protection increase as the SPF value increases (Ref. 7). The European Commission recommends a minimum UVA protection factor equal to at least one-third of the labeled SPF or a critical wavelength of at least 370 nm, but does not recommend that the actual value of the UVA protection factor or critical wavelength be displayed. The European Commission recommends that the main indicator of sun protection be the SPF value. Broad spectrum protection is indicated by a symbol on sunscreen labels—the acronym "UVA" enclosed within a circle the diameter of which should not exceed the height of the SPF value.

We agree with the submissions that the UVA star rating would likely be confusing in conjunction with the numerical SPF rating. We also agree with the submissions requesting that UVA protection should be proportional to the SPF value. We are requiring such proportionality in the broad spectrum test described in this document. Because of this proportionality, there is no longer a need for a separate UVA rating. Instead of a rating, we are requiring a "broad spectrum" statement on the PDP if a product has a critical wavelength equal to or greater than 370 nm. This pass/fail "broad spectrum" statement is consistent with the recommendations in the submissions citing the recommendations of the European Commission.

As noted, several submissions responding to our proposal for a separate UVA rating with stars suggested that consumer comprehension testing should be performed before the proposed labeling is implemented. We agree with the submissions that consumer comprehension data can be very helpful in formulating labeling changes. In fact, in conjunction with our 1993 proposal to allow products to be labeled as "broad spectrum" if they contained sunscreen active ingredients that absorbed UVA radiation (58 FR 28194 at 28233), we requested label comprehension study data to allow us to determine consumer understanding of the terms "broad spectrum," "UVA," and "UVB" (58 FR 28194 at 28243). Unfortunately, the data we received were not sufficient to allow us to determine the level of consumer understanding of these terms (72 FR 49070 at 49081 through 49085), and we received no further consumer comprehension data in response to the 2007 proposal to require the UVA star rating. While we acknowledge the value of consumer comprehension data, for reasons explained below, we conclude that conducting consumer comprehension testing is not necessary in this case in light of the labeling we have selected for the final rule.

First, submissions suggesting consumer testing were responding to the UVA star rating in the proposed rule, the value of which would have been based on the results of two tests (72 FR 49070 at 49081 through 49085). As noted, we agree with the submissions suggesting that the 2007 UVA labeling proposal was likely to be confusing. Elsewhere in the document, we also discuss our final choice of a pass-fail test for establishing UV protection (section VIII.B). As a result of these changes in the underlying test method and the submissions on the proposed labeling, we have incorporated a much simpler labeling statement in this final rule. This statement designates as "broad spectrum" those products that are demonstrated to have a critical wavelength of at least 370 nm, using the test in new 21 CFR 201.327(j).

Second, unlike in 1993 when we first sought consumer data on the term "broad spectrum", and unlike the UVA star rating that we proposed in 2007, consumers are now likely to be familiar with the term "broad spectrum" as included in this document because some sunscreen manufacturers have labeled sunscreen products as "broad spectrum" for over 20 years. For example, the Johnson and Johnson "Sundown Broad Spectrum" line of sunscreens was on the market in 1988 (Ref. 8). As already noted, in our 1993 proposed rule, we not only sought consumer data, but in fact proposed that products be permitted to be labeled as "broad spectrum" if they contained sunscreen active ingredients that absorbed UVA radiation, although we did not at that time propose to require a specific test to demonstrate UVA protection (58 FR 28194 at 28233). We continued to allow this statement in the 1999 sunscreen final rule (64 FR 27666 at 27666 through 27667).

Many consumers may also be familiar with the term "broad spectrum" because

of public health campaigns and news articles about the importance of broad spectrum UV protection over the last two decades. For example, an article appearing in Working Woman magazine in 1990 urged women to "make sure to look for the term 'broad spectrum' on the label of a sunscreen" because "it means you're getting protection from both types of radiation" (Ref. 9).

For consumers not already familiar with the term "broad spectrum," the additional indication statement allowed in this document for certain broad spectrum sunscreen products should help consumers recognize the benefit of these products. Under "Uses" in Drug Facts, broad spectrum sunscreen products with an SPF value of 15 or higher are allowed the following indication statement: "if used as directed with other sun protection measures (see Directions [in bold italic font]), decreases the risk of skin cancer and early skin aging caused by the sun" (new 21 CFR 201.327(c)(2)).

In addition, educational campaigns about sun protection will further inform consumers about the benefits of using sunscreens that include the term "broad spectrum" on their labels and have an SPF value of 15 or higher. We expect consumers to learn that a sunscreen labeled with the statement "Broad Spectrum SPF" 15 or higher, when used as directed with other sun protection measures, offers more comprehensive protection against sun-induced skin damage than that provided by a sunscreen that is not broad spectrum or that are broad spectrum with an SPF value less than 15.

It is important to note that the broad spectrum test required in this document captures both UVB and UVA protection for the effectiveness of a sunscreen product. The broad spectrum test is not limited to UVA wavelengths as was the case with the proposed test (see section VIII.B of this document). By requiring that a broad spectrum sunscreen provide both UVB and UVA protection in a pass/fail test, the amount of UVA protection for a sunscreen product that passes the test must increase as the SPF increases. For example, a Broad Spectrum SPF 40 sunscreen product provides greater protection against both UVB and UVA than a Broad Spectrum SPF 20 sunscreen product. In contrast, an SPF 40 sunscreen product that is not broad spectrum provides more UVB protection than a SPF 20 sunscreen product that is not broad spectrum, but may not provide more UVA protection.

This proportionality between UVB and UVA protection is important because consumers have been accustomed to basing their purchase decision concerning protection level primarily on the SPF value, and only secondarily on indications of whether or not the sunscreen provides broad spectrum protection. For example, a consumer seeking lower protection may have chosen an SPF 15 sunscreen product, whereas a consumer seeking higher protection may have chosen an SPF 40 sunscreen product. By creating a clear and standardized "yes/no" indicator regarding broad spectrum protection, these final labeling requirements will enable consumers to make better and more informed purchase decisions by looking to see if a product has a "Broad Spectrum SPF" value on the label. Thus, the ultimate purchase decision would be based on the numerical value associated with the Broad Spectrum SPF statement. For products offering broad spectrum protection, the Broad Spectrum SPF value on the PDP will not only indicate the relative level of protection against UVB radiation but will also reflect the level of UVA protection, with increasing SPF values indicating greater protection against both UVA and UVB radiation. For broad spectrum products, linking the amount of UVA protection to the SPF value, is consistent with the approach taken in Europe (Ref. 7).

For broad spectrum products, we are requiring the broad spectrum statement on the PDP to appear in combination with the SPF statement. For example, an SPF 40 sunscreen product which passes the broad spectrum test will be labeled "Broad Spectrum SPF 40" in a uniform font style, size, and color and with the same background color. This placement will help consumers recognize that the particular sunscreen product is broad spectrum in conjunction with the SPF value. As previously explained, the broad spectrum statement and SPF value together will provide a relative measure of both UVB and UVA protection. Combining the broad spectrum and SPF statements will help consumers become more aware of the importance of broad spectrum protection.

Under the 2007 proposed rule, if an OTC sunscreen product was not tested for or did not protect against UVA radiation, the statement "No UVA protection" would have been required on the PDP (proposed 21 CFR 352.50(b)(1)). Ten submissions argued against requiring this statement (Ref. 1). Some submissions argued that this statement is misleading because all sunscreen products provide some UVA protection. Submissions also stated that a negative statement is inconsistent with the OTC Drug Review because a drug should only describe the indications for which it is effective. Other submissions suggested that we should require all sunscreen products to provide UVA and UVB protection, making this statement unnecessary.

We have concluded that the "No UVA Protection" statement is not necessary and could be misleading. Under this final rule, the labeling on the PDP of sunscreens no longer refers the type of UV radiation (UVA or UVB) protection offered; rather, products that pass the critical wavelength test in 201.327(j) are labeled with "Broad Spectrum SPF" values. Under this labeling, consumers who see "UVA" on the PDP, even if it is part of the statement "No UVA Protection," may mistakenly believe that the product offers UVA protection. To eliminate this potential misunderstanding, we are not including the "No UVA Protection" statement on the PDP.

In contrast to four submissions requesting that we make the UVA statement less prominent than the SPF statement, we are requiring the SPF and broad spectrum statements to be equally prominent on the PDP by appearing as a combined statement. The four submissions stated that they believe UVB radiation contributes more to skin cancer and photodamage than UVA radiation and argued that more prominence should be given to the SPF statement. However, none of the submissions included data to support this argument. Some submissions suggested that consumers are familiar with SPF ratings and that providing another rating with similar prominence may mislead and confuse consumers.

It is well known that both UVA and UVB radiation contribute to photodamage and skin cancer (Refs. 6–7 and 10–12). Therefore, in our view, providing consumers with information about the effectiveness of a sunscreen product for UVA and UVB radiation protection is equally important. We are requiring that the broad spectrum statement be displayed in combination with the SPF statement. The two statements must not be interrupted with any graphics or text. In addition, the broad spectrum statement must be the same font style, size, and color as the SPF statement with the same background color. It is important for consumers to evaluate both statements when making a purchase decision. By requiring this information to be presented with identical prominence on the PDP, consumers should be able to quickly and easily identify sunscreen products that provide broad spectrum protection, as well as the SPF of all sunscreen products. While we are not requiring a negative statement on the

PDP of products that do not pass the critical wavelength test in new 301.327(j), we caution that such products may be misbranded if they include statements regarding UVA protection; such statements may misleadingly imply that the product provides benefits that are similar or superior to those of products labeled with Broad Spectrum SPF values.

C. Water Resistance Statement

In the 2007 sunscreen proposed rule (proposed 21 CFR 352.52), we allowed the PDP of OTC sunscreen products to contain the statement "water resistant" if a sunscreen product was shown to retain the labeled SPF value after 40 minutes of water immersion, or "very water resistant" if a sunscreen product was shown to retain the labeled SPF value after 80 minutes of water immersion, according to the test in proposed 21 CFR 352.76. We simultaneously proposed that the "Uses" section of labeling (not the PDP) indicate specifically whether the product had been established to be water resistant for 40 minutes or 80 minutes, and included specific directions addressing times for reapplication of each product, dependent on its level of water resistance (proposed 21 CFR 352.52(b)(1)(vii), (b)(1)(viii), (d)(2), and (d)(3); 72 FR 49070 at 49113). In this document, we are revising the PDP to contain the statement "water resistant (40 minutes)" or "water resistant (80 minutes)" as determined by the water resistance test in new 21 CFR 201.327(i)(7). We are removing this information from the indications section of Drug Facts (section IV.B of this document). We continue to include directions based on the duration of water resistance established under the new water resistance test (section IV.D of this document).

One submission stated that including information about water resistance in the indications section as well as in the directions section is "redundant and confusing" (Ref. 1). The submission recommended that we delete the indications statement. We agree with the submission. To eliminate redundancy and simplify the labeling for consumers, we are relocating the information formerly contained within the indication statement to the PDP.

The content of the labeling as a whole is the same as that included in the 2007 proposed rule. However the proposed statement on the PDP did not clearly and accurately convey to consumers the difference between "water resistant" and "very water resistant" sunscreen products. For example, knowing that a sunscreen product is "very water resistant" does not give any indication of how much time a consumer can safely spend in the water. Under the 2007 proposed rule, a consumer would have had to read either the "Uses" or the "Directions" section of the Drug Facts label to determine the duration of water resistance for a sunscreen product (proposed 21 CFR 352.52(b)(1)(vii) and (b)(1)(viii) and proposed 21 CFR 352.52(d)(2) and (d)(3), respectively; 72 FR 49070 at 49113).

Providing, on the PDP, specific information about the actual time (40 or 80 minutes) a consumer can expect a sunscreen product to retain its labeled SPF value is likely to be more helpful to consumers because the information is displayed in one place—on the PDP and not on different parts of the labeling. The revised statements "water resistant (40 minutes)" or "water resistant (80 minutes)" should make it clearer and easier for consumers to understand water resistance as part of their purchase decision. This water resistance information continues to be reinforced by information in the directions regarding reapplication.

D. UVB and UVA Educational Statement

In the 2007 sunscreen proposed rule, we proposed that the following educational statement be included on the PDP of all OTC sunscreen products (proposed 21 CFR 352.50(c)): "UV rays from the sun are made of UVB and UVA. It is important to protect against both UVB and UVA rays to prevent sunburn and other skin damage."

We received four submissions regarding the UVB and UVA educational statement in response to the 2007 sunscreen proposed rule (Ref. 1). The submissions made the following requests:

- Do not require the educational statement on the PDP or
- Combine the educational statement with the sun alert statement and include the combined statement in the "Other Information" section of the Drug Facts label.

We considered including the proposed educational statement on the PDP. We concluded that this information is not critical for effective use of sunscreen products, particularly since we are no longer requiring other PDP statements to refer separately to UVA and UVB protection. An understanding that the sun produces ultraviolet (UV) rays or that there are two types of UV rays that reach the earth's surface is not necessary to ensure the safe and effective use of sunscreen products. The explanation of these concepts on sunscreen labeling is potentially confusing and could raise additional questions about their meaning. We could not determine a succinct educational statement that would not also be potentially misleading. Therefore, we have concluded that an educational statement should not be required on the PDP.

As noted, submissions also requested that the proposed educational statement be combined with proposed sun alert, included in the proposed rule as a warning. In section IV.C of this document, we address submissions on the sun alert warning, and explain our decision to incorporate the information regarding the role of certain sunscreens in reducing the risk of skin cancer and early skin aging into a new indication and accompanying directions for sunscreens with Broad Spectrum SPF values of 15 or higher. We are retaining a modified warning to be included as the first warning on sunscreen products that are either not broad spectrum or that are broad spectrum with an SPF value less than 15. Because we are not requiring an educational statement on the PDP and are either eliminating or modifying the proposed sun alert warning, the request to combine these two statements is no longer relevant.

IV. Drug Facts Labeling

In September 2004 (69 FR 53801), we delayed the May 16, 2005, implementation date for the Drug Facts final rule (21 CFR 201.66) for OTC sunscreen products until further notice). The Drug Facts final rule (21 CFR 201.66) establishes general labeling format and content requirements for all OTC drugs. With the additional exception of certain OTC drug products in "convenience size" packages (see 67 FR 16304 at 16306 (April 5, 2002), other OTC drug products are already required to comply with 201.66. We delayed implementation of 201.66 for sunscreens so as to avoid the potential that sunscreen manufacturers would have to relabel their products twice within a short time period if a final rule specifying labeling for sunscreens published shortly after the original May 2005 implementation date for the general content and format requirements of the Drug Facts final rule. We published the notice of delay for OTC sunscreens' implementation of the Drug Facts final rule so that such products could simultaneously implement both the general labeling provisions of that rule and the specific labeling provisions for sunscreens when we published a sunscreen labeling final rule. We are now lifting the stay on the implementation of the Drug Facts final

rule for OTC sunscreen products. In this document, we are requiring the same implementation date for the regulations set forth in this labeling and testing final rule (21 CFR 201.327) and in the Drug Facts final rule (21 CFR 201.66) as applied to these sunscreen products.

This action will benefit both consumers and manufacturers. Consumers will benefit by having sunscreen labeling presented in the Drug Facts format that they are familiar with. Manufacturers benefit because they will achieve compliance with two rules through one labeling revision (rather than following the more expensive course of making two labeling changes at two different times).

In 2003 (68 FR 33362, June 4, 2003), we also stayed the part of the skin protectant monograph that describes GRASE combinations of skin protectant and sunscreen active ingredients (21 CFR 347.20(d)). Because this document addresses the labeling and testing of sunscreen products and not the GRASE status of individual sunscreen active ingredients, we are not lifting the stay of 21 CFR 347.20(d).

This document requires much of the Drug Facts labeling included in the 2007 proposed rule. However, we have made several revisions to the proposed labeling. These revisions are discussed in detail throughout the remainder of this section. In addition, table 2 of this document summarizes these revisions as follows:

TABLE 2—SUMMARY OF DRUG FACTS LABELING INCLUDED IN THE 2007 PROPOSED RULE AND THIS FINAL RULE

Drug facts section	2007 Proposed rule	This final rule
Active Ingredients/ Purpose.	Name and amount of ingredient(s) followed by "sunscreen"	Name and amount of ingredient(s) followed by "sunscreen."
Uses	• [low, medium, high, or highest] UVB sunburn protection • [low, medium, high, or highest] UVA protection • retains SPF after 80 minutes of activity in the water	• for all sunscreen products: "helps prevent sunburn." • Optional, for sunscreen products with Broad Spectrum SPF values of 15 or higher, "if used as directed with other sun protection measures (see Directions), decreases the risk of skin cancer and early skin aging caused by the sun."
Warnings	*UV exposure from the sun increases the risk of skin cancer, premature skin aging, and other skin damage. It is important to decrease UV exposure by limiting time in the sun, wearing protective clothing, and using a sunscreen.* *For external use only* *Stop use and ask a doctor if* skin rash occurs *When using this product* keep out of eyes. Rinse with water to remove. *Keep out of reach of children.* If swallowed, get medical help or contact a Poison Control Center right away.	For sunscreen products that are not broad spectrum or for products that are broad spectrum with an SPF value less than 15, Skin Cancer/Skin Aging Alert [in bold font]: Spending time in the sun increases your risk of skin cancer and early skin aging. This product has been shown only to help prevent sunburn, not [in bold font] skin cancer or early skin aging. For all sunscreens: *For external use only* *Do not use* on damaged or broken skin *Stop use and ask a doctor if* rash occurs *When using this product* keep out of eyes. Rinse with water to remove. *Keep out of reach of children.* If swallowed, get medical help or contact a Poison Control Center right away.

Directions	*Non-Water Resistant Product*
	• apply liberally [# minutes] before sun exposure
	• reapply at least every 2 hours and after towel drying, swimming, or sweating
	• apply and reapply as directed to avoid lowering protection
	• children under 6 months: Ask a doctor
	Water Resistant Product
	• apply liberally [# minutes] before sun exposure
	• reapply after 40 [or 80] minutes of swimming or sweating and after towel drying. Otherwise, reapply at least every 2 hours.
	• apply and reapply as directed to avoid lowering protection
	• children under 6 months: Ask a doctor
	Water Resistant and Non-Water Resistant Products
	No statement
		Non-Water Resistant Product
		• apply liberally 15 minutes before sun exposure
		• use a water resistant sunscreen if swimming or sweating
		• reapply at least every 2 hours
		• children under 6 months: Ask a doctor
		Water Resistant Product
		• apply liberally 15 minutes before sun exposure
		• reapply:
		  • after 40 [or 80] minutes of swimming or sweating
		  • immediately after towel drying
		  • at least every 2 hours
		• children under 6 months: Ask a doctor
		Water Resistant and Non-Water Resistant Products
		For sunscreens with Broad Spectrum SPF values of 15 or higher:
		• Sun Protection Measures [in bold font]. Spending time in the sun increases your risk of skin cancer and early skin aging. To decrease this risk, regularly use a sunscreen with a Broad Spectrum SPF value of 15 or higher and other sun protection measures including:
		  • limit time in the sun, especially from 10 a.m.–2 p.m.
		  • wear long-sleeved shirts, pants, hats, and sunglasses.

Inactive Ingredients	List inactive ingredients in alphabetical order	List inactive ingredients in alphabetical order.
Other Information	No required statements	• protect this product from excessive heat and direct sun.
Questions?	No required statements	No required statements.

A. Active Ingredients/Purpose

We received one submission regarding the listing of active ingredients and one submission requesting that we provide specific details about what each ingredient does in the product (Ref. 1). One of these submission also requested that we require listing of the percentage of each active ingredient next to the ingredient name.

We are not making any changes to the "Active ingredients/Purpose" section of the Drug Facts label. The general OTC labeling regulations specify that the "quantity of each active ingredient per dosage unit" be listed with the established name of each active ingredient (21 CFR 201.66(c)(2)). Therefore, every sunscreen product is already required to include the active ingredient names followed by the percentage (weight per volume) in the "Active ingredients/Purpose" section, as requested by the first submission.

We are not requiring specific details about what each ingredient does in the product. The function of each active ingredient in an OTC drug product is already required to be listed by 21 CFR 201.66(c)(3), which specifies that the "Active ingredients/Purpose" section of the label list the "general pharmacologic categories or principal intended actions of each active ingredient." There is not currently a requirement to list the purpose of inactive ingredients on OTC drug labels. This information is not needed to safely and effectively use sunscreen products. Therefore, in this document, we are not requiring the purpose of inactive ingredients to be listed on sunscreen labels.

B. Uses

1. Indications Statements Proposed in the 2007 Proposed Rule

The 2007 proposed rule included three indication statements under "Uses" in Drug Facts:

1. Level of UVB sunburn protection (proposed 21 CFR 352.52(b)(1)(i)–(b)(1)(iv))
2. Level of UVA protection (proposed 21 CFR 352.52(b)(1)(v) and (b)(1)(vi))
3. Extent of water resistance (proposed 21 CFR 352.52(b)(1)(vii) and (b)(1)(viii))

The first statement would have appeared on all monograph sunscreen products. The second statement would only have appeared on monograph sunscreen products providing UVA protection. The third statement would only have appeared on monograph sunscreen products that are water resistant for either 40 or 80 minutes. We received numerous submissions from the public concerning these statements following publication of the 2007 proposed rule (Ref. 1).

We are not requiring these indication statements in this final rule. Instead, all sunscreen products covered by this rule will be required to include the indication statement "helps prevent sunburn," as required in the 1999 sunscreen final rule (64 FR 27666; new 21 CFR 201.327(c)(1)). We are requiring this statement instead of the first proposed statement (level of UVB sunburn protection) because we agree with submissions arguing that sunburn is not caused solely by UVB radiation (Ref. 1). We also agree with submissions arguing that the SPF value by itself on the PDP informs consumers of the level of sunburn protection, so a separate description of the level of sunburn protection does not need to be included as an indication.

In addition, sunscreen products covered by this rule that provide broad spectrum protection according to the test in new 21 CFR 201.327(j) and have SPF values of 15 or higher, may include the following indication statement (new 21 CFR 201.327(c)(2)(i)): "if used as directed with other sun protection measures (see Directions), decreases the risk of skin cancer and early skin aging caused by the sun." This statement replaces the second proposed indication statement. We are allowing this statement for certain sunscreens covered by this rule based on available clinical studies, the fact that UV radiation from the sun is harmful, and the scientific understanding that substantially limiting overall UVB and UVA exposure reduces the risk of skin cancer and early skin aging.

As discussed in the remainder of this section of the document, it is critical that the indication statement regarding skin cancer and early skin aging includes information about using the products as directed and following other sun protection measures (listed under the heading Directions). We have concluded that the reference to other sun protection measures is necessary to ensure that the consumer's overall UV exposure is substantially decreased. A consumer who relies on the use of a sunscreen with Broad Spectrum SPF value of 15 or higher alone may not obtain a meaningful net decrease from the risk of skin cancer or early skin aging if, because he or she is wearing the sunscreen, the consumer spends more time in the sun and/or wears less protective clothing. In fact, reliance on sunscreen use alone, without also employing other sun protection measures, could actually result in an increase in the consumer's overall UV exposure. Therefore, if the indication statement regarding decreasing risk of skin cancer and early skin aging does not include the information about using the product as directed, which includes following other sun protection measures, the statement will be considered misleading (and thus make a sunscreen product misbranded) (new 21 CFR 201.327(c)(3)). Similarly, sunscreen products covered by the rule that provide broad spectrum with SPF values between 2 and 15 or do not provide broad spectrum protection should not state or imply that the use of a sunscreen product alone will reduce the risk of skin cancer or early skin aging. Doing so would cause the product to be misbranded.

We are not including the third proposed indication statement (regarding water resistance) in this document. As already discussed, under this final rule, information about water resistance is included on the PDP, as well as under "Directions" in Drug Facts (see sections III.C and IV.D of this document). We conclude that information about the water resistance of a sunscreen product is more effectively and accurately presented on the PDP and as a direction than as an indication statement. The extent of water resistance informs a consumer about how long the SPF value is retained following water exposure and, therefore, how long an interval can elapse before reapplying the sunscreen product (40 or 80 minutes). In addition, the PDP requirements in this document include the time interval as part of the water resistance statement, so that consumers can readily distinguish between products on this basis when making purchasing decisions. Because we include water resistance on the PDP and under "Directions," we are not including a separate indication statement about water resistance in this document.

2. Statement Regarding Skin Cancer and Early Skin Aging

a. Submissions Arguing For a Skin Cancer and Early Skin Aging Indication

As already stated, in this final rule we have adopted, for the first time, an indication for skin cancer and early skin aging for sunscreen products covered by the rule that have Broad Spectrum SPF values of 15 or higher. In our 2007 proposed rule, we had included indication statements that indicated the degree of protection against both UVB and UVA radiation but that linked UVB protection only to sunburn prevention and did not expressly link UVA

protection to any specific health benefit (proposed 21 CFR 352.52(a)). At the same time, however, we had proposed both an educational statement on the PDP stating that UV rays from the sun are made of both UVB and UVA and that it is important to protect against both types of radiation to prevent sunburn and other skin damage (proposed 21 CFR 352.50 (c)). We also proposed a "sun alert" statement as the first warning. This first warning read, "UV exposure from the sun increases the risk of skin cancer, premature skin aging, and other skin damage. It is important to decrease UV exposure by limiting time in the sun, wearing protective clothing, and using a sunscreen." (proposed 21 CFR 352.52(c)(1)).

In response to our proposed rule, we received a total of 12 submissions asking that we include a specific statement regarding reduction in risk of skin cancer and early skin aging as an indication for covered sunscreens (Ref. 1). The submissions asked that we allow an indication statement informing consumers that the regular, consistent, or continued use of a sunscreen product reduces or helps reduce the risk or chance of developing skin damage, early skin aging, and some types of skin cancer (Ref. 1). These submissions also supported our proposed requirement of a "sun alert" on the labeling to inform consumers of the need to limit time in the sun and wear protective clothing. The submissions came from sunscreen manufacturers and public health organizations including the American Academy of Dermatology, the American Cancer Society, and the Skin Cancer Foundation. Many of the submissions provided references to studies that they argued support the inclusion of this indication statement. One submission specifically requested that we allow an anti-aging claim (without mention of skin cancer), and one other submission argued that no sunscreen can claim to prevent cancer (Ref. 1). We received no new data to accompany these requests for a separate indication that the regular use of sunscreen decreases the risk of skin cancer and early skin aging. However, on reconsideration of the data reviewed prior to the 2007 proposed rule, we agree with the argument that the data underpinning our proposed education statement and warning are sufficient to support an appropriately qualified skin cancer and premature skin aging indication for one subset of sunscreens covered by this rule—those that have Broad Spectrum SPF values of 15 or higher. As a result, our final rule provides different labeling for these sunscreens than for sunscreens covered by the rule that are not broad spectrum or that provide broad spectrum with SPF values less than 15. In addition, we conclude that such an indication should not be included in the Warnings section of Drug Facts. We have concluded that, as proposed in 2007, the second sentence of the first warning (*i.e.*, the "Sun Alert" warning) is an implied indication: "It is important to decrease UV exposure by limiting time in the sun, wearing protective clothing, and using a sunscreen." Because it follows a warning that "UV exposure from the sun increases the risk of skin cancer, premature skin aging, and other forms of skin damage," the second sentence implies that using any sunscreen, regardless of SPF value or broad spectrum protection, and following other sun protection measures will decrease the risks of skin cancer, early skin aging, and other consequences of UV exposure to the sun. We have concluded, based on a reconsideration of data previously reviewed in the 2007 proposed rule, that, if consumers use broad spectrum sunscreens with SPF values of 15 or higher and follow other sun protection measures, they can reduce their risk of skin cancer and early skin aging. For these products, we agree with the public submissions that this information is most appropriately placed as an indication (*i.e.*, under Uses) with a reference to the need to use the product as directed with other sun protection measures. For these products, we include under the heading Directions, specific reference not only to regularly use sunscreens with Broad Spectrum SPF values of 15 or higher (the subset of sunscreens for which the indication is allowed) but also to employ the other listed sun protection measures listed under Directions. For sunscreen products covered by this rule that are not Broad Spectrum or that are broad spectrum with an SPF value less than 15, however, we conclude that existing data are insufficient to support an indication for reducing risk of skin cancer or early skin aging. In the sections that follow, we explain the specific scientific basis for our conclusion, as well as explain our rationale for the specific framing of the labeling, as included in the final rule, for both subsets of the sunscreens covered by the final rule—those that have Broad Spectrum SPF values of 15 or higher and those that do not have Broad Spectrum or that are Broad spectrum with SPF values less than 15.

b. Limiting Overall UV Exposure Reduces Risk of Skin Cancer and Early Skin Aging

For drugs subject to OTC monographs, like sunscreen products, indication statements about the effectiveness of the drug products must be supported with scientific data (21 CFR 330.10(a)(4)(ii)). In order for an OTC drug to be considered generally recognized as effective (GRAE), there must be a reasonable expectation that, in a given proportion of the target population, the drug will provide clinically significant relief of the type claimed (21 CFR 330.14(a)(4)(ii)). Based on the available data concerning the harmful effects of UV radiation and sunscreen UV protection, we have concluded that sunscreens, in conjunction with the critical behavioral steps of limiting time in the sun particularly during the midday hours and wearing protective clothing (long sleeve shirt, pants, hat, and sunglasses), provide "clinically significant relief" in reducing the risk of skin cancer and early skin aging. Based on the available data, we have limited this claim to broad spectrum sunscreen products with an SPF value of 15 or higher.

UV radiation from the sun has been associated with nonmelanoma skin cancers since 1927 and with melanomas since 1952 (Ref. 13). It is estimated that as much as 90 percent of melanomas and nonmelanomas are caused by sun exposure (Ref. 5). In 1992, the International Agency for Research on Cancer (IARC), under the auspices of the World Health Organization, identified UV radiation as a human carcinogen [3] (Ref. 14). More recently, broad spectrum UV radiation was listed as a human carcinogen in the National Toxicology Program's 11th Report on Carcinogens issued in 2005 (Ref. 15). It is important to note that this report indicates that UVB and UVA radiation across the spectrum are known human carcinogens, but that either UVB radiation alone or UVA radiation alone is "reasonably anticipated to be a human carcinogen." This classification is due to the fact that the exact wavelengths of UV radiation that cause different harmful effects (*e.g.*, DNA damage or loss of skin elasticity) have not yet been identified. It is clear, though, that broad spectrum UV radiation causes skin cancer. Broad spectrum UV radiation has also been shown to cause other types of skin damage, including early skin aging (Refs. 6 and 16). Therefore, we agree

[3] A carcinogen is anything that is known to cause the development of cancer. UV radiation is known to cause skin cancer.

with the principle that a reduction, of sufficient magnitude, in broad spectrum UV exposure should reduce the risk of harmful effects to the skin, including skin cancer and early skin aging.

Broad spectrum sunscreens, by absorbing UVA and UVB radiation, decrease consumer exposure to both types of UV radiation from the sun that reach the earth's surface. Other critical behavioral steps, such as limiting time in the sun and wearing protective clothing, also decrease consumer exposure to UVA and UVB radiation. After considering the submissions and other available data, we have concluded that a claim for the reduction in risk of skin cancer and early skin aging is appropriate for certain sunscreen products, when the claim also includes the requirement that consumers use the product as directed and the Directions specify other sun protection measures be followed (see section IV.D of this document). We are basing this claim on the scientific understanding of the harm from UVA and UVB radiation and the absorption and/or reflection of that UV radiation by broad spectrum sunscreens, as well as data from studies concerning sunscreen use and the development of skin cancer or precursors of skin cancer (section IV.B.2.c of this document).

For a sunscreen to be effective (*i.e.*, provide "clinically significant relief") in reducing the risk of skin cancer and early skin aging, consumers must not increase their overall exposure to UV radiation by overreliance on sunscreen use. Other behavioral factors could account for such an increase, such as the amount of time spent in the sun and the use of protective clothing. If consumers rely on sunscreen use to spend more time in the sun and/or to wear less protective clothing, then consumers could actually increase their overall UV exposure, which would eliminate the effectiveness of sunscreen use in reducing the risk of skin cancer and early skin aging.

To illustrate this point, it is helpful to consider what has been termed the "compensation hypothesis." As we noted in the 2007 proposed rule, the compensation hypothesis states that consumers who wear high SPF sunscreens generally spend more time in the sun and/or wear less protective clothing (72 FR 49070 at 49086). If the hypothesis is true, consumers would not reduce their risk of skin cancer or early skin aging because their overall UV exposure increases, even though a properly applied (and reapplied) sunscreen absorbs UV radiation and helps prevent sunburn. We cited two retrospective studies which support the compensation hypothesis in the 2007 proposed rule (72 FR 49070 at 49086). Reynolds *et al.* published a study in 1996 finding, in a study of 509 sixth-graders, that adolescents who used sunscreen on both Saturday and Sunday of a Labor Day weekend spent significantly more time in the sun than those who used sunscreen only one day or not at all (Ref. 17). In the second study, parents of 503 children, aged less than 2 to 12 years, were surveyed as to parental attitudes about their children's sun exposure (Ref. 18). The authors reported that "sunscreen use in children was significantly associated with longer duration of sun exposure" (Ref. 18).

Increased overall UV exposure might, in fact, increase the risk of skin cancer and early skin aging, despite the proper use of sunscreens. Likewise, if consumers limit time in the sun, especially during midday, and wear more protective clothing (such as broad brimmed hats, long pants, and long sleeve shirts) while outside, but do not use sunscreens for areas of the skin exposed to the sun (such as parts of face and neck), then the consumer may not decrease the risk of skin cancer and early skin aging for sun-exposed areas. For these reasons, for products that are entitled to include an indication for reducing the risk of skin cancer and early skin aging, we continue to direct consumers to follow a comprehensive sun protection program that includes use of sunscreens with Broad Spectrum SPF values of 15 or higher, limiting time in the sun, and wearing protective clothing, similar to the sun protection measures discussed in the 2007 proposed rule (72 FR 49070 at 49089). Nearly identical multi-step behavioral sun protection programs are advocated by a number of medical and public health organizations, including the American Academy of Dermatology, the Skin Cancer Foundation, and the American Cancer Society.

We have concluded that a comprehensive sun protection approach is critical to ensure that consumers who are seeking to obtain a reduction in the risk of skin cancer and early skin aging limit their overall sun exposure. Without the reduction in consumers' overall UV exposure, even a sunscreen with Broad Spectrum SPF value of 15 or higher may not be effective in decreasing the risk of skin cancer and early skin aging. As discussed below, the available clinical studies do not control for these behavioral factors and, therefore, do not demonstrate that even this subset of sunscreens alone reduce the risk of skin cancer and early skin aging. However, based on the scientific understanding of the harm from UV exposure and our assessment of the study data, we have concluded that if consumers use sunscreens with Broad Spectrum SPF values of 15 or higher, limit time in the sun especially during the midday hours, and wear protective clothing when exposed to the sun, the resulting reduction in overall UV exposure will reduce the risk of skin cancer and early skin aging. Therefore, there is sufficient evidence of "clinically significant relief" to justify the indication and related directions for this subset of products, as set forth in the rule. However, we conclude that the omission of prominent information in the indication regarding the need for other sun protection measures would misbrand the product, as would the omission of the associated direction specifying these measures. Indeed, it would suggest a different indication than that which available evidence supports. Consequently, we have included in this final rule a new provision indicating that "Any labeling or promotional materials that suggest or imply that the use, alone, of any sunscreen reduces the risk of or prevents skin cancer or early skin aging will cause the product to be misbranded under section 502 of the FD&C Act (21 U.S.C. 352)." (new 21 CFR 201.327(c)(3)).

c. Available Scientific Data

We are not aware of any data other than what we reviewed in the 2007 proposed rule that evaluate the effectiveness of sunscreens in reducing the risk of skin cancer or early skin aging for healthy subjects. One more recent study, published in 2009, found that regular use of Broad Spectrum SPF 50+ sunscreen "may prevent" the development of actinic keratoses and non-melanoma skin cancer in immune-compromised organ transplant recipients (Ref. 19). We have not relied on this study in reaching our conclusions regarding OTC sunscreens, because we do not consider the immune-compromised study population to be representative of the general population.

We have re-evaluated the data originally reviewed in preparing the 2007 proposed rule to determine whether those data support allowing the indication for all sunscreen products or only for certain sunscreen products. Based on our re-evaluation, we have concluded that the data is supportive of an indication for broad spectrum sunscreens having SPF values of at least 15. Further, we have determined that, while the existing evidence does not support a claim for the use of any sunscreen alone, it does support an indication that the combination of using

a sunscreen with Broad Spectrum SPF value of 15 or higher along with other sun protection measures, reduces the risk of skin cancer and early skin aging, consistent with other positions in the 2007 proposed rule (72 FR 49070 at 49087 through 49090).

To date, there are no clinical studies demonstrating that use of any sunscreen alone can prevent skin cancer. There are two prospective[4] studies that directly examine the role of sunscreen products in preventing skin cancer. Although it did not show any difference in primary endpoints, a large 1999 study conducted in Australia demonstrated that people who applied a Broad Spectrum SPF 15 sunscreen product on a daily basis over a 4.5 year period had a lower overall incidence of one type of skin cancer, squamous cell carcinoma, on the head, neck, arms, and forearms than study participants who did not apply sunscreen (28 cases in the broad spectrum sunscreen group vs. 46 cases in the group not using broad spectrum sunscreen) (Ref. 20). In an extension of that study, van der Pols *et al.* evaluated the same population of subjects over an additional 8 years, and found that the sunscreen users continued to have a statistically significant lower incidence of squamous cell carcinoma over the entire 12.5 year period (Ref. 21). Neither study found that daily sunscreen use had any measurable effect on the most common form of skin cancer, basal cell carcinoma. Further, we are not aware of any studies examining the effect of sunscreen use on the development of melanoma, which is the deadliest form of skin cancer.

Although data from clinical studies addressing the specific end points of cancer is limited, some prospective studies have evaluated the effects of regular sunscreen use on the development of surrogate skin lesions that can be precursors to cancer: actinic keratoses and melanocytic nevi. A small percentage of actinic keratoses progress to squamous cell carcinomas (Ref. 22). At least four studies have demonstrated that the number of actinic keratoses is lower for individuals regularly using sunscreens with Broad Spectrum SPF values of 15 or higher (Refs. 23 through 26). We are not aware of any studies examining the potential effects on surrogate skin lesions of sunscreens that either are not broad spectrum or are broad spectrum with SPF values less than 15.

Two prospective studies have shown that regular use of a Broad Spectrum SPF 30 sunscreen reduces the risk of developing melanocytic nevi, which can progress into melanomas (Ref. 22). In a 2000 study, Gallagher *et al.* examined the formation of new melanocytic nevi in 393 Canadian school children. The group of children given Broad Spectrum SPF 30 sunscreen product had fewer new nevi over the course of the three year study than did children not given sunscreen products or advice on sunscreen use (Ref. 27). The difference was small (24 v. 28 nevi, respectively), but statistically significant ($p = 0.048$). In a follow-up study published in 2005, Lee *et al.* evaluated the same group of children for differences in melanocytic nevi by location on the body and demographic factors (Ref. 28). These investigators found that the sunscreen group had significantly fewer new nevi on the trunk than the control group ($p = 0.05$).

With respect to the role of sunscreen products in decreasing the risk of early skin aging, we are aware of only indirect evidence that sunscreen use decreases early skin aging. One recent study demonstrated that a broad spectrum sunscreen product can reduce the extent of solar UV-induced damage to factors associated with early skin aging even when the SPF value is less than 10 (Ref. 29). Although this study was small, evaluating only 12 Caucasian subjects, it shows the importance of broad spectrum protection. These findings have been corroborated in a large number of studies using broad spectrum sunscreens with SPF values ranging from 19 to 50, as reported by Fourtanier *et al.* in two recent reviews (Refs. 10 and 30).

Neither those studies evaluating the long term effect of regular sunscreen use on the development of skin cancer and early skin aging nor those evaluating the long term effect of sunscreen use on surrogate markers for these conditions were adequately controlled. Such studies, which must take place over many years, make adequate controls extremely difficult, if not impossible to implement. For example, one cannot control for time and duration of exposure, application and re-application amounts, or use of supplemental behavioral measures such as wearing protective clothing for a study which takes place over several years.

Despite their limitation, the results of the short-term effectiveness studies are consistent with our understanding that measures which significantly reduce UV exposure decrease the risk of skin cancer and early skin aging. UVA and UVB radiation is the only known external risk factor for skin cancer and early skin aging. Therefore, measures that significantly reduce both UVA and UVB exposure should decrease the risk of skin cancer and early skin aging. Based on this understanding, limiting time in the sun, wearing protective clothing and using a broad spectrum sunscreen with an SPF value of 15 or higher should decrease the risk of skin cancer and early skin aging. Using a broad spectrum sunscreen with an SPF value of 15 or higher ensures adequate breadth and magnitude of UVA and UVB protection. For these products, the broad spectrum test measures breadth and SPF test measures magnitude of UV protection. Consistent with this scientific principle, the short-term effectiveness studies demonstrate a decrease in the development of surrogates for skin cancer and early skin aging. Thus, we have concluded that the available evidence supports our finding that sunscreen products, in conjunction with limiting time in the sun and wearing protective clothing, reduce the risk of developing skin cancer or early skin aging.

d. Indication Limited to Covered Sunscreens With Broad Spectrum SPF Values of 15 or Higher

In light of the submissions requesting that we reframe our labeling information regarding sunscreen use and reduced risk of skin cancer and premature skin aging as an indication, we re-evaluated skin cancer and aging studies discussed in the 2007 proposed rule to determine whether the skin cancer and early skin aging indication should apply to all sunscreen products or be limited to certain sunscreen products. Available data support this indication only for broad spectrum sunscreens with SPF values of 15 or higher. Several reports have indicated that UV-induced skin damage associated with both skin cancer and early skin aging can be reduced by the use of broad spectrum sunscreens (Refs. 10 and 29 through 31). In a direct comparison of a broad spectrum sunscreen and a non-broad spectrum sunscreen with the same SPF, Moyal and Fourtanier found that the broad spectrum sunscreen provided significantly better protection from UV radiation-induced immunosuppression, a factor associated with both skin cancer and early skin aging (Ref. 32). Furthermore, the National Toxicology Program classified broad spectrum UV radiation as a known human carcinogen because it is not clear which UVB and/or UVA wavelengths contribute to the development of cancer (Ref. 15).

[4] A prospective study is designed to study subjects under pre-specified conditions. These studies differ from retrospective studies that try to prove hypotheses by assessing past experiences. Generally, prospective studies are superior to retrospective studies in demonstrating drug effectiveness.

Therefore, available data indicate that a broad spectrum sunscreen is necessary to reduce the risk of skin cancer. Likewise, we do not know which UVB and/or UVA wavelengths contribute to early skin aging. Therefore, it is reasonable to conclude that reducing the risk of early skin aging also requires a broad spectrum sunscreen (in conjunction with limiting time in the sun and wearing protective clothing).

With regard to SPF value, the available study data concerning the use of sunscreens in reducing the risk of skin cancer is based on products with SPF values of 15 or higher. The sunscreen product used in the 1999 Australian study on skin cancer (squamous cell and basal cell carcinomas) had a Broad Spectrum SPF value of 16, and those that were found to reduce actinic keratoses and nevi had SPF values ranging from 16 to 46. The studies on early skin aging make it difficult to know for certain whether Broad Spectrum SPF values of 15 or higher are necessary to reduce the risk of early skin aging. However, we conclude that the data regarding the minimum sunscreen protection necessary to reduce the risk of skin cancer can be extrapolated to early skin aging. In many ways, the biological processes that take place in response to UV radiation are similar for both conditions. For both skin cancer and early skin aging, UV radiation causes damage in the skin that is not completely repaired and leads to cancer, fine lines, wrinkles, etc. Because the supporting data for a skin cancer claim are based on products with SPF values of 15 or higher, we are only allowing the skin cancer and early skin aging claim for covered sunscreen products that are broad spectrum and have SPF values of at least 15. This rule does not preclude approval of a new drug application including an indication for reduction in risk of skin cancer and early skin aging for any sunscreen product. To be approved, such an application must be supported by the submission of adequate data. This rule also does not preclude future amendment of the sunscreen monograph in 21 CFR part 352, if additional data are provided to support a similar indication for other types of sunscreens.

e. Precedent for an Indication Statement That Includes Behavior Modification

There is at least one other OTC drug product with an indication statement that describes not only the drug's intended effect but also one or more behavioral measures to ensure the effect. The indication statement on the weight loss aid orlistat states that the product is to be used "for weight loss in overweight adults, 18 years and older, when used along with a reduced-calorie and low-fat diet" (Ref. 33). The behavioral measure of reduced caloric intake is necessary for consumers to experience weight loss. A low-fat diet is necessary for consumers to avoid the undesirable side effect of diarrhea caused by consuming a high-fat diet while taking orlistat.

The need to include reduced caloric intake as part of the indication statement for orlistat is similar to the need for including the use of other sun protection measures as part of the indication statement for sunscreens. Orlistat increases the likelihood of weight loss by preventing fat from being absorbed as food is digested in the stomach and intestines. If consumers take orlistat and decrease their caloric intake, they increase the likelihood of losing weight. However, if consumers increase their caloric intake while taking orlistat, they are less likely to lose weight. Orlistat's effect of preventing fat absorption could be offset by the high number of calories being eaten. Similarly, the reduction in UV exposure afforded by use of broad spectrum sunscreens with SPF values of 15 or higher can be offset if consumers increase their UV exposure by spending more time in the sun and/or wearing less protective clothing. This increased overall exposure could eliminate the effectiveness of sunscreen use in reducing the risk of skin cancer and early skin aging.

The labeling of prescription cholesterol-lowering drug products (*i.e.*, statins) follows a similar principle by emphasizing that reduction of cholesterol levels requires not only use of the drug product but also a healthy diet. The National Institutes of Health (NIH) specifies therapeutic lifestyle changes that can be followed to lower levels of cholesterol in the blood (Ref. 34). These changes include following a diet restricted in saturated fat and cholesterol, exercising regularly, and managing weight. Used in conjunction with cholesterol reducing drugs (currently available only by prescription), these lifestyle changes improve the chance of effectively treating high cholesterol levels.

Prescription cholesterol-lowering drug products include the behavioral step of following a low fat diet in the indication statement (Ref. 35). The body produces cholesterol, which the drug product inhibits to produce the desired drug effect of lowering cholesterol being made by the body. However, the total cholesterol circulating in the blood reflects cholesterol made by the body plus cholesterol absorbed from foods containing fats. Therefore, if consumers use a statin and minimize the amounts of food containing fats in their diet, then they will reduce the total cholesterol level in the blood. However, if consumers do not minimize the amounts of food containing fats in their diet, they may not reduce the total cholesterol in the blood. The decreased cholesterol production in the body caused by the statin may not be significant compared to the high amount of cholesterol derived from food eaten by consumers.

In the same way that regularly taking an OTC weight loss aid or a prescription cholesterol-lowering drug product without also following a healthy diet may not result in the intended health effect, use of a sunscreen with Broad Spectrum SPF value of 15 or higher without also limiting time in the sun and covering sun-exposed areas may not result in a net reduction in the risk of developing skin cancer or early skin aging. For this reason, we are requiring that the indication statement allowed on sunscreens with Broad Spectrum SPF values of 15 or higher include all parts of the sun protection program and not suggest or imply that use of a sunscreen alone reduces the risk of skin cancer or early skin aging.

C. Warnings

We received submissions requesting that we revise warnings included in the 2007 proposed rule and that we add new warnings not included in the 2007 proposed rule (Ref. 1). In section IV.C.1 of this document, we discuss one new and one revised warning included in this final rule. We are adding the new warning "Do not use on damaged or broken skin" (new 21 CFR 201.327(d)(1)). We are revising the warning about skin rash (proposed 21 CFR 352.52(c)(3)): "Stop use and ask a doctor if skin rash occurs" to read "Stop use and ask a doctor if rash occurs."

In section IV.C.2 of this document, we discuss our revision to the proposed "Sun Alert" warning. Under this final rule, the warning proposed for all monograph sunscreens is replaced with an optional indication and required direction on covered sunscreens with Broad Spectrum SPF values of 15 or higher, while covered sunscreens that are broad spectrum with SPF values less than 15 or that do not provide broad spectrum protection will bear a revised warning, called the "Skin Cancer/Skin Aging Alert." (new 21 CFR 201.327(d)(2)).

In section IV.C.3 of this document, we discuss three new warnings that were requested in submissions, but are not

being included in this document. Submissions argued that we should add warnings that the regular use of sunscreen products may cause vitamin D deficiency and may reduce the photoprotective effects of tanning. We also considered adding a warning concerning sunscreen products containing alpha hydroxy acids (AHAs). We are not adding any of these warnings because the available data do not support the need for these warnings.

In summary, this document requires the following warnings on all covered OTC sunscreen products (new 21 CFR 201.327(d)):

- "Do not use on damaged or broken skin"
- "Stop use and ask a doctor if rash occurs"
- "When using this product keep out of eyes. Rinse with water to remove."

For all covered sunscreen products that either are not broad spectrum or are broad spectrum with SPF values less than 15, this final rule also requires a "Skin Cancer/Skin Aging Alert" as the first statement under the heading Warnings. In addition to these warnings, all sunscreen products are required to include the "external use" and "keep out of reach of children" warning statements required on all topical OTC drug products (21 CFR 201.66(c)(5)(i) and (c)(5)(x)).

1. New and Revised Warnings for Damaged or Broken Skin and Rash

The new warning that we are requiring on all covered sunscreen drug products reads, "do not use on damaged or broken skin." We require this warning or a similar warning for other topical OTC drug products:

- Acne treatments (21 CFR 333.350(c)(3))
- Skin protectants (21 CFR 347.50(c)(6))
- Antiperspirants (21 CFR 350.50(c)(1))

The safety data for these ingredients are based on application to intact (*i.e.*, unbroken or undamaged) skin. We do not have data of the safe use of these ingredients if the skin is not intact. For the same reason, the warning appears on sunscreen products marketed under new drug applications (NDAs).[5] Therefore, in this document, we are requiring this warning for all covered OTC sunscreen products, which are marketed without approved applications (new 21 CFR 201.327(d)(1)(i)).

In addition to the new warning, we are revising the warning in proposed 21 CFR 352.52(c)(3): "Stop use and ask a doctor if skin rash occurs." We are deleting the word "skin" so that the new warning reads: "Stop use and ask a doctor if rash occurs" (new 21 CFR 201.327(d)(1)(iii)). We received two submissions arguing that the word "skin" is unnecessary in this warning because every rash is a skin rash (Ref. 1). We agree and are removing the word to make the warning more concise. Consumers will likely understand the warning without the word "skin."

2. Revision of the Proposed "Sun Alert" Warning

In 2007, we proposed a warning, based on the "Sun Alert" statement cited in the 1999 stayed sunscreen final rule (64 FR 27666 at 27679), as the first statement under the heading Warnings for all monograph sunscreen products regardless of SPF value or broad spectrum protection (proposed 21 CFR 352.52(c)(1)). As proposed, this warning would have stated, "UV exposure from the sun increases the risk of skin cancer, premature skin aging, and other skin damage. It is important to decrease UV exposure by limiting time in the sun, wearing protective clothing, and using a sunscreen." Submissions regarding this proposed warning are discussed in section IV.B.2 of this document. As noted there, we agree that, as proposed, this warning included an implied indication that all sunscreens reduce the risk of skin cancer and skin aging. Under this final rule, we are no longer requiring a "Sun Alert" or similar warning on broad spectrum sunscreens with SPF values of 15 or higher covered by the rule. This decision is based on our re-evaluation of the available scientific data. We are now permitting an indication stating that, used as directed with other sun protection measures, these sunscreens reduce the risk of skin cancer and premature skin aging (new 21 CFR 201.327 (c)(2)).

For these products we are also requiring a new direction statement (new 21 CFR 201.327(e)(1)(iv)). The direction states:

> Sun Protection Measures. [in bold font] Spending time in the sun increases your risk of skin cancer and early skin aging. To decrease this risk, regularly use a sunscreen with a Broad Spectrum SPF of 15 or higher and other sun protection measures including: [bullet] limit time in the sun, especially from 10 a.m.–2 p.m. [bullet] wear long-sleeved shirts, pants, hats, and sunglasses

We have concluded that information about decreasing sun exposure and wearing protective clothing is more appropriate in "Directions" than in "Warnings." These measures, in addition to use of a sunscreen with Broad Spectrum SPF value of 15 or higher, are necessary for the consumers' sun protection as part of a comprehensive program.

For covered sunscreen products that do not provide broad spectrum protection or those that do provide broad spectrum protection with SPF values less than 15, we conclude that a warning regarding the risks of skin cancer and skin aging remains necessary. In light of comments received on the "Sun Alert" warning proposed in 2007, however, we are revising the text to read as follows: "Skin Cancer/Skin Aging Alert [in bold font]: Spending time in the sun increases your risk of skin cancer and early skin aging. This product has been shown only to help prevent sunburn, not [in bold font] skin cancer or early skin aging." (new 21 CFR 201.327(d)(2). The title "Skin Cancer/Skin Aging Alert" more accurately and specifically conveys the nature of the warning that follows than the proposed "Sun Alert" warning, particularly since the products that will bear this statement are indicated to help prevent sunburn, one consequence of sun exposure. The first sentence of this warning is a factual statement similar in content to the opening statement of the warning proposed in 2007. Like the proposed "Sun Alert" warning, this statement alerts consumers to risks they continue to incur from sun exposure, the conditions under which they will make use of the product. The second sentence clarifies for users the limits on the benefits that the product in hand has been established to provide, specifying that these products have been shown to help prevent sunburn but have not been shown to reduce the risk of skin cancer or early skin aging. Inclusion of this warning is critical to help ensure that consumers do not mistakenly conclude that all sunscreens have been demonstrated to provide the same benefits. It will reinforce the distinction between sunscreens indicated only for preventing sunburn (those that have broad spectrum with SPF values below 15 or that are not broad spectrum) and sunscreens that have also been shown to reduce the risk of skin cancer and early skin aging when used as directed with other sun protection measures (those with Broad Spectrum SPF values of 15 or higher). This warning serves a similar purpose to one required on cosmetic suntanning preparations that do not contain a sunscreen ingredient, which likewise is intended to assist consumers in distinguishing among products that they might otherwise confuse. (See 21 CFR 740.19).

[5] NDAs 21–501, 21–502, 21–471, and 22–009.

3. Warnings Requested in Submissions But Not Included in This Final Rule

We considered adding the following three warnings:

- Sunscreens may reduce the photoprotective effects of tanning
- Increased sun sensitivity caused by alpha hydroxy acids (AHAs) in sunscreen products
- Regular use of sunscreen products may cause vitamin D deficiency

However, as discussed in this section of the document, we conclude that these warnings are not needed for the safe and effective use of sunscreen products.

We received a submission arguing that we should require the following warning on all OTC sunscreen products containing UVA-protective active ingredients (Ref. 1): "The use of this product will prevent the development of photo-protective facultative pigmentation, a.k.a., a tan." The submission implies that UVA protection is not only unnecessary but harmful to consumers. No data were included in the submission.

We agree that tanning caused by UVA radiation offers some protection against sunburn. However, tanning, particularly when attributable to prolonged exposure to UVA radiation in tanning beds or booths, may also have harmful effects on the skin (Refs. 36 and 37). In addition, one study suggests that the protective effects of tanning are small, as a tan only appears to provide an SPF value of approximately 4 (Ref. 36). As stated in the 2007 proposed rule (72 FR 49070 at 49083), we do not know which UVA wavelengths cause specific types of damage (*e.g.*, skin cancer or early skin aging). We continue to assert, however, that protection against UVA radiation is important for consumers' health (72 FR 49070 at 49083). We have concluded that the warning suggested in the submission is not in the best interest of public health because the warning discourages consumers from using broad spectrum sunscreen products. Therefore, we are not requiring any warning related to tanning.

We are not adding any additional warnings to sunscreen products containing AHAs. In the 2007 proposed rule, we requested comment on the need for additional warnings or directions on sunscreen products combined with AHAs (72 FR 49070 at 49110). We made this request in response to a 2005 guidance that we issued for cosmetic products containing alpha hydroxy acids (70 FR 1721, January 10, 2005). The guidance recommends the following warning be included on cosmetic products containing alpha hydroxy acids: "Sunburn Alert: This product contains an alpha hydroxy acid (AHA) that may increase your skin's sensitivity to the sun and particularly the possibility of sunburn. Use a sunscreen and limit sun exposure while using this product and for a week afterwards."

Many cosmetic products containing alpha hydroxy acids also contain sunscreens because the sunscreen helps protect the skin made sensitive to the sun by the alpha hydroxy acids. The guidance does not address products combining alpha hydroxy acids and sunscreens.

Two submissions stated that additional warnings are not necessary on these products (Ref. 1). We agree with these submissions. We considered added a warning or other labeling to inform consumers that AHAs contained in some sunscreen products may make the consumer more likely to sunburn. However, the sunscreen component of such products would, in fact, protect consumers from sunburn. Furthermore, we have concluded that the addition of sunscreen active ingredients to AHA-containing cosmetic products provides valuable UV protection for consumers. Therefore, at this time, we have concluded that a warning about AHA is not necessary on OTC sunscreen products.

The other new warning requested in submissions relates to vitamin D deficiency. We received six submissions arguing that consumers should be warned that frequent sunscreen use may result in vitamin D deficiency (Ref. 1). The submissions cite articles discussing the negative effects of vitamin D deficiency, such as growth retardation, rickets, and osteoporosis (Ref. 38). The submissions include numerous published articles concerning vitamin D, but only four clinical studies that directly examine the effect of sunscreen use on vitamin D levels. In the remainder of this section, we discuss the four studies included in submissions, as well as three additional studies that we located through a literature search. Collectively, the studies do not demonstrate that the use of sunscreen causes vitamin D deficiency.

The term "vitamin D" refers to several forms of the vitamin, but the two forms important to humans are vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol) (Ref. 39). Vitamin D_2 is obtained by eating vitamin D-rich foods such as fish or food fortified with vitamin D. The skin makes vitamin D_3 when it is exposed to sunlight (Ref. 40) and, therefore, vitamin D production may vary depending on the following factors: (1) Skin pigmentation, (2) age, (3) clothing, (4) season, (5) latitude, (6) time of day, (7) weather conditions, and (8) sunscreen application (Refs. 40–43). Vitamin D deficiency has long been associated with Ricketts, but recent research suggests that vitamin D deficiency may also be associated with other diseases (Ref. 38). However, the threshold of vitamin D blood levels that constitutes a deficiency is currently being re-evaluated by scientific experts (Refs. 40, 44, and 45).

To determine whether sunscreen use causes vitamin D deficiency, we reviewed four clinical studies included in the submissions that explored the effect of sunscreen use on vitamin D levels as well as three studies that we identified in a literature search:

- Matsuoka *et al* 1987 (Ref. 46)
- Matsuoka *et al* 1988 (Ref. 47)
- Marks *et al.* 1995 (Ref. 48)
- Farrerons *et al.* 1998 (Ref. 49)
- Kimlin *et al.*, 2007 (Ref. 50)
- Cusack *et al.*, 2008 (ref. 51)
- Hoesl *et al.*, 2010 (Ref. 52).

All but one of these studies assessed 25-hydroxyvitamin D levels because 25-hydroxyvitamin D is typically used as the biological marker for vitamin D (in the D_2 or D_3 form) (Ref. 53). Much of the data available in the literature involves nonclinical studies, which can be difficult to extrapolate to consumer (human) actual use conditions. Studies with clinical data provide more meaningful results because, if adequately designed, they can be more easily extrapolated to consumer actual use conditions. Therefore, we are focusing discussion in this document on the clinical studies.

In the 1987 study by Matsuoka *et al.*, four subjects applied a sunscreen product with an unknown SPF to the entire body, while four control subjects did not apply any topical product (Ref. 46). All of the subjects were exposed to 1 MED[6] of UV radiation (260–330 nm[7]) and then vitamin D_3 levels were monitored for 15 days. The subjects using sunscreen product applied the sunscreen product 1 hour before UV exposure. The level of vitamin D_3 was determined one day before UV exposure to serve as the baseline measure.

The level of vitamin D_3 in the control group (no sunscreen) increased significantly over baseline 1 day after UV exposure (from ~2 ng/ml[8] to 25 ng/ml) and then decreased gradually, returning to baseline 15 days after UV exposure. In contrast, the levels of vitamin D_3 in the sunscreen group did

[6] MED refers to the lowest dose of UV radiation that produces perceptible reddening of the skin.

[7] Nanometers.

[8] Nanograms per milliliter.

not change significantly from the baseline level (5 ng/ml) at each time point.

Based on this preliminary study, Matsuoka *et al.* conducted another study in 1988 (Ref. 47). This study enrolled 40 subjects from Illinois and Pennsylvania with 20 subjects in the control group and 20 subjects in the sunscreen group. Each time they went outdoors for 1 year, the subjects in the sunscreen group, who had a history of skin cancer, applied a sunscreen product with an unknown SPF to all sun-exposed areas of the body.

Serum 25-hydroxyvitamin D levels were measured in each group at the conclusion of the study and were significantly lower in the sunscreen group than the control group: 40.2 and 91.3 nmol/L,[9] respectively. The difference in 25-hydroxyvitamin D levels between the two groups was statistically significant ($p < 0.001$).

Marks *et al.* conducted a randomized, double-blind controlled clinical study over a summer period in Australia (Ref. 48). In this study, 113 subjects over 40 years old who exhibited at least one solar keratosis (a precursor of carcinoma of the skin) were recruited and divided into two groups. The first group of 56 subjects applied an SPF 17 sunscreen cream. Fifty-five subjects in the control group applied a placebo cream. Subjects in both groups were asked to apply their cream on the head, neck, forearm and dorsal side of each hand once a day in the morning and more frequently if sweating, swimming, or involved in activities that might rub off the cream.

The mean levels of 25-hydroxyvitamin D rose significantly by almost the same amount in both groups over the period of the study. The mean level in the placebo group increased by 12.8 mmol/L, whereas the mean level in the sunscreen group increased by 11.8 mmol/L. The difference between these increases from baseline values was not statistically significant.

In 1998, Farrerons *et al.* carried out a study to examine the effects of sunscreen use on vitamin D levels in elderly individuals (Ref. 49). In this 2-year study, 24 subjects (10 men and 14 women with a mean age of 71 years) were enrolled in the sunscreen group. The subjects had actinic keratosis, basal cell carcinoma, or squamous cell carcinoma. None of the subjects had previously used sunscreen products, but were instructed to apply an SPF 15 sunscreen product to sun-exposed areas of the body each morning, avoid mid-day sun, and wear UV-protective clothing during the spring and autumn. The control group of 19 subjects did not use sunscreen product, but had the same skin characteristics. Mean serum levels of 25-hydroxyvitamin D were measured at eight different time points (four in the autumn and four in the spring) over the two-year study period.

The mean serum levels of 25-hydroxyvitamin D were statistically lower in the sunscreen group as compared to the control group at one spring and one autumn time point ($p < 0.05$). However, the mean serum levels of 25-hydroxyvitamin D were not statistically different between the groups at the other 6 spring and autumn time points.

In 2007, Kimlin *et al.* reported that there was "no association" between use of sunscreens with SPF values higher than 15 and blood levels of 25-hydroxyvitamin D in a study of 126 Australian adults 18–87 years of age (Ref. 50). However, the authors stated that mean levels of 25-hydroxy vitamin D increased with increasing frequency of sunscreen use. Interestingly, study "participants who 'usually' or 'almost always' wore a hat when outdoors" were significantly more likely to have higher serum 25-hydroxy vitamin D levels than those who wore hats less often (Ref. 50). On the other hand, study participants who usually or almost always wore long sleeve shirts or pants while outside were statistically more likely to have lower serum 25-hydroxyvitamin D levels than those who wore these types of protective clothing less often (Ref. 50).

In 2008, Cusack *et al.* reported that decreased levels of 25-hydroxyvitamin D levels were only "weakly correlated" with sunscreen usage in 52 Irish patients with cutaneous lupus erythematosus (Ref. 51). This study population was specifically selected because patients with lupus are particularly sensitive to exposure to the sun. While an analysis of the effects of daily sunscreen use on serum levels of 25-hydroxyvitamin D showed the relationship between these two parameters to be significant, a multivariate analysis of the same data was not significant (Ref. 51).

Most recently, in 2010, Hoesl *et al.* reported "no statistically significant association" between serum levels of 25-hydroxyvitamin D and use of the sunscreen drometrizole trisiloxane in a cohort of 15 patients with Xeroderma pigmentosum (Ref. 52). Like those with lupus ertythematosus, patients with Xeroderma pigmentosum are extremely sensitive to the sun. The authors reported that reductions in serum levels of 25-hydroxyvitamin D are "not associated with any type or duration of sun protection applied by these patients" (Ref. 52).

These seven clinical studies are inconclusive because the results were contradictory. Two studies suggest that sunscreens decrease vitamin D levels and the other five studies suggest that sunscreens do not decrease vitamin D levels. In addition, the studies were relatively small, only enrolling 8 to 126 subjects. The study with the greatest number of participants was inconclusive showing that people who regularly used sunscreens and wore hats had increased levels of vitamin D, whereas people who regularly wore pants outside had decreased levels (Ref. 50).

Because the preponderance of currently available data suggests that sunscreen use does not cause clinically meaningful decreases in vitamin D levels (*i.e.*, decreases that lead to vitamin D deficiency and/or disease caused by low levels of vitamin D), we are not including a warning regarding vitamin D deficiency on OTC sunscreen products. In addition, determining whether decreases in vitamin D levels result in vitamin D deficiency is especially difficult because the threshold of vitamin D blood levels that constitutes a deficiency is currently being re-evaluated by scientific experts (Refs. 38, 44, and 45). We recognize that certain subpopulations may be at increased risk of vitamin D deficiency, as pointed out in one submission. However, there are many factors that determine the amount of sun exposure necessary to ensure adequate vitamin D levels (*e.g.*, geographical location, season, skin pigmentation, dietary vitamin D intake). Because of these many other factors, it is difficult for us to determine a meaningful message in sunscreen product labeling for consumers, especially in the absence of conclusive data. If we become aware of data from adequate and well-controlled studies demonstrating that regular use of sunscreen causes vitamin D deficiency, we will re-evaluate this issue.

D. Directions

We received numerous submissions requesting that we revise directions included in the 2007 proposed rule (Ref. 1). In response to those requests and our reevaluation of OTC sunscreen labeling, we are revising the following directions:

- "Reapply after [select one of the following: '40 minutes of' or '80 minutes of' " for products that satisfy either the water resistant or very water resistant test procedures in proposed paragraphs 352.76(a) and (b), respectively] swimming or [select one of the

[9] Nanomoles per liter.

following: 'sweating' or 'perspiring'] and after towel drying. Otherwise, reapply at least every 2 hours" (proposed 21 CFR 352.52(d)(2)).

- "Reapply at least every 2 hours after towel drying, swimming, or sweating" (proposed 21 CFR 352.52(d)(3)).

These two directions are the reapplication instructions for water resistant and non-water resistant products, respectively. We also received five submissions requesting that we revise the direction: "Apply [select one of the following: 'liberally' or 'generously'] [and, as an option: 'and evenly'] [insert appropriate time interval, if a waiting period is needed] before sun exposure" (proposed 21 CFR 352.52(d)(1)(i)). As discussed in this section, we are not revising this direction statement.

In addition to the revisions to these provisions (described in more detail in this section of the document), we are no longer requiring the following proposed direction: "Apply and reapply as directed to avoid lowering protection" (proposed 21 CFR 352.52(d)(1)(ii)).

As already discussed, for covered sunscreen products with Broad Spectrum SPF values of 15 or higher, we are requiring the following direction:

"Sun Protection Measures. [in bold font] Spending time in the sun increases your risk of skin cancer and early skin aging. To decrease this risk, regularly use a sunscreen with a Broad Spectrum SPF of 15 or higher and other sun protection measures including: [bullet] limit time in the sun, especially from 10 a.m.–2 p.m. [bullet] wear long-sleeved shirts, pants, hats, and sunglasses"

(new 21 CFR 201.327(e)(1)(iv)). For these products, this direction most appropriately conveys the information proposed in the "Sun Alert" warning included in the 2007 proposed rule, and provides the necessary directions to complement the new indication permitted for these products.

In addition to the required directions, we will allow the optional direction heading "for sunscreen use" (new 21 CFR 201.327(e)(1)(i)).

1. Revised Directions

We are revising the directions for water resistant sunscreen products (new 21 CFR 201.327(e)(2)) to read:

- Reapply:
- After 40 [or 80] minutes of swimming or sweating
- Immediately after towel drying
- At least every 2 hours

We are also revising the directions for non-water resistant sunscreen products (new 21 CFR 201.327(e)(3)) to read: "[Bullet] reapply at least every 2 hours [bullet] use a water resistant sunscreen if swimming or sweating." These revisions should clarify the directions. We are removing reapplication directions concerning swimming and sweating from non-water resistant products because these products should not be used when swimming or sweating. Instead, we are requiring more accurate directions instructing consumers to use a different sunscreen product—a water resistant sunscreen product—if swimming or sweating.

We considered revising the 2-hour reapplication timeframe because some of the submissions objected to this specific timeframe (Ref. 1). The submissions argued that we should require the word "often" instead of a 2-hour reapplication timeframe because there are no data supporting this timeframe. The submissions also point out that the American Academy of Dermatology (AAD) no longer supports a 2-hour timeframe, even though we cited AAD as supporting the 2-hour timeframe in the 2007 proposed rule (72 FR 49070 at 49093).

In its submission following the 2007 proposed rule, the AAD does not state its support for the 2-hour timeframe. However, all of the public education materials from AAD instruct consumers to reapply sunscreen at least every 2 hours (Refs. 54 through 58). In addition, other public health organizations such as the Centers for Disease Control and Prevention (CDC) and the U.S. Environmental Protection Agency (EPA) recommend reapplication at least every 2 hours (Refs. 59 and 60).

We disagree with the submissions stating that data do not support this timeframe. In the 2007 proposed rule, we described two studies demonstrating a significantly decreased sunburn risk if sunscreen product were applied at least every 2 hours (72 FR 49070 at 49092 through 49093). Wright *et al.* found that subjects who reapplied sunscreen every 1 to 2 hours and after swimming were not sunburned (Ref. 61). Similarly, Rigel *et al.* reported that people who reapplied sunscreen every two hours or sooner were five times less likely to sunburn compared to those who reapplied sunscreen only after 2.5 hours or longer (Ref. 62).

One of the submissions following the 2007 proposed rule included results from a computer-simulation of sunscreen product reapplication based on a mathematical model (Ref. 1). The results of this simulation suggested that sunscreen products should be reapplied 15 to 30 minutes after sun exposure begins. The results also suggested that further reapplication of sunscreen product is necessary after vigorous activity that could remove sunscreen product, such as swimming, toweling, excessive sweating, or rubbing. No other reapplication time is suggested. The usefulness of this study in determining whether to revise the directions is limited. In particular, we do not know whether this simulation was validated, because it has not been confirmed with clinical studies. Until we receive clinical studies demonstrating that consumers do not experience skin damage when sunscreen is reapplied at longer timeframes, we will continue to require the 2-hour reapplication timeframe. As discussed in the 1999 final rule, manufacturers may seek approval of different reapplication directions by submitting specific and substantive supporting data to us under an NDA deviation (described in 21 CFR 330.11).

2. Proposed Directions Not Being Revised

We are not revising proposed 21 CFR 352.52(d)(1)(i): "Apply [select one of the following: 'Liberally' or 'generously'] [and, as an option: 'And evenly'] [insert appropriate time interval, if a waiting period is needed] before sun exposure." Several submissions requested that we allow "smoothly" to be included in this statement (Ref. 1). However, we continue to consider this word to be vague (72 FR 49070 at 49072 and 49092). Some submissions also requested that we include a specific application amount in place of the terms "generously" and "liberally" (Ref. 1). For example, the submissions suggested that the statement could read "apply 2 tablespoonsful." The submissions argued that more specific directions would lead to consumers applying more sunscreen product, reflecting the 2 milligrams per square centimeter (mg/cm^2) used during the SPF test. However, specifying a certain amount in the directions will not accomplish this goal. The amount of sunscreen product that needs to be applied to reach 2 mg/cm^2 varies for each sunscreen product and depends on the amount of skin surface area being covered. For example, the volume of sunscreen oil applied to the neck and face will differ greatly from the amount needed to apply a sunscreen lotion to every sun-exposed area of the body. Therefore, we are continuing to require the terms "generously" and "liberally."

3. Proposed Directions Not Being Required

We are not requiring the proposed statement "apply and reapply as directed to avoid lowering protection" (proposed 21 CFR 352.52(d)(1)(ii)). We included this statement in the 2007

proposed rule because reapplication time appears to be critical to achieve proper sun protection (72 FR 49070 at 49093). However, we have concluded that this statement is redundant with more specific reapplication directions and may confuse consumers. It is not clear that consumers will understand the intent of this statement to emphasize the need to follow reapplication instructions. Therefore, we are not requiring the statement in this document.

4. New Directions Resulting From Submissions on the Proposed Rule

For covered sunscreens with Broad Spectrum SPF values of 15 or higher, we are requiring a new Directions statement that emphasizes the need not only to regularly use such a sunscreen, but also to follow other sun protection measures. For these sunscreens, the statement will read, "[bullet] Sun Protection Measures. [in bold font] Spending time in the sun increases your risk of skin cancer and early skin aging. To decrease this risk, regularly use a sunscreen with a Broad Spectrum SPF of 15 or higher and other sun protection measures including: [Bullet] limit time in the sun, especially from 10 a.m.–2 p.m. [bullet] wear long-sleeved shirts, pants, hats, and sunglasses (new 21 CFR 201.327(e)(1)(iv)). This statement is taken from the proposed warning "UV exposure from the sun increases the risk of skin cancer, premature skin aging, and other skin damage. It is important to decrease UV exposure by limiting time in the sun, wearing protective clothing, and using a sunscreen." (proposed 21 CFR 352.52(c)(1)). As discussed in section IV.C. of this document, this warning is no longer being required for sunscreens with Broad Spectrum SPF values of 15 or higher. Rather, as discussed in section IV.B of this document, submissions suggested that the information proposed as a warning is better understood as an indication, with the supporting conditions for achieving effectiveness. As described in section IV.B, on reexamination of the scientific data, we agree that an appropriately limited indication for reduction in risk of skin cancer and early skin aging is supported for sunscreens with Broad Spectrum SPF values of 15 or higher. For these products, the direction instructs users how to use the product in a manner that supports that indication.

In this final rule, we are being more specific about the need to limit time in the sun especially during the midday hours of 10 a.m. to 2 p.m. when the intensity of solar radiation is greatest because the sun is at its zenith (*i.e.*, directly overhead). In our 1993 proposed rule, we stated that, "on any day of the year, the intensity of the UV energy of sunlight is greatest between 10 a.m. and 2 p.m." (58 FR 28194 at 28199). We have concluded that this information is important to consumers trying to protect themselves from the sun and are including the information in the new direction statement. This change is also responsive to the concerns of two submissions on the portion of the proposed sun alert that referred to "limiting time in the sun," both of which suggested alternatives intended to provide more concrete information for consumers to act on (Ref. 1).

Several submissions argued that we should allow different Drug Facts labeling for cosmetics containing sunscreens so that consumers will apply the product appropriately for its intended cosmetic use (Ref. 1). For example, the submissions argued that reapplication every 2 hours may not be appropriate for cosmetic-sunscreen products. We disagree with these submissions. Cosmetic-sunscreen combinations that are intended for use as drugs require adequate labeling for their drug use. (See 21 CFR 700.35). The Drug Facts label communicates information to the consumer so that the cosmetic-sunscreen product can be used safely and effectively. To help consumers understand that the sunscreen directions apply to the use of the product as a drug, for sun protection, we are allowing the optional statement "for sunscreen use:" to appear as the first line under "Directions." Consumers who are using these products primarily for cosmetic use will be more likely to understand that they might not receive the intended sun protection if they do not follow the directions in the Drug Facts label.

E. Constitutionality of Labeling Statements Regarding Skin Cancer and Skin Aging

Two submissions questioned the constitutionality of the labeling provisions in the 2007 sunscreen proposed rule. Specifically, the submissions contended that our proposed restriction on any claims about the prevention of skin cancer, early skin aging, and related skin damage would violate the sunscreen manufacturers' commercial speech rights under the First Amendment to the U.S. Constitution.

In the 2007 proposed rule preamble, we had concluded that our proposed restriction on claims about the prevention of skin cancer, early skin aging, and related skin damage would be permissible under the First Amendment, in part, because, at that time, we tentatively concluded that there were insufficient scientific data to support inclusion of such claims in the sunscreen monograph. As described elsewhere in this document, we received numerous submissions in response to the 2007 proposed rule, some of which contained references to clinical studies we had reviewed in preparing the 2007 proposed rule about the effectiveness of sunscreens in protecting against the harmful effects of UV radiation. As already described in section IV.B.2, based in part on our re-evaluation of the data from these studies, as well as the scientific fact that reducing exposure to both UVB and UVA radiation by a substantial amount (*i.e.*, equivalent to that provided by a broad spectrum sunscreen with an SPF value of 15 or higher) decreases the risk of damaging the skin, we find that the science supports the conclusion that one subset of sunscreens covered by this rule, broad spectrum sunscreen products with an SPF value of 15 or higher, in conjunction with limiting time in the sun and wearing protective clothing, reduce the risk of developing skin cancer and early skin aging. Our conclusion is reflected in the permissible indication described in this final rule for covered products with Broad Spectrum SPF values of 15 or higher. Although we have decided to permit a claim about the prevention of skin cancer and early skin aging for certain covered sunscreens, as requested in the submissions, we have nevertheless conducted a First Amendment analysis of our requirements concerning the skin cancer/early skin aging claim in this final rule (hereinafter "skin cancer/early aging indication"), as well as the "Skin Cancer/Skin Aging Alert" required as a warning for covered products that do not provide broad spectrum protection with an SPF value of 15 or higher. For the following reasons, we have concluded that these requirements do not violate the First Amendment.

This rule establishes effectiveness testing methods and labeling that are appropriate for the safe and effective use of OTC sunscreen products covered by this rule. Any covered sunscreen product that deviates from the requirements set forth in this labeling regulation and any other applicable labeling regulation would be considered misbranded under section 502 of the FD&C Act. In particular, sunscreen products covered by this rule would be misbranded if they are labeled with a skin cancer/early aging indication but

do not provide broad spectrum protection with an SPF value of 15 or higher. Such products would also be misbranded if they do not include the "Skin Cancer/Skin Aging Alert" described in this rule (see 21 CFR 201.327(d)(2)). Covered sunscreen products that do provide broad spectrum protection with an SPF value of 15 or higher would be misbranded if they are labeled with the permissible skin cancer/early aging indication but do not include reference to the need to use the product as directed with other sun protection measures (21 CFR 201.327(c)(3)). Manufacturers of covered sunscreen products that comply with the labeling requirements in this document would not be subject to enforcement actions on the basis that the products are misbranded, provided they comply with all other requirements under section 502 of the FD&C Act. Because this rule applies only to products marketed without approved applications, manufacturers who wish to deviate from the testing or labeling requirements in this document may do so by means of a new drug application (NDA) under section 505 of the FD&C Act.

We have concluded that the labeling requirements in this rule satisfy the applicable tests governing commercial speech, as set forth by the Supreme Court. The requirements for the "Skin Cancer/Skin Aging Alert" and the information in the skin cancer/early aging indication about using the product as directed with other sun protection measures, are permissible under the First Amendment because they are reasonably related to the Government's interest in protecting public health (see *Zauderer* v. *Office of Disciplinary Counsel*, 471, U.S. 626, 651 (1985)).

We are requiring covered sunscreen products that do not provide broad spectrum protection with an SPF value of 15 or higher to include the "Skin Cancer/Skin Aging Alert" under the "Warnings" heading on the label to ensure that consumers are aware of the continued risks of skin cancer and early skin aging that occur from sun exposure, the conditions under which they will be using the product, and that they understand that the product has been shown only to help protect against sunburn. Without this warning, consumers could fail to distinguish between these sunscreen products and other sunscreen products that have been proven to help provide protection against skin cancer and early skin aging. Providing this information is important for consumers to be able to make informed choices about the selection and use of sunscreens.

For covered sunscreen products that do provide broad spectrum protection with an SPF value of 15 or higher, we are requiring that the additional information about using the product as directed with other sun protection measures be included in the indication so that consumers are not misled about how to use these sunscreens effectively or about the conditions under which these sunscreens are effective. Use of a sunscreen alone—even a broad spectrum sunscreen with an SPF value of 15 or higher—has not been shown to reduce the risk of skin cancer or early skin aging if a consumer increases overall UV exposure by spending greater time in the sun and/or wearing less protective clothing. The additional information required in the skin cancer/early aging indication about using the product as directed with additional sun protection measures clarifies how the use of sunscreens is part of a comprehensive sun protection program. Displaying this information elsewhere would underemphasize its importance in relation to the use of these sunscreens for protection against skin cancer and early skin aging (see *N.Y. State Rest. Ass'n* v. *N.Y. City Bd. of Health*, 556 F.3d 114 (2d Cir. 2009); see also 21 U.S.C. 352(c)). Thus, these disclosure requirements will promote the proper use of covered sunscreens and are, therefore, reasonably related to the Government's interest in protecting public health.

Our requirements concerning the skin cancer/early aging indication would also be permissible under the First Amendment using the analytical framework provided in *Central Hudson Gas & Electric Corporation* v. *Public Service Commission*, 447 U.S. 557 (1980). Under *Central Hudson*, commercial speech that is false, misleading, or concerns unlawful activity is not entitled to protection under the First Amendment. While commercial speech that concerns lawful activity and is not misleading receives some protection under the First Amendment, it may nonetheless be regulated by the Government if the following conditions are met: (1) The asserted governmental interest is substantial; (2) the regulation directly advances the asserted governmental interest; and (3) the regulation is not more restrictive than necessary to serve that interest (*Id.* at 566). The Supreme Court has explained that the last element of the *Central Hudson* test is not a "least restrictive means" requirement but, rather, requires narrow tailoring (*i.e.*, "a fit that is not necessarily perfect, but reasonable" between means and ends) (*Board of Trustees of the State Univ. of N.Y.* v. *Fox*, 492 U.S. 469, 480 (1989)). In subsequent decisions, the Court has also clarified that "misleading" in the first element of the test refers to speech that is inherently or actually misleading.

Based on the data currently available, we have concluded that the following statements or omissions would be false or inherently misleading: (1) Use of the skin cancer/early aging indication on the labeling of a sunscreen product that does not provide broad spectrum protection with an SPF value of 15 or higher, (2) the omission of the "Skin Cancer/Skin Aging Alert" under the "Warnings" heading of the labeling for sunscreen products that do not provide broad spectrum protection with an SPF value of 15 or higher, and (3) use of the skin cancer/early aging indication that omits the required information about using the product as directed with other sun protection measures.

Use of the skin cancer/premature aging indication on the labeling of covered sunscreen products that do not provide broad spectrum protection with an SPF value of 15 or higher would be false or inherently misleading for several reasons. As discussed elsewhere in this document, only broad spectrum UV radiation is classified as a known human carcinogen, according to the National Toxicology Program. Therefore, covered sunscreen products that do not provide broad spectrum UV protection may not reduce the risk of skin cancer. Furthermore, since the precise wavelengths of UV radiation that cause skin cancer and early skin aging are unknown, a covered sunscreen product that only provides protection against part of the UV spectrum may not ensure a reduction in the risk of developing skin cancer or early skin aging. In addition, all of the scientific data that support the skin cancer/early aging indication for certain covered sunscreens were derived from studies that used sunscreen products with an SPF value of 15 or higher. Therefore, the skin cancer/early aging indication would be false or inherently misleading on covered sunscreen products that do not provide this level of protection, because there is a lack of any evidence demonstrating that these products would reduce the risk of skin cancer or early skin aging. Similarly, omitting the "Skin Cancer/Skin Aging Alert" on these products, which are identified on their labels as "sunscreens," would be inherently misleading because consumers who are using these products for sun protection would not be sufficiently alerted to the fact that these products have been shown only to

protect against sunburn, while sun exposure also increases the risks of skin cancer and early skin aging.

A skin cancer/early aging indication on a covered product with Broad Spectrum SPF value of 15 or higher that omits the required information about using the product as directed with other sun protection measures would also be false or inherently misleading because sunscreen use alone has not been shown to reduce the risk of skin cancer or early skin aging if a consumer increases overall UV exposure by spending greater time in the sun and/or wearing less protective clothing. As discussed above in this section and elsewhere in this document, without the reduction in consumers' overall UV exposure, a covered sunscreen product may not be effective in reducing consumers' risk of skin cancer and early skin aging.

We also conclude that the labeling claims and omissions described above would cause the product to be misbranded and, therefore, relate to an unlawful activity. As described earlier in this section and elsewhere in this document, labeling regulations establish certain requirements that help ensure the safe and effective use of OTC drug products. The false or misleading labeling described above would cause covered products to be misbranded under section 502 of the act. Therefore, such labeling would concern the illegal sale of misbranded drugs. Under the *Central Hudson* test, then, we have not violated the First Amendment with these requirements, which simply prohibit false or inherently misleading labeling.

Although we conclude that the labeling described above would not be entitled to First Amendment protection under the threshold inquiry of the *Central Hudson* test, we conclude that our regulation directly advances a substantial Government interest and is no more extensive than necessary, and therefore would also pass muster under the test's three remaining steps. Under the first remaining step, we have a substantial interest in protecting public health (see *Pearson* v. *Shalala*, 164 F.3d 650, 656 (DC Cir. 1999) (citing *Rubin* v. *Coors Brewing Co.*, 514 U.S. 476, 484–485 (1995)).

Under the second remaining step of the *Central Hudson* test, our labeling requirements discussed in this section directly advance the Government's interests in protecting public health because they help ensure that covered sunscreen products are adequately labeled for safe and effective use by consumers.

As stated previously in this document, scientific evidence only supports the skin cancer/premature aging indication for sunscreen products that provide broad spectrum protection with an SPF value of 15 or higher. Allowing the skin cancer/early aging indication on sunscreen products for which it is not scientifically supported would lead to consumers unjustifiably relying on such products for protection against skin cancer and early skin aging. Furthermore, the "Skin Cancer/Skin Aging Alert" allows consumers to be aware that spending time in the sun increases their risk of skin cancer and early skin aging, and that products on which this alert appears have not been shown to provide this type of protection. The requirement for information in the skin cancer/early aging indication about using sunscreens as directed with sun protection measures also directly advances our interest in protecting public health because these elements are essential for consumers to reduce their overall UV exposure and, consequently, their risk of developing skin cancer and early skin aging. Thus, these requirements directly advance the Government's interest in protecting public health through the safe and effective use of sunscreens.

Under the final remaining step of the *Central Hudson* test, our requirements concerning the skin cancer/early aging indication are not more restrictive than necessary, because there are not numerous and obvious alternatives (*Cincinnati* v. *Discovery Network*, 507 U.S. 410, 418 n. 13 (1993)) to achieve the Government's substantial interests. By permitting the skin cancer/early aging indication only for covered sunscreen products with Broad Spectrum SPF values of 15 or higher, and requiring the "Skin Cancer/Skin Aging Alert" for products that do not offer this level of protection, we are ensuring that consumers do not mistakenly rely on sunscreen products that have not been demonstrated to be effective for protection against skin cancer and early skin aging. In addition, labeling that omits a statement regarding the use of other sun protection measures as directed from the skin cancer/early aging indication could lead to consumers foregoing other sun protection measures, thereby negating the protective effect of the sunscreen. Including a statement in the skin cancer/early aging indication regarding the need to follow other sun protection measures as well as the related directions ensures that consumers understand how to use sunscreens to reduce their risk of skin cancer and early skin aging.

It is important to note that manufacturers of OTC sunscreens covered by this rule have several alternatives for adding labeling information that is not included in this labeling regulation. For example, such manufacturers can file an NDA under section 505 of the FD&C Act or submit a petition under 21 CFR 10.30 to amend the labeling regulation. In either case, the manufacturer need only submit the requisite evidence to support the indication or other labeling for the product that differs from that addressed by the regulation. Therefore, we are not being more restrictive than necessary when these viable alternatives are available for manufacturers.

Reacting to the fact that our proposed rule did not permit any indication statement for any sunscreen regarding prevention of skin cancer and early skin aging, one submission asserted that we must consider use of a disclaimer as an alternative means of addressing the limits of the product's effectiveness. As noted previously in this document, this final labeling regulation permits an appropriately limited indication for broad spectrum sunscreens with SPF values of 15 or higher—one stating that when used as directed with other sun protection measures, such products decrease the risk of skin cancer and early skin aging caused by the sun. The claim is authorized for this subset of covered sunscreen products because available scientific data discussed elsewhere in this document are sufficient to substantiate the claim for these products. Because we have included a skin cancer/early skin aging claim in these labeling regulations, we no longer view the submission's request as being applicable.

In any event, we note that the use of disclaimers on drug labeling to qualify inadequately supported or unapproved indications is not an effective, less restrictive means of achieving FDA's substantial interests in protecting public health and preserving the integrity of its premarket approval systems. Indeed, disclaimers on drug labeling would severely undermine the Government's interests here. For over 100 years, Congress has charged FDA with enforcing misbranding laws to protect public health. In 1962, Congress amended the FD&C Act to require that all new drugs be approved as both safe and effective prior to marketing. Congress found that a premarket approval system, requiring specific types of supporting evidence (see 21 U.S.C. 355(d)), and misbranding provisions, among other requirements, were necessary to avoid further tragedies involving unsafe and ineffective drugs. Using disclaimers for drugs would completely undermine the

regulatory framework established by Congress for the protection of public health. FDA's labeling regulations help ensure the safety and effectiveness of OTC drugs and establish the conditions under which a drug is not misbranded under the FD&C Act. If a manufacturer of a covered sunscreen would like to label its sunscreen product in a way that does not conform to this labeling regulation, it cannot circumvent the premarket NDA process.

In summary, we conclude that the labeling requirements provided in this document do not violate the First Amendment.

F. Other Information

We received submissions requesting that we add a new statement about storage conditions under "Other information" in the Drug Facts label (Ref. 1). The submissions argued that sunscreen products in containers are often exposed to heat when used at the beach, swimming pools, etc. The concern expressed in the submissions was that heat could cause sunscreen formulations inside containers to change, resulting in less sun protection. We agree with the submissions. Sunscreen products within containers should not be exposed to direct sun and can be protected by wrapping them in towels and/or keeping them in shaded environments (*e.g.*, under an umbrella and/or in a purse or bag). Consumers could also store sunscreen product containers in coolers while outside during hot periods. In this final rule we are requiring the following statement in the "Other information" section of the Drug Facts label: "[Bullet] protect the product in this container from excessive heat and direct sun" (new 21 CFR 201.327(f)).

In addition to the statement about storage conditions, we received numerous submissions requesting that we relocate the proposed "sun alert" warning to the "Other information" section of the Drug Facts label. The submissions argued that the "sun alert" is an educational statement and not a warning: "UV exposure from the sun increases the risk of skin cancer, premature skin aging, and other skin damage. It is important to decrease UV exposure by limiting time in the sun, wearing protective clothing, and using a sunscreen."

As already discussed, in light of our re-evaluation of the evidence supporting the indications for sunscreens, we have made changes to the labeling to more accurately convey appropriate information to consumers about the benefits, directions, and limitations of two different groups of products covered by the rule—those that provide broad spectrum protection with an SPF value of 15 or higher, and those that do not. We do not agree that this information belongs under the heading "Other information" but have included it in modified form under the headings Uses and Directions for products with Broad Spectrum SPF values of 15 or higher (new 201.327(c)(2) and (e)(2), and under a revised "Skin Cancer/Skin Aging Alert" under the heading Warnings for other sunscreens (new 201.327(d)(2)).

In this document, we are also removing the optional "Other information" statements in proposed 21 CFR 352.52(e):

1. "Low," "medium," "high" or "highest" "sunburn protection product"
2. "Higher SPF products give more sun protection, but are not intended to extend the time spent in the sun."

According to the 2007 proposed rule, these statements could appear in "Other information" or anywhere outside Drug Facts. However, in this rule, we have revised the labeling and are no longer requiring the principal display panel to characterize the level the sunburn protection. Rather, for broad spectrum products, the rule requires only the statement "Broad Spectrum SPF [fill in tested SPF value]" to appear on the principal display panel. In light of this revised approach to labeling, we are concerned that including the characterizations of the product as providing "low," "medium," "high" or "highest" "sunburn protection would be confusing or misleading, and are no longer including it as an option.

We have concluded that the second statement, although truthful, is not necessary. Consumers likely understand the first part of this statement (higher SPF values represent more sun protection) based on the long-standing inclusion on SPF values on OTC sunscreen products. The second part of the statement (higher SPF products are not intended to extend time spent in the sun) is redundant with the information already provided under "Uses" and "Directions," particularly concerning the need for limiting time in the sun (see sections IV.B and IV.D). Although we are not requiring inclusion of the second statement under "Other information," the statement may appear outside the Drug Facts label because it is truthful and nonmisleading.

G. Reduced Labeling

Five submissions requested changes to our proposed regulations allowing reduced labeling for sunscreen products sold in small packages (*i.e.*, packages which meet the requirements in 21 CFR 201.66(d)(10)) that are labeled for use only on small areas of the face. One submission stated that all cosmetic products labeled with sunscreen indications should be required to include all sunscreen product labeling.

After reassessing the criteria for reduced labeling, we are not allowing the reduced labeling included in the 2007 proposed rule. OTC drug labeling regulations (21 CFR 201.66(d)(10)) allow reduced labeling for any OTC drug product sold in a small package, including sunscreen products. In the 2007 proposed rule, we proposed additional reductions in labeling for three types of sunscreen products sold in small packages and intended for use on small areas of the face:

- Proposed 21 CFR 352.52(f)(1)(i)–(f)(1)(iv): Sunscreen products sold in small packages and labeled for use specifically on the lips, nose, ears, and/or around the eyes (*i.e.*, small areas of the face)
- Proposed 21 CFR 352.52(f)(1)(v): Sunscreen-lip protectant combination products sold in small packages
- Proposed 21 CFR 352.52(f)(1)(vi): Sunscreen products formulated as lipsticks, lip products that prolong wear of lipstick, lip gloss, and lip balms

Three submissions argued that we should not restrict labeling exemptions only to sunscreen products sold in small packages and labeled for use on small areas of the face. The submissions stated that reduced labeling provisions should apply to all sunscreen products sold in small packages whether or not they are labeled for use on small parts of the face. Two of the submissions argued that such a restriction violates the Administrative Procedures Act (APA). The submissions cite *Bracco Diagnostics, Inc.*, v. *Shalala* 963 F. Supp. 20, 27–28 (D.D.C. 1997) as evidence that the courts oppose regulations requiring "two sets of similar products to run down two sets of separate [regulatory] tracks * * * for no apparent reason."

In this document, we continue to allow the reduced labeling specified in 21 CFR 201.66(d)(10). Therefore, if the information listed under Drug Facts requires more than 60 percent of the total available surface area, the Drug Facts labeling can be reduced by making the formatting changes specified in 21 CFR 201.66(d)(10)(i)–(d)(10)(v). However, in contrast to the 2007 proposed rule, we are not allowing additional reductions in labeling for any sunscreen products.

When we proposed the additional reduced labeling, we recognized that many of the sunscreen products sold in

small packages and labeled for use on small areas of the face could not accommodate full Drug Facts labeling. However, in the last several years, manufacturers have introduced new label designs that permit full Drug Facts labeling on very small packages. For example, some stick products, including lip protectant-external analgesic combinations marketed in 0.15 oz. amounts, have been labeled with wrap-around labels that contain full Drug Facts labeling. If these products can be labeled to accommodate full Drug Facts labeling, then all sunscreen products should be able to accommodate full Drug Facts labeling. Requiring full Drug Facts labeling should not discourage manufacturers from including sunscreen ingredients because of limited labeling space, as stated in the 2007 proposed rule (72 FR 49070 at 49075 through 49077). Therefore, in this document, we are eliminating all of the allowances for reduced labeling in proposed 21 CFR 352.52(f). Sunscreen products can only have reduced labeling for formatting if they meet the criteria in 21 CFR 201.66(d)(10).

V. Miscellaneous Labeling Outside Drug Facts

We received several submissions regarding various performance claims, including comments asking us to allow claims for protection immediately upon application (instant protection) and for extended duration between applications (extended wear) and comments asking us not to allow terms such as "sunblock," "waterproof," and "sweatproof" (Ref. 1). These kinds of claims were not included in the 2007 proposed rule (Ref. 1).

We are not including labeling in 21 CFR 201.327 permitting these claims on OTC sunscreen products covered by the rule. The current record does not contain support for any of these kinds of claims. To clarify the status of these kinds of claims, we are finalizing two provisions. We include instant protection and extended wear claims, which are claims that we think may be capable of substantiation, in 21 CFR 310.545(a)(29)(ii). While these claims may not be included on products marketed without approved applications, including them in this provision makes it clear that these claims may be substantiated for an individual product by the submission of adequate data in an NDA.

We agree with the submissions that argue that "sunblock," "waterproof," and "sweatproof" claims are false or misleading, as we have stated in previous sunscreen rulemakings (58 FR 28194 at 28228; 64 FR 27666 at 27676 through 27680). These terms are essentially exaggerations of performance that FDA does not think can be substantiated. Accordingly, in this final rule, we codify these as terms or phrases that would be false or misleading on covered products, and are therefore prohibited (21 CFR 201.327(g)).

In addition to submissions requesting that we allow certain labeling outside Drug Facts, we also received a submission requesting that we require information about the UV index (UVI). A stated in the 2007 proposed rule, we have determined that the usage information provided on OTC sunscreen products applies regardless of the UVI value (72 FR 49070 at 49073). Therefore, we will allow but do not require information about the UV index to be included on sunscreen products outside the Drug Facts label.

A submission requested that we require that the UV index appear on sunscreen product labels because this information would help consumers understand and use the UV index to determine their risk of sunburn. The UV index was developed in 1995 by the National Weather Service, Environmental Protection Agency, and Centers for Disease Control and Prevention to provide a forecast of the expected risk of overexposure to UV rays. The UV index is calculated using ozone data, atmospheric pressure, temperature, and cloudiness. As stated in the 2007 proposed rule, we are not requiring labeling of UV index information because it is not necessary for consumers to understand this index in order to safely and effectively use OTC sunscreen products (72 FR 49070 at 49073). However, manufacturers may include truthful and nonmisleading information about the UV index in the labeling outside of Drug Facts if they choose.

We also received a submission requesting that we allow a claim of "instant protection" and to allow claims for extended periods of protection between applications (*i.e.*, longer than the 2 hours specified in "Directions" in the 2007 proposed rule). The submission argued that several marketed products provide sunburn protection immediately upon application, as demonstrated by test results included in the submissions. In this document, SPF testing requires a 15-minute waiting period between sunscreen application and UV exposure of the test site. It appears that the submitted test method included the same 15-minute waiting period. Therefore, the assertion that this product provides "instant protection" does not appear to be substantiated. We also did not receive any data regarding claims for extended periods of use, so it is not clear whether these claims are truthful. Claims that a product provides for an extended period of protection between applications or immediately upon application would have to be supported by data. Therefore, these claims could be made only under approved new drug applications (NDAs) with the required data.

In this document, we are specifically identifying these claims as not allowed on any OTC sunscreen product, regardless of SPF value or broad spectrum protection, without an approved application containing sufficient substantiation to support the claim. (new 21 CFR 310.545(a)(29)(ii)):

- Instant protection or protection immediately upon application
- Claims for "all-day" protection or extended wear claims citing a specific number of hours of protection that are inconsistent with the directions for application in 21 CFR 201.327.

In addition, we are identifying the terms "sunblock" "waterproof," and "sweatproof" as false and misleading, as we have stated in previous sunscreen rulemakings:

- Sunblock (64 FR 27666 at 27679 and 27680)
- Sweatproof (58 FR 28194 at 28227 through 28228)
- Waterproof (58 FR 28194 at 28227 through 28228).

We have previously identified these claims as ones that would render a product misbranded but are addressing them again in this document because OTC sunscreen products currently marketed without approved applications continue to contain the claims. In this final rule, we are listing these false and misleading terms in 21 CFR 201.327(g). These terms may not be included on any OTC sunscreen products covered by the rule.

Finally, in the 2007 proposed rule, we proposed to specify other optional statements that could be included outside of Drug Facts in proposed 21 CFR 352.52(e)(3):

- "Broad spectrum sunscreen"
- "Provides [select one of the following: 'UVA and UVB' or 'broad spectrum'] protection"
- "Protects from UVA and UVB [select one of the following: 'rays' or 'radiation']"
- "[Select one of the following: 'absorbs' or 'protects'] within the UVA spectrum."

This final rule is not a monograph, and we do not consider it necessary in this rule to codify optional statements for use outside of "Drug Facts." The labeling required in this document

should provide consumers with the information that they need to safely and effectively use the sunscreen products that it addresses. Under this final rule, products marketed without approved applications that provide broad spectrum protection according to the test in new 21 CFR 201.327(j) of this document will be identified on the PDP by use of the term "Broad

Spectrum SPF." In light of this requirement in the rule for use of the term "broad spectrum" on these particular products, including a statement anywhere in the labeling of a product that does not pass the broad spectrum test in 21 CFR 201.327(j) that suggests or implies that the product provides broad spectrum protection would misbrand that product. We likewise caution against references to "UVA" (or "UVA/UVB") protection on products that do not provide broad spectrum protection as demonstrated by the test in 21 CFR 201.327(j). Such labeling would misbrand the products if it misleadingly suggests that the products provide protection that is equivalent or greater to that provided by products labeled with "Broad Spectrum SPF" values or is otherwise false or misleading.

VI. SPF Test Parameters

The 2007 proposed rule included the SPF test from the 1999 final rule with revisions to a few test parameters. In response to the 2007 proposed rule, we received numerous submissions requesting that we revise additional test parameters (Ref. 1). In this document, we have rewritten the regulations describing the SPF test in an effort to make it easier to read and understand and to more closely follow the order in which steps of the SPF testing procedure are conducted. We have also made several revisions to the test parameters. However, we did not make all of the revisions requested in the submissions. Table 4 of this document summarizes test parameters that we considered revising. The table identifies the parameters that we are changing in this document as well as those that we are not changing. Detailed discussion of each test parameter appears throughout the remainder of this section.

TABLE 4—SUMMARY OF SPF TEST PARAMETERS INCLUDED IN THE 2007 PROPOSED RULE AND THIS FINAL RULE

2007 Proposed rule	This final rule
21 CFR 352.70(a). Standard sunscreens	*21 CFR 201.327(i)(2). SPF standard*
Two standards: 8% homosalate (SPF 2—≤15) 7% padimate, 3% oxybenzone (SPF >15) HPLC reference standard: no limits set for accuracy of oxybenzone & padimate O	One standard: 7% padimate, 3% oxybenzone (all SPFs) HPLC reference standard: limit set to within 5% of theoretical for accuracy of oxybenzone & padimate O
21 CFR 352.70(b). Light source (solar simulator)	*21 CFR 201.327(i)(1). UV source (solar simulator)*
Emission spectrum specifications: (1) COLIPA[1] 1994 (Ref. 63) (2) no specifications for UVA Calibration: every 6 months Total irradiance: 1500 Watts/square meter (W/m^2) Beam uniformity: within 20 percent	Emission spectrum specifications: (1) COLIPA[1] 2006 (Ref. 64) (2) specifications for UVA I and UVA II percentages of total UV Calibration: at least annually Total irradiance: 1500 Watts/square meter (W/m^2) Beam uniformity: within 20 percent
21 CFR 352.70(c)(7). Number of subjects	*21 CFR 201.327(i)(3). Test subjects*
SPF < 30: 20–25 subjects; ≥ 20 valid results SPF ≥ 30: 25–30 subjects; ≥ 25 valid results	All SPFs: • 10–13 subjects; ≥10 valid results
21 CFR 352.70(c)(4). Test site delineation/subsite	*21 CFR 201.327(i)(4)(i) and (ii). Test site/subsite*
test site area: ≥ 50 cm^2 test subsite area: ≥ 1 cm^2 Distance between subsites: ≥ 1 cm	test site area: ≥ 30 cm^2 test subsite area: ≥ 0.5 cm^2 Distance between subsites: ≥0.8 cm
21 CFR 352.70(c)(5). Application of test materials	*21 CFR 201.327(i)(4)(iii). Applying test materials*
Application amount: 2 milligrams per square centimeter (mg/cm^2) Presaturation of finger cot: Required Water-resistant statement requirements: 20 minute water immersion times 20 minute drying times	Application amount: 2 milligrams per square centimeter (mg/cm^2) Presaturation of finger cot: not required Water-resistant statement requirements: 20 minute water immersion times 15 minute drying times
21 CFR 352.70(d)(3). Determination of individual SPF values	*21 CFR 201.327(i)(5). UV exposure*
Definitions of MED: (1) MED(PS) = MED for protected skin (2) MED(US) = MED for unprotected skin	Definitions of MED: (1) ssMEDp = MED for skin protected by sunscreen standard (2) tpMEDp = MED for skin protected by test product (3) initial MEDu = MED for unprotected skin prior to testing test product (4) final MEDu = MED for unprotected skin determined when testing test product
UV doses for MED(US):	UV doses for initial MEDu:

TABLE 4—SUMMARY OF SPF TEST PARAMETERS INCLUDED IN THE 2007 PROPOSED RULE AND THIS FINAL RULE—Continued

2007 Proposed rule	This final rule
five doses	number of doses not specified
21 CFR 352.70(c)(8) Response criteria	*21 CFR 201.327(i)(5). UV exposure*
Maximal UV exposure:	Maximal UV exposure:
"no more than twice the total energy of the minimal exposure"	not specified

[1] Draft test method entitled "International Sun Protection Factor (SPF) Test Method" developed by the European Cosmetic, Toiletry and Perfumery Association (COLIPA).

We are not making some of the requested changes to certain test parameters because we lack adequate data to determine whether these changes would change the accuracy or reproducibility of the SPF test. We are making changes to some test parameters based on the following developments since the 2007 proposed rule published:

- New data (submitted by the public or published in the scientific literature)
- Technical improvement of SPF testing equipment
- Accumulating experience in the performance of SPF testing
- Efforts towards international harmonization of SPF testing procedures

In support of the requested changes, several submissions (Ref. 1) cited differences between the SPF test in the 2007 proposed rule and the COLIPA SPF test (Ref. 64). The COLIPA SPF test is a joint effort by the cosmetic industry trade associations in Europe, Japan, South Africa, and the United States to harmonize SPF test procedures. The International Organization for Standardization (ISO) is currently developing an SPF test method. Because harmonization of testing methods is important, we are actively involved in the ISO working group responsible for developing methods for assessing the efficacy of sun protection products.

We are revising our proposed SPF test method to be as consistent as possible with the COLIPA SPF test. We acknowledge the merits of harmonizing test methods and are an active participant in ongoing harmonization efforts. However, some of the test parameters in this document differ from comparable parameters in the COLIPA SPF test because we have concluded that the data do not support using the COLIPA SPF test parameters. Throughout the remainder of this section, we discuss whether test parameters in this document match or do not match those in the COLIPA SPF methods.

A. Solar Simulator

Several submissions recommended adopting the solar simulator specifications in the COLIPA SPF test (Ref. 1). We are revising solar simulator specifications to:

- Allow the use of smaller beam, multiport simulators
- Adjust the relative cumulative erythemal effectiveness (RCEE) range specifications for each wavelength band
- Specify that UVA II (320–340 nm) and UVA I (340–400 nm) irradiance should equal or exceed 20 percent and 60 percent, respectively, of the total UV (290–400 nm) irradiance
- Change the regular calibration period from every 6 months to at least once a year

These changes are consistent with the COLIPA SPF test. More importantly, these revisions will allow the SPF test to continue to be accurate and reproducible. For example, we received calibration data demonstrating that solar simulators and their UV lamps are stable for periods longer than 1 year. Therefore, the requirement in the 2007 proposed rule to calibrate every 6 months is unnecessary. The test results should be the same whether calibration is done annually or every 6 months.

In contrast, we are not changing the following solar simulator specifications because changes to these specifications could reduce test accuracy and/or reproducibility:

- Total irradiance limit of 1500 W/m^2
- Total irradiance range of 250–1400 nm
- 20 percent beam uniformity requirement.

These test specifications differ from the COLIPA SPF test, which recommends a 1600 W/m^2 limit and a 10 percent beam uniformity requirement.

Two submissions (Ref. 1) objected to limiting total solar simulator irradiance to 1500 W/m^2 for all wavelengths between 250 and 1400 nm (proposed 21 CFR 352.70(b)(1)). We proposed the 1500 W/m^2 limit because we were concerned that solar simulators operating above this limit could cause excessive heat. Excessive heat could harm test subjects and/or cause loss of dose reciprocity, the correlation between UV dose and resulting erythema. One submission argued that no data indicate that exceeding 1500 W/m^2 causes excessive heat or affects SPF test results. The submission argued that higher intensities should be allowed as long as they are thermally tolerated by test subjects, because allowing higher intensities enables faster SPF testing.

We are not changing the 1500 W/m^2 total irradiance limit. We do not have data demonstrating that exceeding 1500 W/m^2 leads to loss of dose reciprocity. However, we conclude that the limit should be retained to protect test subjects. The COLIPA SPF test cites a study showing that total irradiance of 1600 W/m^2 induces heat and pain in a majority of test subsites, and recommends keeping total irradiance below 1600 W/m^2 (Ref. 64). Therefore, we are keeping the 1500 W/m^2 total irradiance limit (new 21 CFR 201.327(i)(1)(i)).

One submission also objected to the 250–1400 nm range over which total irradiation should be monitored (Ref. 1). The submission argued that portable spectroradiometers are typically incapable of measuring wavelengths out to 1400 nm. According to the submission, emissions from longer wavelengths have not been shown to affect SPF testing.

We are not changing the requirement that total irradiation be monitored over a range of 250–1400 nm. We have concluded that monitoring over this range of wavelengths helps protect SPF test subjects from being exposed to undesirable, unnecessary radiation. The requirement should not impose undue hardship, because longer wavelengths can be monitored using a thermopile, pyroelectric, or similar detectors.

We received two submissions addressing the requirement in proposed 21 CFR 352.70(b)(2) that a solar simulator have "good beam uniformity (within 20 percent) in the exposure plane" (Ref. 1). One submission argued that advances in equipment and monitoring allow for a stricter beam uniformity requirement (<20 percent), which would result in less variability in SPF test results. Another submission argued that the beam uniformity

requirement is only important for large diameter beams and has no impact on SPF testing using small beams.

We are not changing the 20 percent beam uniformity requirement because accurate determination of SPF values relies upon good beam uniformity for all beam sizes. In the 2007 proposed rule, we described how small diameter beams can be tested for beam uniformity (see 72 FR 49070 at 49098). The submission requesting stricter requirements did not include data showing that current solar simulators can reasonably be expected to have beam uniformity less than 20 percent. We conclude that a 20 percent beam uniformity requirement is adequate to produce reliable SPF results. Therefore, we are keeping the requirement that solar simulators demonstrate good beam uniformity (within 20 percent) in new 21 CFR 201.327(i)(1) (iii).

B. Sunscreen Standards

The 2007 proposed rule include two sunscreen standards for use in SPF testing. The two proposed sunscreen standards were a 7 percent padimate O/3 percent oxybenzone standard (mean SPF value of 16.3) and an 8 percent homosalate standard (mean SPF value of 4.47). For SPF testing of sunscreen products with SPF values of 2 to 15, either the padimate O/oxybenzone standard or the homosalate standard would have been required to be tested along with the test sunscreen product. Tests for sunscreen products with SPF values over 15 would have required use of the padimate O/oxybenzone standard.

We received two requests to include an additional sunscreen standard with an SPF value of 30 or higher to test sunscreen products with SPF values of 30 or more (Ref. 1). Neither request specified any particular sunscreen standard formulation with an SPF in this range. If a particular sunscreen standard formulation were specified, we would also need validation data to support including the additional sunscreen standard in the monograph. Therefore, we are not including a sunscreen standard with an SPF value of 30 or more in this document.

We also received a request to include the JCIA SPF 15 'P3' sunscreen standard containing 0.5-percent avobenzone, 3-percent octyl methoxycinnamate, and 2.78-percent phenylbenzimidazole sulfonic acid. To support including the "P3" standard, the request included a table showing mean, maximum, and minimum SPF values from tests conducted in labs in Europe, Japan, Australia, and South Africa. We recognize that the "P3" standard has been widely used and is included in the COLIPA SPF test, but we are not including the "P3" standard in this document. In the 2007 proposed rule (72 FR 49070 at 49095 to 49095), we requested further data to show that testing using the "P3" standard could be performed with:

- Low level interlaboratory variation
- Sufficient sensitivity to detect experimental error
- A reasonable degree of accuracy

The submitted data (*i.e.* the table of SPF values) fail to show that the "P3" standard meets these performance requirements because they do not show:

- Individual lab results
- The number of tests conducted in each lab
- The number of test subjects used in each test
- Calculated standard errors for each test

Without these data, we cannot assess interlaboratory variability, sensitivity to experimental error, or test result accuracy. In addition, the advantage of using the "P3" standard instead of the padimate O/oxybenzone standard is unclear, because both these standards have approximately the same SPF value of 16. Therefore, we are not including the "P3" standard in this document.

We are also eliminating the proposed homosalate standard with an SPF value of 4.47 because the padimate O/oxybenzone standard with an SPF value of 16.3 is adequate for validating all test methodologies. In the 2007 proposed rule, we stated that the sunscreen standards were "method controls rather than calibration tools." As a method control, the purpose of the sunscreen standard is verifying proper and consistent performance of test equipment and procedures, rather than verifying the accuracy of the SPF value determined for sunscreen test products. Therefore, we conclude that it is not critical for the SPF value of the sunscreen standard to be close to the SPF value of the sunscreen test product. It is more important that the sunscreen standard demonstrate consistency of test performance. Consequently, we have concluded that including multiple sunscreen standards is unnecessary, and that the padimate O/oxybenzone standard is a suitable sunscreen standard for all sunscreen products. We favor including the padimate O/oxybenzone standard over the homosalate standard because the homosalate standard was only proposed for use for SPF testing of sunscreen products with SPF values lower than 15. Because most currently marketed sunscreen products have SPF values of 15 or higher, the padimate O/oxybenzone standard is used much more frequently than the homosalate standard.

We received one submission identifying errors in the "Composition of the Padimate O/Oxybenzone Standard Sunscreen" table that appears in the 2007 proposed rule. As suggested by the submission, we are moving the inactive ingredient "propylparaben" from "Part A" to "Part B," as it appears in the COLIPA SPF test. We are not revising the listing of the inactive ingredient "glyceryl monostearate" to read "glyceryl monostearate (Glyceryl Stearate SE)," as suggested. The United States Pharmacopeia defines "glyceryl monostearate" as an "emulsifying and/or solubilizing agent," which adequately describes the ingredient that is appropriate for use in the formulation.

C. Test Subjects

In the 2007 proposed rule, we proposed requiring the following numbers of test subjects providing valid results:

- 20 to 25 subjects for sunscreen products with SPF less than 30
- 25 to 30 subjects for sunscreen products with SPF value of 30 or more

We explained that a minimum of 20 subjects would be required to provide an acceptably accurate SPF result (*i.e.*, low standard error of the mean). We had concluded that sunscreen products with SPF values of 30 or more required a greater number of test subjects because we suspected higher test result variability for these sunscreen products. However, the data used for determining appropriate test subject numbers were limited and dated. Therefore, we invited submission of additional data demonstrating what subject numbers would be adequate.

Several submissions recommend requiring 10 to 25 test subjects as in the COLIPA SPF test (Ref. 1). These submissions include data demonstrating that SPF testing can be performed with suitable accuracy and precision with as few as 10 test subjects. The submissions further argued that SPF testing using a minimum of 10 test subjects has been practiced globally for many years, even for sunscreen products with high SPF values.

We agree with the submissions and are lowering the number of test subjects required for SPF testing. We are requiring that a test panel produce a minimum of 10 valid test results. A maximum of three subjects may be rejected from the panel. Therefore, if 3 subjects would be rejected, a test panel would have had to include 13 subjects.

We are reducing the number of test subjects in this document because the

data we received demonstrate that SPF testing can be conducted with adequate accuracy and precision using as few as 10 test subjects, even when testing high SPF products. The submissions include SPF test results for several sunscreen formulations using panels of 20 to 25 test subjects. We randomly selected 10 subjects within each of these panels to determine if using fewer subjects significantly decreased test accuracy and precision. For each of these panels, the mean SPF value and standard error calculated from a randomly selected subset of 10 subjects were not significantly different from those calculated from all 20 to 25 subjects in the panel. Therefore, these data indicate that using as few as 10 test subjects will not compromise SPF test accuracy or precision. Consequently, fewer test sites and subsites need to be tested and fewer test results need to be rejected, thereby decreasing the number of test subjects needed. Our revised SPF test subject number requirement is similar to the COLIPA SPF test requirement. The only significant difference related to test subject number is that we are not including a statistical requirement or allowing individual subjects to be added incrementally to a test panel as allowed under the COLIPA SPF test.

D. Test Sites and Subsites

Several submissions requested the following revisions of the minimum size specifications for test sites and subsites proposed in the 2007 proposed rule (Ref. 1):

- Test site: proposed 50 cm^2 revised to 30 cm^2
- Test subsite: proposed 1 cm^2 revised to 0.5 cm^2
- Subsite separation: proposed 1 cm revised to 0.8 cm

According to the submissions, these smaller revised minimum sizes would allow multiport solar simulators to be used, while the larger proposed sizes would not. These revised specifications have also been adopted in the COLIPA SPF test (Ref. 64).

We are revising the test site and subsite size specifications as requested by these submissions. Our previously proposed specifications were based on single port solar simulators. Some new multiport solar simulators cannot meet these proposed specifications. In the 2007 proposed rule, we stated that reducing test site/subsite size specifications would be considered if data were submitted showing that these reductions would not compromise testing accuracy (72 FR 49070 at 49100). New data show that SPF testing can still be accurately performed using the recommended reduced test site/subsite size specifications (Ref. 1). Therefore, we are revising the test site/subsite size specifications to accommodate new equipment and to harmonize our specifications with global SPF test methods.

E. Finger Cot

In the 2007 proposed rule, we proposed that a finger cot, presaturated with sunscreen, be used to apply the sunscreen in the SPF test (proposed 21 CFR 352.70(c)(5)):

> Use a finger cot compatible with the sunscreen to spread the product as evenly as possible. Pretreat the finger cot by saturating with the sunscreen and then wiping off material before application. Preteatment is meant to ensure that sunscreen is applied at the correct density of 2 mg/cm^2.

We received one submission that objected to the use of finger cots because consumers do not typically use finger cots when applying sunscreens (Ref. 1). Other submissions argued that the presaturation requirement for finger cots is unnecessary and introduces variability in applied amounts (Ref. 1). Other submissions requested the optional use of sponge applicators for testing powder formulations, because they argued that sponge applicators distribute powder formulations more evenly than finger cots (Ref. 1). We are not addressing issues regarding the use of sponge applicators for the testing of powders in this rule. Elsewhere in this issue of the **Federal Register**, we publish an advance notice of proposed rulemaking that discusses sunscreen dosage forms, including powders. We may address this issue in a future rulemaking..

While we acknowledge that consumers do not use finger cots to apply sunscreens, we are continuing to require the use of finger cots in the SPF test. The use of finger cots seems to increase reproducibility of test results, which was why we originally proposed requiring use of finger cots (72 FR 49070 at 49100 through 49101). We agree with the submissions that the presaturation requirement is unnecessary and are removing this requirement. We proposed requiring finger cot presaturation to prevent sunscreen product from adhering to the finger cot instead of being transferred to the test subject's skin, resulting in sunscreen product being applied at less than the intended 2 mg/cm^2. We received study results showing that a residual amount of sunscreen product may adhere to non-presaturated finger cots, but the amount was small (approximately 2 percent) (Ref. 1). In this study, each of 100 finger cots (without presaturation) was weighed before and after sunscreen product application at 2 mg/cm^2 (100 mg sunscreen product applied over 50 cm^2). However, the study did not include a comparison to presaturated finger cots. Therefore, it is difficult to determine the effect of presaturation on residual sunscreen amounts.

In addition, we reassessed the basis for presaturation. We are now concerned that performing the presaturation step may lead to overestimation of SPF values, because the residual amount normally left on a finger cot with presaturation may increase the amount of sunscreen applied to the skin This could lead to overestimation of SPF values. Overestimation of SPF may, in turn, lead to increased incidence of sunburn because consumers may anticipate greater protection than a sunscreen product actually provides. This overestimation risk is a sufficient basis to remove the presaturation step from the proposed SPF test method.

We also received data showing that testing without the presaturation step can produce highly reproducible results (Ref. 1). In a test of 20 subjects without the presaturation step, a control sunscreen product yielded a mean SPF value of 4.19 with a standard error of 0.06 (*i.e.*, 1.4 percent error), while a test sunscreen product yielded a mean SPF value of 15.54 with a standard error of 0.22 (*i.e.*, 1.4 percent error). These errors are small, suggesting that the calculated SPF values did not vary significantly between test subjects. If lack of presaturation increased variability, then the errors would be expected to be larger. Therefore, we are removing the presaturation requirement because of the risk of overestimation of SPF values and our conclusion that the removal of the presaturation step will not affect the reproducibility of SPF test results.

F. Application Amount

We are continuing to require that 2 mg/cm^2 sunscreen product be applied for the SPF test (proposed 21 CFR 352.70(c)(5); new 21 CFR 201.327(i)(4)(iii)). Several submissions argued for a lower application amount that better reflects the actual amount used by consumers, which they argued is commonly 1 mg/cm^2 or less (Ref. 1). These submissions argued that the unrealistically high 2 mg/cm^2 application amount results in SPF values that overstate the actual sun protection provided by the amounts consumers typically apply. Other submissions supported the 2 mg/cm^2 application amount (Ref. 1). These submissions argued that SPF values are relative, not absolute, values that allow comparison of sun protection provided

by different sunscreen products. According to the submissions, changing the application amount will affect the ability of consumers to make this comparison.

We are not changing the sunscreen product application amount because we have concluded that the advantages of continuing to require 2 mg/cm² exceed the disadvantages of lowering the amount. Requiring the 2 mg/cm² sunscreen product application amount is consistent with SPF test methods used in other countries. The 2 mg/cm² application amount is being used in Europe, Australia, Canada, Korea, and Japan (Refs. 65–67). If we lower the application amount, sunscreen products available in the United States will have significantly lower SPF values than similar products available in other countries. This discrepancy in SPF values is counterproductive to our global harmonization efforts and would likely mislead consumers traveling to other countries about the SPF protection of foreign sunscreen products.

Another advantage of continuing to require a 2 mg/cm² sunscreen product application amount is greater reproducibility of SPF test results. Bimczok *et al.* compared the SPF values determined using sunscreen product application amounts of 0.5, 1, and 2 mg/cm² (Ref. 68). The SPF values determined using 2 mg/cm² sunscreen product were more reliable and reproducible than SPF values determined using the lower application amounts. A sunscreen product application amount of 2 mg/cm² is a large enough amount to allow visualization of the distribution of sunscreen product as it is applied. This allows for more consistent and uniform application of the sunscreen used in testing. Therefore, the 2 mg/cm² sunscreen product application amount is more likely to generate reproducible results.

G. Water Resistance

In the 2007 proposed rule, sunscreen products tested with two 20-minute immersion periods (*i.e.*, 40 minutes total) would be allowed to include a "water resistant" statement and sunscreen products tested with four 20-minute immersion periods (*i.e.*, 80 minutes total) would be allowed to include a "very water resistant" statement. There is a 20-minute drying period between each immersion period. For example, a "water resistant" sunscreen product would be tested by having test subjects in the water for 20 minutes, out of the water for 20 minutes, and in the water for 20 minutes.

We received various requests to revise the test (Ref. 1). One submission recommended longer water immersion times equal to those in water resistance tests used in Australia and New Zealand. Another submission included data from an in vitro water resistance test to support removing the in vivo water resistance test. A third submission stated the test should be eliminated because it is not validated and requires too much time. Further, the submission argued that directions for frequent reapplication make the test unnecessary.

We are continuing to include a water resistance test because water resistance is an important property of sunscreen products that can benefit consumers. The water resistance test indicates that a sunscreen product's labeled SPF protection is retained for a certain period of time after immersion in water. This is useful information to consumers. Therefore, we conclude that a water resistance statement based on the test should be allowed (see section III.C of this document).

We are not changing the 20-minute water immersion periods or the number of immersion periods required. We based these time periods on marketing data indicating that individuals at the beach or the pool spend an average of 21 minutes in the water and go into the water an average of 3.6 times (43 FR 38206 at 38263, August 25, 1978). We have not received any other data supporting different time periods. We have concluded that more or longer water immersion periods are not needed.

We are, however, reducing the drying period from 20 minutes to 15 minutes. We are making this change to decrease the time required for testing. Shorter testing time may increase test accuracy and reproducibility, especially for high SPF sunscreens that retain their water resistance for 80 minutes. In addition, 15 minutes is adequate time to allow for drying. It is possible that sunscreens may lose water resistance with repeated wetting and drying. However, we have concluded that a 15-minute drying period mimics consumer behavior and ensures that the water resistant properties of a sunscreen do not change with multiple cycles of water immersion and drying.

VII. SPF Test Issues (Other than Test Parameters)

A. Pass/Fail (Binomial) SPF Test

Several submissions requested the optional use of a pass/fail (binomial) test to determine the SPF value of a sunscreen product (Ref. 1). These submissions promote the pass/fail test because it would expose fewer subjects to UV irradiation, cost less, and save time. The pass/fail test is based on the hypothesis that a sunscreen product of a certain SPF has a 50:50 probability of preventing the MED response when irradiated with a UV dose correlated with that SPF. For example, a sunscreen product with an expected SPF value of 30 or more should prevent the MED response in greater than 50 percent of test subsites irradiated with a UV dose equivalent to 30 times the UV dose that causes the MED response on unprotected skin. If a test sunscreen product prevents the MED response in a significant number of the subsites (*i.e.*, significantly more subsites that "pass" versus "fail"), then the test sunscreen product would be allowed to be labeled with the SPF correlated to the UV dose.

We are not including the optional use of a pass/fail test for SPF testing. We considered a pass/fail SPF test in the 2007 proposed rule (72 FR 49070 at 49094 to 49095). We stated that a pass/fail test could be a reasonable substitute for our proposed SPF test for sunscreen products with SPF values of 30 or more if certain modifications were made and validation data demonstrated that the test could be performed similarly between labs.

In response to our invitation for public comment, one submission included two studies comparing a pass/fail SPF test to the proposed SPF test: (1) A single center study of four sunscreen products with different SPF values and (2) a multicenter (four laboratories) study of two high SPF sunscreen products. After reviewing these data, we have determined that the pass/fail test has the following drawbacks:

- Each test subsite evaluation is biased towards "pass" because the evaluator expects that no skin reaction should occur on subsites protected by the test sunscreen product.
- The test fails to reject test sites where all of the subsites show positive responses or all of the subsites show negative responses.
- The validity of treating each subsite as an independent variable is questionable.
- The test endpoint (any observed reaction) differs from the endpoint in the proposed SPF test (clearly defined erythema).
- A passing test result for the sunscreen standard does not demonstrate that the test is being performed correctly.
- Test results do not include data for water resistant sunscreen products.
- Allowing this test as an option would yield products with different UV

protection levels labeled with the same SPF.

- SPF test methods developed by various standards-setting organizations do not include a pass/fail test.
- The study report includes statistical errors that overstate the statistical power of the test to distinguish whether a test sunscreen product provides significant UV protection.

Therefore, we are not including a pass/fail test in the SPF test procedure, because including a pass/fail test would present numerous complications and the available data indicate that a pass/fail test has disadvantages compared to the SPF test included in this document.

B. Photostability

Several submissions expressed concern about the loss of UV protection by sunscreen products due to breakdown of ingredients from exposure to sunlight (Ref. 1). These submissions recommended a test to ensure that sunscreen products exposed to sunlight retain sufficient UV protection. Submitted data show that the composition of sunscreen products can change from exposure to UV radiation. The submissions argue that the published photostability studies are inconclusive because the studies employ artificial test conditions that may not be appropriately extrapolated to actual use of sunscreens:

- Tested sunscreen active ingredients were contained in solutions rather than in typical sunscreen product formulations
- Tested sunscreen products contained active ingredients that are not representative of the active ingredients included in typical sunscreen products
- Products were tested over a limited range of the UV spectrum

The submissions argue that understanding the photostability of sunscreen active ingredients alone is not useful. Rather, the submissions argue that it is critical to understand the photostability of sunscreen active ingredients as part of an overall sunscreen product.

We agree that the available data have limitations. Although the submissions argue that the inconclusive data support including a test for photostability, we have concluded that the data do not justify requiring a photostability test at this time. We are not able to establish specific photostability test procedures or specifications based on the available data. We have not received data validating the performance of a photostability test, nor have we received data demonstrating that the effectiveness of any particular sunscreen product is significantly diminished because of photodegradation. We maintain that the proposed SPF test procedure does account for photostability to some extent, because the SPF test exposes sunscreen products to UV radiation before an SPF value is determined. Consequently, sunscreen products susceptible to photodegradation have correspondingly lower SPF values. One submission argued that the SPF test does not fully account for photostability because the solar simulator emission spectrum is different than natural sunlight. However, this difference is an unavoidable limitation in testing because solar simulators cannot perfectly replicate natural sunlight.

We acknowledge that UV radiation can change the composition of sunscreen products if the products are not photostable, as demonstrated by the submitted data. However, we are not certain that these data are applicable under actual use conditions. The data regarding the effects of UV radiation on the protection provided by sunscreen active ingredients are limited and inconclusive. Therefore, we are not creating a photostability test as part of the SPF test procedure in this document.

C. In Vitro SPF Test

One submission suggested replacing the proposed in vivo SPF test with an in vitro SPF test (Ref. 1). An in vitro SPF test would have advantages of faster performance, lower expense, and no exposure of subjects to UV radiation.

We agree that an in vitro SPF test has these advantages. However, we are not replacing the in vivo SPF test with an in vitro SPF test for the same reasons we stated in the 2007 proposed rule (72 FR 49070 at 49095). One shortcoming of an in vitro test is the lack of data on the performance characteristics of in vitro test substrates, such as quartz or artificial skin. In the 2007 proposed rule, we stated that data failed to show that a substrate adequately mimicked the physiological characteristics of human skin. We stated that we would consider an in vitro test if validating data demonstrated that the performance of the in vitro test was equivalent to the in vivo test. We have not received adequate data to validate an in vitro SPF test. Therefore, we are not including an in vitro test in this document.

D. Anti-Inflammatory Ingredients

One submission recommended requiring a test to verify that sunscreen products do not contain anti-inflammatory ingredients that significantly decrease erythemic response to UV radiation (Ref. 1). The submission did not identify specific anti-inflammatory ingredients. The submission argued that, by decreasing the erythemal response, these ingredients could falsely inflate SPF values determined in SPF testing. In addition, these anti-inflammatory ingredients may increase the likelihood of unwanted harmful effects from sun exposure because sunburn, a cue to avoid sun exposure, would be less evident.

Although the submission raises a serious concern, we are not aware of any data confirming that this problem exists. Therefore, a test to show that anti-inflammatory ingredients may be decreasing erythemic response to UV radiation is not required at this time. It seems unlikely that anti-inflammatory ingredients will affect SPF values because their anti-erythemic effect is relatively short-lived compared to the 16–24 hour interval between UV exposure and erythema observation in the SPF test.

VIII. Broad Spectrum Test

In this document, we are referring to testing involving the UVA part of the spectrum as "broad spectrum testing." The term "broad spectrum" more accurately describes the test as covering the full extent of the terrestrial solar UV spectrum (*i.e.*, UVA and UVB radiation). Section VIII.A. of this document provides our rationale for no longer requiring an in vivo test assessing the persistent pigment darkening associated with UVA radiation. Section VIII.B. of this document explains why the in vitro test should be changed from a modified Diffey-Robson ratio to the critical wavelength test. Section VIII.C. defines the testing parameters to be employed in evaluating the critical wavelength of an OTC sunscreen product.

A. In Vivo Test Method: Not Required

We stated in the 2007 proposed rule that an assessment of UVA protection should include determination of both the magnitude and breadth of absorption in the UVA part of the spectrum (72 FR 49070 at 49102 through 49106). We proposed that an in vivo Persistent Pigment Darkening (PPD) test be used to evaluate the magnitude of absorption and an in vitro test be used to evaluate the breadth of absorption. The PPD test, a modification of the PPD test accepted by JCIA[10] since 1996, is almost identical to the SPF test. It is recognized as a standard for the in vivo assessment of UVA protection by the JCIA and the European Commission

[10] Japanese Cosmetic Industry Association.

(Ref. 7). The most significant differences in the PPD test compared to the SPF test are (1) the light source emits only UVA radiation (320–400 nm) and (2) the endpoint is darkening of the skin (tanning) rather than reddening of the skin (erythema).

We have concluded that the PPD test is not necessary to establish that a sunscreen product provides protection against UVA radiation. The magnitude of absorption over the solar terrestrial UV portion of the spectrum (both UVA and UVB) can be effectively assessed based on the SPF test in combination with a pass/fail broad spectrum in vitro test (see Section VIII.B of this document). If sunscreen products pass the in vitro broad spectrum test, then the amount of UVA radiation protection, as well as UVB radiation protection, must increase as the SPF value increases. For example, a Broad Spectrum SPF 40 sunscreen product must provide more UVB and UVA radiation protection than a Broad Spectrum SPF 20 sunscreen product.

For sunscreen products that pass the in vitro broad spectrum test, we have concluded that the SPF and PPD tests are redundant of each other, but we have reasons to prefer the SPF test. The SPF and PPD tests are both clinical and indicative of the magnitude of absorbance of UV radiation. Furthermore, both tests depend on the skin type of the individual. The SPF test measures skin reddening, which is due primarily to UV radiation in the UVB and UVA II regions (290–340 nm). The PPD test measures skin darkening, which is due primarily to UV radiation in the UVA II part of the spectrum (320–340 nm). Therefore, the UV radiation range covered by the PPD test is also covered by the SPF test. In both tests, the endpoint is indicative of how much UV radiation is absorbed. As the magnitude of UV radiation absorbance increases for a sunscreen product, both the SPF and PPD ratings increase.

We have identified several disadvantages of the PPD test as described in the proposed rule (72 FR 49070 at 49103):

- Human subjects are exposed to high doses of UVA radiation with unknown health consequences.
- Exposure to UVA radiation alone (*i.e.*, in the absence of UVB radiation) is never encountered in nature, and the biological effects of such exposure may differ greatly from those due to exposure to natural sunlight.
- Because it is unclear how tanning relates to the harmful effects of sunlight, it is unclear whether persistent pigment darkening represents a clinically meaningful endpoint.

Other disadvantages are pointed out by Nash *et al.* (Ref. 4):

- The physical properties of sunscreen products may differ when sunscreen products are exposed to UVA radiation alone.
- The PPD test is expensive, time consuming, and labor intensive.
- The ability to identify small differences in pigmentation requires a high degree of expertise and interpretation of pigmentation changes will be dependent on the examiner.
- There may be a high degree of variability in test results between subjects in the same test panel as well as between different test panels for the same sunscreen product.
- The test results may not be reproducible between labs.

Because of these disadvantages of conducting the PPD test, and the fact that information obtained from such tests is already provided by SPF testing for sunscreen products that pass the in vitro broad spectrum test, we are eliminating the requirement to conduct a PPD or any other in vivo UVA test in this final rule.

B. In Vitro Test Method: Critical Wavelength

Many submissions objected to our proposal to use a modification of the Boots adaptation of the Diffey/Robson ratio as an in vitro measure of UVA protection (Ref. 1). The Diffey/Robson ratio evaluates UVA protection relative to UVB protection. The ratio is calculated as the area under the absorbance curve in the UVA region (320–400 nm) divided by the area under the absorbance curve in the UVB region (290–320 nm). As the degree of protection against UVA radiation increases, the ratio increases.

We proposed a modification of this ratio to be calculated as the area under the absorbance curve in the UVA I region (340–400 nm) divided by the area under the absorbance curve over total UVB and UVA range (290–400 nm). We indicated that this modification was necessary because we were concerned that a sunscreen product absorbing strongly in the UVA II region (320–340 nm), but not absorbing strongly in the UVA I region, might produce a disproportionately high ratio value (72 FR 49070 at 49105). We would not consider this sunscreen product to be a good broad spectrum sunscreen product even though it has a high ratio value. We noted the importance of ensuring that protection extends well into the UVA I region (340–400 nm), because neither SPF nor PPD measurements provide much information about the longer wavelengths of UVA radiation. Therefore, we modified the ratio to give more emphasis to the UVA I area under the absorbance curve.

Many submissions argued that we should require a determination of critical wavelength rather than the proposed ratio to determine broad spectrum protection (Ref. 1). We agree with the arguments made in the submissions. Therefore, in this document, we are requiring that broad spectrum protection be assessed by determining the critical wavelength of a sunscreen formulation. The submissions noted the following disadvantages with the proposed ratio:

- The proposed ratio places too much emphasis on the UVA I region, which is not generally considered to contribute significantly to the harmful effects of exposure to UV radiation.
- A large ratio could result if one or more ingredients absorb radiation in the shorter wavelength UVA II region but not at all or only minimally in the longer wavelength UVA I region. For example, oxybenzone absorbs radiation at 340–360 nm, and inclusion of this ingredient at higher concentrations might result in a high ratio even though it does not provide true broad spectrum protection.
- The proposed ratio is not a validated measure of UVA protection and is not used anywhere else in the world.
- To achieve high ratios with existing GRASE active ingredients, the concentrations of ingredients that absorb in the UVB and UVA II parts of the spectrum have to be reduced, lowering protection in these parts of the spectrum (*i.e.*, the SPF has to be lowered to increase the ratio).

We agree that our proposed ratio is not the most appropriate in vitro measure of broad spectrum protection. In agreement with many of the submissions, we have concluded that the ratio places too much emphasis on absorption in the UVA I part of the spectrum. Although there is some evidence that UVA I radiation contributes to immune suppression and an increase in p53-positive cells, the effects of UVA I radiation on these processes are 100 to 1000 times less than the effects attributed to UVB and UVA II radiation (Ref. 4). We also acknowledge that there is no experience using the proposed ratio. Further, we received some data in the submissions that demonstrate the need to reduce SPF values in order to achieve high ratio values. We are concerned that, in an effort to gain UVA protection, consumers may be more susceptible to

sunburn because SPF values could be lower in products with higher ratios.

In agreement with many of the submissions, we have concluded that the critical wavelength method provides a better measure of broad spectrum protection. The critical wavelength (λ_c) is derived from the same data as the modified ratio. The critical wavelength is the wavelength at which the area under the absorbance curve represents 90 percent of the total area under the curve in the UV region. This is expressed mathematically as:

$$\int_{290}^{\lambda c} A(\lambda)d\lambda = 0.9 \int_{290}^{400} A(\lambda)d\lambda$$

In this expression, $A(\lambda)$ is the mean absorbance at each wavelength, and $d\lambda$ is the wavelength interval between measurements.

Like the proposed ratio, the critical wavelength measures the breadth of the UV absorbance curve. Unlike the proposed ratio, the critical wavelength does not emphasize certain parts of the UV spectrum, but is a measure of absorbance across the entire solar terrestrial UV spectrum (UVB and UVA radiation). Sunscreen products offering primarily UVB protection would have a critical wavelength less than 320 nm, whereas those providing both UVB and UVA protection would have critical wavelengths between 320 and 400 nm.

The critical wavelength method is simple, reproducible, and inexpensive. It has been used by sunscreen manufacturers to evaluate UVA protection for over a decade and is one of the most commonly used UVA tests. This is evidenced by the organizations that recommend its use for determining broad spectrum protection, including the European Commission, the American Academy of Dermatology, the American Society for Dermatologic Surgery, and the Skin Cancer Foundation (Ref. 1).

In this document, we are requiring that sunscreen products have a critical wavelength of at least 370 nm (the mean value must be equal to or greater than 370 nm) to be labeled as providing broad spectrum protection (see section VIII.B.). This differs from the tiered rating (low, medium, high, and highest) that we included in the 2007 proposed rule (proposed 21 CFR 352.50(b)(2)). We have concluded that the threshold critical wavelength for a broad spectrum statement should be 370 nm. This wavelength is sufficiently difficult to achieve and will ensure that sunscreen products meeting this threshold provide a significant amount of broad spectrum protection. On the other hand, it is not so difficult to formulate sunscreen products to achieve this critical wavelength that manufacturers cannot develop broad spectrum sunscreen products. We have concluded that UV radiation in the range of 370—400 nm is not very harmful based on the available action spectra for sunburn and skin cancer. We conclude that most of the harmful effects from the sun are caused by UV radiation in the range of 290—370 nm. Further, we conclude that critical wavelength (breadth of UVB and UVA protection) coupled with the SPF value (magnitude of UVB and UVA protection) provides a complete measure of broad spectrum protection provided by a sunscreen product.

C. Critical Wavelength Test Parameters

Although the proposed ratio and critical wavelength calculations are different, both tests are based on the construction of a transmittance curve over the range of UV wavelengths from 290 to 400 nm. We received several submissions requesting that we change or, in some cases, better define aspects of the methodology used to measure transmittance over these wavelengths (Ref. 1). Although the submissions, in most cases, referred specifically to the proposed ratio test, the points made regarding methodology apply equally to the critical wavelength test.

We are making several revisions to the section we referred to as the "UVA in vitro testing procedure" in the 2007 proposed rule (proposed 21 CFR 352.71). To more accurately describe the test as covering both the UVB and UVA regions of the spectrum, we now refer to the test as the "broad spectrum test." The revisions are listed in Table 5 in the order in which they appear in this section of the document.

TABLE 5—SUMMARY OF REVISIONS TO THE PROPOSED IN VITRO BROAD SPECTRUM TEST INCLUDED IN THIS FINAL RULE

Revised test parameter	2007 proposed rule	This final rule
Plate	Quartz plate (21 CFR 352.71(b))	PMMA[1] plate (21 CFR 201.327(j)(1)(i))
Term "spectroradiometer"	Spectroradiometer (21 CFR 352.71(c) and (d))	Spectrometer (21 CFR 201.327(j)(1)(ii), (iv), and (v))
Light source for transmittance measurements.	Solar simulator (21 CFR 352.71(a))	Produce a continuous spectral distribution of UV radiation from 290 to 400 nanometers (21 CFR 201.327(j)(1)(iii)
Input optics: Bandwidth	5 nanometers (21 CFR 352.71(d))	1 nanometer (21 CFR 201.327(j)(1)(iv))
Dynamic range of the spectrometer.	Not specified	Sufficient to measure transmittance accurately through highly absorbing sunscreen (21 CFR 201.327(j)(1)(v))
Application of sunscreen drug product to plate.	2.0 mg/cm^2 with single-phase spreading (21 CFR 352.71(e))	0.75 mg/cm^2 with 2-phase spreading (21 CFR 201.327(j)(2))
Pre-Irradiation dose	Proportional to SPF value (21 CFR 352.71(f))	Fixed at 800 J/m^2-eff (21 CFR 201.327(j)(3))
Number of transmittance measurements.	12 measurements of mean transmittance on 5 different plates (21 CFR 352.71(g) and (i))	5 measurements of mean transmittance on 3 different plates (21 CFR 201.327(j)(4) and (6))
Calculation of critical wavelength.	Not applicable	21 CFR 201.327(j)(7))

[1] Polymethylmethacrylate

We re-organized the broad spectrum test parameters in this final rule so that they are listed in the order that the test is done. This section of the document begins with a description of the plates to be used and the requirements for UV spectrometry. The next section addresses application of the sunscreen product to the plate, and the following section addresses the pre-irradiation procedure. The last sections included under broad spectrum test parameters address measuring the amount of radiation transmitted through the sunscreen product, converting these measurements to absorbance values, and calculating the critical wavelength of a sunscreen product.

All of the proposed test parameters were re-evaluated in the preparation of this document. Some of the parameters did not require revision. Test parameters not revised include:

- Sample holder
- Input optics (other than slit width)
- Light source for pre-irradiation
- Calculation of mean transmittance values
- Calculation of mean absorbance values

The parameters defined in this section are based on our review of submitted data (Ref. 1) and peer-reviewed literature. Wherever possible and consistent with sound science, we have attempted to harmonize the parameters with existing standards, including those of the European Commission (Ref. 7) and COLIPA (Ref. 69). As stated earlier in this document, we are also actively involved in the ISO working group responsible for developing methodologies for assessing sun protection (both UVB and UVA protection).

1. Plate

Many submissions argued that we should specify that roughened PMMA (polymethylmethacrylate) plates be used as a substrate rather than roughened quartz included in the 2007 proposed rule (Ref. 1). The submissions stated that they prefer PMMA plates because these plates are:

- Less expensive than quartz
- Disposable—no need to clean or re-roughen
- Readily available with roughened surface
- Validated in COLIPA ring tests and in widespread use for more than a decade
- Recommended by the European Commission and COLIPA

We agree with these submissions and are specifying, in this document, that PMMA plates be used as the substrate in this document. We are specifying the use of PMMA plates primarily because the vast majority of validation data we have reviewed was collected using PMMA rather than quartz plates. Further, we agree with the submissions noting that PMMA plates are less expensive than quartz and, therefore, can be disposable. The disposability of the PMMA plates will eliminate the requirements for cleaning and re-roughening the surface characteristic of quartz plates.

Consistent with COLIPA, we are also specifying the degree of roughness and size of the application area on these plates. Plates should be roughened on one side to a three-dimensional surface topography measure (Sa) between 2 and 7 micrometers. These Sa values are supported by validation studies (Ref. 70) and are comparable to those recommended by COLIPA (Ref. 69). The application area must be at least 16 square centimeters with no side shorter than 4 centimeters. We are also replacing the word "substrate" with the simpler and more widely used term "plate."

These changes are included in 21 CFR 201.327(j)(1)(i) of this document. Specifying standardized roughness and size parameters will result in more accurate and reproducible intra- and inter-laboratory measurements of broad spectrum photoprotection. Because these PMMA plates of specified roughness and size are already being used in many parts of the world and are recommended by COLIPA, we have concluded that they can be employed in broad spectrum testing in this country with minimal expense or training of personnel.

2. "Spectroradiometer" vs. "Spectrometer"

Four submissions asked us to replace the term "spectroradiometer" with the more generally used term "spectrophotometer" (Ref. 1). We originally chose the term "spectroradiometer" because UV radiation is not detectable by the human eye and, therefore, is not gauged by photometry (which measures visible light). However, the term "spectrophotometer" is often used interchangeably with the term "spectroradiometer." In this document, we are replacing the term "spectroradiometer" with the more inclusive term "spectrometer." Use of the term "spectrometer" allows the use of either a spectroradiometer or spectrophotometer and will make the language more consistent with current COLIPA guidelines (Ref. 69).

3. Light Source for Transmittance Measurements

Four submissions (Ref. 1) asserted that it is inappropriate to specify a solar simulator as the light source for measuring transmittance (proposed 21 CFR 352.71(a)). Three of the submissions argued that radiation emitted from a solar simulator is filtered such that there is very low energy output in the UV region below 300 nm (Ref. 1). One submission noted that a light source filtered in this way cannot provide sufficient energy to measure transmittance through highly absorbing sunscreen products. The same submission suggested that there may not be enough transmittance at wavelengths less than 300 nm to exceed the noise level of the system even in the absence of a sunscreen product (when transmittance should be maximal).

We agree with the submissions and, in 21 CFR 201.327(j)(1)(iii) of this document, are specifying that the light source for transmittance measurements provide continuous, full spectrum radiation from 290 to 400 nanometers. The use of such a light source should maximize instrument transmission properties while retaining full sensitivity. We note that this type of light source is recommended by COLIPA (Ref. 69).

4. Wavelength Interval Between Transmittance Measurements

Two submissions argued that we should reduce the wavelength intervals between transmittance measurements from the proposed 5 nm to 1 nm (Ref. 1). The submissions stated that specifying a smaller interval would produce more accurate results and noted that current spectrometers are capable of making measurements at 1 nm intervals. We agree with the submissions. Additionally, we are aware that the COLIPA guideline (Ref. 69) specifies that transmittance measurements are to be taken at 1 nm intervals. Therefore, we are revising the required input slit bandwidth in this document to specify that it be less than or equal to 1 nm (new 21 CFR 201.327(j)(1)(iv)). We are also revising the measurement interval (new 21 CFR 201.327(j)(4)) to state that transmittance values should be measured at 1 nm intervals.

5. Dynamic Range of the Spectrometer

We are adding new 21 CFR 201.327(j)(1)(v) to specify that the dynamic range of the spectrometer be "sufficient to measure transmittance accurately through a highly absorbing sunscreen product at all UV

wavelengths (between 290 and 400 nm)." The information in this section had been included in the section entitled "Calculation of the spectral transmittance at each wavelength interval" in the proposed rule (proposed 21 CFR 352.71(g)). We considered requiring a minimum dynamic range of 2.2 absorbance units, as specified in the COLIPA guidelines (Ref. 69). However, we have concluded that it is not necessary to include this requirement because nearly all current spectrometers are capable of measuring a dynamic range of 2.2 absorbance units or better.

6. Application of Sunscreen Product to PMMA Plate

Thirteen submissions (Ref. 1) expressed one or more concerns over the method by which we proposed applying sunscreen product to the plate (proposed 21 CFR 352.71(e)). Eleven of the thirteen submissions recommended we reduce the amount applied from 2 milligrams per square centimeter (mg/cm^2) to between 0.75 and 1.2 mg/cm^2. Three submissions suggested we specify that the sunscreen product be applied with a better defined spreading action. Two submissions requested we consider requiring that a saturated fingertip be used to apply the product rather than a gloved finger.

We are reducing the application amount in this document because transmittance of UV radiation through a film of 2 mg/cm^2 thickness is low and, therefore, can result in inaccurate and/or irreproducible measures of UVA protection. UV detectors have a range of UV radiation that they can accurately measure referred to as the dynamic range. If UV radiation is outside the dynamic range (either lower or higher), measurements from the detector become less accurate and often less reproducible. We received validation data demonstrating that application amounts lower than 2 mg/cm^2 are more accurate and reproducible than an application of 2 mg/cm^2 (Ref. 1). The 2007 proposed rule required an application amount of 2 mg/cm^2 because this is the amount specified in the proposed in vivo SPF and PPD tests. We are not including the PPD test in this document and we have concluded that consistency with the SPF test is not warranted given the concerns about inaccurate and/or irreproducible results with an application amount of 2 mg/cm^2 in the in vitro UVA method. A reduced application amount is consistent with the COLIPA guidelines (Ref. 69). Both of these documents specify an application amount of 0.75 mg/cm^2. Data we have reviewed from the Personal Care Product Council demonstrate that application of 0.75 to 1.0 mg/cm^2 results in good transmission within the dynamic range of UV detectors (Ref. 1). Therefore, in this document, we are reducing the application amount to 0.75 mg/cm^2 to ensure the UV radiation transmitted through sunscreens is within the dynamic range of UV detectors (21 CFR 201.327(j)(2)).

We are also specifying the type of spreading action to be employed when applying sunscreen product to a plate. One submission noted that the type of spreading action employed would depend on the type of product being applied. The submission argued that it might take 30 seconds to evenly spread thicker water resistant creams, but only 10 seconds to evenly spread lotions or oils. We recognize that the very light spreading action for 10 seconds we proposed may not be sufficient to evenly distribute all dosage forms on a plate (proposed 21 CFR 352.71(e)). One submission provided data from a ring test involving 7 different laboratories showing that the UVAI/UV absorbance ratio is affected by the amount of pressure applied during application. A second submission referenced a paper by Ferrero *et al.* which shows that light pressure applied to some sunscreen products results in different ratios than application with greater pressure (Ref. 70). Both submissions recommended adopting a two-phase application process like that recommended by COLIPA (Ref. 69).

We agree that a two-phase spreading action is a more effective means of achieving a film of uniform thickness and distribution for a variety of sunscreen dosage forms than is the proposed 10 seconds of light spreading. This type of spreading action is more reflective of actual use than the method we proposed. Therefore, we are harmonizing the standard with the COLIPA guidelines by specifying that a two-phase process be used. Section 201.327(j)(2) in this document specifies that "spreading should be done with a very light spreading action for approximately 30 seconds followed by spreading with greater pressure for approximately 30 seconds."

Two submissions argued that we should specify a saturated fingertip be used rather than a gloved finger. We do not agree for the reasons specified in section VI.E of this document.

7. Pre-Irradiation Dose

Several submissions expressed concern that the pre-irradiation dose we proposed to account for differences in photostability is too high, particularly if we reduce the application amount (Ref. 1). We proposed that the pre-irradiation dose be proportional to the SPF value of a sunscreen product (proposed 21 CFR 352.71(f)). This was to account for the possibility that consumers may spend more time in the sun with higher SPF products. Proportional pre-irradiation dosing is also recommended in the testing procedure published by COLIPA (Ref. 69). In these documents, the pre-irradiation dose is determined relative to the UVA protection factor. Pre-irradiation dose increases as the UVA protection factor increases.

Two submissions suggested that we use a fixed or absolute dose rather than a relative dose proportional to the SPF value of a sunscreen product (Ref. 1). The submissions noted that, at the same time and location on the earth's surface, all sunscreen products are exposed to the same intensity of sunlight. Therefore, sunscreen products with higher SPF values or UVA protection factors should not be exposed to higher pre-irradiation doses.

We agree with these two submissions. It is appropriate to evaluate sunscreen product photostability using a fixed exposure intensity. We have data demonstrating that avobenzone-containing sunscreen products undergo almost complete photodegradation when exposed to doses between 2 and 3 MEDs[11] (Ref. 71). At a dose of 4 MEDs, there were no further decreases in UVB and UVA absorption of five different sunscreen products containing 2.5- to 3- percent avobenzone. These data reflect the worst case scenario for photodegradation because avobenzone appears to be the least photostable active ingredient in the sunscreen monograph. Therefore, all sunscreen products marketed under the monograph are likely to be completely degraded after 4 MEDs. Based on this data, we are specifying a fixed pre-irradiation dose equivalent to 4 MEDs. As we noted in the 2007 proposed rule, one MED for a skin type II individual is 200 J/m^2-eff (72 FR 49070 at 49107). Therefore, in this document, we are specifying a pre-irradiation dose of 4 times 200 J/m^2-eff (800 J/m^2-eff).

8. Number of Transmittance Measurements

Two submissions (Ref. 1) stated that requiring 12 transmittance measurements on each plate as proposed is excessive and not statistically warranted (proposed 21 CFR 352.71(g)). One submission provided data showing that there are no significant differences in UVAI/UV ratios calculated based on 3, 5, 8, or 12

[11] Minimal erythema dose—the lowest UV dose that produces skin reddening (erythema).

sub-sites per plate. The submission argued that we should reduce the number of required test sites per sample to 6. The other submission proposed that we require only one transmittance measurement per plate. The submission suggested that, rather than taking multiple measurements from several small areas on the plate, one measurement could be made over a relatively broad area.

One of the submissions also argued that it is not necessary to evaluate transmittance on five different plates (proposed 21 CFR 352.71(j)). The submission provided data showing that the UVAI/UV ratio for an SPF 15 sunscreen product is not significantly different whether it is measured on 1, 2, 3, or 5 plates (with 12 measurements per plate). We note that the COLIPA guidelines (Ref. 69) recommend that 3 separate plates be used.

We agree with the submissions that requiring 12 discrete measurements on each plate is not necessary to obtain an accurate transmittance spectrum. The submitted data demonstrate that there are no significant differences in UVAI/UV ratios based on 3, 5, 8, or 12 test sites. Similarly, we agree with the submissions that requiring measurements for five plates is not necessary to obtain an accurate transmittance spectrum. Determining 12 transmittance measurements on five plates, as proposed, results in a total of 60 transmittance measurements. Based on the submitted data, a total of 15 transmittance measurements should produce an accurate transmittance spectrum. Therefore, we are requiring 5 or more measurements on at least 3 different plates (21 CFR 201.327(j)(6) in this document.

9. Determination of Critical Wavelength

Critical wavelength is to be determined as described in section VIII.B of this document.

IX. Analysis of Impacts

A. Final Regulatory Impact Analysis

We have examined the impacts of the final rule under Executive Order 12866, Executive Order 13563, the Regulatory Flexibility Act (5 U.S.C. 601–612), and the Unfunded Mandates Reform Act of 1995 (Pub. L. 104–4). Executive Orders 12866 and 13563 direct agencies to assess all costs and benefits of available regulatory alternatives and, when regulation is necessary, to select regulatory approaches that maximize net benefits (including potential economic, environmental, public health and safety, and other advantages; distributive impacts; and equity). OMB has determined that this final rule is a significant regulatory action under Executive Order 12866. Consistent with Executive Order 13563, the approach taken here maintains "flexibility and freedom of choice for the public," above all by providing "information for the public in a form that is clear and intelligible.

The Regulatory Flexibility Act requires agencies to analyze regulatory options that would minimize any significant impact of a rule on small entities. Because we lack information characterizing the number of products by firm-size and because most affected entities are considered small, we conclude that this final rule will have a significant economic impact on a substantial number of small entities.

Section 202(a) of the Unfunded Mandates Reform Act of 1995 requires that agencies prepare a written statement, which includes an assessment of anticipated costs and benefits, before proposing "any rule that includes any Federal mandate that may result in the expenditure by State, local, and tribal governments, in the aggregate, or by the private sector, of $100,000,000 or more (adjusted annually for inflation) in any one year." The current threshold after adjustment for inflation is $136 million, using the most current (2010) Implicit Price Deflator for the Gross Domestic Product. We do not expect this final rule to result in any 1-year expenditure that would meet or exceed this amount.

1. Background

The purpose of this rule is to finalize labeling and testing conditions under which OTC sunscreen drug products marketed without approved applications are not misbranded. This rule addresses labeling and testing requirements for both UVB and UVA radiation protection. The rule modifies the existing SPF test, specifies a test for broad spectrum protection, and requires changes to the product label that affect both the front of the package (the principal display panel or PDP) and the Drug Facts section. In addition, the rule lifts the stay of effective date applied to the 1999 Drug Facts labeling final rule (64 FR 13254) specifically for sunscreen products (66 FR 67485). All manufacturers of sunscreens will incur some labeling costs due to revisions to both the PDP and the Drug Facts section of the product label (see section IX.A.4 of this document). In addition, many manufacturers will incur additional broad spectrum testing costs unless they have already tested their products according to the broad spectrum test required in this rule. Manufacturers of sunscreens will also incur SPF testing costs (see section IX.A.5 of this document). Some manufactures will also have to relabel products that are currently labeled with claims that are not allowed under this final rule (§ 201.327(g) and § 310.545(a)(29)(ii)).

2. Benefits

As discussed in section IV.B of this document, the regular use of a Broad Spectrum SPF 15 or higher sunscreen product, when combined with limiting time in the sun and wearing clothing to protect sun-exposed areas, reduces the risk of skin cancer and early skin aging. The National Cancer Institute estimates that there are more than one million new cases of non-melanoma skin cancer and more than 68,000 new cases of melanoma per year in the United States (Refs. 72 and 73). According to the National Cancer Institute, about 8,700 persons will die of melanoma in 2010. Fatal cases of non-melanoma skin cancer are less common but nonetheless number several hundred per year. The labeling requirements in this rule, in conjunction with implementing the format and content requirements in 21 CFR 201.66, which were stayed for sunscreens but are being lifted in this rule, will provide consumers with clear and concise information about sunscreen use and protection, and about the role of sun exposure in increasing the risk of skin cancer and early skin aging. Consumers will be able to more easily identify products that reduce the risks of skin cancer and early skin aging, when used as directed. The new requirements for product testing will ensure the accuracy of the SPF value and broad spectrum claim on the product label.

Although we are unable to quantify the effects of clear and concise information, the final rule will provide clearer and more consistent information on the benefits of certain sunscreens in regard to skin cancer risk reduction than is available on current labels. By requiring better information on levels of protection, the rule should contribute to reduced exposure to UVB and UVA radiation and thereby reduce the incidence of skin cancer.

The benefits from reduced incidence of skin cancer will equal the value of the illnesses averted. The most appropriate measure of that value is based on the average willingness to pay to reduce the probability of skin cancer. We would then multiply the value per illness averted by the likely number of illnesses averted to determine the benefits of this final rule. Because we lack estimates of the likely numbers of illnesses averted, we present estimates of the value per

illness averted to illustrate the gains per averted case.

We estimated the value per case of preventing skin cancer for fatal and non-fatal cases of melanoma and non-melanoma skin cancer. The estimated average medical cost of treatment, lost productivity, and willingness to pay to avoid some symptoms and other effects represents a plausible lower bound on willingness to pay to avoid a non-fatal case of skin cancer. For melanoma, the estimated total cost is about $2,860 per non-fatal case; for non-melanoma skin cancer, the total cost is about $1,400 per non-fatal case; (Refs. 74 and 75).

The largest potential public health gains from this final rule would likely come from averted deaths. We can calculate the monetary value of averted fatal cases as either the value of statistical lives saved or the value of statistical life-years saved. Although skin cancers occur at all ages, most cases occur at older ages. For that reason, we estimate the benefit from preventing fatal cases using the value of life years saved. According to the National Cancer Institute, the average age of death from melanoma is 68 (Ref. 73); life expectancy for a person between the ages of 68 and 69 is about 16 years (Ref. 76). If we discount the average years of life saved for averted fatal melanoma with rates of 3 and 7 percent, we get discounted statistical life-years saved equal to 12.6 and 9.4 years. The various studies of fatal cases of non-melanoma skin cancer find mean or median ages of death in the 77 to 82 range (Refs. 77–79). The life expectancy for someone between the ages of 79 and 80 is about 9 years (Ref. 76). If we discount the average years of life saved for fatal non-melanoma skin cancers with discount rates of 3 and 7 percent, we get discounted years saved equal to 7.9 and 6.5 years.

In other analyses of life-years saved, we have used values for a statistical life-year in the $107,000 to $322,000 range (74 FR 33030, July 9, 2009; updated to current prices). For this illustrative analysis, we use a medium value of $214,000 per statistical life-year. We multiply the value of a statistical life-year by the discounted life-years saved per fatal case of melanoma, which yields $2.69 million using a 3 percent rate of discount and $2.02 million using a 7 percent rate of discount. If we multiply the value of a statistical life-year by discounted life-years saved per fatal case of non-melanoma skin cancer, we get $1.67 million using a 3 percent rate of discount and $1.39 million using a 7 percent rate of discount.

The development of melanoma and non-melanoma skin cancer from chronic exposure to sunlight, as well as any preventative effects of sunscreen (or any other intervention), occur with a long lag. To estimate the monetary value of an averted case of melanoma or non-melanoma skin cancer through combining other protective measures with increased broad spectrum and at least SPF 15 protection, we adjust for the lag between increased protection and a decrease in the incidence of non-melanoma skin cancer. The only available long-term study finds a minimum lag of 5 years before any significant risk reduction would occur (Refs. 20 and 21). Substantial reductions occur with a much longer lag, probably 15 to 25 years; we use a 20-year lag in this illustrative analysis. With a 20-year lag discounted at 3 percent, the value per averted statistical case of non-fatal melanoma is $1,586; if we discount for at 7 percent, the value per averted case is $740. With a 20-year lag discounted at 3 percent per year, the monetary value per averted statistical case of non-melanoma skin cancer is $773; if we discount at 7 percent, the value per averted case is $361.

For fatal cases, with the 20-year lag discounted at 3 percent per year, the monetary value per averted statistical case of fatal melanoma is $1.49 million; discounted at 7 percent, the value per averted fatal case is $520,000. With a 20-year lag and a 3 percent rate of discount, the discounted value per averted case of non-melanoma skin cancer is $920,000 million; with a 7 percent rate of discount, value per averted fatal case is $360,000.

We have four estimates of the discounted value per averted cases of melanoma and non-melanoma skin cancer, with values corresponded to non-fatal and fatal cases. The annual benefits of this final rule will be the numbers of cases of each type averted multiplied by the value of each type. We do not, however, have estimates of the numbers of actual or statistical cases that will be averted. Although there is wide agreement among experts that the use of more effective sunscreens reduces the risk of sun-related skin cancer, we are unaware of any studies that quantify the reduced risk. Without quantitative estimates of the risk reduction associated with broad spectrum protection, we are unable to quantity the overall effects of this final rule on public health.

3. Number of Products Affected

Estimating the number of products affected by this rule is difficult because we do not have complete data on the number of OTC sunscreen products currently marketed. Our Drug Listing System does not have accurate information on the number of marketed OTC sunscreen products. In the 2007 proposed rule (72 FR 49070 at 49108), we estimated that there were about 3,000 OTC sunscreen drug products, including cosmetic products containing sunscreen, with about 12,000 SKUs.[12]

In response to the 2007 proposed rule, we received a submission arguing that our estimates of the number of products and SKUs were low but the submission did not suggest a corrected value. We contracted with the consulting firm Eastern Research Group (ERG) to profile the sunscreen market and assess the cost to reformulate a sunscreen product. ERG's full report can be found in Docket No. FDA–1978–N–0018 (Ref. 80). ERG did an extensive search using the internet and other sources and found fewer dosage forms and SKUs than we had estimated. ERG estimates that there are about 3,065 to 3,600 SKUs. More recently, the new FDA labeling cost model estimates that about 3,591 sunscreen SKUs are marketed, with up to 2,348 different formulations. Because these data are based on a recent survey of the market, we conclude that they are more representative of the number of products affected than the estimates in the proposed rule. For this analysis, we therefore use 3,591 SKUs to represent the number of affected sunscreen labels and 2,348 for the number of formulations.

To comply with the rule, sunscreen products currently marketed as providing broad spectrum protection that were already tested using the test method in this rule will have to be re-labeled but will not have to be retested for broad spectrum protection. Other products will be tested for broad spectrum protection and, if they pass and, will be relabeled with the broad spectrum protection claim. Manufacturers may also choose to reformulate their products to pass the test or discontinue production of the products.

We have not attributed any reformulation costs to this final rule but realize that some manufacturers may choose to reformulate their product if it does not pass the broad spectrum test.

4. Cost To Relabel Sunscreen Products

The cost to relabel varies greatly depending on the printing method and number of colors used. In the 2007 proposed rule, we stated that the majority of sunscreen products are packaged in plastic bottles or tubes with the label printed directly on the

[12] SKUs refers to "stock keeping units," which are individual products, packages, and sizes.

container or applied as a decal or paper label during the packaging process.

The labeling requirements in this rule will change both the PDP and the Drug Facts section of the package and are considered a major redesign. Frequent label redesigns are typical for OTC sunscreen products, with redesigns generally implemented every 1 to 2 years. If a scheduled redesign coincides with relabeling required by this rule, the incremental labeling cost will be lower than if the labeling change takes place before scheduled changes. To estimate the cost to relabel, we are assuming that all products will be relabeled and none are discontinued.

In the 2007 proposed rule, we used a model developed for us by the consulting firm RTI International to derive an estimate of the cost to relabel sunscreen products (Ref. 81). The model was developed to estimate the cost of food labels, which are similar to the labels on the products affected by this final rule. In response to the 2007 proposed rule, we received a submission disagreeing with our estimates of how sunscreens are packaged and the cost to relabel these products (Ref. 1). The submission argued that many sunscreen products, particularly sunscreen-cosmetic combinations, have a secondary container and, therefore, an additional label. The submission also argued that some sunscreen products would require a fold-out label or new secondary carton to accommodate the labeling required in this rule. Furthermore, the submission argued that relabeling these products would cost $15,000 to $17,000 per SKU. The submission did not include any data or information to support its estimate.

We agree that cosmetic packaging and labeling is generally more costly than OTC drug labeling. We also agree that manufacturers of sunscreen-cosmetic products would use the packaging norm of the cosmetic industry because those are the products they are competing with. The cost estimates we are using now demonstrate a large variation in the price per SKU to account for the differences in packaging. If the standard content and format changes required by the OTC labeling final rule (64 FR 13254) are being implemented for the first time, there could be increases in the size of container and carton labels. Since we are allowing, in this rule, for a compliance period of 1 year for most products but 2 years for products with low sales volume ($25,000 annually), inventory losses for unused packaging and labels are minimized and accounted for in this analysis.

For this final rule, we use the new FDA labeling cost model developed by RTI International, which includes estimates for changing sunscreen labels. The one-time costs for a major labeling change to sunscreen labels are $7,454 to $18,785, depending on the type of labeling and packaging. The medium estimate is $11,572 per major labeling changes. These costs include mostly labor and materials, with some cost for lost inventory.

We estimate that the timing of scheduled relabeling will coincide with the relabeling required by this rule for 50 percent of the 3,591 SKUs . We estimate the total labeling cost for the SKUs with coinciding scheduled redesign would be minimal administrative costs or about $550 ($310 to $790). Therefore, the total one-time cost for relabeling would be about $13.9 million to $35.1 million, with a medium estimate of $21.8 million (1,796 × $11,572 + 1,796 × $550).

5. Cost To Test or Retest Products To Determine SPF Values

Manufacturers will incur SPF testing costs because the rule requires labeling for OTC sunscreen products to include SPF values determined in accordance with the specific test method that it describes. We will publish draft guidance entitled "Guidance for Industry: Enforcement Policy—OTC Sunscreen Drug Products Marketed Without An Approved Application" that describes our intended enforcement policy regarding these OTC sunscreen products. In the draft guidance, we propose to exercise enforcement discretion for a period of 2 years after the publication of this final rule with regard to the SPF testing requirements for certain OTC sunscreen products on the market prior to June 17, 2011. We estimate that 65 to 75 percent of sunscreen reformulations, or 1,526 to 1,761 will require SPF retesting. The cost of an SPF test depends on whether the product is also making water resistance claims and the SPF value being tested; the cost of water resistant testing is much higher than static testing (see Table 6). In their analysis of the sunscreen market ERG found that about 5 percent of products claimed water resistance and SPF values less than 30, 3 percent of products claimed water resistance with SPF greater than 30, while the remaining 92 percent could use the static SPF test. We use those percentages to estimate total SPF testing costs of $3.2 to $5.9 million (see Table 6). The midpoint of estimated SPF testing costs is $4.6 million.

TABLE 6—COST OF SPF TESTING

Type of test	Estimated number of formulations		Cost of test		*Total cost*	
	Low	High	Low	High	Low	High
Water resistant, SPF < 30	76	88	$4,500	$4,860	$343,395	$427,923
Water resistant, > 30	46	53	4,500	5,130	260,037	271,018
SPF static test	1,404	1,620	1,900	3,240	2,667,798	5,249,189
Total Cost for SPF testing					3,217,230	5,948,130

6. Cost to Test or Retest Products for Broad Spectrum Protection

In the proposed rule, we estimated that about 75 percent of sunscreen products would need to be tested for broad spectrum protection. We received a submission arguing that our estimate was too low and that at least 90 percent of products would need to be tested (Ref. 1). The argument in the submission was based on the four-tier UVA star rating in the proposed rule. The submission stated that sunscreen products with "low," one-star protection would need to be tested. We have now changed the rating criteria to pass-fail, where a critical wavelength of at least 370 nm is necessary to make the broad spectrum statement. Over the years, there has been a steady increase in the number of products with claims of broad spectrum protection. A recent survey of marketed products found that 65 percent of the products surveyed met the criteria for the broad spectrum statement (Ref. 82). Products that were tested in accordance with the broad

spectrum test in this rule would not need to be re-tested.

Because the broad spectrum test in this rule is different than the proposed test, we assume that all affected products would need to be tested. In the 2007 proposed rule, we estimated a one-time testing cost of approximately $5.4 million for products that have broad spectrum protection claims. This estimate was based on 2,250 sunscreen products (75 percent of marketed products) being tested with a test cost of $2,400. The test costs were estimated as $2,200 for the proposed in vivo test and $200 for the proposed in vitro test. In this rule, we are not requiring the in vivo test.

In response to the proposed rule, we received two submissions arguing that our estimate of $200 for the cost of the in vitro test was too low (Ref. 1). The first submission states that the cost of an in vitro test is $500, and the second states that the cost is $800. The first submission, from a sunscreen manufacturer, states that $500 is the price charged by an independent testing laboratory to test its product. The second submission does not provide any basis for its estimate. Although the in vitro test in this rule is different than the in vitro test in the 2007 proposed rule, the cost to conduct the tests is the same. ERG found that the cost of the test ranges from $300 to $800 (Ref. 80). Assuming all affected marketed product formulations (1,526 to 1,761 formulations) will be tested for broad spectrum protection at a cost ranging from $300 to $800, the total cost to test sunscreen products for broad spectrum protection is estimated to be $457,860 to $1,408,800 [(1,526 × $300) to (1,761 × $800)].

7. Total Incremental Costs

Because we took steps earlier to mitigate the impact of labeling changes on the sunscreen industry by staying the requirements in earlier rules, the labeling costs in this rule incorporate the labeling costs from three final rules:

1. 1999 OTC drug labeling final rule (64 FR 13254)
2. 1999 Sunscreen final rule (64 FR 27666)
3. This rule.

Manufacturers were able to postpone compliance costs when we chose to stay the labeling requirements for the 1999 final rule that standardized the format and content requirements for labeling OTC drug products (21 CFR part 201), which would have become effective for all sunscreens by 2005 (69 FR 53801). We include, as part of labeling costs, the cost of increased container labels and package size to accommodate the Drug Facts format.

The estimated total one-time incremental cost of this rule range $17.6 to 42.5 million [($13.9 million labeling cost + $3.2 million SPF testing cost + $0.5 million broad spectrum testing cost) to ($35.1 million labeling cost + $5.9 million SPF testing cost + $1.4 million broad spectrum testing cost)]. The medium estimated one-time incremental costs are $27.3 million. Annualized over 10 years, the costs are $2.1 to $5 million using a 3 percent rate of discount and $2.5 to $6.1 million using a 7 percent rate of discount. Annualized medium costs are $3.2 million using a 3 percent rate of discount and $3.9 million using a 7 percent rate of discount. If some manufacturers of sunscreen products have already complied with the 1999 final rule and would not otherwise have to relabel products as a result of this final rule, then these estimates may overstate actual total costs.

8. Analysis of Alternatives

The principal alternatives we identified were the inclusion of several provisions from the 2007 proposed rule. In the 2007 proposed rule, we required in vivo and in vitro tests for determining UVA protection. In this rule, we have eliminated the in vivo test requirement, reducing compliance costs by about $5 million. We also proposed labeling on the PDP that would indicate the level of UVA protection. In this rule, we changed the in vitro test to one that measures both UVB and UVA protection (*i.e.*, broad spectrum protection). We also established a pass/fail broad spectrum protection statement on the PDP in place of a UVA rating.

We considered requiring a negative statement on the PDP indicating that a product did not have broad spectrum protection if it failed the in vitro test. Numerous submissions from manufacturers opposed this requirement, and we are concerned that the statement could be misinterpreted by consumers. Moreover, as noted previously, this alternative is beyond the scope of this final rule, which applies only to products that do provide broad spectrum protection.

B. Small Business Impact (Final Regulatory Flexibility Analysis)

We estimate that about 78 percent of the approximately 100 domestic companies that manufacture OTC sunscreen products would be considered small business entities (defined by the Small Business Administration as having fewer than 750 employees). Because most affected entities are considered small, we conclude that this final rule will have a significant economic impact on a substantial number of small entities. Consequently, this analysis, together with other relevant sections of this document, serves as the Final Regulatory Flexibility Analysis, as required under the Regulatory Flexibility Act.

The average one-time incremental cost per firm will be about $185,000 to $445,000, with a medium of about $285,000. This burden, described in more detail in section IX.A of this document, includes labeling costs, SPF testing costs, and broad spectrum testing costs. The economic impact will vary by firm, depending on the number of products requiring testing and the number of SKUs requiring labeling. Also, firm-specific impact will vary inversely with the product sales; the per firm burden will be lower for firms with products with high sales volumes. Because the relative economic impact of product retesting is greater for products with lower sales volume, which could disproportionately affect smaller firms, we are providing a longer implementation period (2 years) for products with annual sales of less than $25,000. Because the OTC drug industry is highly regulated, all firms are expected to have access to the necessary professional skills on staff or to have contractual arrangements to comply with the testing requirements of this rule.

X. Paperwork Reduction Act of 1995

This final rule contains certain information collection provisions that are subject to review by the Office of Management and Budget (OMB) under the Paperwork Reduction Act of 1995 (44 U.S.C. 3501–3520). Specifically, the final rule establishes requirements for SPF labeling based on specified testing of covered products, (21 CFR 201.327(a)(1) and (i)). This rule also lifts the delay of implementation date for § 201.66 (21 CFR 201.66), the general OTC Drug Facts labeling format regulation, which has applied to all OTC sunscreen products (69 FR 53801). The information collections associated with § 201.66 have been approved in accordance with the PRA under OMB Control Number 0910–0340, but this approval does not currently include application of these provisions to OTC sunscreens. (76 FR 9022, February 16, 2011). The lifting of the stay of effective date of § 201.66 for OTC sunscreens will modify this information collection.

Elsewhere in this issue of the **Federal Register**, in accordance with section 3506(c)(2)(A) of the PRA (44 U.S.C.

3506(c)(2)(A)), we are publishing a 60-day notice soliciting public comment on the collections of information resulting from this final rule and will then submit these information collection provisions to OMB for approval. These requirements will not be effective until we obtain OMB approval. We will publish a notice concerning OMB approval of these requirements in the **Federal Register** prior to the effective date of this final rule.

With the exceptions noted above, we conclude that the other provisions of this rule are not subject to OMB review under the PRA. Section 201.327 contains specific labeling information, including directions and warnings, which are a "public disclosure of information originally supplied by the Federal Government to the recipient for the purpose of disclosure to the public" (5 CFR 1320.3(c)(2)) and, therefore, are not collections of information. The requirements for obtaining certain medical history information and informed consent from test subjects (21 CFR 201.327(i)(3)(ii) and (i)(3)(iv)) are not collections of information because information collected from subjects of clinical testing does not constitute information under 5 CFR 1320.3(h)(5). There are no recordkeeping provisions associated with the SPF and broad spectrum testing (*i.e.*, effectiveness testing) described in this rule. The burdens of SPF testing as relevant to labeling (third party disclosures) are addressed in the notice published elsewhere in this issue of the **Federal Register**.

XI. Environmental Impact

FDA has determined under 21 CFR 25.31(a) that this action is of a type that does not individually or cumulatively have a significant effect on the human environment. Therefore, neither an environmental assessment nor an environmental impact statement is required.

XII. Federalism

FDA has analyzed this final rule in accordance with the principles set forth in Executive Order 13132. Section 4(a) of the Executive order requires agencies to "construe * * * a Federal statute to preempt State law only where the statute contains an express preemption provision or there is some other clear evidence that the Congress intended preemption of State law, or where the exercise of State authority conflicts with the exercise of Federal authority under the Federal statute." The sole statutory provision giving preemptive effect to the final rule is section 751 of the FD&C Act (21 U.S.C. 379r). We have complied with all of the applicable requirements under the Executive order and have determined that the preemptive effects of this rule are consistent with Executive Order 13132.

XIII. References

The following references are on display in the Division of Dockets Management (HFA–305), Food and Drug Administration, 5630 Fishers Lane, rm. 1061, Rockville, MD 20857, under Docket No. FDA–1978–N–0018 (formerly 1978N–0038) and may be seen by interested persons between 9 a.m. and 4 p.m., Monday through Friday. (FDA has verified all Web site addresses, but FDA is not responsible for any subsequent changes to the Web sites after this document publishes in the **Federal Register**.

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List of Subjects

21 CFR Part 201

Drugs, Incorporation by reference, Labeling, Reporting and recordkeeping requirements.

21 CFR Part 310

Administrative practice and procedure, Drugs, Labeling, Medical devices, Reporting and recordkeeping requirements.

Therefore, under the Federal Food, Drug, and Cosmetic Act and under authority delegated to the Commissioner of Food and Drugs, 21 CFR part 201 is amended as follows:

PART 201—LABELING

■ 1. The authority citation for 21 CFR part 201 continues to read as follows:

Authority: 21 U.S.C. 321, 331, 351, 352, 353, 355, 358, 360, 360b, 360gg–360ss, 371, 374, 379e; 42 U.S.C. 216, 241, 262, 264.

■ 2. Section 201.327 is added to subpart G to read as follows:

§ 201.327 Over-the-counter sunscreen drug products; required labeling based on effectiveness testing.

The following provisions apply to sunscreen products containing aminobenzoic acid, avobenzone, cinoxate, dioxybenzone, ensulizole, homosalate, meradimate, octinoxate, octisalate, octocrylene, oxybenzone, padimate O, sulisobenzone, titanium dioxide, trolamine salicylate, or zinc oxide, alone or in combination. The provisions do not apply to sunscreen products marketed under approved new drug applications or abbreviated new drug applications.

(a) *Principal display panel.* In addition to the statement of identity in paragraph (b) of this section, the following labeling shall be prominently placed on the principal display panel:

(1) *Effectiveness claim.* (i) *For products that pass the broad spectrum test in paragraph (j) of this section.* (A) The labeling states "Broad Spectrum SPF [insert numerical SPF value resulting from testing under paragraph (i) of this section]".

(B) *Prominence.* The Broad Spectrum SPF statement shall appear as continuous text with no intervening text or graphic. The entire text shall appear in the same font style, size, and color with the same background color.

(ii) *For sunscreen products that do not pass the broad spectrum test in paragraph (j) of this section.* The labeling states "SPF [insert numerical SPF value resulting from testing under paragraph (i) of this section]". The entire text shall appear in the same font style, size, and color with the same background color.

(2) *Water resistance statements.* (i) *For products that provide 40 minutes of water resistance according to the test in paragraph (i)(7)(i) of this section.* The labeling states "Water Resistant (40 minutes)".

(ii) *For products that provide 80 minutes of water resistance according to the test in paragraph (i)(7)(ii) of this section.* The labeling states "Water Resistant (80 minutes)".

(b) *Statement of identity.* The labeling of the product contains the established name of the drug, if any, and identifies the drug as a "sunscreen."

(c) *Indications.* The labeling of the product states, under the heading "Uses," the phrases listed in this paragraph (c), as appropriate. Other truthful and nonmisleading statements, describing only the uses that have been established and listed in this paragraph (c), may also be used, as provided in § 330.1(c)(2) of this chapter, subject to the provisions of section 502 of the Federal Food, Drug, and Cosmetic Act (the FD&C Act) relating to misbranding and the prohibition in section 301(d) of the FD&C Act against the introduction or delivery for introduction into interstate commerce of unapproved new drugs in violation of section 505(a) of the FD&C Act.

(1) For all sunscreen products, the following indication statement must be included under the heading "Uses": "[Bullet] helps prevent sunburn". See § 201.66(b)(4) of this chapter for definition of bullet.

(2) For sunscreen products with a Broad Spectrum SPF value of 15 or higher according to the tests in paragraphs (i) and (j) of this section, the labeling may include the following statement in addition to the indication in § 201.327(c)(1): "[Bullet] if used as directed with other sun protection measures (see Directions [in bold italic

font]), decreases the risk of skin cancer and early skin aging caused by the sun".

(3) Any labeling or promotional materials that suggest or imply that the use, alone, of any sunscreen reduces the risk of or prevents skin cancer or early skin aging will cause the product to be misbranded under section 502 of the FD&C Act (21 U.S.C. 352).

(d) *Warnings.* The labeling of the product contains the following warnings under the heading "Warnings".

(1) *For all sunscreen products.* (i) The labeling states "Do not use [bullet] on damaged or broken skin".

(ii) The labeling states "When using this product [bullet] keep out of eyes. Rinse with water to remove."

(iii) The labeling states "Stop use and ask a doctor if [bullet] rash occurs".

(2) *For sunscreen products that are broad spectrum with SPF values of at least 2 but less than 15 according to the SPF test in paragraph (i) of this section or that do not pass the broad spectrum test in paragraph (j) of this section.* The first statement under the heading "Warnings" states "Skin Cancer/Skin Aging Alert [in bold font]; Spending time in the sun increases your risk of skin cancer and early skin aging. This product has been shown only to help prevent sunburn, not [in bold font] skin cancer or early skin aging."

(e) *Directions.* The labeling of the product contains the following statements, as appropriate, under the heading "Directions." More detailed directions applicable to a particular product formulation may also be included.

(1) *For all sunscreen products.* (i) As an option, the labeling may state "For sunscreen use:".

(ii) The labeling states "[bullet] apply [select one of the following: 'Liberally' or 'generously'] [and, as an option: 'And evenly'] 15 minutes before sun exposure".

(iii) As an option, the labeling may state "[bullet] apply to all skin exposed to the sun".

(iv) The labeling states "[bullet] children under 6 months of age: Ask a doctor".

(2) *For sunscreen products with a Broad Spectrum SPF value of 15 or higher according to the tests in paragraphs (i) and (j) of this section.* The labeling states "[bullet] Sun Protection Measures. [in bold font] Spending time in the sun increases your risk of skin cancer and early skin aging. To decrease this risk, regularly use a sunscreen with a Broad Spectrum SPF value of 15 or higher and other sun protection measures including: [Bullet] limit time in the sun, especially from 10 a.m.–2 p.m. [bullet] wear long-sleeved shirts, pants, hats, and sunglasses".

(3) *For products that satisfy the water resistance test in paragraph (i)(7) of this section.* The labeling states "[bullet] reapply: [Bullet] after [select one of the following determined by water resistance test: '40 minutes of' or '80 minutes of'] swimming or sweating [bullet] immediately after towel drying [bullet] at least every 2 hours".

(4) *For products that do not satisfy the water resistance test in paragraph (i)(7) of this section.* The labeling states "[bullet] reapply at least every 2 hours [bullet] use a water resistant sunscreen if swimming or sweating".

(f) *Other information.* The labeling of the product contains the following statement under the heading "Other information:" "[bullet] protect the product in this container from excessive heat and direct sun".

(g) *False and misleading claims.* There are claims that would be false and/or misleading on sunscreen products. These claims include but are not limited to the following: "Sunblock," "sweatproof," and "waterproof." These or similar claims will cause the product to be misbranded under section 502 of the FD&C Act (21 U.S.C. 352).

(h) *Labeling of products containing a combination of sunscreen and skin protectant active ingredients.* Statements of identity, indications, warnings, and directions for use, respectively, applicable to each ingredient in the product may be combined to eliminate duplicative words or phrases so that the resulting information is clear and understandable. Labeling provisions in § 347.50(e) of this chapter shall not apply to these products.

(i) *SPF test procedure.* (1) *UV source (solar simulator).* (i) *Emission spectrum.* A single port or multiport solar simulator should be filtered so that it provides a continuous emission spectrum from 290 to 400 nanometers (nm) with a limit of 1,500 Watts per square meter (W/m^2) on total irradiance for all wavelengths between 250 and 1,400 nm.

(A) The solar simulator should have the following percentage of erythema-effective radiation in each specified range of wavelengths:

SOLAR SIMULATOR EMISSION SPECTRUM

Wavelength range (nm)	Percent erythemal contribution [1]
< 290	< 0.1
290–300	1.0–8.0
290–310	49.0–65.0
290–320	85.0–90.0
290–330	91.5–95.5
290–340	94.0–97.0
290–400	99.9–100.0

[1] Calculation of erythema action spectrum described in § 201.327(i)(1)(ii) of this section.

(B) In addition, UVA II (320–340 nm) irradiance should equal or exceed 20 percent of the total UV (290–400 nm) irradiance. UVA I (340–400 nm) irradiance should equal or exceed 60 percent of the total UV irradiance.

(ii) *Erythema action spectrum.* (A) Calculate the erythema action spectrum weighting factor (V_i) at each wavelength λ:

(*1*) $V_i(\lambda) = 1.0$ ($250 < \lambda \leq 298$ nm)

(*2*) $V_i(\lambda) = 10^{0.094 * (298 - \lambda)}$ ($298 < \lambda \leq 328$ nm)

(*3*) $V_i(\lambda) = 10^{0.015 * (140 - \lambda)}$ ($328 < \lambda$ 400 nm)

(B) Calculate the erythema-effective UV dose (E) delivered by a solar simulator as follows:

$$E = \sum_{250}^{400} V_i(\lambda) * I(\lambda) * t$$

Where $V_i(\lambda)$ = erythema action spectrum weighting factor at each wavelength λ
$I(\lambda)$ = irradiance (Watts per square meter) at each wavelength λ
t = exposure time (seconds)

Erythema-effective dose (E) is expressed as effective Joules per square meter (J/m^2-eff).

(C) The emission spectrum must be determined using a handheld radiometer with a response weighted to match the spectrum in ISO 17166 CIE S 007/E entitled "Erythemal reference action spectrum and standard erythema dose," dated 1999 (First edition, 1999–12–15; corrected and reprinted 2000–11–15), which is incorporated by reference in accordance with 5 U.S.C. 552(a) and 1 CFR part 51. You may obtain a copy from the ISO Copyright Office, Case Postale 56, CH–1211, Geneva 20, Switzerland, telephone +41–22–749–01–11 or fax +41–22–74 -09–47. *http://www.iso.org.* You may inspect a copy at the Center for Drug Evaluation and Research, 10903 New Hampshire Ave., Bldg. 22, Silver Spring, MD 20993, call 301–796–2090, or at the National Archives and Records Administration (NARA). For information on the availability of this material at NARA, call 202–741–6030, or go to: *http://*

www/archives.gov/federal_register/code_offederal_regulations/ibr_locations.html. The solar simulator output should be measured before and after each phototest or, at a minimum, at the beginning and end of each test day. This radiometer should be calibrated using side-by-side comparison with the spectroradiometer (using the weighting factors determined according to paragraph (i)(1)(ii)(A) of this section) at the time of the annual spectroradiometric measurement of the solar simulator as described in paragraph (i)(1)(iv) of this section.

(iii) *Operation.* A solar simulator should have no significant time-related fluctuations (within 20 percent) in radiation emissions after an appropriate warm-up time and demonstrate good beam uniformity (within 20 percent) in the exposure plane. The delivered dose to the UV exposure site must be within 10 percent of the expected dose.

(iv) *Periodic measurement.* To ensure that the solar simulator delivers the appropriate spectrum of UV radiation, the emission spectrum of the solar simulator should be measured at least annually with an appropriate and accurately calibrated spectroradiometer system (results should be traceable to the National Institute for Standards and Technology). In addition, the solar simulator must be recalibrated if there is any change in the lamp bulb or the optical filtering components (*i.e.*, filters, mirrors, lenses, collimating devices, or focusing devices). Daily solar simulator radiation intensity should be monitored with a broadband radiometer with a response weighted to match the erythema action spectrum in ISO 17166 CIE S 007/E entitled "Erythemal reference action spectrum and standard erythema dose," which is incorporated by reference in paragraph (i)(1)(ii)(C) of this section. If a lamp must be replaced due to failure or aging during a phototest, broadband device readings consistent with those obtained for the original calibrated lamp will suffice until measurements can be performed with the spectroradiometer at the earliest possible opportunity.

(2) *SPF standard.* (i) *Preparation.* The SPF standard should be a formulation containing 7-percent padimate O and 3-percent oxybenzone.

COMPOSITION OF THE PADIMATE O/OXYBENZONE SPF STANDARD

Ingredients	Percent by weight
Part A:	
Lanolin	4.50
Cocoa butter	2.00
Glyceryl monostearate	3.00
Stearic acid	2.00
Padimate O	7.00
Oxybenzone	3.00
Part B:	
Purified water USP	71.60
Sorbitol solution	5.00
Triethanolamine, 99 percent	1.00
Methylparaben	0.30
Propylparaben	0.10
Part C:	
Benzyl alcohol	0.50
Part D:	
Purified water USP	QS [1]

[1] Quantity sufficient to make 100 grams.

Step 1. Add the ingredients of Part A into a suitable stainless steel kettle equipped with a propeller agitator. Mix at 77 to 82 °C until uniform.

Step 2. Add the water of Part B into a suitable stainless steel kettle equipped with a propeller agitator and begin mixing at 77 to 82 °C. Add the remaining ingredients of Part B and mix until uniform.

Step 3. Add the batch of Step 1 to the batch of Step 2 and mix at 77 to 82 °C until smooth and uniform. Slowly cool the batch to 49 to 54 °C.

Step 4. Add the benzyl alcohol of Part C to the batch of Step 3 at 49 to 54 °C. Mix until uniform. Continue to cool batch to 35 to 41 °C.

Step 5. Add sufficient water of Part D to the batch of Step 4 at 35 to 41 °C to obtain 100 grams of SPF standard. Mix until uniform. Cool batch to 27 to 32 °C.

(ii) *HPLC assay.* Use the following high performance liquid chromatography (HPLC) procedure to verify the concentrations of padimate O and oxybenzone in the SPF standard:

(A) *Instrumentation.* (*1*) Equilibrate a suitable liquid chromatograph to the following or equivalent conditions:

(*i*) Column	C–18, 250 millimeters (mm) length, 4.6 mm inner diameter (5 microns)
(*ii*) Mobile Phase.	85:15:0.5 methanol: water: acetic acid
(*iii*) Flow Rate	1.5 milliliters (mL) per minute
(*iv*) Temperature.	Ambient
(*v*) Detector	UV spectrophotometer at 308 nanometers
(*vi*) Attenuation.	As needed

(*2*) Use HPLC grade reagents for mobile phase.

(B) *Preparation of the HPLC reference standard.* (*1*) Weigh 0.50 gram (g) of oxybenzone USP reference standard into a 250-mL volumetric flask. Dissolve and dilute to volume with isopropanol. Mix well.

(*2*) Weigh 0.50 g of padimate O USP reference standard into a 250-mL volumetric flask. Dissolve and dilute to volume with isopropanol. Mix well.

(*3*) Pipet 3.0 mL of the oxybenzone solution and 7.0 mL of the padimate O solution into a 100-mL volumetric flask. Dilute to volume with isopropanol and mix well.

(C) *HPLC system suitability.* (*1*) Make three replicate 10-microliter injections of the HPLC reference standard (described in paragraph (i)(2)(ii)(B) of this section). The relative standard deviation in peak areas should not be more than 2.0 percent for either oxybenzone or padimate O.

(*2*) Calculate the resolution (R) between the oxybenzone and padimate O peaks from one chromatogram as follows:

$$R = \frac{2 * (to - tp)}{Wo + Wp}$$

Where t_o = retention time for oxybenzone
t_p = retention time for padimate O
W_o = oxybenzone peak width at baseline
W_p = padimate O peak width at baseline

If the resolution (R) is less than 3.0, adjust the mobile phase or replace the column.

(D) *SPF standard assay.*

(*1*) The SPF standard is diluted to the same concentration as the HPLC reference standard according to the following steps:

(i) Step 1. Weigh 1.0 g of the SPF standard (described in paragraph (i)(2)(i) of this section) into a 50-mL volumetric flask.

(ii) Step 2. Add approximately 30 mL of isopropanol and heat with swirling until contents are evenly dispersed.

(iii) Step 3. Cool to room temperature (15 to 30 °C) and dilute to volume with isopropanol. Mix well.

(iv) Step 4. Pipet 5.0 mL of the preparation into a 50-mL volumetric flask and dilute to volume with isopropanol. Mix well.

(*2*)(*i*) Inject 10-microliter of diluted SPF standard from paragraph (i)(2)(D)(*1*) of this section and calculate the amount of oxybenzone and padimate O as follows:

$$\text{Percent Oxybenzone} = \frac{\text{Peak area of oxybenzone in sunscreen standard}}{\text{Peak area of oxybenzone in HPLC reference standard}} * 100$$

$$\text{Percent Padimate O} = \frac{\text{Peak area of padimate O in sunscreen standard}}{\text{Peak area of padimate O in HPLC reference standard}} * 100$$

(ii) The percent of oxybenzone and padimate O in the SPF standard should be between 95 and 105.

(3) *Test subjects.* (i) *Number of subjects.* A test panel should include enough subjects to produce a minimum of 10 valid test results. A maximum of three subjects may be rejected from this panel based on paragraph (i)(5)(v)) of this section.

(ii) *Medical history.* (A) Obtain a medical history from each subject with emphasis on the effects of sunlight on the subject's skin. Determine that each subject is in good general health with skin type I, II, or III as follows:

(*1*) Always burns easily; never tans (sensitive).

(*2*) Always burns easily; tans minimally (sensitive).

(*3*) Burns moderately; tans gradually (light brown) (normal).

(*4*) Burns minimally; always tans well (moderate brown) (normal).

(*5*) Rarely burns; tans profusely (dark brown) (insensitive).

(*6*) Never burns; deeply pigmented (insensitive).

(B) Skin type is based on first 30 to 45 minutes of sun exposure after a winter season of no sun exposure. Determine that each subject is not taking topical or systemic medication that is known to alter responses to UV radiation. Determine that each subject has no history of sensitivities to topical products and/or abnormal responses to sunlight, such as a phototoxic or photoallergic response.

(iii) *Physical examination.* Conduct a physical examination to determine the presence of sunburn, suntan, scars, active dermal lesions, and uneven skin tones on the areas of the back to be tested. A suitable source of low power UVA, such as a Woods lamp, is helpful in this process. If any of these conditions are present, the subject is not qualified to participate in the study. The presence of nevi, blemishes, or moles will be acceptable if, in the physician's judgment, they will neither compromise the study nor jeopardize a subject's safety. Subjects with dysplastic nevi should not be enrolled. Excess hair on the back is acceptable if the hair is clipped. Shaving is unacceptable because it may remove a significant portion of the stratum corneum and temporarily alter the skin's response to UV radiation.

(iv) *Informed consent.* Obtain legally effective written informed consent from all test subjects.

(4) *Sunscreen application.* (i) *Test site.* Test sites are locations on each subject's back, between the beltline and the shoulder blades (scapulae) and lateral to the midline, where skin responses to UV radiation are determined. Responses on unprotected skin (no test material applied) and protected skin (sunscreen test product(s) or SPF standard applied) are determined at separate unprotected and protected test sites, respectively. Test sites should be randomly located in a blinded manner. Each test site should be a minimum of 30 square centimeters and outlined with indelible ink.

(ii) *Test subsite.* Test subsites are the locations to which UV radiation is administered within a test site. At least five test subsites should receive UV doses within each test site. Test subsites should be at least 0.5 square centimeters (cm^2) in area and should be separated from each other by at least 0.8 cm. Each test subsite should be outlined with indelible ink.

(iii) *Applying test materials.* Apply the sunscreen test product and the SPF standard at 2 milligrams per square centimeter (mg/cm^2) to their respective test sites. Use a finger cot compatible with the sunscreen to spread the product as evenly as possible.

(iv) *Waiting period.* Wait at least 15 minutes after applying a sunscreen product before exposing the test sites to UV radiation as described in paragraph (i)(5)) of this section. For water resistant sunscreen products, proceed with the water resistance testing procedure described in paragraph (i)(7) of this section after waiting at least 15 minutes.

(5) *UV exposure.* (i) *Definition of minimal erythema dose (MED).* The minimal erythema dose (MED) is the smallest UV dose that produces perceptible redness of the skin (erythema) with clearly defined borders at 16 to 24 hours after UV exposure. The MED for unprotected skin (MED_u) is determined on a test site that does not have sunscreen applied. The MED for protected skin (MED_p) is determined on a test site that has sunscreen applied. An MED_p is determined for the SPF standard ($ssMED_p$). An MED_p is determined for the sunscreen test product ($tpMED_p$).

(ii) *UV exposure for initial* MED_u. For each test subject, administer a series of UV radiation doses expressed as J/m^2-eff (as determined according to paragraph (a)(2) of this section) to the test subsites within an unprotected test site using an accurately calibrated solar simulator. Select doses that are a geometric series represented by 1.25^n (*i.e.*, each dose is 25 percent greater than the previous dose).

(iii) *UV exposure for final* MED_u, $ssMED_p$, and $tpMED_p$. For each subject, determine the final MED_u, $ssMED_p$, and $tpMED_p$ by administering a series of five UV doses to the appropriate test sites. The middle dose (X) in each of these dose series (*i.e.*, the third dose) should equal the initial MED_u times the expected SPF. Note that the expected SPF equals 1 and 16.3 for the final MED_u and $ssMED_p$, respectively. The remaining UV doses in the series depend upon the expected SPF value of the sunscreen test product(s).

For products with an expected SPF less than 8, administer UV doses that increase by 25 percent with each successive dose (*i.e.*, 0.64X, 0.80X, 1.00X, 1.25X, and 1.56X). For products with an expected SPF from 8 to 15, administer UV doses that increase by 20 percent with each successive dose (*i.e.*, 0.69X, 0.83X, 1.00X, 1.20X, and 1.44X). For products with an expected SPF higher than 15, administer UV doses that increase by 15 percent with each successive dose (*i.e.*, 0.76X, 0.87X, 1.00X, 1.15X, and 1.32X).

(iv) *Evaluation of test subsites.* In order that the person who evaluates the test subsites is not biased, he/she should not be the same person who applied the sunscreen drug product to the test site or administered the UV doses. After UV doses are administered, all immediate responses should be recorded. These may include an immediate darkening or tanning, typically grayish or purplish in color, which fades in 30 to 60 minutes; an immediate reddening at the subsite, due to heating of the skin, which fades rapidly; and an immediate generalized heat response, spreading beyond the subsite, which fades in 30 to 60

minutes. After the immediate responses are noted, each subject should shield the exposed area from further UV radiation until the MED is determined. Determine the MED 16 to 24 hours after UV exposure. Because erythema is evaluated 16 to 24 hours after UV exposure, the final MED_u, $ssMED_p$, and $tpMED_p$ are typically determined the day following determination of the initial MED_u. Evaluate the erythema responses of each test subsite using either tungsten or warm white fluorescent lighting that provides at least 450 lux of illumination at the test site. For the evaluation, the test subject should be in the same position as when the test site was irradiated.

(v) *Invalid test data.* Reject test data for a test subject if erythema is not present on either the unprotected or protected test sites; or erythema is present at all subsites; or the responses are inconsistent with the series of UV doses administered; or the subject was noncompliant (*e.g.*, the subject withdraws from the test due to illness or work conflicts or does not shield the exposed testing sites from further UV radiation until the MED is determined).

(6) *Determination of SPF.* (i) Calculate an SPF value for each test subject (SPF_i) as follows:

$$SPFi = \frac{MEDp}{MEDu}$$

(ii) Calculate the mean

$$SPF\ (\overline{SPF})$$

and the standard deviation (s) from the SPF_i values. Calculate the standard error (SE), which equals $s/\sqrt{n}$ (where n equals the number of subjects who provided valid test results). Obtain the t value from Student's t distribution table corresponding to the upper 5-percent point with n—1 degrees of freedom. Determine the labeled SPF value, which equals the largest whole number less than

$$\overline{SPF} - (t * SE).$$

In order for the SPF determination of a test product to be considered valid, the SPF value of the SPF standard should fall within the standard deviation range of the expected SPF (*i.e.*, 16.3 ± 3.43).

(7) *Determination of water resistance.* The following procedure should be performed in an indoor fresh water pool, whirlpool, and/or hot tub maintained at 23 to 32 °C. Fresh water is clean drinking water that meets the standards in 40 CFR part 141. The pool and air temperature and the relative humidity should be recorded.

(i) *Water resistance (40 minutes).* The labeled SPF should be determined after 40 minutes of water immersion using the following procedure:

(A) Step 1: Apply the sunscreen as described in paragraph (d) of this section.

(B) Step 2: Perform moderate activity in water for 20 minutes.

(C) Step 3: Rest out of water for 15 minutes. Do not towel test site(s).

(D) Step 4: Perform moderate activity in water for 20 minutes.

(E) Step 5: Allow test sites to dry completely without toweling.

(F) Step 6: Apply the SPF standard as described in paragraph (d) of this section.

Step 1. Expose test sites to UV doses as described in paragraph (e) of this section.

(ii) *Water resistance (80 minutes).* The labeled SPF should be determined after 80 minutes of water immersion using the following procedure:

(A) Step 1: Apply the sunscreen as described in paragraph (d) of this section.

(B) Step 2: Perform moderate activity in water for 20 minutes.

(C) Step 3: Rest out of water for 15 minutes. Do not towel test site(s).

(D) Step 4: Perform moderate activity in water for 20 minutes.

(E) Step 5: Rest out of water for 15 minutes. Do not towel test site(s).

(F) Step 6: Perform moderate activity in water for 20 minutes.

(G) Step 7: Rest out of water for 15 minutes. Do not towel test site(s).

(H) Step 8: Perform moderate activity in water for 20 minutes.

(I) Step 9: Allow test sites to dry completely without toweling.

(J) Step 10: Apply the SPF standard as described in paragraph (d) of this section.

(K) Step 11: Expose test sites to UV doses as described in paragraph (e) of this section.

(j) *Broad spectrum test procedure.* (1) *UV Spectrometry.* (i) *Plate.* Use optical-grade polymethylmethacrylate (PMMA) plates suitable for UV transmittance measurements. The plate should be roughened on one side to a three dimensional surface topography measure (Sa) between 2 and 7 micrometers and must have a rectangular application area of at least 16 square centimeters (with no side shorter than 4 cm).

(ii) *Sample holder.* The sample holder should hold the PMMA plate in a horizontal position to avoid flowing of the sunscreen drug product from one edge of the PMMA plate to the other. It should be mounted as close as possible to the input optics of the spectrometer to maximize capture of forward scattered radiation. The sample holder should be a thin, flat plate with a suitable aperture through which UV radiation can pass. The PMMA plate should be placed on the upper surface of the sample holder with the roughened side facing up.

(iii) *Light source.* The light source should produce a continuous spectral distribution of UV radiation from 290 to 400 nanometers.

(iv) *Input optics.* Unless the spectrometer is equipped with an integrating sphere, an ultraviolet radiation diffuser should be placed between the sample and the input optics of the spectrometer. The diffuser will be constructed from any UV radiation transparent material (*e.g.*, Teflon® or quartz). The diffuser ensures that the radiation received by the spectrometer is not collimated. The spectrometer input slits should be set to provide a bandwidth that is less than or equal to 1 nanometer.

(v) *Dynamic range of the spectrometer.* The dynamic range of the spectrometer should be sufficient to measure transmittance accurately through a highly absorbing sunscreen product at all terrestrial solar UV wavelengths (290 to 400 nm).

(2) *Sunscreen product application to PMMA plate.* The accuracy of the test depends upon the application of a precisely controlled amount of sunscreen product with a uniform distribution over the PMMA plate. The product is applied at 0.75 mg per square centimeter to the roughened side of the PMMA plate. The sunscreen product should be applied in a series of small dots over the entire PMMA plate and then spread evenly using a gloved finger. Spreading should be done with a very light spreading action for approximately 30 seconds followed by spreading with greater pressure for approximately 30 seconds. The plate should then be allowed to equilibrate for 15 minutes in the dark before the pre-irradiation described in paragraph (c) of this section.

(3) *Sunscreen product pre-irradiation.* To account for lack of photostability, apply the sunscreen product to the PMMA plate as described in paragraph (b) of this section and then irradiate with a solar simulator described in section 352.70(b) of this chapter. The irradiation dose should be 4 MEDs which is equivalent to an erythemal effective dose of 800 J/m² (*i.e.*, 800 J/m²-eff).

(4) *Calculation of mean transmittance values.* After pre-irradiation described in paragraph (c) of this section, mean transmittance values should be

determined for each wavelength λ over the full UV spectrum (290 to 400 nanometers). The transmittance values should be measured at 1 nanometer intervals. Measurements of spectral irradiance transmitted for each wavelength λ through control PMMA plates coated with 15 microliters of glycerin (no sunscreen product) should be obtained from at least 5 different locations on the PMMA plate [C1(λ), C2(λ), C3(λ), C4(λ), and C5(λ)]. In addition, a minimum of 5 measurements of spectral irradiance transmitted for each wavelength λ through the PMMA plate covered with the sunscreen product will be similarly obtained after pre-irradiation of the sunscreen product [P1(λ), P2(λ), P3(λ), P4(λ), and P5(λ)]. The mean transmittance for each wavelength,

$$\overline{T(\lambda)},$$

is the ratio of the mean of the C(λ) values to the mean of the P(λ) values, as follows:

$$\overline{T(\lambda)} = \frac{\sum_1^n P(\lambda)/n}{\sum_1^n C(\lambda)/n}$$

Where $n \geq 5$

(5) *Calculation of mean absorbance values.* (i) Mean transmittance values,

$$\overline{T(\lambda)},$$

are converted into mean absorbance values,

$$\overline{A(\lambda)},$$

at each wavelength by taking the negative logarithm of the mean transmittance value as follows:

$$\overline{A(\lambda)} = -\log \overline{T(\lambda)}$$

(ii) The calculation yields 111 monochromatic absorbance values in 1 nanometer increments from 290 to 400 nanometers.

(6) *Number of plates.* For each sunscreen product, mean absorbance values should be determined from at least three individual PMMA plates. Because paragraph (d) of this section requires at least 5 measurements per plate, there should be a total of at least 15 measurements.

(7) *Calculation of the critical wavelength.* The critical wavelength is identified as the wavelength at which the integral of the spectral absorbance curve reaches 90 percent of the integral over the UV spectrum from 290 to 400 nm. The following equation defines the critical wavelength:

$$\int_{290}^{\lambda c} A(\lambda)d\lambda = 0.9 \int_{290}^{400} A(\lambda)d\lambda$$

Where λc = critical wavelength
A(λ) = mean absorbance at each wavelength
dλ = wavelength interval between measurements

A mean critical wavelength of 370 nm or greater is classified as broad spectrum protection.

PART 310—NEW DRUGS

■ 4. The authority citation for 21 CFR part 310 continues to read as follows:

Authority: 21 U.S.C. 321, 331, 351, 352, 353, 355, 360b–360f, 360j, 361(a), 371, 374, 375, 379e; 42 U.S.C. 216, 241, 242(a), 262, 263b–263n.

■ 5. Section 310.545 is amended by revising paragraphs (a)(29) and (d)(31) and by adding new paragraph (d)(40) to read as follows:

§ 310.545 Drug products containing certain active ingredients offered over-the-counter (OTC) for certain uses.

(a) * * *

(29) *Sunscreen drug products.*

(i) *Ingredients.*

Diethanolamine methoxycinnamate

Digalloyl trioleate

Ethyl 4-[bis(hydroxypropyl)] aminobenzoate

Glyceryl aminobenzoate

Lawsone with dihydroxyacetone

Red petrolatum

(ii) Any ingredients labeled with any of the following or similar claims. Instant protection or protection immediately upon application.

Claims for "all-day" protection or extended wear claims citing a specific number of hours of protection that is inconsistent with the directions for application in 21 CFR 201.327.

* * * * *

(d) * * *

(31) December 31, 2002, for products subject to paragraph (a)(29)(i) of this section.

* * * * *

(40) June 18, 2012, for products subject to paragraph (a)(29)(ii) of this section. June 17, 2013, for products with annual sales less than $25,000.

Dated: June 9, 2011.

Leslie Kux,

Acting Assistant Commissioner for Policy.

[FR Doc. 2011–14766 Filed 6–14–11; 8:45 am]

BILLING CODE 4160–01–P

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration

21 CFR Parts 201 and 310

[Docket No. FDA–2010–D–0509]

Draft Guidance for Industry on Enforcement Policy for Over-the-Counter Sunscreen Drug Products Marketed Without an Approved Application; Availability

AGENCY: Food and Drug Administration, HHS.

ACTION: Notice of availability.

SUMMARY: The Food and Drug Administration (FDA) is announcing the availability of a draft guidance for industry entitled "Enforcement Policy—OTC Sunscreen Drug Products Marketed Without an Approved Application." The draft guidance is intended to inform manufacturers of over-the-counter (OTC) sunscreen products about our enforcement policy for certain OTC sunscreen products marketed without an approved new drug application. The draft guidance describes our intended approach to enforcement for certain OTC sunscreen products prior to an effective final monograph.

DATES: Although you can comment on any guidance at any time (see 21 CFR 10.115(g)(5)), to ensure that the Agency considers all comments on this draft guidance before it begins work on the final version of the guidance, submit either electronic or written comments on the draft guidance by August 16, 2011. Submit written comments on the proposed collection of information by August 16, 2011.

ADDRESSES: Submit written requests for single copies of the draft guidance to the Division of Drug Information, Center for Drug Evaluation and Research, Food and Drug Administration, 10903 New Hampshire Ave., Bldg. 51, rm. 2201, Silver Spring, MD 20993–0002. Send one self-addressed adhesive label to assist that office in processing your requests. See the **SUPPLEMENTARY INFORMATION** section for electronic access to the draft guidance document.

Submit electronic comments on the draft guidance to *http://www.regulations.gov.* Submit written comments to the Division of Dockets Management (HFA–305), Food and Drug Administration, 5630 Fishers Lane, rm. 1061, Rockville, MD 20852.

FOR FURTHER INFORMATION CONTACT: Reynold Tan, Center for Drug Evaluation and Research, Food and Drug Administration, 10903 New

In vitro assessment of the broad-spectrum ultraviolet protection of sunscreen products

Article *in* Journal of the American Academy of Dermatology · January 2001

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In vitro assessment of the broad-spectrum ultraviolet protection of sunscreen products

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Background: There are considerable data to suggest that protection from solar ultraviolet (UV) radiation will reduce the risk of acute and chronic skin damage in humans. Whereas the sun protection factor (SPF) provides an index of protection against erythemally effective solar UV, largely confined to the UVB (290-320 nm) and short-wavelength UVA (320-340 nm) region, there is currently no agreed-upon method to measure broad-spectrum protection against long-wavelength UVA (340-400 nm).

Objective: The objective of these studies was to assess the potential of in vitro UV substrate spectrophotometry and subsequent calculation of the "critical wavelength" value as a measure of broad-spectrum UV protection and as a routine, practical procedure for classification of sunscreen products.

Methods: The spectral absorption of 59 commercially available sunscreen products and multiple experimental formulas with one or more UV filters was measured. Sunscreen product, 1 mg/cm^2, was applied to a hydrated synthetic collagen substrate, preirradiated with a solar simulator, and then subjected to UV substrate spectrophotometry. Multiple determinations from 5 independent samples per product were used to calculate the critical wavelength value, defined as the wavelength at which the integral of the spectral absorbance curve reached 90% of the integral from 290 to 400 nm.

Results: We found that a recognized long-wave UVA active ingredient such as titanium dioxide, zinc oxide, or avobenzone is a necessary but insufficient product requirement for achieving the highest proposed broad-spectrum classification, that is, critical wavelength of 370 nm or more. Although SPF and critical wavelength are largely independent of each other, UVA absorbance must increase commensurate with SPF to maintain the same critical wavelength value. Substrate spectrophotometry and the calculation of critical wavelength can readily account for sunscreen photostability by UV preirradiation. Finally, there is also a strong positive relationship between critical wavelength and a currently available in vivo measure of UVA protection.

Conclusion: Determination of critical wavelength by means of UV substrate spectrophotometry provides a rapid, inexpensive, and reliable measure of broad-spectrum protection, which is largely independent of SPF, yet ensures long-wavelength UVA protection commensurate with SPF. The procedure provides a routine, sensitive means of differentiating and classifying sunscreen products and, importantly, obviates the need to subject volunteers to acute exposures of high-dose, nonterrestrial UV, the health risks of which are still poorly understood. (J Am Acad Dermatol 2000;43:1024-35.)

The spectral distribution of solar UV radiation reaching the surface of the earth encompasses wavelengths between 290 and 400 nm. Commonly defined ranges for specific bands of the terrestrial UV spectrum are UVB (290-320 nm) and UVA (320-400 nm). In addition, the UVA waveband is often further divided into UVA2 (320-340 nm) and UVA1 (340-400 nm), generally reflecting the higher erythemogenic efficiency of shorter UVA wavelengths (UVA2).[1] Importantly, the division between these UV wavebands is arbitrary and anthropogenic; human skin is exposed primarily to solar UV, which includes all UV wavebands.

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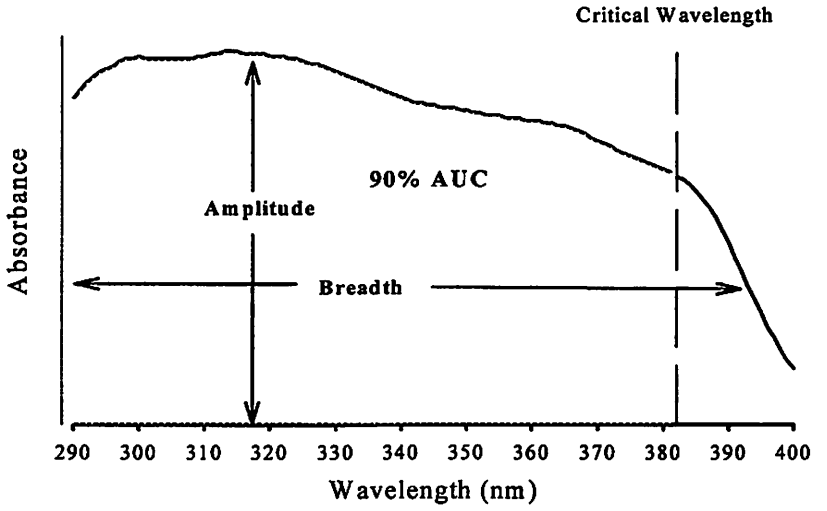

Fig 1. Absorption spectrum for hypothetical sunscreen product. UV attenuation is determined at fixed intervals across UV spectrum using substrate spectrophotometry. Wavelength below which 90% of the area under the whole absorption spectrum from 290 to 400 nm falls is the critical wavelength. The shape of the absorption spectrum is independent of application density.

Strategies aimed at reducing overexposure to sunlight include the use of topical sunscreens.[2,3] To date, sunscreen product protection has been defined by efficacy in prevention of UV-induced erythema, determined directly by the standard in vivo sun protection factor (SPF) procedure.[4] The action spectrum for UV-induced erythema is well known[5,6] and largely confined to wavelengths from 290 to 330 nm. In addition, the erythema action spectrum is nearly identical to that proposed for DNA damage[7,8] and the induction of nonmelanoma skin tumors in mice and, by extension, humans.[9] Thus the SPF test provides a clinically relevant in vivo measure of sunscreen product efficacy, which appears also to be a surrogate for chronic skin damage.

It has become apparent that longer wavelengths of solar UV can contribute to skin damage. This assertion is based on both clinical evidence and theoretical considerations. The studies by Lavker et al,[10,11] Lavker and Kaidbey,[12] and Lowe et al[13] provide evidence that repeated exposure to an artificial source of long-wavelength UVA produces morphologic changes in human skin indicative of photodamage. These data corroborate studies in animals in which exposure to UVA was reported to accelerate photodamage[14,15] and the induction of skin tumors.[16,17] Because the overwhelming majority of sunscreen products available to consumers provide protection primarily limited to UVB and short-wavelength UVA2 (320-340 nm), it has even been hypothesized that the use of such products may paradoxically increase exposure to long-wavelength UVA1 (340-400 nm)[18] by selectively changing the spectrum of solar sunlight received by the skin.[19]

Abbreviations used:

AVO:	avobenzone
HSAL:	homosalate
OCTO:	octocrylene
OMC:	octyl methoxycinnamate
OPABA:	octyl dimethyl *para*-aminobenzoic acid (padimate O)
OSAL:	octyl salicylate
OXY:	oxybenzone
PBSA:	2-phenylbenzimidazole-5-sulfonic acid
PPF:	phototoxic protection factor
SPF:	sun protection factor
UV:	ultraviolet

Given the need for long-wavelength UVA protection and the absence of meaningful information regarding such protection on currently marketed sunscreen products,[20] there is an urgent demand for a reliable, versatile, and universally applicable method that provides purposeful, SPF-independent information regarding UVA photoprotection. Several in vivo methods using human subjects have been proposed[21-23] but are not widely accepted. Although there are several reasons for this, the limitation of all proposed human studies of UVA photoprotection is the absence of an end-point measure that is a true surrogate marker for UVA-induced skin damage, especially carcinogenesis and photoaging. From a more practical perspective, the existing human studies uti-

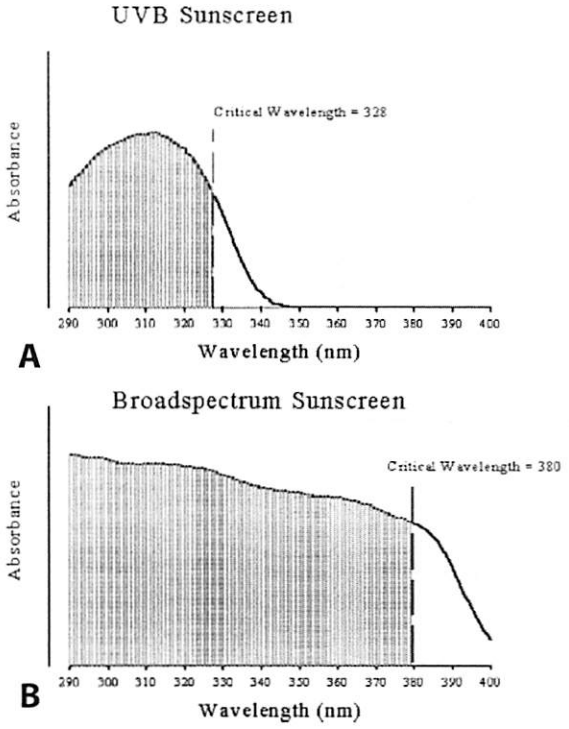

Fig 2. Absorption spectra for UVB **(A)** and broad-spectrum **(B)** sunscreen products. *Shaded areas* represent 90% of the area under the absorption curves from 290 to 400 nm. The critical wavelength is the singular point below which 90% of the whole absorption spectrum falls.

lize end points that (1) are redundant with SPF testing (ie, erythema)[24]; (2) are oxygen and, by definition, UV dose-rate dependent[25-27]; (3) are skin-type dependent; and (4) in some cases require extraordinary exposures to an artificial UVA source, the human health consequences of which are as yet unknown.

Thus several in vitro alternatives to such approaches have been developed.

One such method proposed by Diffey[28] makes no assumptions regarding the action spectra for UVA-induced acute or chronic skin damage and obviates the need for human subjects utilizing clinical end points with indeterminate value in relation to protection from sunlight. This proposed in vitro method is based on the absorption spectrum of a sunscreen product, illustrated in Fig 1, which is obtained by means of UV substrate spectrophotometry. The absorption spectrum is reduced to a single index termed *critical wavelength*, defined as the wavelength at which the integral of the spectral absorbance curve reached 90% of the integral from 290 to 400 nm. Importantly, the critical wavelength value is based on the inherent shape of the absorbance curve, not its amplitude, and therefore is independent of application thickness and other undesirable variables characteristic of in vitro calculations of absolute protection factors. As well, the critical wavelength determination does not promote the false notion of UVB and UVA as separate entities, but rather as part of a continuous electromagnetic spectrum. Examples of the critical wavelength determination are illustrated in Fig 2. The shaded areas in Fig 2 represent 90% of the area-under-the-absorption curve from 290 to 400 nm. The critical wavelength for a UVB sunscreen (Fig 2, *A*) is less than that of a broad-spectrum product (Fig 2, *B*).

Therefore the objective of the present studies was to evaluate the accuracy, versatility, and in vivo relevance of in vitro UV substrate spectrophotometry and the calculated critical wavelength as a usable measure of UVA photoprotection.

MATERIAL AND METHODS

Substrate preparation

Synthetic collagen substrate with simulated skin topography (Vitro-Skin, Innovative Measurement Solutions Inc, Milford, Conn) was placed in a constant environment chamber (22°C ± 2°C; 80%-90% relative humidity), roughened side up, for at least 24 hours. After this controlled hydration step, the collagen sheet was cut into 9 × 10.2 cm rectangles and returned to the constant environment chamber to maintain homeostasis.

Product samples and application

Commercially available sunscreen products were purchased in June 1997 from retail stores located in Cincinnati, Ohio. Model sunscreen creams were also prepared as oil-in-water emulsions using a similar formula matrix containing varying concentrations of UV filters, solvents, or emollients as needed. Product, 1 mg/cm^2 (absolute quantity, 91.8 mg), was applied uniformly to the roughened side of the hydrated synthetic collagen with a presaturated finger cot. The product film was then allowed to dry under ambient conditions (22°C ± 2°C) for 15 minutes.

UV source

Samples were irradiated with broad-band UV radiation from an Oriel 1000 W Xenon Arc Solar Simulator (Oriel Corp, Stratford, Conn), the output of which was filtered (dichroic mirror, Oriel filters 81017 and 81018) to simulate a solar UV spectrum. The mean UV irradiance (290-400 nm) from the solar simulator was 6.1 mW/cm^2, as measured at sample height using a calibrated spectroradiometer (model 754, Optronics Co, Orlando, Fla).

Preirradiation of sunscreen products

Except for the photostability study, all sunscreen samples applied to collagen substrate were preirra-

Table I. Critical wavelength (CW) for 59 commercially available sunscreen products in the United States

	UVB/UVA2 filters†								UVA1 filters‡			
SPF*	OMC	OXY	OSAL	HSAL	OCTO	OPABA	MAN	PBSA	AVO	ZnO	TiO_2	CW (nm)
4						X						315
4	X					X						327
4						X						330
4	X						X					344
4	X	X										345
4		X		X								350
4	X	X										351
8	X					X						330
8	X	X										347
8		X				X						350
8	X	X										350
8	X											352
8	X	X										352
8	X	X										353
8	X	X										357
8											X	**370**
15	X							X				344
15	X							X			X	346
15	X	X	X									351
15	X	X	X									352
15	X							X				352
15	X		X		X						X	353
15	X		X								X	354
15	X										X	354
15	X	X										356
15	X	X										357
15	X	X										358
15	X	X	X									358
15	X	X				X						359
15	X	X	X									360
15	X	X			X						X	363
15	X	X									X	365
15	X						X				X	368
15	X									X		**374**
15											X	**374**
15	X									X		**375**
15											X	**378**
15	X	X							X			**380**
30	X		X								X	353
30	X	X	X									355
30	X	X	X									357
30	X	X	X									358
30	X	X	X									358
30	X	X	X									358
30	X	X	X									358
30	X	X	X									359

CW, Critical wavelength.
CWs marked in bold indicate those products with CW of 370 nm or greater.
*Labeled SPF.
†UVB/UVA2 filters: *OMC,* octyl methoxycinnamate; *OXY,* oxybenzone; *OSAL,* octyl salicylate; *HSAL,* homosalate; *OCTO,* octocrylene; *OPABA,* padimate O; *MAN,* menthyl anthranilate; *PBSA,* 2-phenylbenzimidazole-5-sulfonic acid.
‡UVA1 filters: *AVO,* avobenzone; *ZnO,* zinc oxide; *TiO_2,* titanium dioxide.

Continued on page 1028

Table I. Cont'd.

	UVB/UVA2 filters†								UVA1 filters‡			
SPF*	OMC	OXY	OSAL	HSAL	OCTO	OPABA	MAN	PBSA	AVO	ZnO	TiO_2	CW (nm)
30	X		X		X		X					360
30	X	X	X									360
30	X	X	X									361
30	X	X	X		X							361
30	X	X	X									362
30	X	X	X									362
30	X	X	X									367
45	X	X	X									351
45	X		X								X	358
45	X	X	X		X							359
45	X	X	X	X								360
45	X	X	X	X								361
45	X	X	X		X						X	362

diated with a total UV dose in joules per square centimeter numerically equal to one third the labeled (commercial products) or expected (model sunscreen formulations) product SPF (eg, for an SPF 15 product, preirradiation was 5 J/cm^2). Product preirradiation was performed to account for changes that can occur in the absorption spectrum of UV filters. In the photostability study, product samples were preirradiated with solar-simulated UV at total doses ranging from 0 to 30 J/cm.^{2}

Absorbance measurements

Immediately after preirradiation, the UV absorbance of the product film was measured using a Labsphere UV-1000S UV transmittance analyzer (Labsphere Inc, North Sutton, NH). Reference absorbance measurements were performed on the 9 × 10.2 cm rectangular piece of untreated, hydrated Vitro-Skin with the Labsphere UV-1000S. After application, the product was allowed to dry. UV absorbance of the product film was measured at 8 different sites on the collagen substrate. For each product sample, 5 independent replicate product films were prepared and evaluated as described above. The measurement performed on the product sample was corrected for the untreated reference and the resulting absorbance curve used to calculate the critical wavelength.

Critical wavelength determination

The critical wavelength was calculated with the following equation:

$$\int_{290}^{\lambda c} A(\lambda)d\lambda = 0.9 \int_{290}^{400} A(\lambda)d\lambda$$

where A is absorption and λ wavelength. For each absorption spectrum, the integrals, which represent the area-under-the- product absorbance curve, were estimated using trapezoidal integration. For each product film, the critical wavelength was the average of 8 determinations. The final critical wavelength value for each product or model sunscreen was the 95% lower confidence limit computed from the 5 individual replicates.

Experimental design

Study I. The critical wavelength values of 59 sunscreen products marketed in the United States were measured according to the procedures described above. The products included creams, lotions, and oils intended for daily or recreational use.

Study II. The relationship between the critical wavelength and SPF was studied with two model sunscreens. Oil-in-water emulsions containing 6% octyl methoxycinnamate (OMC) and either 3.5% ZnO or 4% TiO_2 were prepared with an estimated SPF equal to 15. Similar products with an estimated SPF equal to 30 were prepared by the addition of 10% octocrylene (OCTO), a UVB/UVA2 absorber. The estimated SPF was determined by comparing the in vitro absorbance curves for the model sunscreens with those from marketed products in which SPF had been determined in vivo. The absorption spectrum was measured and critical wavelength value determined for all 4 model sunscreen products.

Study III. Model sunscreen creams were prepared with each of the 11 UV filters currently in use. The absorption spectrum of each model cream was then measured and the critical wavelength determined. A model sunscreen product containing the

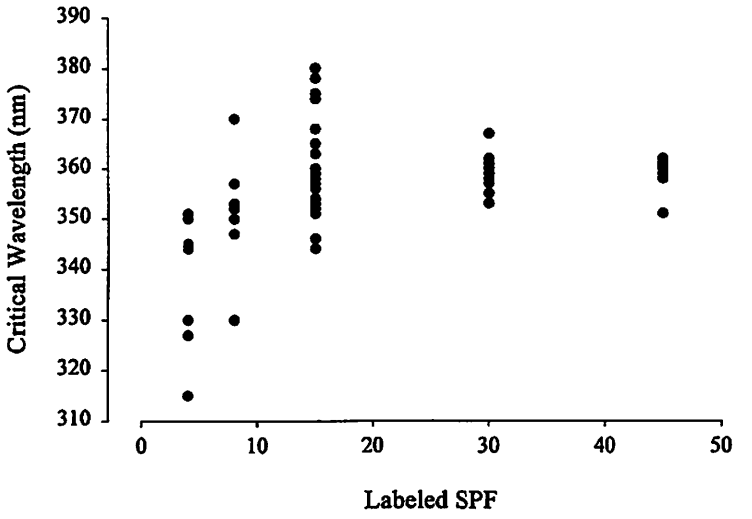

Fig 3. Relationship between labeled SPF and critical wavelength for 59 commercially available products.

UV filters with the lowest and highest critical wavelength values, 2-phenylbenzimidazole-5-sulfonic acid (PBSA) and AVO, respectively, was also prepared. The absorption spectrum and critical wavelength were then determined.

Study IV. The spectral photostability of 4 model sunscreens was determined by means of substrate spectrophotometry. Model SPF 15 sunscreens with different combinations of UV filters were prepared. The absorption spectrum and resulting critical wavelength were determined initially and then after irradiation with 10, 20, and 30 J/cm^2 of solar simulated UV.

Study V. The relationship between in vivo determinations of UVA protection and critical wavelength was investigated. Phototoxic protection factors (PPFs) for several experimental sunscreen products were obtained from Gange et al,[29] Lowe et al,[30] and Lowe.[31] PPF is determined by measuring the minimal phototoxic dose of UVA in protected versus unprotected skin. The phototoxic skin response (ie, erythema) is obtained by topical administration of 8-methoxypsoralen followed by irradiation with an artificial light source filtered to emit a continuous spectrum of UVA (320-400 nm) radiation. Model sunscreen products were prepared with the use of combinations and concentrations of the UV filters reported in these studies. The absorbance spectrum was measured for each product and the critical wavelength determined.

RESULTS

Study I

Table I presents the labeled SPF, UV filters, and critical wavelength value for each of the 59 commercial products evaluated. The products included those intended for recreational and daily use in lotion, cream, and oil forms ranging in SPF from 4 to 45 (ie, 7 [SPF 4], 9 [SPF 8], 22 [SPF 15], 15 [SPF 30], and 6 [SPF 45]). The products contained 11 different sunscreen active ingredients. The 3 most widely used UV filters were OMC, oxybenzone (OXY), and octyl salicylate (OSAL) found in 88%, 66%, and 46% of the 59 commercial products, respectively. More than 80% of the products referred to UVA on their package label with 98% of sunscreens with SPFs of 15 to 45 claiming some UVA protection.

Only 10% (6/59) of the commercial products had a critical wavelength of 370 nm or more. The products that achieved a critical wavelength of 370 nm or more contained a recognized UVA1 filter (ie, titanium dioxide [TiO_2], zinc oxide [ZnO], or AVO). Not all products with these sunscreens achieved a critical wavelength greater than or equal to 370 nm. This demonstrates that effective concentration and appropriate formulation are essential and that simply including UVA1 filters in a formulation does not ensure a true broad-spectrum product. All of the commercial products having critical wavelengths of 370 nm or greater were SPF 8 or SPF 15.

Analysis of variance (Kruskal-Wallis test) showed no dependence (P = .98) of critical wavelength on SPF for products of SPF15 and higher (Fig 3). However, as illustrated in Fig 3, when all SPF groups were included (SPF 4-45), there was a tendency for the critical wavelengths of low SPF products (SPF 4 and 8) to be generally lower than high (SPF $\geq$ 15) products.

Table II. Effect of doubling the sun protection factor (SPF) on the critical wavelength

Sunscreen actives			
UVB/UVA2	UVA1	Estimated SPF*	Critical wavelength (nm)
6% OMC	4% TiO_2	15	372
6% OMC, 10% OCTO	4% TiO_2	30	364
6% OMC	3.5% ZnO	15	376
6% OMC, 10% OCTO	3.5% ZnO	30	368

OCTO, Octocrylene; *OMC*, octylmethoxycinnamate; *TiO_2*, titanium dioxide; *ZnO*, zinc oxide.
*Estimated SPF was determined by comparing the in vitro absorption curves for the model sunscreens to those of marketed products in which the SPF had been determined in vivo.

Table III. Absorption bands and critical wavelength for the most commonly used UV filters

UV filter	Concentration (%)*	Wavelength (nm) 290 300 310 320 330 340 350 360 370 380 390 400	Critical wavelength (nm)
PBSA (2-phenylbenzimidazole -5-sulfonic acid)	4		324†
OSAL (octyl salicylate)	5		327
HSAL (homosalate)	15		328
OPABA (octyldimethyl PABA)	8		330
OMC (octyl methoxycinnamate)	7.5		339
OCTO (octocrylene)	10		356
OXY (oxybenzone)	6		361
MAN (menthyl anthranilate)	5		363
TiO_2 (titanium dioxide)	25		379‡,§
ZnO (zinc oxide)	25		382‡
AVO (avobenzone)	3		383

Solid bar represents UV attenuation (expressed in nanometers squared) and is based on substrate spectrophotometry determinations. Filters were prepared in a representative oil-in-water emulsion. *Shaded bar* represents peak absorbance.
*The maximum concentration established in the Sunscreen Drug Products for Over-the-Counter Human Use; final monograph.
†Determined at 2% oil-in-water emulsion.
‡Determined at 15% oil-in-water emulsion.
§Shape of the UV attenuation spectra varies with particle size.

Study II

The effect of doubling the product SPF on critical wavelength is presented in Table II. In the two examples evaluated, products estimated to provide an SPF of 15 were created by combining OMC with either TiO_2 or ZnO. The critical wavelength for each product was 372 and 376 nm, respectively. When 10% OCTO was added to the formulations to increase the UVB/UVA2 absorbance and provide an estimated SPF of 30, the critical wavelength values were markedly reduced to below 370 nm.

Study III

Table III presents the critical wavelength, UV attenuation, and peak absorbance for 11 sunscreen active ingredients in model creams. All of the ingredients were evaluated at their current maximum allowable concentration except PBSA, which was incorporated at a concentration of 2%, and the two inorganic active ingredients, TiO_2 and ZnO, which were incorporated at concentrations of 15%, because of limitations with the standard formulation matrix to accommodate greater amounts of these sunscreen active ingredients. Model sunscreen creams containing the UV filters PBSA, OSAL, homosalate (HSAL), octyl dimethyl *para*-aminobenzoic acid (OPABA), OMC, OCTO, and OXY had critical wavelengths below 370 nm. Model sunscreen creams containing the recognized long-wavelength UVA1 filters, AVO, TiO_2, and ZnO had critical wavelengths of 370 nm or greater.

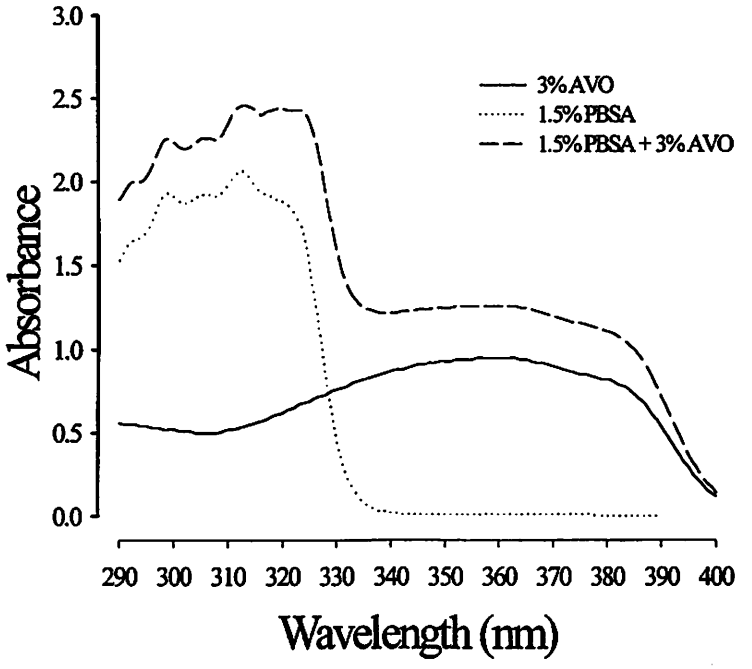

Fig 4. Absorption spectrum and critical wavelength for 1.5% PBSA (critical wavelength = 324 nm), 3% AVO (critical wavelength = 383 nm), and their combination (critical wavelength = 378 nm). PBSA, AVO, and their combination were prepared as oil-in-water emulsions and the absorption spectrum determined by means of substrate spectrophotometry. PBSA has the lowest critical wavelength and AVO the highest. The UV filters alone and in combination have broad absorption spectra.

The UV absorption for the combination of 1.5% PBSA and 3% AVO is presented in Fig 4. These UV filters represent the extremes of UV absorption ranges for the 11 UV filters evaluated and, consequently, the largest difference in critical wavelength values. As illustrated in Fig 4, both PBSA and avobenzone have relatively broad-band UV attenuation. When these 2 UV filters are combined, there are no obvious "holes" or "gaps" in the full absorption spectrum.

Study IV

The effect of increasing doses of UV before irradiation (0, 10, 20, 30 J/cm^2) on the critical wavelength value of 4 model sunscreens is presented in Fig 5. The model sunscreen products consisting of OMC + TiO_2, OMC + ZnO, and OCTO + AVO had the same critical wavelength after preirradiation up to 30 J/cm^2. In contrast, a prototype product with a combination of active ingredients comprising OMC, OSAL, and AVO had significantly lower critical wavelengths concurrent with increasing doses of UV preirradiation. The critical wavelength decreased from 379 nm with no preirradiation to 357 nm after 30 J/cm^2 of UV preirradiation; these spectrally derived data were indicative of photoinstability on a molecular level.

Study V

The relationship between the phototoxic protection factor (PPF) and critical wavelength is presented in Fig 6. Values for PPF were obtained from Gange et al,[29] Lowe et al,[30] and Lowe.[31] There was a linear correlation between the PPF and critical wavelength for products with an SPF of 3 or 4 (r = 0.99) and those with SPFs of 9 to 15 (r = 0.98).

DISCUSSION

From these studies, it appears that most current sunscreen products available to US consumers do not provide significant broad-spectrum UV protection even though the majority of products evaluated (80% of SPF 4-45 and 98% of SPF 15-45) claim some UVA protection. This is primarily due to the lack of a coherent broad-spectrum test and labeling system, a situation that is extant in Europe. As a direct consequence, UVA or broad-spectrum claims may currently be made for sunscreen products simply on the basis of inclusion of a UVA1 or UVA2 filter, regardless of amplitude or breadth of wavelength protection

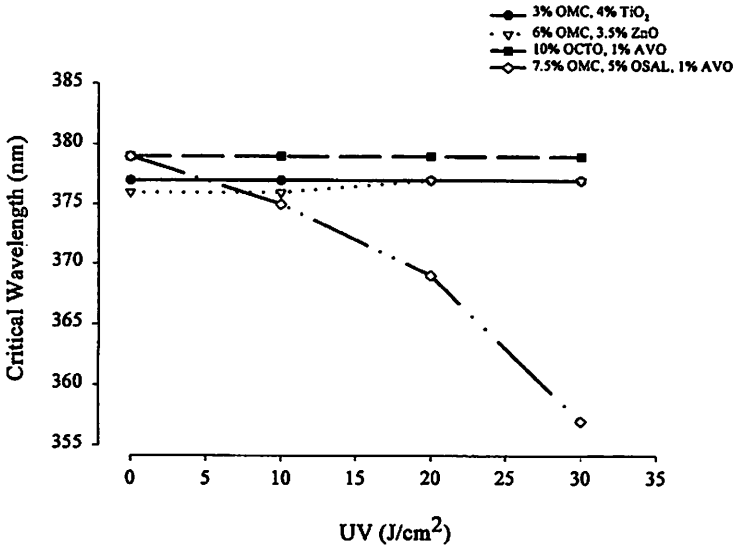

Fig 5. Photostability of model sunscreen products after increasing doses of solar simulated UV. Each product was exposed to 0, 10, 20, or 30 J/cm^2 solar simulated radiation. Each value is the mean of 5 independent samples. The critical wavelength was calculated from the absorption spectrum obtained by means of substrate spectrophotometry.

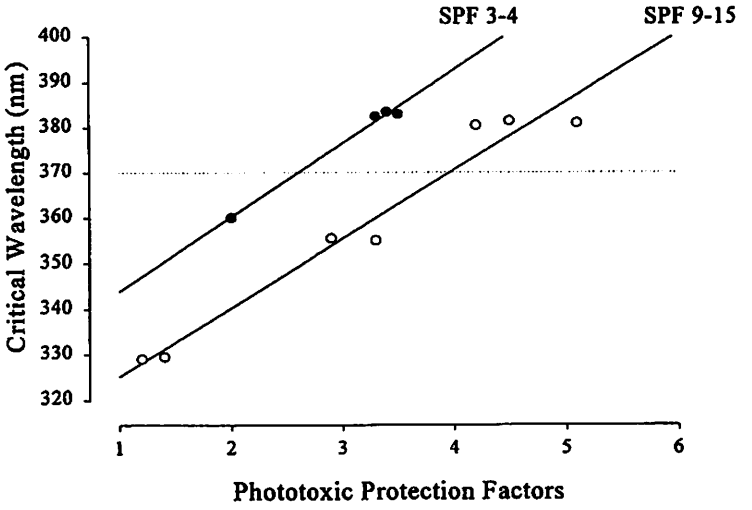

Fig 6. Relationship between phototoxic protection factor and critical wavelength.

across the spectral interval of 290 to 400 nm. Whereas a well-equipped testing laboratory can routinely determine such parameters, the consumer, unfortunately, cannot.

To address this insufficiency, some measure of broad-spectrum efficacy is needed that is versatile, reliable, and independent of SPF, yet ensures broad-spectrum protection commensurate with SPF. Moreover, such information needs to be communicated to the consumer in an understandable and meaningful way. In this regard, our comprehensive evaluation of the sunscreen market in the United States, together with a study demonstrating that photostability may be easily accounted for, and a positive correlation with in vivo measures of UVA photoprotection, all support the view that UV substrate spectrophotometry and calculation of the critical wavelength provide a convenient, reproducible, and adapt-

able procedure for evaluating the breadth of UV protection. Moreover, when coupled with the in vivo SPF value, critical wavelength provides a simple, transparent, and explicit means of communicating broad-spectrum photoprotection to the consumer.

In the seminal publication proposing the critical wavelength method,[28] 5 categories of broad-spectrum protection were proposed. The difference between wavelengths dividing these 5 categories was logarithmic, based on the well-characterized UV-induced biologic response patterns. The highest classification was a critical wavelength greater than or equal to 370 nm. From the evaluation of 59 commercial products, the overwhelming majority (93%) had a critical wavelength value greater than 340 nm. However, only 10% of the 59 products evaluated achieved a critical wavelength of 370 nm or greater. More importantly, these products contained a recognized long-wavelength UVA1 filter. In addition, the same filters formulated in model sunscreen creams achieved critical wavelength values of more than 370 nm. Based on these data, a more conservative product classification would be the selection of a single critical wavelength at which a sunscreen product would either pass or fail to achieve a broad-spectrum classification. In this regard, a critical wavelength of greater than or equal to 370 nm appears to be a rigorous minimum that sunscreen products would need to achieve to be labeled broad-spectrum. In addition, such a simple pass/fail approach would readily accommodate advances in scientific knowledge regarding UVA end points and subsequent methods.

The absorption spectrum is the most relevant attribute of any sunscreen product. This can be measured with relatively inexpensive, readily available equipment by applying a sunscreen product evenly over a substrate. The resulting absorbance curve comprises 2 key attributes, namely, the amplitude and shape. The amplitude of the absorption curve reflects the degree of protection. However, the overall amplitude of the absorbance curve can vary significantly across repeated measurements, primarily because of the intrinsic link of this parameter to product application density and substrate topography.[32] This is precisely why attempts to develop in vitro measures of SPF have not succeeded; interlaboratory and intralaboratory variance is simply too high. The other key attribute of the absorption spectrum is the shape of the curve, that is, the efficiency at which the product attenuates one UV wavelength relative to another. In practice, the relative wavelength absorption efficiency is generally very consistent across repeated measurements, resulting in an absorbance-by-wavelength fingerprint, characteristic of the sunscreen active system.

From the absorption curve, the efficiency for which a sunscreen product attenuates UV can be expressed in several ways. For example, Sayre and Agin[33] measured the spectral absorbance of several sunscreen products and calculated UVA protection factors by using the product of the standard solar spectrum and the CIE erythemal action spectrum[6] convoluted with the sunscreen absorption spectrum. However, this erythemally weighted measure is strongly associated with SPF[34] and is therefore a redundant measure. Other uses of the absorption spectrum to estimate UVA photoprotection include methods based on a ratio of UVA/UVB absorbance (eg, the UK "Boots" Star Method[35]) and the critical wavelength method as proposed by Diffey.[28] The latter approach can be distinguished from others on the basis of its total reliance on the shape of the absorption curve. To date, however, it is true to say that the rigor and versatility of this approach have been largely unreported outside of cross-industry methods task forces.

A commonly voiced concern with in vitro procedures is their relationship to an in vivo end point. In the case of long-wavelength UVA protection, there is currently no end point or surrogate that can be used with confidence. The importance of this should not be underestimated. For example, there are several in vivo measures of UVA photoprotection using erythema or pigmentation as the end point. Erythema is already accounted for in SPF testing and is heavily weighted in the short UVA wavelengths. Arguably the most well-studied and published of the in vivo UVA photoprotection methods is the so-called immediate pigment darkening (IPD) method. IPD is the transient gray-brown discoloration of the skin that develops immediately after exposure to predominantly long-wavelength UVA. It was first described by Hausser[36] and more recently proposed as an in vivo method to evaluate UVA photoprotection.[37,38] The efforts to gain widespread use of this method have largely stalled, in part related to questions of the biologic significance of the end-point measure. Moreover, pigmentation, whether immediate or persistent, is reported to be an oxygen-dependent phenomenon,[39] which, by definition, would make such an end-point measure UV dose-rate dependent. Thus, unlike SPF testing in which UV-dose reciprocity has been reported to exist,[40] UVA-induced pigment changes have been reported to be both irradiance dependent[26] and independent.[37] Regardless, we believe the present in vivo approaches for UVA photoprotection have multiple shortcomings, thereby limiting their potential usefulness.

To establish the biologic relevance of in vitro measures, we determined the relationship between criti-

cal wavelength values and previously published PPF values. The PPF method using 8-methoxypsoralen as a topical or oral sensitizer has also been used to evaluate UVA photoprotection. Because psoralens are photogenotoxic,[41] their routine use for evaluating sunscreen products is ethically untenable. Nonetheless, we used results from historical studies to establish the relationship between critical wavelength values and in vivo UVA photoprotection. There was a very positive relationship between the PPF and the critical wavelength for sunscreen products with similar SPFs. This relationship establishes the in vivo significance of the critical wavelength when in vivo SPF is taken into account and further supports the meaningful information derived from this measure.

Finally, we found that the procedure can readily account for photoinstability changes through UV preirradiation. Because of known photochemical processes, this accounting for potential photoinstability can only be appropriately and reliably accomplished through full-spectrum UV (290-400 nm) product irradiation; this is a unique characteristic of this approach versus other procedures that irradiate using sources filtered to give UVA only.

The results presented herein highlight that a sunscreen product's critical wavelength value must always be considered in conjunction with its corresponding in vivo SPF. To be explicit, if two products (A and B) share the same critical wavelength but exhibit differing in vivo SPF values (15 and 30, respectively), then according to the critical wavelength calculation, product B must have been formulated with significantly more long-wavelength UVA protection than product A (ie, commensurate with SPF). In short, a combination of in vivo SPF and critical wavelength provides a complete description of a product's inherent photoprotective characteristics; SPF describes the amplitude of protection (at a given application rate) and critical wavelength provides a reliable measure of the product's spectral absorption capability. No other efficacy measures are needed.

The conclusion from this comprehensive assessment of the critical wavelength procedure is that it provides a rapid, inexpensive, and reliable measure of broad-spectrum protection, which is independent of SPF, yet ensures broad-spectrum protection commensurate with SPF. The method also provides a routine yet sensitive means of differentiating and classifying sunscreen products and, importantly, eliminates the need to subject volunteers to acute exposures of high-dose, nonterrestrial UV, the human health risks of which are still poorly understood.

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Prevalence and Costs of Skin Cancer Treatment in the U.S., 2002–2006 and 2007–2011

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Abstract

Background—Skin cancer, the most common cancer in the U.S., is a major public health problem. The incidence of nonmelanoma and melanoma skin cancer is increasing; however, little is known about the economic burden of treatment.

Purpose—To examine trends in the treated prevalence and treatment costs of nonmelanoma and melanoma skin cancers.

Methods—This study used data on adults from the 2002–2011 Medical Expenditure Panel Survey full-year consolidated files and information from corresponding medical conditions and medical event files to estimate the treated prevalence and treatment cost of nonmelanoma skin cancer, melanoma skin cancer, and all other cancer sites. Analyses were conducted in January 2014.

Results—The average annual number of adults treated for skin cancer increased from 3.4 million in 2002–2006 to 4.9 million in 2007–2011 ($p<0.001$). During this period, the average annual total cost for skin cancer increased from $3.6 billion to $8.1 billion ($p=0.001$), representing an increase of 126.2%, while the average annual total cost for all other cancers increased by 25.1%. During 2007–2011, nearly 5 million adults were treated for skin cancer annually, with average treatment costs of $8.1 billion each year.

Conclusions—These findings demonstrate that the health and economic burden of skin cancer treatment is substantial and increasing. Such findings highlight the importance of skin cancer prevention efforts, which may result in future savings to the healthcare system.

Introduction

Skin cancer, the most commonly diagnosed cancer in the U.S., is increasingly a major public health problem. An estimated 3.5 million cases of nonmelanoma skin cancer (NMSC) were treated in 2006,[1] and more than 60,000 melanomas were diagnosed in 2010.[2] The incidence

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of NMSC and melanoma is increasing,[3,4] although little is known about the economic burden of treatment. The purpose of this study is to examine trends in the number of adults treated for NMSC and melanoma, as well as the associated annual costs of treatment.

Methods

Data on adults from the 2002–2011 Medical Expenditure Panel Survey (MEPS) full-year consolidated files were used, as well as information from corresponding medical conditions and medical event files. The MEPS combines household-reported data on use and costs, and provider-reported data on costs, to provide nationally representative estimates among the U.S. civilian noninstitutionalized population. Because data used in these analyses cannot be used to personally identify individuals, this study was exempt from IRB review. The Clinical Classification Software (CCS) category was used to classify types of cancer as nonepithelial cancer of the skin (code 23), melanomas of the skin (code 22), or other cancers (codes 11–21 and 24–25).[5] Owing to the relatively small number of people reported in MEPS as receiving treatment for melanoma (unweighted average of about 150 annually) and the skewed distribution of healthcare expenditures, annual estimates among population subgroups (e.g., age/gender categories) in general were subject to less statistical precision. To allow for a comparison over time and improve the statistical precision of the estimates, two 5-year periods of data were created (2002–2006 and 2007–2011). SAS, version 9.2, complex survey analysis procedures were used to produce average annual national estimates that properly accounted for the MEPS sample design and survey nonresponse. Reported *p*-values in the tables are based on simple *t*-tests of differences between estimates for the two time periods.

Individuals were classified as being treated for NMSC, melanoma, or other cancers if they had any ambulatory visits (office-based and hospital outpatient), inpatient stays, home health visits, or prescribed medication purchases associated with the corresponding CCS code. Costs were defined as expenditures from all sources for healthcare services reported in the survey, including out of pocket, private insurance, Medicare, Medicaid, and other miscellaneous sources. Costs by source of payment and type of service are not reported for melanoma because of small sample sizes. All costs were adjusted to 2011 U.S. dollars using the Personal Health Care Expenditure Price Index.[6] Analyses were conducted in January 2014.

Results

The average annual number of adults treated for any skin cancer (NMSC or melanoma) increased from 3.4 to 4.9 million between 2002–2006 and 2007–2011 ($p<0.001$), while the average number treated for all other cancers increased from 7.8 to 10.3 million ($p<0.001$, Table 1). Subgroup analyses indicated increases among adults aged 65 years and older for NMSC ($p<0.001$) and melanoma ($p<0.001$), and women aged 18–64 years for melanoma ($p=0.006$).

Between 2002–2006 and 2007–2011, the average annual total cost for skin cancer increased by 126.2%, from $3.6 billion to $8.1 billion ($p=0.001$), while the average annual total cost

for all other cancers increased by 25.1%, from $63.7 billion to $79.7 billion ($p$=0.005, Table 2). Average annual total treatment costs during 2007–2011 were $4.8 billion for NMSC and $3.3 billion for melanoma. During 2007–2011, nearly three quarters of annual NMSC costs were attributable to office-based visits compared to one third among all other cancer sites (excluding skin cancers). During the same period, private health insurance paid for 43.4% of all skin cancer treatment costs while Medicare paid for 41.1%. Among all other cancer sites (excluding skin cancer), private health insurance paid for 45.2% of treatment costs, while Medicare paid for 36.1%.

Discussion

The number of adults treated for skin cancer increased between 2002–2006 and 2007–2011 to nearly 5 million adults annually. Average annual total treatment costs for skin cancer also increased substantially between these periods to $8.1 billion annually. Increased skin cancer treatment costs resulted from an increase in the number of people treated for skin cancer and an increase in per person treatment costs. Annual spending increased more rapidly for skin cancers than for other cancers, suggesting that the economic burden of skin cancer is a particular cause for concern. These findings underscore the importance of prevention and early detection of skin cancer.

Although this study demonstrates the substantial costs of skin cancer treatment, it also highlights the potential for savings through prevention efforts. Primary prevention efforts have been shown to reduce skin cancer incidence, mortality, and healthcare expenditures.[7–9] For example, the Sunwise Program, a health and environmental education program that teaches children and their caregivers how to protect themselves from overexposure to the sun, could avert nearly 11,000 skin cancer cases, while saving $2–$4 in medical care costs and lost productivity for each dollar invested in the program.[7] Similarly, in Australia, the SunSmart public education program promoting sun protection and skin cancer prevention messages through structural, environmental, and legislative initiatives was estimated to save 22,000 life years, while saving approximately $2 for every dollar invested.[8] Reducing indoor tanning, which is associated with an increased risk of NMSC and melanoma,[10,11] is also an important strategy for decreasing the burden of skin cancer.[9] In Australia, it was estimated that stricter indoor tanning regulations, including age restrictions among minors aged ≤18 years, could prevent approximately 24 melanoma cases, 226 squamous cell carcinoma cases, and save $256,000 in medical costs per 100,000 persons.[9] According to the U.S. Preventive Services Task Force,[12] there is currently insufficient evidence to recommend for or against regular skin cancer screening, including self-examination for early detection of skin cancer in the adult general population. However, screening among individuals at increased risk for melanoma may be cost-effective. For example, one-time screening among high-risk individuals in the U.S. was associated with a small increase in life expectancy and was reasonably cost-effective.[13]

Limitations of this study include its reliance on self- or household-reported survey data, which are subject to measurement errors. In addition, because institutionalized adults and adults in the military are not sampled in the MEPS, the results only apply to the

noninstitutionalized civilian adult population, which may result in an underestimation of the treated prevalence and treatment costs of skin cancer among adults.

In summary, this study demonstrates that the health and economic burden of skin cancer treatment is substantial and increasing. These findings highlight the importance of skin cancer prevention and early detection efforts. Such efforts are needed to reduce the increasing burden of skin cancer in the U.S.

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Table 1

Annual Estimated Number of Adults with Treatment for Skin Cancer and Other Cancers in the U.S.

	All skin cancer (melanoma or nonmelanoma)			Nonmelanoma skin cancer			Melanoma skin cancer			All cancer sites (excluding skin cancer)		
	2002–2006, *n* (SE)	2007–2011, *n* (SE)	*p*-value[a]	2002–2006, *n* (SE)	2007–2011, *n* (SE)	*p*-value[a]	2002–2006, *n* (SE)	2007–2011, *n* (SE)	*p*-value[a]	2002–2006, *n* (SE)	2007–2011, *n* (SE)	*p*-value[a]
Persons with cancer treatment	3,419,507 (170,507)	4,914,910 (243,528)	<0.001	3,090,442 (154,887)	4,301,338 (224,361)	<0.001	372,536 (44,081)	700,647 (59,659)	<0.001	7,809,643 (236,563)	10,345,779 (311,917)	<0.001
	% (SE)	% (SE)		% (SE)	% (SE)		% (SE)	% (SE)		% (SE)	% (SE)	
PERCENT WITH CANCER TREATMENT												
Aged ≥18 years												
Male and female	1.55 (0.07)	2.12 (0.09)	<0.001	1.40 (0.06)	1.85 (0.09)	<0.001	0.17 (0.02)	0.30 (0.03)	<0.001	3.55 (0.08)	4.46 (0.10)	<0.001
Male	1.76 (0.10)	2.40 (0.12)	<0.001	1.58 (0.09)	2.12 (0.12)	<0.001	0.21 (0.03)	0.32 (0.04)	0.019	3.14 (0.12)	4.07 (0.15)	<0.001
Female	1.36 (0.08)	1.85 (0.11)	<0.001	1.24 (0.08)	1.60 (0.10)	0.003	0.13 (0.02)	0.28 (0.03)	<0.001	3.92 (0.11)	4.83 (0.13)	<0.001
Age 18–64 years												
Male and female	0.81 (0.05)	0.94 (0.06)	0.081	0.70 (0.04)	0.79 (0.05)	0.202	0.11 (0.02)	0.17 (0.02)	0.068	2.26 (0.06)	2.74 (0.08)	<0.001
Male	0.84 (0.07)	0.89 (0.08)	0.617	0.72 (0.05)	0.77 (0.07)	0.56	0.13 (0.03)	0.12 (0.02)	0.874	1.55 (0.08)	1.97 (0.10)	0.001
Female	0.78 (0.07)	0.99 (0.08)	0.042	0.68 (0.06)	0.80 (0.07)	0.194	0.10 (0.02)	0.21 (0.03)	0.006	2.96 (0.10)	3.48 (0.13)	0.001
Aged ≥65 years												
Male and female	5.23 (0.27)	7.66 (0.35)	<0.001	4.87 (0.26)	6.86 (0.32)	<0.001	0.44 (0.06)	0.94 (0.11)	<0.001	9.87 (0.31)	12.56 (0.37)	<0.001
Male	7.01 (0.47)	10.53 (0.53)	<0.001	6.46 (0.45)	9.34 (0.49)	<0.001	0.67 (0.11)	1.38 (0.20)	0.002	12.17 (0.53)	15.31 (0.70)	<0.001
Female	3.89 (0.31)	5.46 (0.36)	0.001	3.67 (0.30)	4.97 (0.35)	0.004	0.26 (0.06)	0.61 (0.12)	0.011	8.15 (0.41)	10.46 (0.40)	<0.001

Note: Boldface indicates statistical significance ($p<0.05$). Estimates are based on weighted data from the 2002–2011 Medical Expenditure Panel Survey. Estimates of persons treated for "all skin cancers" are slightly lower than the sum of nonmelanoma and melanoma because a small number of persons were reported as treated for both types.

[a]Difference from 2002–2006 and 2007–2011.

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Table 2

Annual Estimated Treatment Costs for Skin Cancer and Other Cancer Sites in the U.S.

	All skin cancer (melanoma or nonmelanoma)			Nonmelanoma skin cancer			Melanoma skin cancer			All cancer sites (excluding skin cancer)		
	2002–2006	2007–2011	*p*-value[a]	2002–2006	2007–2011	*p*-value[a]	2002–2006	2007–2011	*p*-value[a]	2002–2006	2007–2011	*p*-value[a]
Total annual national costs ($) in millions[b]	3570 (354)	8075 (1357)	**0.001**	2726 (243)	4752 (382)	**<0.001**	864 (223)	3349 (1317)	0.063	63,720 (3,513)	79,713 (4,431)	**0.005**
Average annual costs per person ($)[b]	1044 (95)	1643 (280)	**0.043**	882 (68)	1105 (84)	**0.04**	2320 (540)	4780[c] (1840)	0.2	8,159 (391)	7,705 (379)	0.405
Median annual costs per person ($)[b]	307 (15)	325 (16)	0.409	309 (16)	323 (17)	0.547	285 (51)	347 (40)	0.339	814 (51)	755 (48)	0.399
	% (SE)	% (SE)		% (SE)	% (SE)					% (SE)	% (SE)	
Costs by source of payment												
Private health insurance	35.8 (3.1)	43.4 (8.4)	0.396	33.8 (2.9)	37.1 (4.0)	0.508				46.3 (2.5)	45.2 (2.6)	0.761
Medicare	35.5 (3.9)	41.1 (8.5)	0.551	34.8 (3.4)	41.8 (4.0)	0.182				35.7(2.3)	36.1 (2.2)	0.898
Out-of-pocket	13.2 (1.8)	6.7 (1.3)	**0.003**	14.2 (2.1)	9.6 (1.4)	0.076				7.2 (1.3)	4.9 (0.3)	0.086
Medicaid/CHIP	4.4[c](2.0)	1.9[c](0.7)	0.248	3.2[c](1.5)	2.4[c](0.9)	0.621				5.0 (0.6)	5.6 (1.0)	0.603
Other	11.1 (2.6)	7.0 (1.9)	0.192	14.0 (3.4)	9.1 (2.7)	0.256				5.9 (0.8)	8.3 (1.1)	0.095
Costs by type of service												
Office-based medical provider	67.7 (3.6)	52.1 (7.3)	0.054	71.9 (3.7)	73.2 (4.1)	0.806				27.2 (1.5)	32.7 (2.0)	**0.029**
Outpatient department	19.9 (2.7)	10.5[c](4.2)	0.060	17.6 (2.8)	10.7[c](3.6)	0.122				19.1 (1.6)	17.4 (1.1)	0.387
Hospital inpatient	8.0[c](2.7)	32.3 (9.4)	**0.014**	6.7[c](2.9)	10.1[c](3.3)	0.438				44.8 (2.1)	36.1 (2.0)	**0.002**
Prescription medication	3.7 (0.9)	3.3[c](1.0)	0.730	3.1 (0.4)	3.4 (0.9)	0.812				4.4 (0.7)	9.5 (1.1)	**<0.001**
Other	0.7[c](0.3)	1.9[c](0.9)	0.196	0.7[c](0.4)	2.7[c](1.1)	0.095				4.5 (0.5)	4.3 (0.6)	0.805

Note: Boldface indicates statistical significance ($p<0.05$). Estimates are based on weighted data from the 2002–2011 Medical Expenditure Panel Survey. All costs are in 2011 U.S. dollars. Other type of service includes home health and emergency room. Costs by source of payment and type of service are not available for melanoma due to small sample size and unreliable estimates.

[a] Difference from 2002–2006 and 2007–2011.

[b] Values in parentheses are SEs.

[c] Estimates with a relative SE >0.30 are considered unreliable.

CHIP, Children's Health Insurance Program.

Am J Prev Med. Author manuscript; available in PMC 2016 February 01.

5. National Cancer Institute: Cancer Trends Progress Report Sun-Protective Behavior
Last Updated: January 2017.
Available at https://progressreport.cancer.gov/prevention/sun_protection

Sun-Protective Behavior Last Updated: January 2017

In 2015, 70.8% of adults said they usually or always protect themselves from the sun by practicing at least one of three sun protection behaviors.

Introduction
Avoiding sunburns and intermittent high-intensity sun exposure (especially in children, teens, and young adults) reduces the chances of getting melanoma skin cancer. Engaging in sun protective behaviors when outside can reduce one's exposure to UV radiation and sunburn. For example, broad spectrum sunscreen (protects against UVA and UVB) should be used and applied appropriately (e.g., proper amount applied, sunscreens should be applied prior to exposure, and sunscreen should be reapplied for prolonged UV exposure). In recent years, the Food and Drug Administration has improved standards for sunscreen content and labeling. Seeking shade can also reduce the risk of sunburn and one of the goals of the Surgeon General's Call To Action to Prevent Skin Cancer is to increase the availability of shade in outdoor recreation, education, and workplace environments.

Addition behaviors such as wearing sunglasses and sun protective clothing (e.g., long sleeve shirt, long pants, and wide brim hat) can help prevent excessive exposure to UV. Sun protective behaviors are most needed when UV intensity is greatest, which occurs during the summer time and between 10 am and 4 pm. However, for some regions of the US, such as the southeast and southwest, UV intensity is high year round. To help maximize one's protection, multiple sun protective behaviors should be practiced.

Measure
The percentage of adults aged 18 years and older who reported that they usually or always practice at least one of three sun-protective behaviors - using sunscreen, wearing protective clothing (a long-sleeve shirt, and/or wide brimmed hat shading the face, ears, and neck, and/or long pants/long skirt), or seeking shade when going outside on a sunny day for more than an hour.

Beginning in 2005, the question on hat use (as part of protective clothing) was modified to more accurately distinguish baseball caps (which do not fully protect the face, neck, and ears) from other types of fully protective hats. Graphic illustrations of different hats were used, and respondents were asked a separate question about baseball cap and sun visor use. Also, long pants/long skirt was an item added in 2005.

In certain sections of this report, the protective clothing and sunscreen measures were defined according to the Healthy People 2020 (HP2020) objectives with data available since 2005, allowing for only short-term trends. HP2020 defines use of protective clothing as wearing one or more of the following -- a wide-brimmed hat that shades the face, ears, and neck, long sleeves, and long pants or long skirt. HP2020 guidelines for sunscreen use refer to sunscreens with a sun protective factor (SPF) of 15 or higher.

Healthy People 2020 Target

Increase to 11.2 percent the proportion of adolescents in grades 9 through 12 who follow protective measures that may reduce the risk of skin cancer.

Increase to 73.7 percent the proportion of adults aged 18 years and older who follow protective measures that may reduce the risk of skin cancer.

Healthy People 2020 is a set of goals set forth by the Department of Health and Human Services.

Data Source
Centers for Disease Control and Prevention, National Center for Health Statistics. National Health Interview Survey
NCI and CDC co-sponsored Cancer Control Supplement, 1992-2010, 2005–2015.

Dermatitis. 2013 Jul-Aug;24(4):176-82. doi: 10.1097/DER.0b013e3182983845.

Patch test reactions associated with sunscreen products and the importance of testing to an expanded series: retrospective analysis of North American Contact Dermatitis Group data, 2001 to 2010.

Warshaw EM1, Wang MZ, Maibach HI, Belsito DV, Zug KA, Taylor JS, Mathias CG, Sasseville D, Zirwas MJ, Fowler JF Jr, DeKoven JG, Fransway AF, DeLeo VA, Marks JG Jr, Pratt MD, Storrs FJ.

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Abstract

BACKGROUND:

Both active and inactive ingredients in sunscreen may cause contact dermatitis.

OBJECTIVES:

This study aimed to describe allergens associated with a sunscreen source.

METHODS:

A cross-sectional analysis of patients patch tested by the North American Contact Dermatitis Group between 2001 and 2010 was performed.

RESULTS:

Of 23,908 patients patch tested, 219 (0.9%) had sunscreen coded as an allergen source. Patients who were male, with occupational dermatitis, or older (older than 40 years) had significantly lower rates of allergic reactions to sunscreens; the most commonly affected areas were the face and exposed sites ($P < 0.0001$). The top 3 most frequent allergens in sunscreens were benzophenone-3 (70.2% for 10% concentration, 64.4% for 3% concentration), DL-alpha-tocopherol (4.8%), and fragrance mix I (4.0%). Less than 40% of positive patch test reactions were detected by the North American Contact Dermatitis Group screening series of 65 to 70 allergens.

CONCLUSIONS:

A supplemental antigen series is important in detecting allergy to sunscreens.

EUROPEAN COMMISSION

Health & Consumer Protection
Directorate-General

OPINION ON

BENZOPHENONE-3

COLIPA N° S38

Opinion adopted by the SCCP during the 10th plenary of 19 December 2006

ACKNOWLEDGEMENTS

Members of the working group are acknowledged for their valuable contribution to this opinion. The members of the working group are:

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Keywords: SCCP, scientific opinion, UV filters, benzophenone-3, S38, Directive 768/76/EEC, CAS 131-57-7

TABLE OF CONTENTS

1. BACKGROUND

Submission I on the UV-filter Oxybenzone (the INN name), also known as Benzophenone-3 or 2-hydroxy-4-methoxybenzone, has been submitted by COLIPA[1].

Benzophenone-3 is proposed to be continued for use in sunscreen products at a maximum concentration at 10% weight/weight.

The substance is currently regulated in the cosmetics directive in annex VII, part 1 list of permitted UV filters which cosmetic product may contain. The regulation demands a warning on the label "contains oxybenzone".

According to the preamble to annex VII the authorised UV-filters *"may be added to other cosmetic products within the limits and under the conditions laid down in this annex."*

2. TERMS OF REFERENCE

1. *Does the SCCP consider the use of 2-hydroxy-4-methoxybenzone in a concentration up to 10% w/w in sunscreen products safe for the consumer?*

2. *Does the SCCP consider the use of 2-hydroxy-4-methoxybenzone in a concentration up to 10% w/w in other products than sunscreen products safe for the consumer?*

3. *Does the SCCP foresee any other restrictions to the safe use of 2-hydroxy-4-methoxybenzone?*

3. OPINION

3.1 CHEMICAL AND PHYSICAL SPECIFICATIONS

3.1.1. Chemical identity

3.1.1.1 Primary name and/or INCI name

INCI name: Benzophenone-3

Ref.: 4, 5, 6, 46, 66

3.1.1.2 Chemical names

Oxybenzone (INN name)
2-hydroxy-4-methoxybenzophenone
(2-Hydroxy-4-methoxyphenyl)phenyl methanone
2-Benzoyl-5-methoxyphenol

Ref.: 46, 66

3.1.1.3 Trade names and abbreviations

Aduvex 24 Escalol 567 Seesorb 101

[1] COLIPA - European Cosmetics Toiletry and Perfumery Association

Advastab 45	Eusolex® 4360	Spectra-Sorb UV 9
Anuvex	MOB	Sunscreen UV 15
ASL 24	Neo Heliopan BB	Uvasorb MET/C
Chimassorb 90	NSC 7778	Uvinul® M 40
Cyasorb UV 9	Ongrostab HMB	Uvistat 24
Cyasorb UV 9 Light Absorber	Onzone	Viosorb 110

COLIPA n° S38

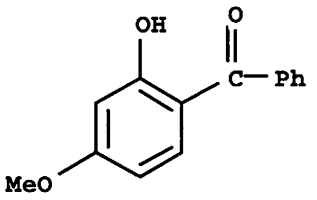
Ref.: 46

3.1.1.4 CAS / EINECS number

CAS: 131-57-7
EINECS: 205-031-5

Ref.: 4, 5, 6, 46, 66

3.1.1.5 Structural formula



3.1.1.6. Empirical formula

Molecular formula: C14H12O3

Ref.: 4, 5, 6, 46, 66

3.1.2. Physical form

White yellowish, cream coloured powder

Ref.: 46

3.1.3. Molecular weight

228.26 g/mol

Ref.: 5, 6, 46, 63, 65, 66

3.1.4. Purity, composition and substance codes

Assay (GC): ≥ 99%*
IR-spectrum: conform**
UV-spectrum: conform**

Ref.: 4, 5, 6, 46

* Capillary Gas Chromatography, chromatogram available, batch nr. stated; no identification of 3 impurities at 0.1%)

** Just a mention in a Technical Data Sheet or Material Safety Data Sheet, no full description of test (standard UV-spectrum available, without batch nr. tested).

3.1.5 Impurities / accompanying contaminants

Organic solvents:	< 0.01% Xylene
Polycyclic aromatic hydrocarbons:	< 10 ppb (total)
Benzo(a)-pyrene:	< 1 ppb
Heavy metals:	< 10 ppm (guaranteed for all batches, with corresponding limits) [46]

3.1.6 Solubility

Water:	0.0037 g/l (20°C)
Glycerin:	< 0.01%
Abil® AV 8853:	2.0%
Jojoba oil:	6.0%
Ethanol:	6.0%
Isostearyl stearate:	7.0%
Isostearyl neopentanoate:	8.0%
Olive oil:	9.0%
Peanut oil:	9.0%
Cetiol® V:	9.0%
Isopropyl stearate:	9.0%
Isopropanol, butanol:	10.0%
Isopropyl myristate:	11.0%
Miglyol® 812:	14.0%
Finsolv® TN:	15.0%
Cetiol® HE:	17.0%
Citroflex® 2:	> 20.0%
Aceton:	> 20.0%
Chloroform:	> 20.0%

Ref.: 5, 6, 46

Note
These values are taken out of Technical Data Sheets or Material Safety Data Sheets.

3.1.7. Partition coefficient (Log Pow)

> 3.7 (n-octanol/water)

Ref.: 46

Note
This value is taken out of a Material Safety Data Sheet.

3.1.8. Additional physical and chemical specifications

Melting point:	62° - 65°C
Solidification point:	62° - 65°C
Loss on drying (40° C):	< 2%
Relative density:	1.32 at 25°C
Ash content at 650°C:	0.1% (upper limit)
Colour number (Gardner):	< 4
K-Value:	64 – 67
Odour:	almost odourless or faint characteristic
Flash point:	> 100°C
Extinction (UV/VIS spectrum in methanol)	400 (0.10 mg/ml cuvette 0.1 cm)
Specific absorbance:	630 - 670 (at 287 nm; 1%, 1 cm, methanol)

Ref.: 5, 6, 46, 63, 65, 66

Note
These values are taken out of Technical Data Sheets or Material Safety Data Sheets.

3.1.9. Stability

Shelf life:	at least 2 years
Stability in distilled water:	at least 96 hours*
Stability in DMSO:	at least 4 hours*
Stability in corn oil:	at least 10 days**
Stability in acetone:	at least 3 weeks***
Stability in oily lotion:	at least 3 weeks***

* determined within recent photomutagenicity studies (full description of stability study under GLP available)
** determined within recent prenatal developmental toxicity study, performed under GLP
*** determined within the oral and dermal US National Toxicology Program (NTP) studies
Ref.: 6, 11, 15, 30, 46, 63

General comments on chemical and physical specifications

Although it is acknowledged that Benzophenone-3 has been used for many years in several types of applications and that the chemical and physical specifications have been extensively studied in the past, it is the opinion of the SCCP that characterization and determination of purity should be based upon raw data instead of simple mentions in material technical/safety data sheets. At least the solubility in water, partition coefficient and the chemical characterisation, including the UV-spectrum, should be given for a recently tested batch.

3.2 FUNCTION AND USES

Benzophenone-3 is used as a broad-band UV filter in concentrations of up to 10% in sunscreen products alone or in combination with other UV filters.

Beside the usage in sunscreens, Benzophenone-3 is incorporated in other types of cosmetic products at concentrations ranging between 0.05 - 0.5% for product protection (photoprotection).

Ref. : 5, 46, 66

3.3 TOXICOLOGICAL EVALUATION

3.3.1 Acute toxicity

A number of acute toxicity studies are briefly described and generated the following values :

LD_{50}-oral-rat	> 6,000 mg/kg	(1953)
LD_{50}-oral-rat	= 11,600 mg/kg	(1964)
LD_{50}-oral-rat	> 12,800 mg/kg	(1972)
LD_{50}-dermal-rabbit	> 16,000 mg/kg	(1953)

Comment
Due to their dates of execution, the studies were not performed according to the current guidelines and GLP practices. Nevertheless it does not appear appropriate from an ethical point of view to request more recent acute toxicity data on Benzophenone-3.

Ref.: 35, 44, 58

3.3.2. Irritation and corrosivity

3.3.2.1 Skin irritation - rabbit

In a report of 1965, Benzophenone-3 is declared to be non-irritant to the rabbit skin (tested in 6 rabbits, all scores remained 0) [59]. This finding is reinforced by a second report of 1976 [1].
A third report of 1979 mentions that 0.5 ml of a sunscreen called *Protective Eye Cream* was also tested on the skin of 3 rabbits and was found to be non-irritating (all scores = 0) [16].

Comment
These 3 reports were very short (1-2 pages) and provided limited data information.

Ref.: 1, 16, 59

3.3.2.2 Mucous membrane irritation - rabbit

In a report of 1965, Benzophenone-3 is declared to be non-irritant to the rabbit eye (tested in 6 animals, all scores remained 0) [60]. This finding was reinforced by two other reports, respectively from 1953 [35] and 1976 [1].
A report of 1979 mentions that 0.1 ml of a sunscreen called *Protective Eye Cream* was instilled in the eye of 3 rabbits and was found to be non-irritating (all scores = 0) [16].

Comment
Again the 4 available reports are very short (1-2 pages) and provide only limited data information.

Ref.: 1, 35, 16, 60

3.3.2.3. Overall conclusion on irritation

The available studies on the skin and mucous irritation potential of Benzophenone-3 are old and therefore not conform to current guideline requirements. Nevertheless they may provide some useful information, since more animals than currently required were used, and in the skin irritation studies the test substance was also applied to the abraded skin representing a worst case situation. Since all results are consistent with each other and taking into consideration the aspect of animal welfare, it does not seem appropriate to ask for new skin and/or eye irritation studies with Benzophenone-3. The compound was shown to have no irritating potential for the skin and eyes of rabbits.

3.3.3. Skin sensitisation

3.3.3.1. Magnusson Kligman Maximisation test - Guinea pig

Date of study:	July 1978
Guideline/method:	Maximization test according to Magnusson and Kligman (1969), precursor of Annex V to Dir. 67/548/EEC, Method B.6 and OECD Guideline 406
Species/strain:	Guinea pig/Hartley white
Group size:	30 female animals in the control, 20 female animals in the test groups
Test substance:	Uvinul M-40 (Benzophenone-3)
Dosage levels:	Induction: 10%, Challenge 2.5%
Route:	Intradermal (induction) and percutaneous (booster and challenge)
Batch:	Not stated

Purity:	Not stated
GLP:	Study performed prior to implementation of GLP

In a dose-range finding study concentrations of 0.25%, 2.5%, 5% and 10% of Benzophenone-3 in petrolatum and the pure compound were applied on the shaved flanks of the animals. Based on the results of this study the animals were intradermally injected with 0.05 ml of 5% Benzophenone-3 in corn oil or 5% Benzophenone-3 in 50% aqueous Freund's complete adjuvant into the shaven shoulder region of 10 animals. One week after the induction injection, a topical booster patch consisting of 0.1 ml of 10% test substance in petrolatum was occlusively applied on the induction site for 48 hours. Prior to the booster, 10% aqueous Sodium Lauryl Sulfate was applied unoccluded to the induction sites of all animals.
The challenge was performed by occlusive epicutaneous application of 0.1 ml of 2.5% test material in petrolatum to a previously untreated site for 24 hours. The application sites were scored 24 and 48 hours after removal of the patch.

At readings 48 hours after challenge 18/20 animals showed no evidence of any effect and 2/20 revealed a barely perceptible erythema. At the 72 hour readings 1/20 showed no skin reaction and 3/20 barely perceptible erythema (different animals to the first reading). No skin reactions were observed in the control group at challenge, while the animals of the positive control group (2% phenylacetaldehyde in petrolatum) showed clear skin reactions as indication of sensitization.

The study authors conclude that Benzophenone-3 did not exhibit any potential to induce dermal sensitization in the performed Guinea pig Magnusson Kligman Maximization test.

Ref.: 2

3.3.3.2 Local Lymph Node Assay - mouse

Date of study:	September 2005
Guideline/method:	Annex V to Dir. 67/548/EEC, Method B.42; OECD Guideline 429
Species/strain:	Mouse, CBA/CaOlaHsd
Group size:	4 female animals per treated and control group
Test substance:	Benzophenone-3
Batch:	101
Purity:	99.8% (GC-FID)
Dosage levels:	0 - 12.5 - 25 - 50% (w/v) in dimethylformamide (DMF)
Route:	Epidermal (topical) application on the dorsal ear lobe surface
GLP/QAU:	Signed documents available

Three groups each of four female mice were treated daily with Benzophenone-3 at concentrations of 12.5, 25 and 50% (w/v) in dimethylformamide (DMF) by topical application to the dorsum of each ear lobe (left and right) for three consecutive days. A control group of four mice was treated with the vehicle (DMF) only. Five days after the first topical application the mice were injected intravenously into a tail vein with radio-labelled thymidine (^{3}H-methyl thymidine). Approximately five hours after intravenous injection, the mice were sacrificed, the draining auricular lymph nodes excised and pooled per group. Single cell suspensions of lymph node cells were prepared from pooled lymph nodes which were subsequently washed and incubated with trichloroacetic acid overnight. The proliferative capacity of pooled lymph node cells was determined by the incorporation of ^{3}H-methyl thymidine measured in a β-scintillation counter.

A test item is regarded as a sensitizer in the LLNA if the exposure to one or more test concentrations results in a 3-fold or greater increase in incorporation of ^{3}HTdR compared with concurrent controls, as indicated by the Stimulation Index (S.I.). The estimated concentration of test item required to produce a S.I. of 3 is referred to as the EC_3 value.

All treated animals survived the scheduled study period.
Stimulation Indices of 1.64, 1.33 and 1.61 were determined with the test item at concentrations of 12.5, 25 and 50% (w/v) in DMF.

The study authors conclude that the test item Benzophenone-3 was not a skin sensitizer under the described conditions.

Ref.: 13

3.3.4. Dermal / percutaneous absorption

3.3.4.1 *In vitro* dermal / percutaneous absorption - human skin

In a publication of 1999, Benzophenone-3 formed part of a battery of five UV filters for which standard operating procedures for their rapid analysis in various skin layers, were established. Benzophenone-3 was included at 4.9% in a cosmetic formulation (composition not stated) applied at 3 mg/cm² on fresh dermatomed (± 344 µm) human skin (6 samples from different donors) put on static diffusion cells. The 3 ml receptor fluid (pH 7.4) was maintained at 32°C and consisted of 1% bovine serum albumin, 0.9% NaCl, 0.02% KCl and 0.04% gentamycin in distilled water. The transepidermal water loss (TEWL) was recorded at each site with a Tewameter. After an exposure time of 16 hours, the skin was washed and dried with cotton swabs. The receptor fluid was collected and 16 strippings were carried out on the skin surface to determine the stratum corneum (SC) content and subsequently the epidermis was separated from the dermis. Analysis was preformed by isocratic RP-HPLC[2] with UV detection.

Benzophenone-3 quantification led to the following results:

Total amount applied	147 µg/cm² (3mg cream/cm², 4.9% Benzophenone-3)
Stratum corneum (SC)	8.5 ± 3.3 µg/cm²
Epidermis	0.3 ± 0.2 µg/cm²
Dermis	0.4 ± 0.1 µg/cm²
Receptor fluid	1.0 ± 0.4 µg/cm²
Washing solution	85.7% ± 4.5%
Recovery	93.4% ± 3.1%

The results indicate that the SC adsorbed the greatest proportion of the applied amount (5.8%), while about 0.5% was absorbed in the viable skin and 0.7% was analyzed in the receptor fluid.
According to the study authors the test can be considered as valid since the recovery was in the accepted range of above 90%.

They estimate the dermal absorption of Benzophenone-3 in respect to bioavailability after topical application to freshly dermatomed human skin for 16 hours as 1.7 µg/cm² (1.0 µg/cm² receptor fluid, 0.4 µg/cm³ dermis, 0.3 µg/cm² epidermis), corresponding to 1.16% of the applied dose.

Ref.: 54

Comment
The following shortcomings can be noted:
- The test concentration of 4.9% is lower than the maximum allowed level of 10%.
- The solubility of the Benzophenone-3 in the receptor fluid at 32°C is not stated. This is essential, since the compound's solubility in water is very low (0.0037g/l at 20°C).

[2] Reverse Phase - High Performance Liquid Chromatography

- The composition of the cosmetic formulation is unknown.
- Not all details on preservation and storage of skin are given.
- The purity of the test substance is not stated.
- Only one concentration of the test substance is used.
- 16 hours of contact is rather unusual. Normally, contact time is 24 hours.
- 6 samples from different donors is less than the requested amount.
- Only measurements after 16h are available; no intermediate sampling has been performed.

Several other studies were published dealing with *in vitro* skin penetration or certain aspects thereof using Benzophenone-3 (labelled or unlabelled) or Benzophenone-3 containing products. New or modified penetration models, different analytical methods as well as newly composed and/or different formulations were investigated and/or compared. The different working groups used human skin (full thickness or split thickness), pig skin (full thickness or dermatomed) or artificial membranes as test systems.
Each study has its own limitations since either no complete penetration but only penetration in certain skin compartments were investigated and reported (stratum corneum, epidermis, dermis), the application duration was changing (ranging from 30 minutes up to 10 hours at maximum) or several deficiencies in respect to methodology and/or reporting, when compared to guideline requirements exist.
Therefore only general statements can be derived from these studies, such as the fact that the dermal absorption of Benzophenone-3 appears to be low, that the major proportion is adsorbed by the stratum corneum and that certain formulations (o/w emulsion, w/o emulsion, gels, oils, creams) can influence the absorption rate in respect to time-course and amount of absorption.

Ref.: 10, 26, 27, 32, 54, 71, 72

3.3.4.2 *In vivo* dermal / percutaneous absorption - human

The published human studies (2002-2003) all concern the tape stripping methodology and they differ in duration of application, analytical methods, calculation of adsorption/absorption and in the composition of the formulations used. Therefore, no quantitative conclusion for the *in vivo* dermal absorption of Benzophone-3 is possible. Qualitatively it can be stated that, as was the case in the *in vitro* studies, the stratum corneum adsorbed the greatest fraction of the applied Benzophenone-3 and that only small amounts could be considered as absorbed and systemically bioavailable. In addition, the type of preparation/formulation had a clear influence on the extent of dermal absorption.

Ref.: 10, 27, 71

Comment
The following recent study on dermal absorption of Benzophenone-3 in volunteers has been added by the SCCP:

In 2006, after the submission of the dossier, Gonzalez et al. published another human study on the dermal absorption of Benzophenone-3 after repeated whole-body applications, with and without UV irradiation. 25 volunteers applied 2 mg/cm² of a sunscreen containing 4% of Benzophenone-3 to their whole body surface area, twice daily for 5 consecutive days. The amount of sunscreen per application varied between the participants and ranged from 26 g to 47 g. The volunteers were divided in two groups, of which one received UV-irradiation. During the 5 days of application, all urine was collected and analyzed for Benzophenone-3 concentration through high-performance liquid chromatography with UV detection.
The test results indicate that there was a large variation in the total amount of Benzophenone-3 excreted in the urine, even after compensation for the differences in body surface area. However, UV-irradiation did not affect the urinary secretion of the compound. The mean value of Benzophenone-3 found in the urine was 3.7% (1.2% - 8.7%). Other excretion routes were not investigated, thus this value may still be an underestimation of the total dermally absorbed percentage of Benzophenone-3. The volunteers excreted

Benzophenone-3 many days after the last application, which could be expected viewing the lipophilicity of the molecule.

Ref.: D

3.3.5 Repeated dose toxicity

3.3.5.1 Repeated dose (28 days) oral / dermal / inhalation toxicity - rat/mouse

A. Subacute oral administration to rats and mice

A report dated 1953 describes a 27-day study in rats (10 animals per group) with dosages of 7.2, 75 and 789 mg/kg bw/day. Food consumption and body weight gains of the test groups were comparable to those of the control rats. There were no deaths and no significant gross pathologic changes in any of the animals which could be attributed to the administration of the test substance.

Ref.: 34

14-day oral toxicity in rat (1985-88)

Five F344/N rats per sex and group received Benzophenone-3 in concentrations of 0, 3,125, 6,250; 12,500; 25,000 and 50,000 ppm in the diet for 2 weeks. The dietary test substance preparations were analyzed for stability and proved to be stable in the diet for at least 3 weeks. Clinical examinations covering clinical signs, mortality, body weight and food consumption were performed in all animals. At termination of treatment, all animals were sacrificed and macroscopically examined, organs were weighed and comprehensive histopathology was performed.

The following effects were noted (dietary levels were converted to dosages):

303 mg/kg bw/day:	increased liver weights in males and females; increased kidney weights in males
576 mg/kg bw/day:	increased liver weights in males and females associated with the presence of cytoplasmic vacuolisation of hepatocytes; increased kidney weights in males
1,132 mg/kg bw/day:	increased liver weights in males and females associated with the presence of cytoplasmic vacuolisation of hepatocytes; increased kidney weights in males
2,238 mg/kg bw/day:	increased liver weights in males and females associated with the presence of cytoplasmic vacuolisation of hepatocytes; increased kidney weights in males
3,868 mg/kg bw/day:	reduced feed consumption in males and females; reduced body weight gain in males; increased liver weights in males and females associated with the presence of cytoplasmic vacuolisation of hepatocytes; increased kidney weights in males; focal dilatation of renal tubules in the cortex and/or medulla

Study authors' conclusion: NOAEL (14d-oral) = 295/311 mg/kg bw/day for the male/female rat.

Ref.: 30

14-day oral toxicity in mouse (1985-88)

Benzophenone-3 was administered in the diet to five B6C3F1 mice per sex and group at concentrations of 0, 3,125; 6,250; 12,500; 25,000 and 50,000 ppm for 2 weeks. The dietary test substance preparations were analyzed for stability and proved to be stable in the diet for at least 3 weeks. Clinical examinations covering clinical signs, mortality, body weight and food consumption were performed in all animals. At termination of treatment, all

animals were sacrificed and macroscopically examined, organs were weighed and comprehensive histopathology was performed.

The following effects were noted (dietary levels were converted to dosages):

1,021 mg/kg bw/day:	increased liver weights in males and females
2,041 mg/kg bw/day:	increased liver weights in males and females associated with the presence of cytoplasmic vacuolisation of hepatocytes
4,430 mg/kg bw/day:	increased liver weights in males and females associated with the presence of cytoplasmic vacuolisation of hepatocytes
8,648 mg/kg bw/day:	increased liver weights in males and females associated with the presence of cytoplasmic vacuolisation of hepatocytes; decreased kidney weight in males
20,796 mg/kg bw/day:	reduced body weight gain; increased liver weights in males and females associated with the presence of cytoplasmic vacuolisation of hepatocytes; decreased kidney weight in males

Study authors' conclusion: NOAEL (14d-oral) = 992/1050 mg/kg bw/day for the male/female mouse.

Ref.: 30

B. Subacute dermal administration to rats and mice

14-day dermal toxicity in rat (1985-88)

Five F344/N rats per sex and group received Benzophenone-3 at dose levels of 0, 1.25, 2.5, 5.0, 10.0, 20.0 mg/rat in acetone or lotion as vehicle for 5 days per week for 2 weeks. A constant volume of 0.25 ml/rat was applied over a fixed standard area (10%) of the interscapular region. The area was clipped 24 hours prior to initial application and weekly thereafter. The preparations in acetone and the lotion were analyzed for stability and proved to be stable in the diet for at least 3 weeks. Clinical examinations covering clinical signs, mortality, body weight and food consumption were performed in all animals. At termination of treatment, all animals were sacrificed and macroscopically examined, organs were weighed and comprehensive histopathology was performed.

The following effects were noted (dietary levels were converted to dosages):

7.0 mg/kg bw/day:	no adverse effects noted
13.6 mg/kg bw/day:	no adverse effects noted
27.7 mg/kg bw/day:	slightly increased liver weights in females
54.9 mg/kg bw/day:	slightly increased liver weights in female
110 mg/kg bw/day:	slightly increased liver weights in females; slightly increased kidney weights

Study authors' conclusion: NOAEL (14d-dermal) = 100/140 mg/kg bw/day for the male/female rat.

Ref.: 30

28-day dermal toxicity in rat (1985-88)

A publication of 1995 describes how 6 male Sprague-Dawley rats per group received Benzophenone-3, formulated in petroleum jelly base, at dosage levels of 0 and 100 mg/kg bw/day, twice daily for 4 weeks. Clinical examinations covering clinical signs and body weight were performed in all animals. Blood samples for haematology and clinical chemistry were taken prior to the start of treatment and on day 16. At termination, the animals were sacrificed, organs were weighed (liver, kidney, testes) and liver, kidneys, testes and skin from treated and untreated area were collected and histopathologically

examined. In addition, on day 16 of treatment, blood samples for GSH determination were collected.
No animal died premature. There was no substance-related effect on body weight, relative organ weights, haematological and clinical-chemical parameters. Physical examination of the skin revealed no substance-related changes. Histopathological examination of livers, kidney and testes revealed no significant difference between control and treated animals and no abnormalities were observed in the skin from treated or untreated areas.

Ref.: 51

14-day dermal toxicity in mouse (1985-88)

Five B6C3F1mice per sex and group received Benzophenone-3 preparations at dose levels of 0, 0.5, 1.0, 2.0, 4.0, 8.0 mg/mouse in acetone or lotion as vehicle for 5 days per week for 2 weeks.
A constant volume of 0.1 ml was applied over a fixed standard area (10%) of the interscapular region. The area was clipped 24 hours prior to initial application and weekly thereafter. The preparations in acetone and the lotion were analyzed for stability. Clinical examinations covering clinical signs, mortality, body weight and food consumption were performed in all animals. At termination of treatment, all animals were sacrificed and macroscopically examined, organs were weighed and comprehensive histopathology was performed.

The following effects were noted (applied doses were converted to dosages):

24.8 mg/kg bw/day: none
48.4 mg/kg bw/day: none
100 mg/kg bw/day: none
196 mg/kg bw/day: increased liver weight
388 mg/kg bw/day: increased liver weight

Study authors' conclusion: NOAEL (14d-dermal) = 384/432 mg/kg bw/day for the male/female mouse.

Ref.: 30

3.3.5.2 Sub-chronic (90 days) oral / dermal / inhalation toxicity - rat/mouse

A. **S**ub-chronic oral administration to rats and mice

90-day oral toxicity in rat (1) (1972)

Benzophenone-3 (source and batch not cited) was examined in 12 male and 12 female rats per sex and group at dosages of approximately 0, 20, 100, 500 and 1,000 mg/kg bw/day for 13 weeks. The animals were observed for clinical findings and body weight and food consumption was determined weekly. Blood samples for haematology were collected in the 6^{th} and 12^{th} week. In addition, at termination liver enzyme activities and clinical chemistry parameters were examined in six rats per sex and dosage group. All animals were sacrificed and macroscopically examined, organs were weighed and comprehensive histopathology was performed.

The following effects were noted:

No deaths occurred.
20 mg/kg bw/day: no adverse effects noted
100 mg/kg bw/day: no adverse effects noted
500 mg/kg bw/day: reduced body weights and body weight gains; reduced haemoglobin and leukocytosis (with an increase in lymphocytes and decrease in neutrophils) after 6 weeks in females; anaemia,

	lymphocytosis and reduced number of granulocytes after 12 weeks in females; decreased absolute weights of the thymus and heart in males and females; decreased relative weights of the pituitary gland, thymus, heart and adrenal glands in males and females; first stages of degenerative nephritis in kidneys of males and females
1,000 mg/kg bw/day:	reduced body weights and body weight gains; ruffled fur and rigid limbs (reversible within 4 weeks); reduced haemoglobin and leukocytosis (with an increase in lymphocytes and decrease in neutrophils) after 6 weeks in females; anaemia, lymphocytosis and reduced number of granulocytes after 12 weeks in females; decreased absolute weights of the thymus, heart, pituitary gland, lungs, spleen, adrenal glands, and gonads in males and females; decreased relative weights of the pituitary gland, thymus, heart and adrenal glands in males and females; decreased relative weights of the lungs and spleen in females; increased relative thyroid weight in females; degenerative nephritis in kidneys of males and females

Study authors' conclusion: NOEL (90d-oral) = 100 mg/kg bw/day for male and female rats.
Ref.: 44

90-day oral toxicity in rat (2) (1985-1988)

Ten F344/N rats per sex and group received Benzophenone-3 in concentrations of 0; 3,125; 6,250; 12,500; 25,000 and 50,000 ppm in the diet for 13 weeks. The dietary test substance preparations were analyzed for stability and proved to be stable in the diet for at least 3 weeks. Clinical examinations covering clinical signs, mortality, body weight and food consumption were performed in all animals at regular intervals. Samples for haematological and clinical-chemical examination and urinalysis were taken on days 3 and 15 and in week 12 of treatment. Sperm morphology/motility and vaginal cytology examinations were performed at dietary levels of 0; 3,125; 12,500 and 50,000 ppm. At termination of treatment, all animals were sacrificed and macroscopically examined, organs were weighed and comprehensive histopathology was performed.

The following effects were noted (dietary levels were converted to dosages):

204 mg/kg bw/day:	coloured urine; increased liver weight
411 mg/kg bw/day:	coloured urine; increased liver weight; disturbed serum protein levels
828 mg/kg bw/day:	coloured urine; increased liver weight; disturbed serum protein levels
1,702 mg/kg bw/day:	decreased growth and body weight gain in males and females; coloured urine; enlarged kidneys with abnormal shape and granular surface; increased absolute and relative kidney weights in females; dilatation of renal tubules; increased liver weight; increased platelet counts; disturbed serum protein levels
3,458 mg/kg bw/day:	decreased growth and body weight gain in males and females; coloured urine; enlarged kidneys with abnormal shape and granular surface; increased absolute and relative kidney weights in males and females; dilatation of renal tubules; mild to moderate inflammation with fibrosis in the renal interstitium; increased liver weight; increased platelet counts; disturbed serum protein levels; reduced sperm motility in males, increase in estrous cycle of females

Study authors' conclusion: NOAEL (90d-oral) = 429/393 mg/kg bw/day for the male/female rat.

Ref.: 30

90-day oral toxicity in mouse (1985-88)

Ten B6C3F1 mice per sex and group received Benzophenone-3 in concentrations of 0, 3125, 6,250; 12,500; 25,000 and 50,000 ppm in the diet for 13 weeks. The dietary test substance preparations were analyzed for stability and proved to be stable in the diet for at least 3 weeks. Clinical examinations covering clinical signs, mortality, body weight and food consumption were regularly performed. Sperm morphology/motility and vaginal cytology examinations were performed at dietary levels of 0; 3,125; 12,500 and 50,000 ppm. At termination of treatment, all animals were sacrificed and macroscopically examined, organs were weighed and comprehensive histopathology was performed.

The following effects were noted (dietary levels were converted to dosages):

554 mg/kg bw/day:	no adverse effects noted
1,246 mg/kg bw/day:	increased liver weight
2,860 mg/kg bw/day:	increased liver weight
6,780 mg/kg bw/day:	decreased body weight gain in males and females; increased liver weight; minimal cytoplasmic vacuolisation of hepatocytes
16,238 mg/kg bw/day:	decreased body weight gain in males and females; minimal renal lesions in males; increased liver weight; minimal cytoplasmic vacuolisation of hepatocytes; decreased sperm density and increased abnormal sperm in males; increased estrous cycle length in females

Study authors' conclusion: NOAEL (90d-oral) = 1068/1425 mg/kg bw/day for the male/female mouse.

Ref.: 30

B. Sub-chronic dermal administration to rats and mice

90-day dermal toxicity in rat (1985-88)

Ten F344/N rats per sex and groups received Benzophenone-3 preparations at dosage levels of 0, 12.5, 25, 50, 100, 200 mg/kg bw/day in acetone for 5 days per week over a period of 13 weeks. A constant volume of 0.25 ml/rat was applied over a fixed standard area (10%) of the interscapular region. The area was clipped 24 hours prior to initial application and weekly thereafter. The preparations in acetone were analyzed for stability. Clinical examinations covering clinical signs, mortality, body weight and food consumption were performed in all animals at regular intervals.
Samples for haematological and clinical-chemical examination and urinalysis were taken on days 3 and 15 and in week 12 of treatment. Sperm morphology/motility and vaginal cytology examinations were performed at dosage levels of 0, 12.5, 50 and 200 mg/kg bw/day. At termination of treatment, all animals were sacrificed and macroscopically examined, organs were weighed and comprehensive histopathology was performed.

The following effects were noted (dietary levels were converted to dosages):

12.5 mg/kg bw/day:	decreased reticulocyte count
25 mg/kg bw/day:	non-dosage related increase in relative kidney weight in females; decreased reticulocyte count
50 mg/kg bw/day:	non-dosage related increase in relative kidney weight in females; decreased reticulocyte count; increased platelet count
100 mg/kg bw/day:	non-dosage related increase in relative kidney weight in females; decreased reticulocyte count; increased platelet count

200 mg/kg bw/day: non-dosage related increase in relative kidney weight in females; decreased reticulocyte count; increased platelet count; increased whole blood cell count produced by lymphocytosis

Study authors' conclusion: NOAEL (90d-dermal) = 200 mg/kg bw/day for male and female rats.

Ref.: 30

90-day dermal toxicity in mouse

Ten B6C3F1 mice per sex and group received Benzophenone-3 preparations at dosage levels of 0, 22.8, 45.5, 91, 182, 364 mg/kg bw/day in acetone for 5 days per week over a period of 13 weeks. A constant volume of 0.1 ml was applied over a fixed standard area (10%) of the interscapular region. The area was clipped 24 hours prior to initial application and weekly thereafter. The preparations in acetone were analyzed for stability. Clinical examinations covering clinical signs, mortality, body weight and food consumption were performed in all animals at regular intervals. Sperm morphology/motility and vaginal cytology examinations were performed at dosage levels of 0, 22.8, 91, 364 mg/kg bw/day. At termination of treatment, all animals were sacrificed and macroscopically examined, organs were weighed and comprehensive histopathology was performed.

At all dosage levels, a mild increase in relative kidney weight in males, together with a decrease in epididymal sperm density, was noted. No other abnormalities were observed.

Study authors' conclusion: NOAEL (90d-dermal) = 364 mg/kg bw/day for male and female mice.

Ref.: 30

3.3.5.3 Chronic (> 12 months) toxicity

No data.

3.3.5.4 Overall conclusion of the submission authors on repeated dose toxicity

The toxicity of Benzophenone-3 after repeated application was comprehensively examined in subacute up to subchronic studies in rats and mice using the oral and dermal application route.
The systemic toxicity after repeated oral application was low and effects could mainly be observed at dose levels which were in the range or clearly above the current internationally accepted limit dose level of 1000 mg/kg bw/day for repeated toxicity studies. Beside unspecific signs of systemic toxicity in the form of reduced food consumption and retarded body weight gain, the identified target organs were the kidney and liver, partly associated with changes in clinical chemistry at high dose levels. Very often the most susceptible parameter was the increase in liver weight. However, this effect without any histopathological correlate does not reflect an adverse effect per se but is considered as an adaptive metabolic response, which is known to be reversible.

At very high dose levels clearly >3000 mg/kg bw/day in rats and >13000 mg/kg bw/day in mice after subchronic oral treatment, an impairment of selective reproductive parameters was noted. However, these are finally assessed in the reproduction section within this dossier (section 3.3.8 Reproductive toxicity).
Repeated dermal application of up to 13 weeks in rats and mice did not lead to any reliable substance-related local or systemic findings up to the highest dose level investigated in each case.
Finally, the reliable No-Adverse-Effect-Level (NOAEL) for subchronic toxicity after oral treatment was 6250 ppm (429/393 mg/kg bw/day in males/females) in rats and 6250 ppm (1068/1425 mg/kg bw/day in males/females) in mice.

For subchronic dermal treatment in each case the highest dose level was the reliable No-Adverse-Effect-Level (NOAEL), namely 200 mg/kg bw/day in rats and 364 mg/kg bw/day in mice.

3.3.6 Mutagenicity / genotoxicity

3.3.6.1 Mutagenicity/Genotoxicity *in vitro*

A. *In vitro* bacterial mutation assay (Ames test)

A number of publications (1980-1992) describe the results of Benzophenone-3 studied in the Ames test. The level of detail provided in these publications ranges from a summary and a title page [36] to a more extensive description of the materials and methods [73].
Generally the test substance was tested for mutagenicity in the reverse mutation assay on bacteria both, with and without metabolic activation (S9 mix from the liver of Aroclor 1254-induced male Sprague-Dawley and male Syrian hamster or rat livers). The mixes were prepared immediately prior to use and contained 10% S9. The tested Salmonella typhimurium strains were combinations of TA97, TA98, TA100, TA1535, TA1537 and/or TA1538 and were exposed to the test substance at various concentrations of Benzophenone-3 with and without S9 mix. Positive controls were included to demonstrate the sensitivity and validity of the test system used.
Bacteriotoxicity was reported [73] to be observed at a varying degree at and above 333 µg/plate and at 1000 µg/plate in all tested strains (TA98, TA100, TA1535, TA1537). In that same publication, Salmonella Typhimurium TA97 was additionally tested and showed a weak mutagenic response using 30% of S9 hamster mix.
However, no effect was noted using 10% hamster or 10% and 30% rat S9 mix compared to the respective controls, the increase in the numbers of revertants was less than 2-fold compared to the solvent control and there was no dose-response relationship. Benzophenone-3 showed to be negative for the induction of revertants in all other strains at the tested concentration range between 3 and 333 µg/plate. All other studies (not including TA97 in their testing battery) showed Benzophenone-3 to be negative in the Ames test.
Considering the fact that Benzophenone-3 did not induce gene mutations by base pair changes or frame shifts in the genome of the Salmonella typhimurium strains used in the presence and absence of S9-mix, the compound is considered to be non-mutagenic in the Ames test. The single weak positive response in one strain with a very high concentration of one specific S9 mix, is considered irrelevant by the authors of the submission.

Ref.: 8, 30, 36, 73

B. *In vitro* chromosome aberration test in Chinese Hamster Ovary cells

A US National Toxicology program report (1992) briefly describes the results of cytogenetic tests with Chinese hamster ovary cells, in which Benzophenone-3-induced sister-chromatid exchanges (effective dose range 5-50µg/ml) and chromosomal aberrations (20-45µg/ml) in the presence of Aroclor 1254-induced male Sprague Dawley rat liver S9.
One trial delivered a questionable result, while another trial appeared to be clearly negative.

Ref.: 30

Note
The cited reference for the chromosome aberration test (i.e. the NTP report) does not provide the level of detail displayed in the submission summary. Probably these details were extracted out of additional test descriptions / references not included in the submission.

3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

A. *In vivo* micronucleus test (Follow-up of the 90-day oral study in the mouse)

A US National Toxicology program report (1992) mentions that peripheral blood smears from the mice used in the 90-day studies as described under 3.3.5.2.A, were analyzed for the frequency of micronucleated normochromatic erythrocytes. No increase was noted in either male or female mice treated with up to 16,238 mg/kg bw/day of Benzophenone-3 administered orally.

Ref.: 30

B. *In vivo* rat bone marrow chromosome aberration test

A publication of 1995 describes how Benzophenone-3 was examined for its cytogenic potential *in vivo* in male and female Sprague-Dawley rats after a single or repeated (5 consecutive days) oral application by gavage. The test substance was dissolved in corn oil and was applied either once in dose levels of 0; 500; 1,670 and 5,000 mg/kg bw/day or for the repeated application at dose levels of 0 and 5000 mg/kg bw/day. Cclophosphamide was used as positive control substance and was orally administered as a single bolus or on five consecutive days at a dose level of 20 mg/kg bw/day. Since previously performed cell cycle kinetic studies investigating bromodeoxyuridine (BrdU) incorporation demonstrated that Benzophenone-3 did not affect the average generation time after single treatment of 5,000 mg/kg bw/day or after daily application for 5 days, bone marrow collection times were set at 8 and 12 hours after single application and at 12 hours after repeated administration.
Bone marrow smears were prepared, stained by fluorescence-plus – Giemsa and a total of 50 metaphase spreads from each animal were scored for chromosomal aberrations. In addition, the mitotic index was determined and the percentages of polyploid and endoreduplicated cells were analyzed.

No substance-related mortality occurred. The single treatment or the treatment for 5 consecutive days did not disturb the cell cycle and had no effect on the average generation time. No increase in the frequency of chromosomal aberrations was observed in bone marrow cells of male and female rats. Sex differences were not noted. The positive control substance caused chromosomal aberrations confirming the sensitivity of the test system.

The authors conclude that, since neither single nor repeated treatment up to 5,000 mg/kg bw/day in male and female Sprague-Dawley rats caused chromosomal aberrations, Benzophenone-3 was shown to be exhibit no clastogenic potential *in vivo*.

In that same publication, Benzophenone-3 is also reported to be investigated in the Drosophila somatic mutation and recombination test (SMART). Larvae from mated multiple wing hair females with heterocygotous flare males were fed a diet containing the test substance at concentrations of 0; 3,000 and 3,500 ppm for 72 hours. None of the fed larvae produced flies with significantly more single or multiple wing spots than negative controls. In contrast, positive control larvae produced flies with significantly more single or multiple wing spots than negative controls and confirmed the sensitivity of the test system. Finally, it was shown that Benzophenone-3 did not induce mutations, chromosome damage or genetic recombination in Drosophila using the SMART procedure.

Ref.: 55

3.3.6.3 Overall conclusion of the submission authors on mutagenicity/genotoxicity

Benzophenone-3 was tested in bacterial and mammalian test systems *in vitro*. No genotoxic/mutagenic potential was noted in three bacterial gene mutation assays in *Salmonella typhimurium* strains in the presence or absence of metabolic activation. The reported effect in a single strain after metabolic activation with an unusual high proportion was finally considered as irrelevant due to reporting and assessment deficiencies.

In mammalian cells systems, Benzophenone-3 showed no clastogenic potential and no ability to induce SCEs in the absence of metabolic activation. With metabolic activation a slight and/or non-concentration related increase in structural aberrations and the SCE rate were reported.

In vivo Benzophenone-3 was shown to be negative in the mouse micronucleus test after dietary administration for 13 weeks and in a chromosome aberration test in rats. Thus, the questionable effect observed *in vitro* was shown to posses no relevance for the *in vivo* situation.

Furthermore, Benzophenone-3 did not induce mutations, chromosome damage or genetic recombination in Drosophila using the SMART procedure.

3.3.7. Carcinogenicity

No data.

3.3.8. Reproductive toxicity

3.3.8.1 Reproduction toxicity screening tests - rat/mouse

A. Oral reproduction toxicity screening tests

Reproductive toxicity pre-screening at the end of the 90-day oral toxicity in rat

Ten F344/N rats per sex and group received Benzophenone-3 in concentrations of 0; 3,125; 12,500 and 50,000 ppm in the diet for 13 weeks (stability of test substance in diet proven to be at least 3 weeks). At termination of the study, some specific examinations were performed to screen the potential reproductive toxicity of the substance. Observations in the male animals consisted of testicular, epididymal and caudal weights, sperm motility and morphology and sperm number per caudal tissue weight. Observations in the females included vaginal cytology examinations and estrual cyclicity.

The following effects were noted (dietary levels were converted to dosages):

204 mg/kg bw/day:	no deviations among the reproductive parameters studied
828 mg/kg bw/day:	no deviations among the reproductive parameters studied
3,458 mg/kg bw/day:	males: decreased right caudal, testicular and epididymal weights; decreased sperm number per caudal tissue females : prolonged cycle length.

Study authors' conclusion: NOAEL (reproduction) = 828 mg/kg bw/day for male and female rats.

Ref.: 24, 30

Reproductive toxicity pre-screening at the end of the 90-day oral toxicity in mouse

Ten B6C3F1 mice per sex and group received Benzophenone-3 in concentrations of 0; 3,125; 12,500 and 50,000 ppm in the diet for 13 weeks (stability of test substance in diet proven to be at least 3 weeks). At termination of the study, some specific examinations were performed to screen the potential reproductive toxicity of the substance. Observations in the male animals consisted of testicular, epididymal and caudal weights, sperm motility and morphology and sperm number per caudal tissue weight. Observations in the females included vaginal cytology examinations and estrual cyclicity.

The following effects were noted (dietary levels were converted to dosages):

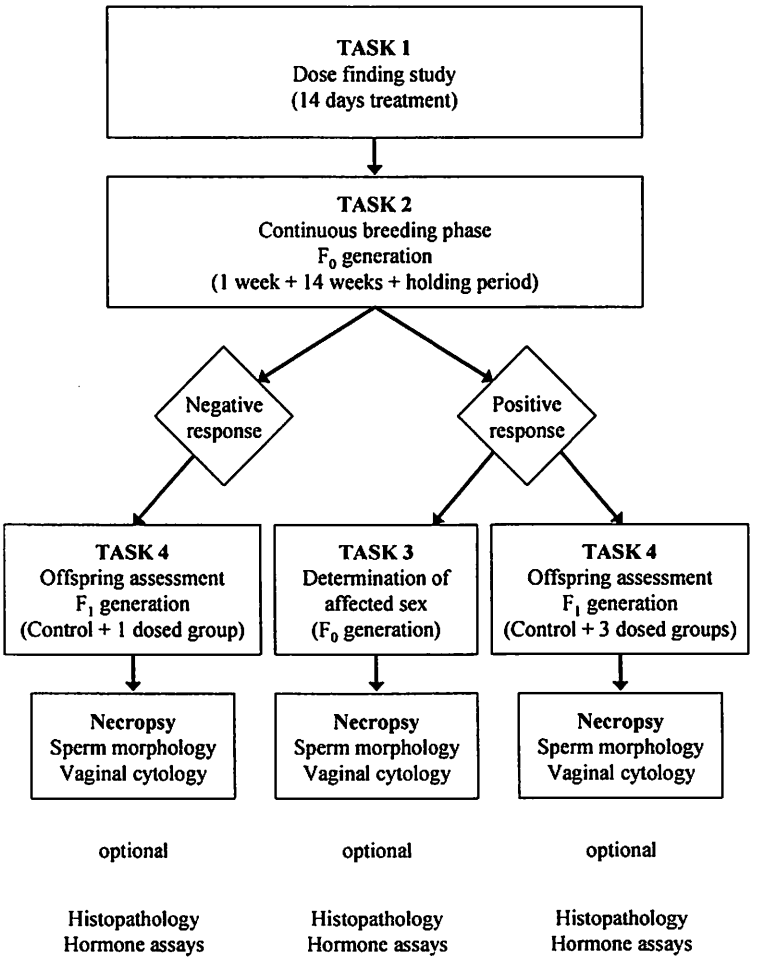
554 mg/kg bw/day:	no deviations among the reproductive parameters studied
2,860 mg/kg bw/day:	no deviations among the reproductive parameters studied
16,238 mg/kg bw/day:	decreased sperm number per caudal tissue and increased incidence of abnormal sperm in the males

Study authors' conclusion: NOAEL (reproduction) = 2,860 mg/kg bw/day for male and female mice.

Ref.: 24, 30

Reproductive Assessment by Continuous Breeding (RACB) in the mouse (1991)

The "Continuous Breeding Protocol" is a test design used by the US National Toxicology Program. It consists of four related tasks (see figure below), which are not all necessarily performed for every compound tested.



In the performed study, **Task 1** consisted of administering Benzophenone-3 in the diets[3] of groups of 8 CD-1 mice per sex at dosages of 0; 1,000; 2,100; 4,700; 10,200 and 15,700 mg/kg bw/day for 14 days. During the continuous breeding phase (**Task 2**), groups of 20 mice per sex were fed diets containing the test substance at dosage levels of about 1,850; 3,950 and 9,050 mg/kg bw/day, while the controls consisted of 40 animals per sex fed with unsupplemented diet. Feeding was started 1 week prior to mating and was continued for another 21 weeks. During this period the mice were cohabitated for 14 weeks (study week 2 to 17). Five litters were delivered during the whole study. The endpoints comprised clinical signs, body weight and food consumption, reproductive and fertility parameters and developmental endpoints in the progeny. The 1 week cross-over mating trial to determine the affected sex (**Task 3**) was not performed in this study, since no noted impairment of fertility occurred.

Finally, in **Task 4**, the development of the offspring was investigated using the last litter from Task 2. This progeny was reared, weaned and kept until mating (at 74 ± 10 days). At sexual maturity, a male and female from different litters were mated. The examinations in this phase were identical to the parents with the addition for checking the presence of a copulatory plug. The body weights were measured and estrous cyclicity was monitored by vaginal lavage 12 days prior to necropsy. In the males, epididymal sperm motility, sperm morphology and sperm count were investigated. At termination, necropsy was performed, organs were weighed and preserved for histopathology (especially reproductive organs).

The following effects were noted:

1,850 mg/kg bw/day:	5 dams died unexpectedly during the Task 2 20-day period
3,950 mg/kg bw/day:	reduced number of pups / litter; reduced dam weights; 4 dams died unexpectedly during the Task 2 20-day period
9,050mg/kg bw/day:	reduced number of pups / litter; reduced dam weights; 9 dams died unexpectedly during the Task 2 20-day period

Study authors' conclusion: NOAEL (fertility) = 8600/9500 mg/kg bw/day for male/female mice.

Ref.: 9, 31

B. Dermal reproduction toxicity screening tests

A short communication published in 1993 describes the assessment of the reproductive toxic potential of Benzophenone-3 in male B6C3F1 mice after a 13-week repeated dermal application of dosages of 0, 20, 100 and 400 mg/kg bw/day. Reproductive organ weights, cauda epididymal sperm concentration, proportion of motile and abnormal sperm and testicular spermatid concentration were determined and testicular histology was evaluated. Since neither any effect on body weight gain nor any abnormalities in the measured reproductive parameters were noted, the study authors conclude that topically applied Benzophenone-3 has no reproductive toxic potential in male B6C3F1 mice at dosages up to 400 mg/kg bw/day.

Study authors' conclusion: NOAEL (reproduction) = 400 mg/kg bw/day for male and female mice.

Ref.: 21

Finally, the results of the examinations of sperm morphology/motility and vaginal cytology in the dermal sub-chronic toxicity studies as described under 3.3.5.2.B, revealed a decrease in epididymal sperm density in the male mice. However, this finding is considered incidental

[3] Stability of Benzophenone-3 in the diet : at least 3 weeks

since there was no other effect on reproductive organs in the study, neither in the other selective reproductive parameters, weight of the reproductive organs nor histopathological examination. Therefore the NOAEL for the investigated reproductive effects in male and female rats and mice was in each case considered the highest dose level applied, more specifically 200 mg/kg bw/day for the rat and 364 mg/kg bw/day for the mouse.

Ref.: 30

3.3.8.2. Teratogenicity - rat - oral

Date of study:	01-16 November 2004
Guideline/method:	Annex V to Dir. 67/548/EEC, Method B.31; OECD Guideline 414
Species/strain:	Crl : WI(Han) Wistar rat
Group size:	25 mated females per dosage group
Test substance:	Benzophenone-3
Batch:	101
Purity:	99.8% (GC-FID)
Dosage levels:	0, 40, 200 and 1000 mg/kg bw/day
Route:	Oral (by gavage)
GLP/QAU:	Signed documents available

Benzophenone-3 was administered as a suspension in corn oil to 25 time-mated female rats per group by gavage at dosages of 40; 200 and 1,000 mg/kg bw/day on day 6 through day 19 post coitum (p.c.). A standard dose volume of 5 ml/kg body weight was used for each group. The control group, consisting of 25 females, was dosed with the vehicle (corn oil) in parallel. The oily test substance preparations were analyzed for stability prior to the study and for correct concentrations and homogeneity.
Food consumption and body weights of the animals were recorded regularly throughout the study period. The state of health of the animals was checked on a daily basis.
On day 20 post coitum all females were sacrificed and assessed by gross pathology (including weight determinations of the unopened uterus and the placenta). For each dam, corpora lutea were counted and number and distribution of implantation sites (differentiated as resorptions, live and dead foetuses) were determined. The foetuses were removed from the uterus, sexed, weighed and further investigated for external findings. Thereafter, nearly one half of the foetuses of each litter were examined for soft tissue findings and the remaining foetuses for skeletal (inclusive cartilage) findings.

The following effects were noted:

40 mg/kg bw/day:	no test-substance related effects on dams, gestational parameters or foetuses
200 mg/kg bw/day:	transient salivation in 3/25 rats immediately after dosing; no test-substance related effects on gestational parameters or foetuses
1,000 mg/kg bw/day:	transient salivation immediately after dosing; stained/reddish coloured urine; reduced food consumption and body weight (gain); slightly increased rates of foetuses/litter with skeletal variations (incomplete ossification of different skull bones and cervical arch, supernumerary 14th ribs(s)) and as a consequence increased rates of total variations; no test-substance related effects on gestational parameters

The performing laboratory concludes that Benzophenone-3 did not display any teratogenic effect and that the NOAEL for maternal and prenatal developmental toxicity is 200 mg/kg bw/day.

Ref.: 11

3.3.8.3 Overall conclusion of the submission authors on reproductive toxicity

Benzophenone-3 was shown to have no effect on fertility when tested up to very high dose levels within a RACB (Reproductive Assessment by Continuous Breeding) study in mice. The NOAEL for fertility was the highest investigated dosage level of 8600/9500 mg/kg bw/day in male/female mice. Within this study the reproductive performance was affected in the form of a slightly lower number of live pups at birth. Signs of developmental toxicity consisted of impaired body weight/body weight gain of the pups. However, in any case these effects were only noted at a dosage level with overt parental toxicity. Consequently, the NOAEL for systemic, reproductive and developmental toxicity was 1800/1900 mg/kg bw/day in males/females.

Repeated **oral** application of Benzophenone-3 for 13 weeks to rats and mice caused only slight effects in selective parameters accompanied with overt systemic toxicity at the highest investigated dosage levels of 3656/3261 mg/kg bw/day in male/female rats and 13937/18539 mg/kg bw/day in male/female mice). In rats, the epididymal sperm count was reduced and a decreased absolute cauda, epididymal and testis weight as a consequence of the reduced body weight was noted. In female rats, an increase in the length of the oestrous cycle was noted. In mice, a decrease in the epididymal sperm count and an increase the incidence of abnormal sperm was recorded, while female mice (as in rats) revealed an increase in the length of the oestrous cycle. However, in rats and in mice, the oestrous cyclicity was not affected. In any case, the next lower investigated dose level was a clear NOAEL for the investigated reproductive parameters.

After subchronic **dermal** application for 13 weeks no clear or reliable effect on selective reproduction parameter was noted and therefore, the NOAEL was in each case the highest dose level applied, namely 200 mg/kg bw/d in rats and 364 mg/kg bw/d in mice. Although in mice a decrease in the epididymal sperm count was reported at all investigated dose levels, a relation to treatment is considered as very unlikely since in the oral study the administration of dose levels up to 16-fold higher had no effect. Moreover, repeated dermal application of Benzophenone-3 to male mice of another strain for the same period led to no signs of reproductive toxicity up to the slightly higher dose level of 400 mg/kg bw/d. Thus, the reported effect on the sperm count was finally considered as incidental.

A recent prenatal developmental toxicity study performed according to valid test guideline and under GLP conditions with characterized test material resulted in marginal effect on few components of the skeleton was at the high dose level in association with overt maternal toxicity, the achieved NOAEL was 200 mg/kg bw/d for maternal and prenatal developmental toxicity.

3.3.9. Toxicokinetics

In a study published in 1986, the disposition of Benzophenone-3 in rats dosed orally, intravenously and topically, has been investigated.
[^{14}C]Benzophenone-3 was administered orally at dosages of 3, 28, 293 and 2570 mg/kg, dermally at approximate dosages of 0.2, 0.6, 0.8 and 3.2 mg/kg and intravenously at a dosage of 4.6 mg/kg. The dermal dosage of 0.6 mg/kg involved the use of a sunscreen lotion as vehicle, while the other dermal dosage levels concerned alcoholic solutions of the compound.
Through all routes and dosages, Benzophenone-3 appeared to be well-absorbed and urinary secretion clearly showed to be the major route of elimination, followed by the faecal route. Only trace amounts appeared to be measured in tissues after 72 hours.

The absorption rates did not differ between topical application of the compound in ethanol compared to the sunscreen lotion, indicating that there is no major vehicle effect on the dermal absorption of Benzophenone-3.

Five metabolites were identified and mainly consisted of glucuronide and sulfate conjugates.
Ref.: 23, 30

A US research group published three papers in 1993-94 describing the metabolism and disposition of Benzophenone-3 when administered orally in rats and mice and dermally in the rat at a uniform single dosage of 100 mg/kg bw.
The same metabolites are detected in all cases: 2,4-Dihydroxybenzone (DHB), 2,2'-dihydroxy-4-methoxybenzone (DHMB) and 2,3,4-trihydroxybenzophenone (THB). They have been identified in their free and conjugated (glucuronidated or sulfonated) forms.
However, some species-differences became very clear. More specifically, in dermally or orally exposed rats, the primary elimination route clearly is urine, followed by feces, whereas in mice, the excretion was divided between urine and fecal routes. In addition, the elimination from the plasma occurs via a biphasic model in the rat, while the mouse obeys a one-compartment elimination model. There also appears to be a higher level of accumulation in the rat liver and kidney compared to the mouse.
The authors attribute the major disparities in metabolism to the species-differences in cytochrome P-450 isoenzyme expression patterns that exist between rats and mice.
In both species and for both exposure routes tested, Benzophenone-3 was rapidly absorbed, metabolized and distributed.

Ref.: 49, 50, 52

The submission contains a paper of 2004 investigating the human dermal absorption and effect on reproductive hormone levels of 3 UV-filters after a whole body topical application of a highly concentrated sunscreen. In this study, 32 volunteers were treated with 2 mg/cm^2 of a basic cream formulation on a daily basis for 4 days during the first week, followed by the same treatment regime with a sunscreen containing 30% of UV-filters in total (10% 4-Methylbenzylidene Camphor, 10% Benzophenone-3 and 10% Ethylhexyl Methoxycinnamate) during the second week. Blood was collected at several time intervals on the first day of treatment and subsequently on a daily basis.

All three compounds were detected in their parent forms both in plasma (Benzophenone-3 up to 300 ng/ml) and urine, showing that there is a substantial skin penetration, dermal uptake and urinary excretion in humans. The systemic concentrations achieved did not affect any hormone level measured (testosterone, follicle-stimulating hormone, sex hormone binding globulin, luteinizing hormone, estradiol) under the conditions of the test.
Ref.: 38

The results of a study of 2002 investigating the urinary content of Benzophenone-3 after topical application to human volunteers can be considered as an indication for low bioavailability. A commercial available sunscreen containing 4% Benzophenone-3 was topically applied in an amount of 40 g to the average body area of 2.0 m^2 of each of 11 volunteers and urine samples were collected subsequently during 48 hours. Although the urine is known as the major excretion route for absorbed and bioavailable material, only 0.4% (corresponding a median of 9.8 mg/volunteer) of the applied Benzophenone-3 dose was recovered in the urine within the 48 hours sampling period.

Ref.: 33

3.3.10 Photo-induced toxicity

3.3.10.1 Phototoxicity/photoirritation and photosensitisation

A. *In vitro* phototoxicity

In the ECVAM[4] validation study of the 3T3 Neutral Red Uptake Phototoxicity Test (3T3 NRU PT), Benzophenone-3 was included in the battery of UV-filters employed to check the accuracy and repeatability of the proposed *in vitro* assay.
The 3T3 NRU PT test makes use of Balb/c 3T3 mouse fibroblasts which are incubated for 60 minutes with several concentrations (usually eight) of the test compound. Thereafter the cells are exposed to a sun simulator for 50 minutes. After 24 hours the neutral red uptake (NRU) is measured and the respective EC_{50} values are defined as the concentrations of the test material which cause 50% reduction of NRU compared to the untreated control cultures. Subsequently the Photo Irritation Factor (PIF) is calculated. The PIF is defined as the factor generated by comparing two equally effective cytotoxic concentrations (EC_{50}) of the test chemical obtained in the absence (-UV) and in the presence (+UV) of a noncytotoxic irradiation with UVA/vis light [PIF = EC_{50}(-UV) / EC_{50}(+UV)]. If the PIF is ≥ 5, the substance is considered phototoxic. If a chemical is only cytotoxic +UVA and not when tested -UVA, the Mean Photo Effect (MPE) is calculated by a special computer software. In case the MEP, a measure which is based on comparison of the complete concentration response curves, is ≥ 0.1, the substance is considered phototoxic.
The validation study states that for Benzophenone-3 no animal data are available for comparison. Out of the 11 laboratories who have tested the compound through the *in vitro* 3T3 NRU PT protocol, the results for Benzophenone-3 were below the respective cut-off criteria for phototoxicity with the exception of one single PIF value of >2.7 and one MPE value of 0.195 obtained by one single lab. This single event was considered as incidental.

Ref.: 61

Benzophenone-3 was tested in another 3T3 NRU phototoxicity test in Balb/c fibroblasts and was found to be not phototoxic.
This conclusion was reinforced by the results of two separate photohemolysis and haemoglobin photo-oxidation tests, respectively performed with human erythrocytes and sheep red blood cells. Both assays showed that Benzophenone-3 had no phototoxic potential.

Ref.: 48, 53

Some other *in vitro* methods including the use of Saccharomyces Cerevisiae or Escherichia Coli plasmids as test organisms, showed Benzophenone-3 to be non-phototoxic.

Ref.: 40, 47

In vivo phototoxicity in guinea pigs

In a publication of 1999, the authors refer to a study published in 1974 in which the phototoxic potential of Benzophenone-3 was examined *in vivo* in guinea pigs. Five albino Hartley guinea pigs received a 0.05 ml aliquot of a 10% solution of Benzophenone-3 in petrolatum on two dorsal sites of the previously shaven and depilated skin. One application site was covered with aluminium foil. After 30 minutes, the uncovered site was irradiated with ultraviolet light (UV-A) for 60 minutes. Skin reactions were assessed 24 and 48 hours after irradiation, according to standard scoring systems (grade 0-4). No skin reactions were seen in any animal at any application site at any time point.

Ref.: 48

[4] European Centre for the Validation of Alternative Methods

B. *In vitro* Photosensitisation

In vitro Photosensitisation (mechanistic *in vitro* test)

Viewing the fact that the 3T3 NRU PT study does not enable to make the distinction between phototoxicity and photosensitisation, Benzophenone-3 was tested for its human serum albumin photobinding and histidine photo-oxidation potential in a newly proposed mechanistic *in vitro* test for the discrimination of the photo-allergic and photo-irritant potential of various test substances. Benzophenone-3 revealed no phototoxic and no photo-allergenic potential in this test.

Ref.: 45

In vivo Photosensitisation in rabbits

Although a short description of the test is provided in the submission summary, the appropriate reference [25] is missing in the dossier.
The test was claimed to be negative.

Ref.: 25

Phototoxicity and photosensitisation : human data

The dossier contains very brief descriptions of three unpublished reports (1978-1980) on the clinical safety assessment of sunscreens or other cosmetic products containing Benzophenone-3 up to 3.5% (full compositions are unknown). These older reports were supplied and assessed by the cosmetic ingredient review panel and published in the "Final Report on the Safety Assessment of Benzophenones-1, -3, -4, 5, -9, and -11 (J. American Coll. Toxicol., 2, 1983)". The conclusion of these reports is that Benzophenone-3 appears to have no phototoxic or photoallergenic potential when used as a cosmetic ingredient.

Ref.: 16, 29, 69

Finally, a number of publications of dermatological departments are available in which the authors display the result of a photopatch test with a number of UV filters including Benzophenone-3, in a population of patients with a history of photosensitisation. The results of these studies are summarized in the following table:

Survey period	# of patients	Country code	UV-filters tested	pos.* reactions	Remarks	Ref
1985-1990	187	USA	Benzophenone-3	9	- most reactions to sunscreens observed in last 3 years - trend towards increasing allergic response to Benzophenone-3 over time	22
			PABA**	1		
			Pentyl dimethyl PABA	2		
			Octyl Dimethyl PABA	5		
			Butyl Methoxy-Dibenzoylmethane	0		
1990-1993	108	IT	Benzophenone-3	4	The authors conclude that photocontact dermatitis caused by Benzophenone-3 is becoming more frequent confirming its increasing diffusion.	70
			PABA	1		
			Octyl Dimethyl PABA	0		
			Butyl Methoxy-Dibenzoylmethane	0		
			Isopopropyl Dibenzoylmethane	2		
			Ethylhexyl-*p*-methoxycinnamate	0		
			Isoamyl-methoxycinnamate	0		
			4-MBC	0		

Survey period	# of patients	Country code	UV-filters tested	pos.* reactions	Remarks	Ref
1982-1992	283	DK	Benzophenone-3	35	As in previous studies, Benzophenone-3 is the major cause for photocontact dermatitis in tested patients and photocontact dermatitis is much more frequent than contact dermatitis (practically not observed).	67
			PABA	3		
			Octyl Dimethyl PABA	14		
			Butyl Methoxy-Dibenzoylmethane	0		
			Isopopropyl Dibenzoylmethane	5		
			Ethylhexyl-*p*-methoxycinnamate	3		
			Isoamyl-*p*-methoxycinnamate	0		
			4-MBC	0		
1981-1996	402	DE	Benzophenone-3	9	Most of the photoallergic reactions appear to occur in the category of UVA absorbers. The authors advise to put in place a registry for adverse reporting of sunscreen agents in general.	57
			PABA	2		
			Octyl Dimethyl PABA	2		
			Butyl Methoxy-Dibenzoylmethane	13		
			Isopopropyl Dibenzoylmethane	32		
			Ethylhexyl-*p*-methoxycinnamate	4		
			Isoamyl-*p*-methoxycinnamate	10		
			4-MBC	5		
1990-1996	355	SV	Benzophenone-3	15	Photocontact reactions by far outnumbered contact reactions.	7
			PABA	2		
			Butyl Methoxy-Dibenzoylmethane	6		
			Isopopropyl Dibenzoylmethane	8		
			Ethylhexyl-*p*-methoxycinnamate	3		
			4-MBC	0		
1990-1994	370	FR	Benzophenone-3	21	Most of the positive cases to Benzophenone-3 were diagnosed before 1993. In France, the UV-filter is not being used in sunscreens any more; only in other cosmetics, such as daily moisturisers.	39
			PABA	1		
			Octyl Dimethyl PABA	1		
			Butyl Methoxy-Dibenzoylmethane	4		
			Isopopropyl Dibenzoylmethane	7		
			Ethylhexyl-*p*-methoxycinnamate	2		
			Isoamyl-*p*-methoxycinnamate	0		
			4-MBC	1		
2002	1	USA	Benzophenone-3	1	One specific case where both immediate and delayed hypersensitivity against Benzophenone-3 occurred.	43
1991-1997	1261	Central EU	Benzophenone-3	8		62
			PABA	3		
			Octyl Dimethyl PABA	0		
			Butyl Methoxy-Dibenzoylmethane	2		
			Isopopropyl Dibenzoylmethane	7		
			Ethylhexyl-*p*-methoxycinnamate	2		
			Isoamyl-*p*-methoxycinnamate	5		
			4-MBC	1		

* Positive reactions are restricted to photocontact allergy. Direct contact allergy is not taken into account in these figures.
** PABA = Para-amino Benzoic Acid

Ref.: 22, 70, 67, 57, 7, 39, 43, 62

Conclusion of the submission authors

* *Phototoxicity/photoirritation in vitro and in vivo*

In vitro Benzophenone-3 was comprehensively examined for its phototoxic potential within the frame work of the EU/COLIPA validation process. In respect to the weight of evidence Benzophenone-3 was proven to be not phototoxic in the 3T3 NRU assay, in the red blood cells assay or in human primary keratinocytes in the presence or absence of artificial sunlight. The failure to induce photohemolysis or photohaemoglobin oxidation was also confirmed independently in a further published red blood cell phototoxicity test.
In vivo there exists also no indication for a phototoxic potential in guinea pigs or mice. However, as the animals number in the guinea pig study was low and in mice, not primarily the phototoxic effect but the protective effect of broad-spectrum sunscreens was investigated, these investigations serve only for information.

* *Photosensitization in vivo*

The study investigating this endpoint is only available as secondary citation and the results should therefore be treated with caution. However, no photosensitization was reported after topical treatment of albino rabbits with a sunscreen containing 6% Benzophenone-3.
Finally, Benzophenone-3 can be regarded to be of no concern for photo-induced toxicity for humans.

Comment
The following publications were not included in the submission and have been added by the SCCP:

Survey period	# of patients	Country code	UV-filters tested	pos.* reactions	Remarks	Ref
1983-1998	2175	UK	Benzophenone-3	14	The authors conclude that, despite the large increase in the use of UV-filters over the last years, the development of photo-allergic reactions remains rare. Photopatch test series should be regularly reviewed and updated, but meanwhile, there is no evidence that photo-allergic reactions represent a common clinical problem.	A
			Benzophenone-10	10		
			PABA	5		
			Octyl Dimethyl PABA	5		
			Amyl Dimethyl PABA	2		
			Butyl Methoxy-Dibenzoylmethane	4		
			Isopopropyl Dibenzoylmethane	6		
			Isoamyl-methoxycinnamate	2		
			Ethyl-methoxycinnamate	2		
			Ethylhexyl-methoxycinnamate	2		
2000-2002	1155	UK IRL NL	Benzophenone-3	21	The publication discusses the methodology of photopatch testing in general and does not provide an assessment of the potential photo-allergy of the compounds tested.	B
			Benzophenone-4	6		
			PABA	1		
			Octyl Dimethyl PABA	2		
			Butyl Methoxy-Dibenzoylmethane	17		
			Isoamyl-methoxycinnamate	11		
			Ethylhexyl-methoxycinnamate	6		
			4-MBC	3		
2001-2003	82	Colombia	Benzophenone-3	22		C
			Octyl Dimethyl PABA	1		
			4-MBC	1		

Survey period	# of patients	Country code	UV-filters tested	pos.* reactions	Remarks	Ref
			Ethylhexyl-methoxycinnamate	8		

* Positive reactions are restricted to photocontact allergy. Direct contact allergy is not taken into account in these figures.

3.3.10.2 Photomutagenicity / photoclastogenicity

A. *In vitro* photomutagenicity : bacterial mutation assay

Date of study:	October - December 2004
Guideline/method:	Annex V to Dir. 67/548/EEC, Method B.13/14; OECD Guideline 471
Test system:	Salmonella Typhimurium strains TA1537, TA98, TA100 and TA102
Test substance:	Benzophenone-3
Batch:	101
Purity:	99.8% (GC-FID)
Doses tested:	3; 10; 33; 100; 333; 1,000; 2,500 and 5,000 µg/plate
GLP/QAU:	Signed documents available

Benzophenone-3 was investigated for its potential to induce gene mutations under irradiation with artificial sunlight according to the plate incorporation test (experiment I) and the preincubation test (experiment II) using the Salmonella typhimurium strains TA 1537, TA 98, TA 100, and TA 102. The test was performed in two independent experiments. Each concentration, including the controls, was tested in triplicate and concentrations of 3; 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate in experiment I of 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate in experiment II were investigated. The test substance was dissolved in DMSO. Prior to the main experiments, the induction of toxicity and mutagenicity was investigated in a pre-experiment with all strains.

Toxic effects evident as a reduction in the number of revertants, were observed in the preexperiment (without irradiation) and in both main experiments in nearly all strains used. The plates incubated with the test item showed normal background growth up to 5000 µg/plate in all strains used. No substantial increase in revertant colony numbers of any of the four tester strains was observed following treatment with Benzophenone-3 under irradiation with artificial sunlight at any dose level. There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance.
The sensitivity and validity of the test system used was demonstrated by the expected induction of a significantly increased number of revertants with the appropriate positive controls.

The study authors conclude that Benzophenone-3 did not induce gene mutations by base pair changes or frameshifts in the genome of the bacterial strains used and was therefore shown to be non-photomutagenic in this Salmonella typhimurium photomutagenicity test.

Ref.: 14

B. *In vitro* photomutagenicity : chromosome aberration test

Date of study:	November 2004 - February 2005
Guideline/method:	Annex V to Dir. 67/548/EEC, Method B.10; OECD Guideline 473
Test system:	V79 Chinese Hamster lung cell lines
Test substance:	Benzophenone-3
Batch:	101
Purity:	99.8% (GC-FID)
Doses tested:	3.1; 6.3; 12.5; 25.0; 50.0 and 75.0 µg/ml
GLP/QAU:	Signed documents available

Benzophenone-3 was investigated for its potential to induce structural chromosomal aberrations in V79 Chinese Hamster cells in the absence and the presence of artificial sunlight in two independent experiments. A xenon burner with an additional special filter glass, emitting visible light and UVA/UVB light (ratio: about 30:1) > 290 nm was used as light source. The cultures were pre-incubated with the test item for 30 min. After pre-incubation, the cultures were exposed to 225 mJ/cm² UVA (Exp. I and II) or 375 mJ/cm² UVA (Exp. II). Three hours after start of treatment, the cultures were washed. Corresponding cultures with the test item were kept in the dark for the 3 hrs exposure period. The chromosomes were prepared 18 hrs (Exp. I) and 28 hrs (Exp. II) after start of treatment. Two parallel cultures were investigated and at least 100 metaphase plates were scored for structural chromosome aberrations in each culture, except for the positive controls, where only 50 metaphase plates were scored.
The highest applied concentration in the pre-test on toxicity (2,230 µg/ml, approx. 10 mM) was chosen with regard to the molecular weight of the test item in line with requirements of the current OECD Guideline 473. Dose selection for the cytogenetic experiments was performed considering the toxicity data and the occurrence of test item precipitation.

In the absence and the presence of irradiation, toxic effects were observed in both experiments as indicated by clearly reduced mitotic indices or cell numbers of below 50 % of control. However, partly concentrations showing clear cytotoxicity could not be scored for cytogenetic damage. In Experiment I and II, in the absence and the presence of irradiation, no biologically relevant increase in the number of cells carrying structural chromosomal aberrations was observed after treatment. The statistically significant differences to the solvent control were observed occasionally in this study but were considered as biologically irrelevant due to the lack of dose-dependency and the values were clearly within the respective historical control data ranges.
No relevant increase in the frequencies of polyploid metaphases was found after treatment with the test item when compared to the controls and the range of the historical control data.
The sensitivity of the system was demonstrated since the positive controls induced statistically significant increases in cells showing structural chromosome aberrations.

The study authors conclude that Benzophenone-3 did not induce structural chromosome aberrations in the absence or presence of artificial sunlight as determined by the chromosomal aberration test in V79 Chinese Hamster cells and was thus shown to be non-clastogenic in this chromosomal aberration photomutagenicity test when tested up to cytotoxic concentrations.

Ref.: 12

C. Additional information

A publication of 2001 describes the plasmid-relaxation assay as a rapid screening system for the detection of photogenotoxic chemicals. Benzophenone-3 showed to be negative in this assay.

Ref.: 47

D. Conclusion of the submission authors with regard to photomutagenicity/photoclasto-genicity

Benzophenone-3 was tested in bacterial and mammalian test systems according to valid testing guidelines and under GLP conditions with the characterized test material. No photogenotoxic/photomutagenic potential was noted in the bacterial gene mutation assays in *Salmonella typhimurium* strains and no photoclastogenic potential was recorded in the chromosome aberration test in Chinese hamster V79 cells, both with and without irradiation.
In addition, a published screening test revealed no indication that Benzophenone-3 may cause DNA damage with or without irradiation.

3.3.11 Human data

A human patch test (1965) showed that Benzophenone-3 was non-irritating to human skin after 24 hours of patching [59].

In a modified Draize Shelanski Repeat Insult Patch Test performed in 1979, approximately 300 mg of the test material *UV-9*[5] was applied to patch sites to the backs or volar forearms of 100 subjects at a concentration of 25% in petrolatum for ten alternate day 24 hour periods under occlusion. After a seven day rest period, challenge patches with 25% of *UV-9* in petrolatum were applied in the same manner to fresh sites on the backs or volar forearms of all subjects for 24 hours. There were no instances of irritation or sensitisation from the material in this test (scores remained 0 at all times) [41].

In 1978, a Human Repeat Insult Patch Test was performed with 2 sunscreen products called *Sun Tan Lotion* and *Protective Face Cream*[6]. 24 hour occlusive patches with about 200 mg of test substance were applied on the skin of 56 subjects, 10 times with a resting period of 24 hours in between. Ten to fourteen days after the last patch, the challenge patch was applied. None of the products was considered capable of inducing significant irritation or sensitisation [29].

Ref.: 59, 41

The submission summary contains a description of more than 10 human volunteer studies, not performed with Benzophenone-3 as such, but with representative products (mostly sunscreens) with a varying concentration of Benzophenone-3 (from 5% up to 10%). The main purpose of these studies is described to be the investigation of the safe usage of these products under enhanced and comprehensive use conditions. No irritation, allergic reaction, photo-irritation or photo-allergy related to the use of the tested sunscreens was noted. The full references of these studies are stated to be "available upon request".

Ref.: 76, 77, 78, 79, 80, 81, 82, 83, 85, 86, 87, 88, 89

3.3.12 Special investigations

Estrogenic potential

The submission authors state that this endpoint was not considered within this dossier since there is a very intensive evaluation of the SCCNFP (Opinion on the Evaluation of Potentially Estrogenic Effects of UV-filters adopted during the 17th plenary meeting of 12 June 2001) available. The final conclusion was that based on the actual scientific knowledge, the SCCNFP is of the opinion that the organic UV-filters used in cosmetic sunscreen products, allowed in the EU market today, have no estrogenic effects that could potentially affect human health.

Ref.: 56

Moreover, a recent study investigated whether 10% of Benzophenone-3 in a sunscreen formulation and 10% of other UV filters were absorbed and influenced endogenous reproductive hormone levels in humans after topical application. In this blinded study 32 healthy volunteers (15 young males and 17 postmenopausal females) received whole-body topical application of 2 mg per cm^2 of basic cream formulation without (week 1) and with (week 2) the sunscreens at 10% (wt/wt) daily. Benzophenone-3 was absorbed and maximum plasma concentrations were 200 ng/ml for females and 300 ng/ml for males. In

[5] Exact identity not stated.

[6] Composition unknown for both products

the urine, approximately 60 ng/ml was detected in females and 140 ng/ml in males The exposure of the sunscreen containing 10% Benzophenone-3 caused no effect on either of the examined hormones (FSH, LH, SHBG, estradiol, inhibin B, testosterone). Minor variations observed were considered to reflect the known and normal biological variations.
Ref.: 38

3.3.13 Safety evaluation (including calculation of the MoS)

Not applicable.

3.3.14 Discussion

The safety of Benzophenone-3 for its usage in sunscreen products for over the counter (OTC) products was first peer reviewed by the US FDA in 1978 [16]. Based on the data available at that time the FDA expert panel classified Benzophenone-3 as safe and effective. Subsequently, published and unpublished information on Benzophenone-3 including other Benzophenones were reviewed by an expert panel and published as cosmetic ingredient review (CIR) in 1983 [17]. The expert panel concluded on the basis of all available data and clinical human experience that Benzophenone-3 is safe for topical application to human skin in the present practices of use and concentrations in cosmetics.

Benzophenone-3 is a widespread UV-filter for which over the years a large amount of data have been generated, many of them between 1970 and 1988. This is reflected in the identification and physicochemical data section. The majority of the data are statements out of the technical and material data sheets. Only a number of determinations (quantification through capillary gas chromatography and part of the stability studies) have been performed according to GLP, clearly mentioning the batch tested and accompanied by a full description of the method. All other parameters are not individually referenced. Two of the references with regard to the identification of the substance (74, 75), are only "available upon request". They should have been included in the submission.

The quality of the toxicological dossier suffers from the fact that studies are often outdated and/or only available as publications in journals, with the result that on several occasions batch number and purity of the test substance are not mentioned, compositions of tested formulations are unknown, etc. Nevertheless the submission summary provides a comprehensive and well-structured overview of the available test descriptions and publications.

The UV-filter displays a low acute toxicity profile with oral and dermal LD_{50}-values exceeding the classification limit of 2000 mg/kg.

Benzophenone-3 is not considered as being irritating to the skin and the eyes. The studies to support this statement are unfortunately outdated and not performed according to current guidelines and GLP, but the human data with the compound under in-use conditions do not provide any indication of skin and eye irritation due to Benzophenone-3. Therefore additional testing in this area does not appear to be necessary.

Benzophenone-3 has been extensively tested for its photoirritating potential *in vitro* during the validation of the 3T3 NRU PT test and was found negative in the majority of cases.

With regard to the sensitising potential of the compound, two animal tests are available : a guinea pig Magnusson Kligman Maximisation test of 1978 and a LLNA of 2005. Both indicate that Benzophenone-3 is non-sensitising.

About 14 additional references, mainly consisting of repeated insult patch tests with Benzophenone-3 containing test formulations, are stated to be "available upon request" and

are described in the submission's summary. However, they do not add additional arguments to the discussion.

In addition, the submission contains a number of reports of clinical trials with regard to the photoallergenic potential of UV-filters in general. In each of these, a number of clear positive reactions to Benzophenone-3 are described. In the current report, some extra references on this issue have been added by the SCCP to the ones included in the submission. Looking at the positive photoallergic reactions to Benzophenone-3, it must be emphasized that the study population in all tests consisted of patients with a suggested history of photocontact allergy. As a general rule, results of clinical trials should be followed up in order to detect potential trends towards an increasing incidence of (photo)allergic reactions to specific compounds.
In the case of Benzophenone-3, the presented publications clearly indicate that the UV-filter is a photoallergen.

As far as the dermal absorption of Benzophenone-3 is concerned, some diverging results have been obtained. An *in vitro* study of 1999 generates a dermal absorption value of 1.7 µg/cm² or 1.16% of the applied dose, but the test suffers several shortcomings (tested concentration too low, solubility in receptor fluid not stated, composition of the tested formulation unknown, skin preservation and storage details not given, purity of test substance not stated, only one concentration tested, unusual contact time, insufficient skin samples and no intermediate sampling). All other *in vitro* studies indicate "low dermal absorption", but do not allow any quantitative determination.

Looking at the available *in vivo* human data, it is clear that Benzophenone-3 is absorbed through the skin to a certain extent, but again quantification is impossible. In one human study, where a 4% Benzophenone 3 sunscreen was applied at 2 mg/cm² on the whole body surface, the absorption was considered to be as low as 0.4 %. In another study, in which a sunscreen containing 10% Benzophenone-3 together with 10% 4-MBC and 10% Ethylhexyl Methoxycinnamate, the absorption appears to be higher, but no exact values are stated and the combination of the three UV-filters in one sunscreen at such high concentrations might influence the result.

A more recent study added by the SCCP and not included in the submission, shows that a sunscreen containing 4% of Benzophenone-3, could lead to a mean urinary excretion of 3.7% (1.2%-8.7%) of Benzophenone-3. However, in this study, again the compound was combined with two other UV-filters, it was not tested at the maximum requested concentration of 10% and other routes of excretion have not been considered.
Therefore, no conclusion can be drawn with regard to the dermal absorption of Benzophenone-3.

After repeated oral administration of Benzophenone-3 in rats and mice, the most frequently encountered adverse effects consist of some unspecific signs of systemic toxicity in the form of reduced food consumption and retarded body weight gain, together with some effects on the identified target organs being the kidney and the liver. These effects were partly associated with changes in clinical chemistry. Very often the most susceptible parameter was the increase in liver weight. The latter, however, without any histopathological correlate, is not considered by the submission authors to reflect an adverse effect per se but should be considered as an adaptive metabolic response which is known to be reversible. Therefore, according to the submission, the oral NOAEL corresponds to 411 mg/kg bw/day.

With regard to the results of the dermal repeated dose studies, a dermal NOAEL of 200 mg/kg bw/day is put forward, on the assumption that deviations without dose-response relationship and without correlated histopathological findings (e.g. the decreased reticulocyte count, increased relative kidney weight, increased platelet count and whole blood cell count in the 90d dermal study in rat) should not be taken into account.

It should be noted that, taking the complete set of oral and dermal subacute and subchronic toxicity studies together, the choice of the dosages may raise some questions. In the oral studies, the dosages appear to be extremely high (up to 20,796 mg/kg bw/day) whereas the dosage levels in the dermal studies appear to be very low (down to 7 mg/kg bw/day). Even though the results indicate that Benzophenone-3 causes adverse effects at lower dosages through the dermal route compared to oral administration, the dermal dosages remain at the low side and this is also confirmed by the absence of clear toxicity signs at the highest levels tested (as requested in the official EC B.9/OECD 410 and EC B.28/OECD 411 testing guidelines).

A recently performed and well-described teratogenicity study in rat showed Benzophenone-3 to be non-teratogenic under the conditions of the test. Only at the highest dosage level, which also caused maternal toxicity, some skeletal aberrations were noted. The NOAEL-value for maternal and developmental toxicity was 200 mg/kg bw/day.

Instead of a 2-generation study, the submission contains some specific reproductive toxicity parameter measurements made at the end of the subchronic toxicity studies described earlier, together with the description of a reproduction screening assay according to the "Continuous Breeding Protocol". Out of these results, a NOAEL value of 400 mg/kg bw/day for reproductive toxicity, was extracted. Although the test is not commonly performed within the EU regulatory framework and although a number of animals in all dosage groups unexpectedly died, it does not seem to be acceptable from an ethical point of view, to request a new 2-generation study with Benzophenone-3.

Toxicokinetic studies indicate that Benzophenone-3 is readily biotransformed into its three major metabolites 2,4-Dihydroxybenzone (DHB), 2,2'-dihydroxy-4-methoxybenzone (DHMB) and 2,3,4-trihydroxybenzophenone (THB), which have been identified in their free and conjugated (glucuronidated or sulfonated) forms. Excretion in the rat primary occurs via the urine, while in the mouse the fecal route appears to be equally important.

As far as the (photo)mutagenic/(photo)genotoxic potential of Benzophenone-3 is concerned, the presented *in vitro* and *in vivo* assays indicate that the substance does not possess (photo)mutagenic of (photo) genotoxic properties. With regard to the studies mentioned in the US National Toxicology Program report, the full text references of the summarised tests should have been provided.

4. CONCLUSION

It is the opinion of the SCCP that insufficient data are presented to calculate the Margin of Safety of Benzophenone-3 under the proposed conditions of use.

The following additional information is required:

- A dermal absorption study with Benzophenone-3 under its in-use concentrations (up to 10%) according to OECD Guideline 428 combined with SCCP/0970/06.

These data are requested before end of March 2007.

5. MINORITY OPINION

Not applicable

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Cosmetic components causing contact urticaria: a review and update†

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Summary

Immediate skin reactions are common in dermatological practice, but may often be overlooked. The main objective of this article is to provide an update of the literature concerning immediate-type reactions or contact urticaria/contact urticaria syndrome caused by cosmetic ingredients in terms of immediate clinical symptoms, positive reactions following open, scratch or, most often, prick testing, and sometimes the detection of specific IgE antibodies. To this end, a selective search in different medical literature databases was performed. This yielded a list of cosmetic ingredients causing immediate reactions, including hair dyes and bleaches, preservatives, fragrance and aroma chemicals, sunscreens, hair glues, plant-derived and animal-derived components, permanent makeup and tattoos, glycolic acid peel, lip plumper, and alcohols. Many of the reported cases, however, lack appropriate controls and detailed investigation. Contact urticaria may occur with or without systemic symptoms, which are sometimes life-threatening.

Key words: contact urticaria; contact urticaria syndrome; cosmetics; IgE antibodies; immediate-type hypersensitivity; proteins; skin testing.

The epidemiology of immediate skin reactions or contact urticaria (CU) is not clear; although the symptoms are easily recognizable when wheal-and-flare reactions are present, milder reactions are difficult to diagnose (1, 2). Such reactions are common in dermatological practice (3, 4), but possibly underdiagnosed, with the exception of natural rubber latex. The other trigger factors have been documented on the basis of isolated cases or small patient series (5).

The main objective of this article is to provide an update of a review, which we published earlier (6), concerning CU and CU syndrome (CUS), caused by cosmetic ingredients that have provoked immediate clinical symptoms as well as positive skin reactions following open, scratch but, most often, prick testing. Sometimes the diagnosis was also based on the detection of specific IgE antibodies.

Immediate skin reactions comprise:

(1) Non-immunological CU (NICU), that is, CU without previous sensitization, with skin lesions generally being restricted to the site of contact, and systemic manifestations rarely being observed (1). The eliciting substances do not cause non-specific histamine release from mast cells, as antihistamines cannot inhibit such reactions. Examples of causal agents are benzoic acid, cinnamic acid, cinnamal, methyl nicotinate, and dimethylsulfoxide (7).
(2) Immunological CU (ICU), a type I hypersensitivity reaction in a previously sensitized individual (8). This condition is probably less often observed in clinical practice than NICU, but its mechanism is better understood: the pathogenesis is identical to that of other types of immediate hypersensitivity reaction, and involves coupling of percutaneously absorbed antigens with specific IgE molecules on

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the surfaces of mast cells (9), the symptoms resulting from the release of histamine. Pre-existing conditions, such as atopic dermatitis, may favour this condition. Generalized reactions and/or extracutaneous reactions are frequent, and this is referred to as CUS.

(3) CUS (10), first defined in 1975 by Maibach and Johnson (11). Since then, numerous cases have been reported with a broad spectrum of clinical manifestations that can be strictly limited to the areas of cutaneous contact, or present as generalized urticaria with concurrent involvement of internal organs. The four stages of the syndrome are:

- local symptoms ranging from non-specific symptoms such as itching, tingling and a burning sensation to a wheal-and-flare response restricted to the area of contact (stage 1)
- generalized urticaria following local cutaneous contact (stage 2), which also includes angioedema
- extracutaneous manifestations, which may include the respiratory (bronchial asthma or rhinoconjunctivitis), orolaryngeal or gastrointestinal tract (stage 3)
- anaphylactic reactions (stage 4).

(4) CU of unknown origin, which comprises reactions with mixed features, or for which mechanisms and pathophysiological features are not well understood (9).

Immediate reactions usually start within 30–60 min following skin exposure, and clear completely within 24 h; however, delayed-onset reactions may appear within 4–6 h. The mechanism of these is unknown; slower skin penetration could offer an explanation. In case of an immune-mediated reaction, the measurement of specific IgE in serum is useful, if technically possible, for both small and large molecules (proteins). The basophil activation test, which is based on the demonstration by flow cytometry of CD63 expression following exposure to allergens, is still experimental (2, 12).

Methods

Different databases were searched: MEDLINE and CDESKPRO (an in-house literature database). A literature review was performed with the search terms (contact urticaria syndrome) AND (cosmetics), (contact urticaria syndrome) AND (fragrances), (persulfate) OR (ammonium persulfate) OR (potassium peroxymonosulfate), (contact urticaria OR systemic) AND hair dyes, (IgE OR immediate) AND cosmetics, (contact urticaria) OR (type 1), and (contact urticarial) OR (immediate).

Cosmetic components causing CU and/or CUS

Adverse reactions to cosmetics include irritant, allergic and photo-allergic contact dermatitis, and CU (13); the last of these is addressed in this review. Many of the reported cases, however, lack appropriate controls and detailed investigation. Table 1 provides a list of cosmetic ingredients causing ICU, NICU, and/or CUS, along with their CAS number, if known, and the respective literature references.

Hair care products

There are a few reports of hair dyes causing immediate-type hypersensitivity, some with anaphylaxis or respiratory symptoms: *p*-phenylenediamine (PPD) and its derivatives, such as *p*-aminophenol and *p*-methylaminophenol (26), and toluene-2,5-diamine (28). The reactions seem to occur only after oxidation by H_2O_2, and are attenuated when the antioxidant sodium sulfite is added to the mix (28). Goldberg et al. (18) identified Brandowski base (CAS no. 20048-27-5), an oxidation product of PPD, as a culprit. The natural permanent hair dye henna, derived from the leaves of the shrubs *Lawsonia alba* or *Lawsonia inermis*, is also a rare cause of rhinitis, conjunctivitis, sneezing, urticaria, and asthmatic symptoms (2). Sensitization seems to occur mainly through inhalation of henna powder dispersed in the air (34). Temporary tattoos, especially those that contain black dyes, have become extremely popular among teenagers in recent years. Most of these tattoos, in addition to hair dyes, contain PPD (156). Haluk Akar et al. (156) reported a case of a 15-year-old adolescent female who had been unaware of being previously sensitized to PPD from a black henna tattoo, with an angioedema-like reaction that occurred after her first exposure to hair dye. Delayed allergic reactions to PPD are known to have the potential to elicit severe facial oedema - thus mimicking type I immediate reactions (157).

Moreover, also direct hair dyes, for example Basic Blue 99 and Basic Brown 17 (39), and patent blue dye (36), have been causes of contact urticaria, mainly acting through occupational exposure.

Persulfate salts have a strong oxidizing action that accelerates the bleaching process and also makes the hair easier to dye (9). Currently, potassium persulfate is more frequently used than the ammonium salt, because the latter has an unpleasant odour (53). It is a potential cause

Table 1. Cosmetic components causing contact urticaria and/or its syndrome

Products and components	CAS no.	Reactions	Authors
Hair care products			
p-Phenylenediamine	106-50-3	ICU, asthma, angioedema, anaphylaxis	Mavroleon, 1998 (14); Calnan, 1963 (15); Edwards, 1984 (16); Temesvari, 1984 (17); Goldberg, 1987 (18); Fukunaga, 1996 (19); Belton, 1997 (20); Sahoo, 2000 (21); Wong, 2003 (22); Birnie, 2007 (23); Helaskoski, 2014 (24)
p-Aminophenol/ *p*-methylaminophenol	123-30-8/55-55-0	Anaphylaxis	Nagano, 1991 (25); Oshima, 2001 (26); Uehara, 2014 (27)
Toluene-2,5-diamine sulfate	615-50-9	Anaphylaxis	Pasche-Koo, 1998 (28); Taniguchi, 2000 (29)
N,N''-(2,5-diamino-2,5-cyclohexadiene-1,4-diylidene) bis-1,4-benzenediamine (Bandrowski's base)	20048-27-5	Anaphylaxis	Goldberg, 1987 (18)
Henna	83-72-7/84988-66-9	ICU, conjunctivitis, rhinitis, asthmatic symptoms	Pepys, 1976 (30); Starr, 1982 (31); Cronin, 1996 (32); Majoje, 1996 (33); Bolhaar, 2001 (34); Oztas, 2001 (35); Davari, 2011 (36)
Basic Blue 99	68123-13-7	ICU, oral and auricular swelling and itching	Wigger, 1996 (37); Jagtman, 1996 (38); Davari, 2011 (36); Vanden Broecke, 2014 (39)
Basic Brown 17	68391-32-2	ICU	Vanden Broecke, 2014 (39)
Ammonium persulfate and potassium persulfate	7727-54-0/7727-21-1	NICU, asthma, anaphylaxis	Calnan, 1963 (15); Brubaker, 1972 (40); Fisher, 1976 (41); Pepys, 1979 (30); White, 1982 (42); Kellett, 1985 (43); Blainey, 1986 (44); Pankow, 1989 (45); Schwaiblmair, 1990 (46); van Joost, 1991 (47); Parra, 1992 (48); Wrbitzky, 1995 (49); Yawalker, 1999 (50); Borelli, 1999 (51); Perfetti, 2000 (52); Roderiguez, 2001 (53); Aalto-Korte, 2003 (9); Munoz, 2003 (54); Babilas, 2005 (55); Moscato, 2005 (56); Harth, 2006 (57); Munoz, 2008 (58); Bregnhoj, 2009 (59); Becker, 2010 (60); Hoekstra, 2012 (61); Hougaard, 2012 (62)
m-Aminophenol	591-27-5	CUS	Tsunoda, 1993 (63)
o-Aminophenol	95-55-6	CUS	Tsunoda, 1993 (63)
2,4-Diaminophenoxyethanol-HCl	66422-95-5	Generalized pruritus and erythema, anaphylaxis	Nosbaum, 2012 (64)
Glyceryl monothioglycolate	30618-84-9	Type I and IV	Engasser 2000 (65)
Antimicrobial agents and preservatives			
Chlorhexidine	55-56-1	ICU, Quincke's oedema, dyspnoea, anaphylaxis	Ohtoshi, 1986 (66); Okano, 1989 (67); Evans, 1992 (68); Torricelli, 1996 (69); Thune, 1998 (70); Snellman, 1999 (71); Krautheim, 2004 (72); Garvey, 2007 (73); Nagendran, 2009 (74); Sharma, 2009 (75); Silvestri, 2013 (76); Wittczak, 2013 (77)
2-Phenoxyethanol	122-99-6	ICU, anaphylaxis	Bohn, 2001 (78); Hernandez, 2002 (79); Birnie, 2006 (80); Lujan, 2009 (81); Núñez Orjales, 2010 (82)
Polyaminopropyl biguanide	32289-58-0/27083-27-8/28757-47-3/ 133029-32-0	Anaphylaxis	Kautz, 2012 (83); Creytens, 2014 (84)
Sodium benzoate	532-32-1	CUNS	Munoz, 1996 (85)
p-Chloro-*m*-cresol	59-50-7	Angioedema, hives	Walker, 2004 (86)
Sorbic acid	110-44-1	CUNS	Rietschel, 1978 (87)
Triclosan	3380-34-5	ICU, angioedema	Özkaya, 2013 (88)
Sunscreens			
Benzophenone-3	131-57-7	Anaphylaxis	Ramsay, 1972 (89); Berne, 1998 (90); Emonet, 2001 (91); Yesudian, 2002 (92); Landers, 2003 (93); Bourrain, 2003 (94); Spijker, 2008 (95)
Fragrance components			
Myroxylon pereirae (balsam of Peru)	8007-00-9	CUNS	Rudzki, 1976 (96); Forsbeck, 1977 (97); Temesvári, 1978 (98); Katsarou, 1999 (99); Cancian, 1999 (100); Tanaka, 2004 (101)

Table 1. Continued.

Products and components	CAS no.	Reactions	Authors
Fragrance mix	–	CUNS	Cancian, 1999 (100); Temesvari , 2002 (102); Tanaka, 2004 (101)
Cinnamal	104-55-2	CU type C, CUNS	Diba, 2003 (103); Mathias, 1980 (104)
Cinnamomum cassia oil	84961-46-6	CUNS	Rudzki, 1976 (96); Rietschel, 1978 (87)
Geraniol	106-24-1	Oedema	Yamamoto, 2002 (105)
Toothpaste flavours			
Menthol Mentha piperita oil	1490-04-6/2216-51-5/ 89-78-1/15356-70-4 8006-90-4/84082-70-2	ICU, rhinitis, asthma, anaphylaxis	Holmes, 2001 (106); Andersson, 2007 (107); Paiva, 2010 (108)
Carvone	6485-40-1/99-49-0/ 2244-16-8	Angioedema	Hansson, 2011 (109)
Plant-derived and animal derived cosmetic ingredients			
Rubber latex and musa paradisiaca fruit (banana)	9006-04-6	Anaphylaxis	Cogen, 2002 (110); Smith, 1998 (111)
Aesculus hippocastanum extract (chestnut)	8053-39-2	Anaphylaxis	Seitz, 2011 (112)
Avena sativa (oat)	84012-26-0	ICU, oral allergy syndrome	De Paz Arranz, 2002 (113); Boussault, 2007 (114); Vansina, 2010 (115)
Wheat and hydrolysates	94350-06-8/222400-28-4/70084-87-6	ICU, anaphylaxis, WDEIA CUNS, conjunctivitis, rhinitis, bronchospasm	Kousa, 1990 (116); Freeman, 1996 (117); Pasche-Koo, 1996 (118); Niinimäki, 1998 (119); Varjonen, 2000 (120); Pecquet, 2002 (121); Laurière, 2006 (122); Olaiwan, 2010 (123); Fukutomi, 2011 (124); Chinuki, 2011 (125); Hiragun, 2011 (126); Ota, 2012 (127); Chiniku, 2012 (128); Barrientos, 2012 (129); Ishii, 2012 (130); Airaksinen, 2013 (131); Leheron, 2013 (132); Yokooji, 2013 (133); Kobayashi, 2015 (134); unpublished case (Fig. 2); McFadden, 2000 (135); Varjonen, 2000 (120); Pasche-Koo, 1996 (118); Pecquet, 2002 (121); Laurière, 2006 (122); Olaiwan, 2010 (123)
Glycine soja oil	8001-22-7	Facial erythema and swelling	Shaffrali, 2001 (136)
Sesamum indicum seed oil (sesame oil)	8008-74-0	Generalized CUNS, anaphylaxis	Birnbaum, 1997 (137); Smith, 1998 (111); Pecquet, 1998 (138)
Fish-derived elastin	–	ICU	Nishida, 2012 (139)
Hydroxypropyltrimonium-hydrolysed collagen (Crotein Q®)	83138-06-1	ICU, angioedema, bronchospasm	Kousa, 1990 (116); Pasche-Koo, 1996 (118); Freeman, 1996 (117); Niinimäki, 1998 (119)
Citrus seed/citrus limon fruit oil	1180-71-8/8008-56-8	Anaphylaxis	Glaspole, 2007 (140)
Chamomilla recutita extract and *Mangifera indica* fruit (mango)	84082-60-0/90063-86-8	Oral allergy syndrome, angioedema	Rudzki, 1999 (141); Rudzki, 2003 (142)
Chamomilla recutita extract and *Tilia cordata*	84929-52-2	Itching and erythema, rhinoconjunctivitis	Subiza, 1990 (143); Foti, 2000 (144); Krakowiak, 2004 (145); Smith, 1998 (111)
Equae lac (mare's milk)	–	ICU	Verhulst, 2016 (146)
Hen egg	–	CUNS	Antonicelli, 2011 (147)
Honey	8028-66-8	–	Katayama, 2016 (148)
Other			
Tattoo ink	–	NICU	Lee-Wong, 2009 (149)
Glycolic acid peel	–	Anaphylaxis	Vishal, 2012 (150)
Lip plumper	–	NICU, irritant contact dermatitis	Firoz, 2009 (151)
Alcohol contact urticaria syndrome	–	Urticarial papules	Rilliet, 1980 (152); Wong, 2011 (153)
Panthenol	81-13-0/16485-10-2	CUNS	Schalock, 2000 (154)
Rouge	–	CUNS	de Groot, 1983 (155)

CU, contact urticaria; CUNS, non-specified; CUS, contact urticaria syndrome; ICU, immunological contact urticaria; NICU, non-immunological contact urticaria; WDEIA, wheat-dependent exercise-induced anaphylaxis.

of contact dermatitis, urticaria, rhinitis, and asthma, the last of these mainly by inhalation in an occupational context (6). Asthmatics seem to be particularly susceptible to developing such reactions (52). Some studies have shown specific binding of IgE to persulfates by two methods, namely, the immunospot test and radioallergosorbent test (RAST); hence, the mechanism of immediate hypersensitivity seems to be IgE-mediated, at least in some patients (9). Yawalker et al. (50) provided evidence that T lymphocytes specific for such low molecular weight compounds may be directly involved in mediating inflammatory processes in the airways, rather than only acting through induction of IgE synthesis in persulfate-triggered occupational asthma.

Antimicrobial agents and preservatives

Chlorhexidine is a biguanide topical antiseptic and disinfectant with broad antimicrobial efficacy. It is increasingly being used in instillation gels for urinary catheters, and in contact lens solutions, but also in many cosmetic products (72, 76, 158, 159), in which it may be used as a preservative or an antimicrobial agent at a concentration up to 0.3%, according to the European Cosmetics Directive (now Regulation) (159). Urticaria following application to intact skin or mucosae, in some cases accompanied by dyspnoea, angioedema, syncope, or anaphylaxis, has been described (76), via the mucosal route at much lower concentrations than elsewhere, generally as low as 0.05% (67, 72). Polyaminopropyl biguanide (INCI; syn. polyhexanide, polyhexamethylene biguanide) is a widely used antiseptic, for example in contact lens solutions and wound dressings, but also in cosmetics (83). It has been shown to elicit IgE-mediated reactions when it is present in wound-care products and wet wipes (83, 84), and may partly cross-react with chlorhexidine (83). A positive basophil activation test result has been described (84).

Phenoxyethanol is commonly used in cosmetics, most often in combination with other preservatives such as parabens and formaldehyde releasers (82). Lujan et al. (81) reported one case of CU in a male patient, resulting from the use of an aftershave product containing phenoxyethanol. Three other cases of contact urticaria caused by cosmetics have been reported (78–80), but the presence of immunological IgE-mediated reactions could not be confirmed, as specific antibodies could not be identified (79).

Sodium benzoate decreases the amount of dental plaque at concentrations between 1% and 4%, and is a well-known cause of NICU in toothpaste (85). Figure 1 shows positive reactions occurring a few minutes following the application on a test chamber to the preservative agents benzoic acid, sodium benzoate, and sorbic acid (as well as to *Myroxylon pereirae* and cinnamal), all of which are known to cause NICU (7).

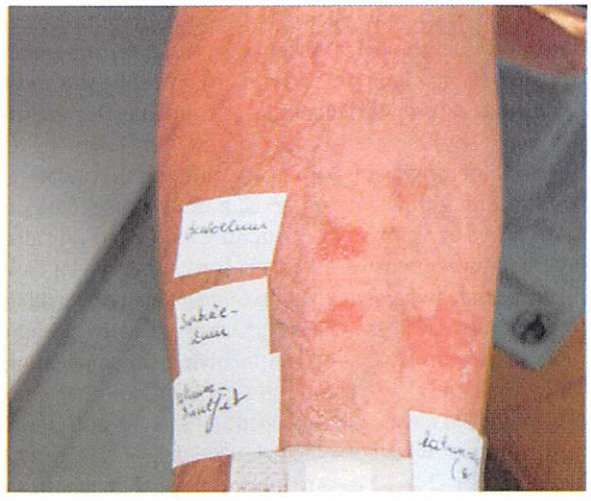

Fig. 1. Non-immunological contact urticaria caused by the preservatives benzoic acid, sodium benzoate, and sorbic acid (sodium metabisulfite remaining negative).

p-Chloro-*m*-cresol is present in a large number of topical preparations, and is a rare cause of allergic contact dermatitis and also CU; the mechanism of this remains uncertain (86). Triclosan, which is used in cosmetics, such as soaps, shampoos, mouthwashes, and deodorants, has provoked a case of severe immunological contact/consort CU and angioedema, owing to its presence in a metronidazole cream (88).

Sunscreens

Skin reactions to sunscreen chemicals include CU, and allergic and photo-allergic contact dermatitis (93); reactions have been found to, for example, benzophenone-3 (INCI; syn. 2-hydroxy 4-methoxy benzophenone, oxybenzone), a common ultraviolet (UV) A/UVB sunscreen, the presence of which also needs to be labeled separately on the cosmetic packaging (92). CU and even contact-mediated anaphylaxis caused by benzophenones are, however, rare (92, 94). The severity of the clinical reaction depends partly on the area of exposed skin; therefore, patch testing does not necessarily elicit anaphylaxis (95). Benzophenones are also added to protect against discolouration of cosmetics (textiles and plastics) that are potentially exposed to sunlight (92).

Fragrance components and aroma chemicals

M. pereirae (balsam of Peru) and fragrance mix (FM) I (a mixture of eight fragrance components in the

baseline series) may elicit CU by both immunological and non-immunological mechanisms (100). Cinnamal, an ingredient common to both, is probably the most important causal ingredient in this context (103). Mathias et al. (104) reported a case of lip swelling following its use in a mouthwash by a patient suffering from allergic rhinitis and asthma. We observed a similar case (data on file) caused by a cinnamal-containing toothpaste and also by cinnamon in pastries. Rietschel (87) described a case of immediate hypersensitivity to *Cinnamonum cassia* oil in toothpaste (as well as to sorbic acid, a preservative present in a shampoo). Facial oedema following the application of cosmetic products containing geraniol, another constituent of FM I, was also described (105): a 20-min closed test showed a wheal response, but no delayed hypersensitivity after 24–72 h. An immunological mechanism has been suggested, because the patient had developed widespread urticaria and flare-up reactions on the face and neck by the day 3 reading of the patch test. Glaspole et al. (140) described a case of anaphylaxis after an individual had used lemon-scented soap when showering; this patient also reported laryngeal oedema, generalized urticaria and asthma within minutes after ingestion of juice prepared from whole crushed oranges, citrus seeds, peanuts, and tree nuts, which seems to be an unusual clinical phenomenon. The antibodies reacting with citrus seeds were suggested to have caused the cross-reactive immune response.

As reported by Holmes et al. (106) regarding type I allergy to mint-flavoured toothpaste, CU should be considered in cases of persistent undiagnosed cheilitis, and both prick tests and patch tests should be carried out with suspected products and allergens. The results of IgE-mediated allergy to mint or menthol include urticaria, rhinitis, asthma, and/or anaphylaxis (107, 108). *R*-carvone, the main ingredient in spearmint oil, is also present in toothpastes; it may cause delayed-type allergy resulting in cheilitis, but occasionally also immediate reactions such as angioedema of the lips appearing within minutes (109), with an open test resulting in an immediate and strong reaction to this compound.

Plant-derived and animal-derived cosmetic ingredients

It is becoming increasingly popular to apply matching hair to the scalp, thereby changing both the length and style of the hair, by the use of bonding glues that contain high concentrations of soluble (natural rubber) latex antigen. Repeated glue exposure may potentially sensitize consumers (110). Pumphrey et al. (160) recently described the anaphylactic death of a 28-year-old British fashion designer immediately following a hair extension procedure. The patient had a history of nut allergy and inhalant atopy, and a known strongly positive prick test reaction to natural rubber latex (160). Moreover, this type of bonding glue may also be used in the application of artificial eyelashes (110).

Emollients and moisturizers are widely used in the treatment of atopic dermatitis, and a recent trend in the cosmetics industry is the use of plant protein derivatives (e.g. from soy, wheat, oat, or sesame). Oat proteins, in particular, are used because of their alleged anti-inflammatory, antioxidant and antipruritic properties. Both allergic contact dermatitis and ICU (114, 115) resulting from the use of emollient creams containing oat extract have occurred. In the latter case the patient later experienced an oral allergy syndrome when eating oatmeal-containing biscuits and bread. The diagnosis was confirmed by prick tests and enzyme-linked immunosorbent assay testing.

Generally, the route of sensitization to proteins can be gastrointestinal, respiratory, and percutaneous, although the penetration of proteins through intact stratum corneum is very limited. However, an impaired skin barrier, skin inflammation and the potential for elevated IgE levels to occur in atopic individuals are predisposing factors.

Most cases of IgE-mediated hypersensitivity to chestnut (*Castanea sativa*), a member of the Fagaceae family, have been attributed to the so-called latex-fruit syndrome, in which ingestion of, for example, avocado, kiwi, banana [the latter a potentail culprit in hair conditioners, (111)] and, less often, chestnut leads to urticaria and anaphylaxis in natural rubber latex-sensitized individuals. This syndrome is caused by cross-reactivity between class I chitinases with a hevein-like domain, such as Mus a 1 (banana), Pers a 1 (avocado), Cas s 5 (chestnut), and Hev b6.02 (late hevein). However, chestnut allergy may occur independently, with Cas s 8, a lipid transfer protein, as the offending allergen. With an increasing number of food proteins being included in so-called natural cosmetics, new cases may appear in the literature, such as contact anaphylaxis induced by cosmetic facial peel containing chestnut (112).

Shaffrali et al. (136) reported on a patient reacting to a cosmetic cream with delayed-type contact allergy to all dilutions of pure soybean extract, and also an immediate response to its 20% dilution, suggesting a possible type I hypersensitivity reaction. However, no allergen-specific IgE for soybean was found, and the patient had previously eaten soy without adverse reactions. It has been shown, however, that the RAST may yield a false-negative result in 27% of cases. Hence, a negative test result for IgE for

soybean does not preclude a diagnosis of type I hypersensitivity. Reports of immediate allergy and anaphylaxis caused by ingested sesame seed or sesame oil have been published. The latter is a known contact allergen in topical pharmaceutical products and cosmetics (138). Despite its widespread use, to our knowledge, only 2 cases of immediate-type reactions induced by cosmetic products have been reported (137, 138).

Protein hydrolysates of collagen, keratin, elastin, milk, wheat, almond, and silk, added to hair conditioners to 'repair' broken hair and to provide a more voluminous appearance, are also causes of CU (119), and are capable of producing reactions through a type 1 mechanism in atopic dermatitis patients in particular (135). Hydrolysed wheat proteins are also widely used in many other cosmetic products, for which several cases have been reported in the literature, including the induction of wheat-dependent exercise-induced anaphylaxis (125, 161). Wheat contains a variety of proteins, which can be divided into salt-soluble proteins of the albumin and globulin type and salt-insoluble proteins, the latter being referred to as gluten (gliadins and glutenins) (122, 125). Their widespread use in food and non-food products aggravates the risk of sensitization. New epitopes may appear during hydrolysis, or the additives used may act as allergens (120). In the case report of Pecquet et al. (121), gluten-derived products were responsible for immediate hypersensitivity through both cutaneous and oral contact. Although the primary route of sensitization is uncertain, the order of reactions in this case favours the cutaneous route. It has been shown that the hydrolysed wheat proteins composed of large polypeptide aggregates possibly induce sensitization to a greater degree than the lower molecular weight compounds (125, 162). Leheron et al. (132) even described IgE-mediated CU in a child caused by hydrolysed wheat proteins and macadamia nuts – both of which were contained in a moisturizing cream used by the mother – with probable sensitization by proxy via maternal skin contact facilitated by atopic dermatitis (132).

Figure 2 shows a 24-year-old non-atopic beautician who developed urticarial lesions on the hands (a) shortly after application of an anti-ageing solution to a client's face. A prick test (b) with the product resulted in an urticarial reaction, but prick tests gave negative results with commercial extracts of cereals, grasses, and wheat flour diluted in water; RASTs (Unicap Pharmacia, Upsala, Sweden) with wheat, grasses and gluten also gave negative results. The cosmetic manufacturer provided the ingredients of the cosmetic products, and positive prick test reactions were observed to hydrolysed wheat proteins only. Additionally, immunoblots were carried out, and

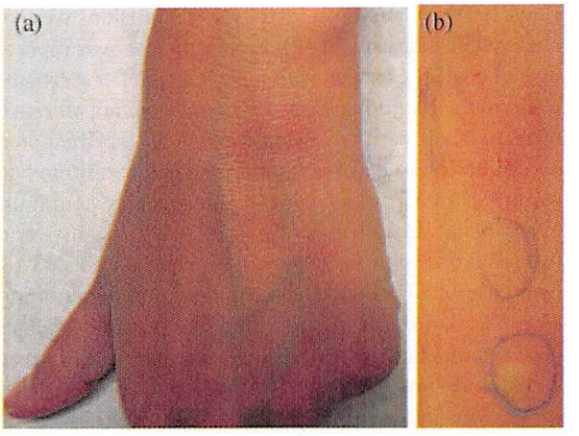

Fig. 2. Immunological contact urticaria caused by hydrolysed wheat proteins in a beautician. (a) Immediate reaction on the hands. (b) Positive prick test reaction to the hydrolysed wheat proteins. (Courtesy: case Dr S. Kerre, Aarschot, Belgium)

showed IgE antibodies in the patient's serum against various fractions of hydrolysed wheat protein.

Beside plant-, also animal-derived protein allergens in cosmetics have been reported as allergenic culprits: for example, an immediate-type reaction to a fish-derived elastin-containing cosmetic cream in a patient with a history of respiratory distress when inhaling smoke from grilled fish (139), and a case of rhinitis, choking and systemic urticaria (syndrome) following the ingestion of egg in a patient who treated her hair weekly with a homemade egg-white based mask (147). Recently, a case of ICU caused by α-lactalbumin from mare's milk-containing cosmetic cream has also been reported (146). Katayama et al. (148) reported the first case of CUS stage 3 caused by honey, induced by sensitization during skin care treatment.

A study by Niinimäki et al. (119) showed hydroxypropyl trimonium-hydrolysed collagen (stearyltrimonium hydroxyethyl-hydrolysed collagen; Crotein Q) to be an especially potent cause of immediate skin reactions, with positive prick test reactions to very low concentrations and specific IgE antibodies against Crotein Q (119).

Chamomile and mango were reported as culprits in a patient with a personal history of childhood eczema and oral allergy syndrome, with hypersensitivity to different kinds of fruit (141). Subiza et al. (143) presented 7 hay fever patients who suffered from conjunctivitis; 2 of them also had angioedema of the lids after washing their eyes with chamomile tea, a folk remedy used to treat conjunctivitis and other ocular reactions. All presented with positive prick test reactions to the tea extract; it is thought that *Matricaria chamomilla* L. [syn: *Chamomilla recutita* (L.)

Rauschert] pollens contained in these infusions were the responsible allergens (143). A similar case was reported by Foti et al. (144), also with a positive prick test reaction to German chamomile (*Matricaria chamomilla*). Moreover, a cosmetician with recurrent itching and erythematous lesions on the backs of her hands and rhinoconjunctivitis resulting from contact with depilatory wax containing *Tilia cordata* and *Matricaria chamomilla*, with positive prick test reactions to these flowers, has been described (145). One case of CUS caused by multiple components in a cosmetic skin mask was reported by West and Maibach (163); immediate open testing showed an extensive wheal-and-flare reaction to whole egg, and to *Melissa* sp. extract 1% in physiological saline. Neither passive transfer nor a RAST was performed to clarify which of the above was the most likely culprit.

Permanent makeup and tattoos

Tattoos and permanent makeup are becoming increasingly prevalent in Western society. Lee-Wong et al. (149) reported a case of anaphylaxis with an immediate skin reaction to purple and blue ink. Unfortunately, many tattoo ink manufacturers are not required to state ingredients on their labels, making it difficult to identify the actual culprit.

Glycolic acid peel

The various complications of chemical peeling that can occur are post-inflammatory hyperpigmentation, infections, scarring, allergic reactions, milia, persistent erythema, and textural changes. Vishal et al. (150) reported a case of CU limited to the area of contact with the glycolic acid peel in a patient with a history of severe acne vulgaris. Apparently, there had been no prior contact with such products in the past.

Lip plumper

New topical agents on the market are designed to increase lip volume within minutes to days following their application; the mechanisms by which they act include vasodilatation secondary to either irritant contact dermatitis or NICU. Common ingredients are essential oils of cinnamon and cayenne pepper (*Capsicum frutescens*), that is, spices that are classified as both irritants and urticants. Firoz et al. (151) reported a case of a young boy who developed an urticarial plaque of rapid onset on the right cheek following a kiss from his mother after she had applied a lip plumper 1 h earlier; the active ingredients of this included benzyl nicotinate and *C. frutescens* resin.

Alcohol urticaria syndrome (AUS)

Angioedema following ingestion of alcohol may be caused by different agents contained in the beverages, such as yeast. AUS is a rarely reported and poorly understood entity that may be triggered either by primary alcohols or alcohol metabolites, that is, aldehydes and acetic acid (153, 164), and that seems to be more common among persons of East Asian descent who have aldehyde dehydrogenase deficiency, leading to increased serum aldehyde levels in the course of alcohol metabolism (153). Case reports of CU caused by local application of alcohol seem to be very rare, and include the following examples: a patient who noted a diffuse pruritic rash after drinking alcoholic beverages, with ethanol applied to the skin provoking an erythematous reaction in ~20 min (165); allergic CU caused by ethanol and isopropyl alcohol (166); urticaria-like lesions provoked by ethanol and stearyl alcohol, associated with delayed dermatitis (167); and a nurse with ethanol-induced CU following the application of perfume and a hand sanitizer, who developed generalized reactions after drinking ethanol (153). Rilliet et al. (152) reported a case in which immediate reactions with most of the primary alcohols were positive, and there was a positive passive transfer test result, with a method corresponding to that of Prausnitz-Küstner (152, 153).

Conclusion

This updated review confirms that various cosmetic components can cause CU with or without systemic symptoms, the latter sometimes being life-threatening. However, such cases might be more common, because patients probably lack awareness. Physicians should therefore continue to look out for possible new causes. Anaphylactic reactions provoked by patch testing with the allergen are rare, but, in patients with a severe history of CUS, emergency measures remain necessary.

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Benzophenones

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Abstract: **Benzophenones are ultraviolet light filters that have been documented to cause a myriad of adverse cutaneous reactions, including contact and photocontact dermatitis, contact and photocontact urticaria, and anaphylaxis. In recent years, they have become particularly well known for their ability to induce allergy and photoallergy. Topical sunscreens and other cosmetics are the sources of these allergens in most patients, but reports of reactions secondary to use of industrial products also exist. Benzophenones as a group have been named the American Contact Dermatitis Society's Allergen of the Year for 2014 to raise awareness of both allergy and photoallergy to these ubiquitous agents.**

Benzophenones are chemical ultraviolet light absorbers. They primarily absorb light in the UV-B range (290–320 nm), whereas 2 benzophenones (benzophenone-3 and benzophenone-4) also absorb UV-AII light (321–340 nm).[1] Benzophenones-1 through benzophenones-12 are substituted derivatives of 2-hydroxybenzophenone and are currently being used for a wide variety of purposes in the United States. These aromatic ketones are planar molecules that are capable of photoabsorption and resonance stabilization, which account for their ability to protect human skin and commercial products from damaging ultraviolet radiation. All benzophenones have slightly different properties based on specific molecular substitutions (Table 1).

Benzophenones were initially used as preservatives in industrial products such as paints, varnishes, and plastics to extend shelf life and reduce photodegradation. In the 1950s, benzophenones were introduced into sunscreens.[2] Although 6 different benzophenones were initially used as sunscreens, benzophenones-3, -4, -8, and -10 are now the 4 agents most commonly used in personal care products.[3] The amount of benzophenone-3 used in US sunscreens is more than all other benzophenones combined; in a 2011 study of the prevalence of known contact allergens in cosmetic and skin care products, benzophenone-3 was found in 68% of the 201 sunscreens assessed.[4] Cosmetic and toiletry products such as moisturizers, hair sprays, hair dyes, perfumes, shampoos, detergent bars, and nail polishes may also contain benzophenones. Other benzophenones continue to be used in industry, with applications ranging from incorporation into plastic lens filters for color photography, aerosol sprays to protect color prints, transparent shades to protect window displays, and many polystyrene, acrylic, and rubber products to prevent darkening and loss of structural integrity.[3]

Benzophenones have been documented to cause a myriad of adverse cutaneous reactions, including contact and photocontact dermatitis, contact and photocontact urticaria, and anaphylaxis.[5–7] In recent years, they have become particularly well known for their ability to induce allergy and photoallergy. Topical sunscreens and other cosmetics are the sources of these allergens in most patients, but reports of reactions secondary to use of industrial products also exist.

ALLERGIC CONTACT DERMATITIS

Allergic contact dermatitis to benzophenone-3 was first documented in 1972.[8] Today, it is not only the most common benzophenone to cause positive patch test reactions, but also it is the most common UV filter, overall, to cause allergy. The most recent 10-year retrospective analysis of the North American Contact Dermatitis Group Data (NACDG; 2001-2010) found that of the 219 of 23,908 patch tested patients with sunscreen listed as an allergen source, 70.2% had positive patch test reactions to benzophenone-3.[9] This finding is consistent with data from other geographic locations. Not only was benzophenone-3 the leading allergen in an Australian retrospective analysis of 6292 patients patch tested with suspicion for allergy to sunscreen, causing 28% of positive reactions,[10] but also recent European multicenter studies and a Canadian single-center study have also found benzophenone-3 to be among the most significant sunscreen allergens.[11–13]

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TABLE 1. Benzophenone Properties, Products, and Patch Test Concentrations

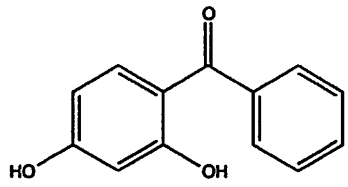
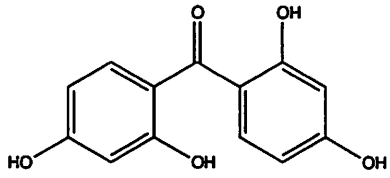
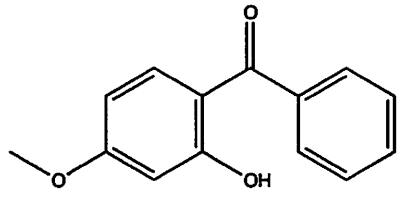
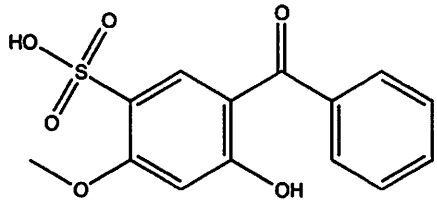
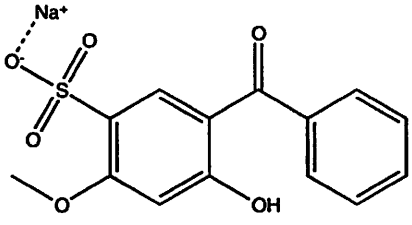
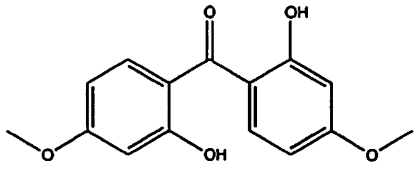
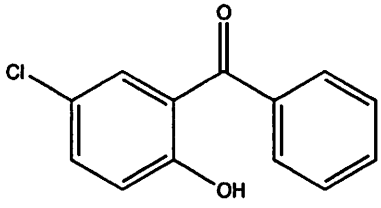
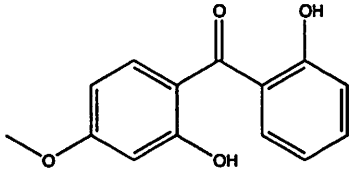
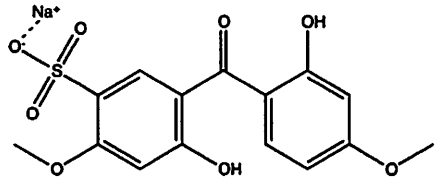
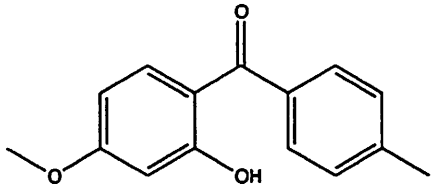
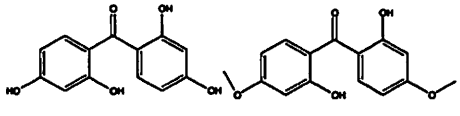
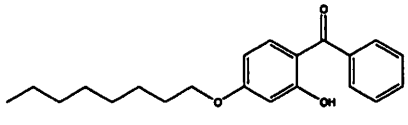
	Other Names	Structure	Properties
Benzophenone-1	2,4-Dihydroxybenzophenone benzoresorcinol, 4-benzoyl resorcinol, (2,4-dihydroxyphenyl)- phenylmethanone, resbenzophenone		-light yellow powder insoluble in water
Benzophenone-2	2,2',4, 4'-Tetrahydroxybenzophenone		-yellow crystalline solid -slightly soluble in water and toluene
Benzophenone-3	Oxybenzone 2-hydroxy- 4-methoxybenzophenone		-light cream powder -soluble in most organic solvents and insoluble in water -can cross the epidermal barrier -metabolites are excreted in the urine after widespread topical application
Benzophenone-4	Sulisobenzone, 2-hydroxy- 4-methoxybenzophenone- 5-sulfonic acid		-pale ivory powder -soluble in water
Benzophenone-5	2-Hydroxy- 4-methoxybenzophenone- 5-sodium sulfonate		-water soluble

TABLE 1.

Source	Patch Test Concentration	Allergic Contact Dermatitis Author[ref#]-Reactions	Photoallergic Contact Dermatitis Author[ref#]-Reactions
-personal care products	1% pet*	NR	NR
-personal care products -herbicides	1% pet.†	NR	1 *total reaction;* 1 study Shaw et al[30]−1
-personal care products -agricultural films (such as polyvinyl chloride)	10% pet.‡	45 *total reactions; 15 studies* Torres and Correia[23]−1 Bilsland and Ferguson[24]−2 Szczurko et al[32]−1 Rademaker[25]−1 Darvay et al[26]−8 Crouch et al[27]−1 Nedorost[20]−1 Kiec-Swierczynska et al[36]−1 Bryden et al[31]−9 Rodríguez et al[35]−2 Hughes and Stone[14]−3 Scalf et al[17]−3 Travassos et al[18]−5 Sasseville et al[38]−1 EMCPPTS[12]−6 Greenspoon et al[13]−17	*153 total reactions; 13 studies* Szczurko et al[32]−35 Horn et al[39]−1 Darvay et al[26]−14 Crouch et al[27]−3 Nedorost[20]−1 Kiec-Swierczynska et al[36]−1 Bryden et al[31]−27 Rodríguez et al[35]−22 Scalf et al[17]−5 Cardoso et al[34]−3 Shaw et al[30]−1 Victor et al[28]−3 EMCPPTS[12]−37 Greenspoon et al[13]−12
-personal care products -hair care products	10% pet* 2% pet.†	*46 total reactions; 6 studies* Ramsay[8]−1 Bryden et al[31]−18 Hughes and Stone[14]−13 Scalf et al[17]−6 Travassos et al[18]−7 Sasseville et al[38]−1 Greenspoon et al[13]−3	*26 total reactions; 6 studies* Bryden et al[31]−7 Rodríguez et al[35] Scalf et al[17] Cardoso et al[34]−3 Victor et al[28]−3 EMCPPTS[12]−3
-personal care products	NR	NR	NR

(Continued on next page)

TABLE 1. *(Continued)*

	Other Names	Structure	Properties
Benzophenone-6	2,2'-Dihydroxy−4, 4'-dimethoxybenzophenone, bis(2-hydroxy-4-methoxyphenyl) methanone		-light yellow solid -insoluble in water
Benzophenone-7	5-Chloro-2-hydroxybenzophenone, 2-hydroxy-5-chlorobenzophenone		
Benzophenone-8	Dioxybenzone, 2,2'-dihydroxy-4-methoxybenzophenone		-yellow crystalline solid -slightly soluble in water
Benzophenone-9	Sodium 2,2'-dihydroxy-4, 4'-dimethoxy-5-sulfobenzophenone		-light yellow powder -water soluble
Benzophenone-10	Mexenone, 2-hydroxy-4-methoxy-4'-methylbenzophenone		
Benzophenone-11	(mixture of benzophenone-6 and other tetra-substituted benzophenones)		-yellow or tan powder
Benzophenone-12	Octabenzone, 2-hydroxy-4-(octyloxy)benzophenone, (2-hydroxy-4-(octyloxy) phenyl)phenylmethanone		

*De Groot.
†As seen in Shaw et al[30] 2010.
‡Chemotechnique recommended primary patch test concentration.

TABLE 1. *(Continued)*

Source	Patch Test Concentration	Allergic Contact Dermatitis Author[ref#]-Reactions	Photoallergic Contact Dermatitis Author[ref#]-Reactions
-personal care products	2% pet*	NR	NR
-commercial grain fungicide	NR	NR	NR
-personal care products	2% pet.*	*2 total reactions; 2 studies* Pariser[19]–1 Sasseville et al[38]–1	NR
-personal care products -grain insecticide	NR	NR	NR
-personal care products	10% pet.†	*33 total reactions; 8 studies* Millard and Barrett[21]–2 English et al[22]–6 Torres and Correia[23]–1 Bilsland and Ferguson[24]–6 Rademaker[25]–1 Darvay et al[26]–13 Crouch et al[27]–3 Hughes and Stone[14]–1	*16 total reactions; 7 studies* Burry[29]–1 Torres and Correia[23]–1 Szczurko et al[32]–1 Darvay et al[26]–9 Kiec-Swierczynska et al[36]–1 Rodríguez et al[35]–2 Cardoso et al[34]–1
-personal care products	2% pet.*	NR	NR
-food stabilizer in petroleum wax -antioxidant/stabilizer in olefin polymers	NR	NR	NR

Allergic contact dermatitis to the other benzophenones commonly used in personal care products is also well documented. Benzophenone-4 was "an emerging allergen in cosmetics and toiletries" 10 years ago because the reports of sensitized patients continued to surface secondary to its incorporation into sunscreens, hair care products, and shower gels.[14,15] This UV filter has also recently been implicated in allergic contact dermatitis to printing ink, highlighting the importance of considering these compounds in occupational settings.[16] Recent literature now points to benzophenone-4 as having "emerged" because it is repeatedly one of the leading 5 sunscreen allergens in large multicenter patch test studies and cross-sectional surveys.[18,31] A few smaller studies have found more positive patch reactions to benzophenone-4 than benzophenone-3.[14,17]

Although benzophenones-8 and -10 were commonly used in topical sunscreens in the past, the literature regarding adverse reactions to these agents is scarce. Only 2 case reports of allergic contact dermatitis to benzophenone-8 have been published.[17,18] Although this may reflect the relative less frequent use of this chemical filter in sunscreen preparations today, it may also be due to difficulty in obtaining the allergen for testing.[7] A number of positive patch reactions to benzophenone-10 have been reported,[13,19–25] although more recent studies in both the United States and the European Union have not documented reactions to this agent.[12,26]

The remainder of the benzophenones has not been documented in the literature as eliciting allergic contact dermatitis. This could be due to infrequent use in products or lack of routine testing to these agents.

PHOTOALLERGIC CONTACT DERMATITIS

Photoallergic contact dermatitis to benzophenone-3 was first reported in 1980, 8 years after the first report of non–light-related contact dermatitis.[27] Benzophenone-3 is implicated in more PACD reactions than any other UV filter available. Although photoallergy to this agent has been demonstrated in studies conducted in the United States,[16,27,28] the number of positive photopatch reactions in Europe has been even greater.[11,12,29] Benzophenone-3 was also the leading UV filter photoallergen in a recent 10-year retrospective Canadian study, causing reaction rates similar to those of known strong photoallergens promethazine and chlorpromazine.[13] It is also important to note that reactions to this agent often stem from its use in cosmetics not marketed as sunscreens. One study found that most patients with positive photopatch reactions to benzophenone-3 had their daily moisturizer as the allergen source.[30] Another recently reported case of PACD to benzophenone-3 was secondary to contact with magazine printing ink. The patient developed positive reactions to benzophenone-3, octocrylene, and ketoprofen on photopatch testing, but of these, only "benzophenones" was a component of the ink used in the making of the magazine that she was reading on the beach.[31]

A number of large photopatch studies have suggested that benzophenone-4 is also a leading cause of photoallergy in patients with adverse reactions to sunscreens, both in the United States and abroad.[27,30,32] In fact, 1 recent US study found benzophenone-4 to be the most frequently implicated photoallergen of the 11 tested UV filters in 182 photopatch tested patients with suspected photodermatoses or sunscreen allergy.[16]

Benzophenone-10 was the second leading photoallergen in a European retrospective study investigating the results of photopatch testing in 2715 patients from 1983 to 1998.[25] However, the authors note that benzophenone-10 was rarely used in sunscreen manufacture by the time they published these results. This is reflected in the relatively small number of case reports documenting photoallergy to this substance.[22,28,31–34]

Benzophenone-2 has been reported to cause a photopatch test reaction in a patient with self-proclaimed "sunscreen allergy."[16] No reports of photoallergy to benzophenone-8 have been documented. However, neither of these agents is routinely included in photopatch testing series.

A literature search found no reports of photoallergic reactions to benzophenones -1, -5, -6, -7, -9, -11, or -12.

PATCH TESTS

The US Food and Drug Administration now regulates the concentrations of ultraviolet filters allowed in topical sunscreen preparations. The Food and Drug Administration Code of Federal Regulations for Sunscreen Drug Products for Over-the-Counter Human Use lists maximum concentrations in personal care products, which are as follows: benzophenone-3 up to 6%, benzophenone-4 up to 10%, and benzophenone-8 up to 3%.[35] Neither benzophenone-2 nor -10 are approved for use in topical sunscreens. The recommended concentrations of each benzophenone for patch testing differ from the product concentrations. Suggested patch test concentrations are listed in Table 1. A literature search found no recommended patch test concentrations for benzophenones-5, -7, -9, or -12.

CROSS-REACTIONS

Because of the shared chemical structure, cross-reactions within the benzophenone family are plausible, whereas an extensive literature search found no confirmed documented case.[19,37] Although there are a few reports that demonstrated multiple allergies to benzophenones, 1 author postulated that this could be due to separate exposure to each implicated allergen or photoallergen, whereas the significance of reaction to benzophenone-8 could not be determined.[36] Given the ubiquitous nature of benzophenones-3 and -4 in cosmetics and toiletries, exposure to both agents in separate products is likely. Most patients patch tested to multiple benzophenones only react to one.

Also of importance, benzophenone-3 shows high rates of cross-reactivity with octocrylene (a UV filter structurally similar to sunscreen agents in the cinnamate family) and ketoprofen (a topical nonsteroidal anti-inflammatory drug [NSAID]). A large European multicenter study across 12 countries found ketoprofen to cause the most photopatch reactions of all agents

tested. It is notable that octocrylene and benzophenone-3 were the third and fourth leading photosensitizers in this study, perhaps reflective of cross-reactivity rather than separate sensitization.[12] Not only do these 3 chemicals have similar structures, but also ketoprofen is broken down into various benzophenones structurally related to benzophenone-3 when it is irradiated with sunlight.[37] Benzophenone's cross-reaction with octocrylene may be of increasing interest in the future, given the increasing use of octocrylene in sunscreens as a stabilizer for butyl methoxy dibenzoylmethane (Parsol 1789). All 3 of these chemicals have been labeled as strong photosensitizers. Other topical and systemic agents containing a benzophenone nucleus may result in cross-reactivity. These include other topical NSAIDs (eg, tiaprofenic acid) and the cholesterol-lowering agent fenofibrate.[38] Topical NSAIDs are frequently used in European countries but are far less common in the United States.

BENZOPHENONES AND SYSTEMIC REACTIONS

Systemic exposure to UV filters is a result of dermal or gastrointestinal absorption. Studies have shown bioaccumulation of UV filters in wildlife (eg, fish) because of the lipophilic nature of these chemicals. Benzophenone-3 and its metabolites have been detected in human urine 4 hours after widespread topical application.[5] Benzophenone-3 has also been detected in human breast milk after topical application.[39] At least 2 cases of anaphylaxis from topical application of benzophenone-3 have been published. Both cases resulted in a generalized wheal and flare reactions and syncope after widespread application of a sunscreen or sunless tanning product with this filter. Contact urticaria developed after more limited exposure to benzophenone-3 in both cases.[5,40] The evidence of bioaccumulation in wildlife and humans also raises the possibility of long-term exposure, including effects on reproduction and ontogeny. Benzophenone-3, in particular, has displayed significant dose-dependent estrogenic activity.[41] More recent studies have also demonstrated the estrogenic effects of other benzophenone derivatives. Kerdivel et al[42] recently examined the effects of 10 different benzophenones on the proliferation of estrogen receptor–positive breast cancer cells and on the transcriptional activity of E2 target genes and found that among the 10 benzophenones tested, benzophenone-8 was high activity about proliferation potential and benzophenone-2 was of moderate activity. Another recent study suggested that exposure to elevated benzophenone-1 levels may be associated with increased odds of an endometriosis diagnosis.[43] A comprehensive literature search did not reveal any evidence that systemic absorption of benzophenone-3 or any other of the benzophenones has been linked to dermatitis or photosensitivity.

SUMMARY

The benzophenones are a group of chemical UV filters, which protect against UV-B and some UV-A. Although the general public has become more aware of the dangers of both UV-A and UV-B radiation, these broad-spectrum agents have increasingly been incorporated into topical sunscreens and various cosmetics. This expanded use has undoubtedly contributed to the increased prevalence of benzophenone sensitization seen when comparing the positive patch test rates to benzophenone-3 in 1995–1996 NACDG data with 2009-2010 NACDG data.[44,45] Benzophenone-3 is the most common sunscreen contact and photocontact allergen in North America. Allergic and photoallergic contact dermatitis to benzophenone-4 is also increasing, both in the United States and abroad. This underscores the importance of inclusion of these allergens in routine screening series, such as the ACDS Core Screening Series.[46] Benzophenones as a group have been named the American Contact Dermatitis Society's Allergen of the Year for 2014 to raise awareness of both allergy and photoallergy to these ubiquitous agents.

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Viewpoint

The 500 Dalton rule for the skin penetration of chemical compounds and drugs

Bos JD, Meinardi MMHM. The 500 Dalton rule for the skin penetration of chemical compounds and drugs.
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Abstract: Human skin has unique properties of which functioning as a physicochemical barrier is one of the most apparent. The human integument is able to resist the penetration of many molecules. However, especially smaller molecules can surpass transcutaneously. They are able to go by the corneal layer, which is thought to form the main deterrent. We argue that the molecular weight (MW) of a compound must be under 500 Dalton to allow skin absorption. Larger molecules cannot pass the corneal layer. Arguments for this "500 Dalton rule" are; 1) virtually all common contact allergens are under 500 Dalton, larger molecules are not known as contact sensitizers. They cannot penetrate and thus cannot act as allergens in man; 2) the most commonly used pharmacological agents applied in topical dermatotherapy are all under 500 Dalton; 3) all known topical drugs used in transdermal drug-delivery systems are under 500 Dalton. In addition, clinical experience with topical agents such as cyclosporine, tacrolimus and ascomycins gives further arguments for the reality of the 500 Dalton rule. For pharmaceutical development purposes, it seems logical to restrict the development of new innovative compounds to a MW of under 500 Dalton, when topical dermatological therapy or percutaneous systemic therapy or vaccination is the objective.

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Key words: drug design – skin – topical drugs – transdermal

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Introduction

Human skin has many functions and its most apparent is that of a defense organ, both physical and biological (1). Penetration from outside into the body of any compound is primarily prevented by the corneal layer of the epidermis. This outer layer is just a few micrometers thick, but effectively forms a barrier that is indeed preserving life. Although absorption is not only dependent on penetration, but also on other variables such as skin metabolism, insufficient release from the carrier, partitioning in an unwanted reservoir, without penetration nothing happens. It is important to realize that the human skin has unique properties in this respect and that penetration studies performed in animal models are of limited use for our understanding of the human skin barrier.

Essentially, the corneal layer consists of apoptotic keratinocytes that have transformed themselves into keratin-rich, lipoprotein-containing envelopes and lipid bilayers with hydrophilic regions in between. Most medicaments will pass the epidermal barrier through the intercellular route. As a consequence of its hydrophobic nature, the stratum corneum barrier will allow the penetration of lipid soluble molecules more readily than water-soluble compounds. Strong lipophilic compounds will however be hampered by the hydrophilic regions in the bilayer. Water-soluble molecules may penetrate through an alternative way, the openings of sweat glands and hair follicles. The total surface of these openings amounts to 0.1% of the total skin surface area, making it probably not significant.

The only way to circumvent the properties of the corneal layer is by disrupting it, for example with ultrasound, a method also known as phonopheresis (2), or with high-voltage electrical pulsing, also known as electroporation (3, 4). Alternative methods such as stripping the corneal layer using adhesive tape have also been advocated but are not reliable. The use of skin penetration enhancers such as dimethylsulphoxide or carriers such as liposomes have never been confirmed to make a difference. Iontopheresis (5) using low-voltage has been developed for increasing the flux of particular

Table 1. Molecular weight of compounds included in the ICDRG European Patch Testing Standard Series

Compound	Test concentration	Molecular weight (Dalton)
Potassium **Dichromate**	0.5% pet	294
Neomycin Sulphate	20% pet	**712**
neomycin (dimer of neamine **322**)		**614**
Thiuram Mix	1% pet	
Dipentamethylenethiuram Disulphide	0.25% pet	318
Tetraethylthiuram Disulphide	0.25% pet	295
Tetramethylthiuram Disulphide (TMTD)	0.25% pet	240
Tetramethylthiuram Monosulphide (TMTM)	0.25% pet	208
p-Phenylenediamine	1% pet	108
Cobalt Chloride (6H2O)	1% pet	130
Benzocaine	5% pet	165
Formaldehyde in water	1% aqua	30
Colophony (90% abeitic acid)	20% pet	302
Chloquinol	5% pet	306
Pinus (**Balsam of Peru**) (60% cinnamein)	25% pet	148
IPPD	0.1% pet	216
Lanolin (**Wool Alcohols**)	30% pet	
Mercapto Mix	1% pet	
Dibenzothiazyl Disulphide	0.333% pet	332
Morpholinylmercaptobenzothiazole	0.333% pet	252
N-Cyclohexylbenzothiazyl-Sulphenamide	0.333% pet	308
Epoxy Resin	1% pet	
Paraben Mix	16% pet	
Butylparaben (Butyl Parahydroxybenzoate)	4% pet	194
Ethylparaben (Ethyl Parahydroxybenzoate)	4% pet	166
Methylparaben (Methyl Parahydroxybenzoate)	4% pet	152
Propylparaben (Propyl Parahydroxybenzoate)	4% pet	180
Paratertiarybutyl Phenol Formaldehyde Resin	1% pet	
Fragrance Mix	8% pet	
alpha-Amyl-Cinnamaldehyde	1% pet	202
Cinnamaldehyde	1% pet	132
Cinnamyl Alcohol	1% pet	134
Eugenol	1% pet	164
Geraniol	1% pet	154
Hydroxycitronellal	1% pet	154
Isoeugenol	1% pet	164
Oak Moss Absolute	1% pet	
Quaternium-15	1% pet	201
Nickel Sulphate, 6H2O	5% pet	155
Methyl(chloro)isothiazolinone (**Kathon CG**)	0.01% aqua	184
Mercaptobenzothiazole	2% pet	167
Sesquiterpene Lactone Mix (allantolactone)	0.1% pet	232
Primin	0.01% pet	209
Wood Tar Mix	12% pet	
Methyldibromoglutaronitrile (**Euxyl K400**)	0.5% pet	266
1,2 Benzisothiazolin-3-On (**BIT**)	0.1% pet	151
Tixocortol-21-Pivalate	1% pet	463
Budesonide	1% pet	431
4,4'-Diaminodiphenylmethane	0.5% pet	198

(pet=petrolatum.)

compounds, but only of low MW. Thus, when large molecules have to be absorbed after topical application, only phonopheresis and electroporation are available, but these techniques are far from practical and not suitable for routine use.

In a recent review, it was stated that "optimal absorption will occur for molecules that are small, have low melting points . . . and have few pendant groups capable of H-bonding" (6). But what is small? The subject of this contribution is the upper molecular weight (MW) limit for chemical compounds and drugs enabling absorption through the human skin barrier. An answer to this question is of use for those developing epicutaneous application of compounds to the human skin for destinations varying from topical to systemic treatment to vaccination. We have therefore looked at the MW of common contact allergens and commonly used topical drugs. We propose the 500 Dalton rule, which says that with a MW increasing over 500 Dalton, absorption of molecules through normal human skin rapidly declines.

Dermato-allergology: chemicals causing allergic contact dermatitis are under 712 Dalton

The variety of chemicals that are known to lead to allergic contact dermatitis in persons exposed to them forms a true encyclopedia of modern society. Thousands of molecules have been described to be associated with the induction and maintenance of allergic contact dermatitis in a limited or more extensive number of persons. The intrinsic sensitizing capacity of molecules is varying widely. Some compounds rarely lead to clinical contact dermatitis. Others are virtually sensitizing any person whose skin is exposed to it. The routine patch test series, advised by the International Contact Dermatitis Research Group (ICDRG), is used for the diagnosis of contact allergy, and it is composed of the most common sensitizing agents known to mankind.

Taking this series of single chemical compounds as well as mixtures of sensitizing agents together, a look at their MW might give us a clue to what size allows penetration through the human skin barrier. A molecule that comes into contact with human skin, but that cannot penetrate the skin barrier in sufficient quantities, will not be a sensitizing agent. The MWs of the ICDRG patch test series compounds were identified using the Merck Index or using software (the Molecular Weight Calculator for Windows 95 – version 4.1 by Matthew Monroe – http://www.unc.edu/~monroen/). They are summarized in Table 1.

Often, the test allergens are mixes and where possible, the MW of individual compounds in these mixes is given (thiuram mix, mercapto mix, paraben mix, fragrance mix). In other instances, the main constituents of a given crude extract is taken (colophony=90% abietic acid: Balsam of Peru=60% cinnamein; sesquiterpene lactone mix= allantolactone). MWs could not be identified for mixes such as lanolin (wool alcohols), epoxy resin,

paratertiarybutyl phenol formaldehyde resin, oak moss absolute, and wood tar mix.

The most common allergens have a MW under 500 Dalton (Table 1). The only exception to the 500 Dalton rule is neomycin sulphate, which has a MW of 712 Dalton. However, this molecule is a dimer of two neamine molecules, each having a MW of 322 Dalton, and it may well be that it is the monomer that sensitizes. All other components of the ICDRG patch test series are under 500 Dalton, with tixocortol-21-pivalate and budesonide being the largest having MWs of 463 and 431 Dalton respectively.

Topical immunosuppressants: cyclosporin's 1202 Dalton is too large

Late in the 1980s, cyclosporin (MW 1202 Dalton) was introduced as a systemic agent for the treatment of dermatological disease, especially psoriasis (7). It soon became known that the drug was highly effective in skin diseases known to respond favorably to topical or systemic corticosteroids. It also was immediately conceived that topical treatment with this new class of cyclic immunosuppressants might potentially revolutionize topical dermatological therapy. However, the topical use of cyclosporin was found to be ineffective in psoriasis as well as in atopic dermatitis and allergic contact dermatitis (8). When injected intralesionally however, cyclosporin is effective in psoriasis (9, 10). Thus, a MW of 1202 Dalton apparently prohibits sufficient skin penetration.

Subsequently, the focus was redirected at other inflammatory cytokine inhibitors, such as tacrolimus (822 Dalton) and the ascomycin derivative SDZ ASM 981 (811 Dalton). Tacrolimus had been found to be effective as a systemic therapy in psoriasis (11). There is one abstract indicating its efficacy in psoriasis when applied under occlusion (12). A controlled study could not detect efficacy in an open application approach (13). Ascomycin derivatives have also been tested for possible topical efficacy. After a first encouraging study in which the ascomycin derivative SDZ 281–240 was found to be effective in psoriasis when used under occlusion (Finn chamber) (14), SDZ ASM 981 was also found to be effective in psoriasis when applied topically under occlusion (15). No reports have been published confirming efficacy in psoriasis using non-occluded applications.

Ascomycin derivative SDZ ASM 981 (16) and tacrolimus (17) do however work when topically applied in atopic dermatitis. It seems that atopic dermatitis patients are the exception to the rule and can, as a result of a somewhat defective barrier, absorb molecules of slightly over 800 Dalton, such as tacrolimus and ascomycin.

Thus, with the exception of atopic dermatitis where 1202 Dalton is too large a molecule to be topically effective, but where 822 Dalton molecules are absorbed, the human skin does not allow penetration of molecules around 800 Dalton, fitting with the 500 Dalton rule.

Molecular weight of the most commonly used topical drugs in dermatotherapy

In dermatological therapy, a wide variety of different active molecules is available for topical treatment of individual skin lesions. A list of the most commonly used topical drugs is presented in Table 2. Their molecular weights were identified using the Chemfinder Webserver (http://chemfinder.camsoft.com). Again, the most commonly used and effective topical drugs in dermatotherapy all have a molecular weight under 500 Dalton, the only exceptions being fusidic acid which is slightly larger with 517 Dalton, and ketoconazole which has a MW of 531 Dalton.

Table 2. List of the world's most commonly used topical drugs and their molecular weight

Compound	Molecular weight (Dalton)
Topical antifungals:	
Ketoconazole	531
Clotrimazole	345
Terbinafine	291
Miconazole	416
Topical corticosteroids	
Hydrocortisone acetate	404.5
Bethamethasone valerate	477
Diflucortolone valerate	394
Clobetasol propionate	467
Mometasone fuorate	?
Topical anti-infectives	
Fucidic acid	517
Gentamycin	478
Acyclovir	225

Table 3. List of drugs available in transdermal drug-delivery systems (Langer 1998)

Compound	Molecular weight (Dalton)
Scopolamine	305
Nitroglycerine	227
Nicotine	162
Clonidine	230
Fentanyl	336
Oestradiol	272
Testosterone	288

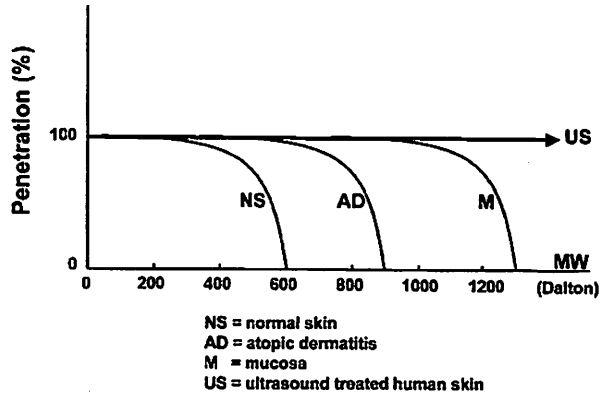

Figure 1. Estimated penetration barrier characteristics for normal human skin, atopic dermatitis skin, mucosa, and phonopheretically disrupted skin.

Molecular weight of drugs used in transdermal drug-delivery systems

Certain drugs for systemic use are delivered through the skin for reasons varying from avoiding the liver (destroying drugs when taken orally) to enabling sustained release. Patches with transdermal drug delivery systems are available for at least 7 different drugs (18). In Table 3, these compounds and their molecular weights are summarized, using the same MW finding strategy as for the topical drugs described earlier. As may be seen from the Table, MWs of drugs used in transdermal drug-delivery systems are all well under 500 Dalton, in fact they are all smaller than 350 Dalton.

Arguments brought forward against the 500 Dalton rule

In discussions of the upper limit of skin absorption, several arguments might be brought forward against the suggested 500 Dalton rule. The first argument is that it is not a penetration problem but a formulation challenge. That is, with the use of the appropriate pharmaceutical formulation, the drug will be absorbed, taking into account its lipophilic or hydrophilic character, adjusting the vehicle in such a way that the drug prefers to leave it and go into the integument. However, authors are not aware of any formulation that contains a drug with a MW well over 500 Dalton, that is clinically effective in any skin condition. Penetration enhancers then would solve the problem, but again, there is no proof for this hypothesis. In fact, the only way described thus far to overcome the epidermal barrier is by exposing skin to phonopheresis or electroporation, as described earlier. These disrupt the corneal layer and allow subsequent penetration of very large proteins. From a practical point of view however, these approaches are not feasible.

A completely different type of argument is the existence of latex allergy. Contact urticaria and contact dermatitis may indeed occur after skin exposure to latex, which is generally believed to consist of high MW molecules. Immediate type reactions have been described and are believed to be IgE mediated. Allergic contact dermatitis has also been reported and is believed to be T-cell mediated. However, it is also known that IgE molecules as well as T-cell receptors do not recognize large proteins, but only peptide epitopes, generally being 6–8 amino acid derivatives. It is unacceptable to believe that latex allergy sufferers have greatly diminished skin barriers allowing large proteins (over 50,000 Dalton) to penetrate. More acceptable is the explanation that latex proteins are degraded by proteases present on the skin surface, allowing smaller peptides derived from it to penetrate and to lead to allergic reactions. Alternatively, latex might naturally contain small molecules that are the immunogenic compounds.

The same might be said about the atopy patch test, where equally large proteins are used epicutaneously to detect allergy, and the superantigen skin challenge, where bacterial superantigens are used for topical provocation of skin lesions. These must most probably be first degraded into smaller molecules to enable penetration and subsequent binding to the molecules that can attach to them, or already contain these smaller breakdown products.

Finally, some investigators believe that larger molecules do indeed penetrate the skin but are quickly metabolized, making them clinically ineffective (19). However, the same mechanism would then apply to smaller molecules, that are metabolized as well, and would not be effective either.

Concluding remarks

The human skin is indeed an effective barrier but it cannot prevent smaller molecules to enter. In Fig. 1, the estimated penetration barrier characteristics for normal human skin, atopic dermatitis skin, mucosa, and phonopheretically disrupted skin are indicated. Somewhere around 500 Dalton is the start of a rapid decline in skin absorption due to molecular size. The barrier is formed by the corneal layer since when absent, such as in mucous membranes, larger molecules may penetrate and thus be effective. Topical treatment of mucosal lichen planus with cyclosporin (1202 Dalton) is an example (20), although this is not without controversy (21). Atopic dermatitis forms the exception to the 500

Dalton rule, since it can be managed by topical application of tacrolimus and ascomycin derivatives (822 and 811 Dalton respectively).

For pharmaceutical development purposes, it seems logical to restrict the development of new innovative compounds to a MW of under 500 Dalton, when topical dermatological therapy or percutaneous systemic therapy or vaccination is the objective. We therefore propose the 500 Dalton rule for the skin penetration of chemical compounds and drugs. We believe that in drug development, a maximum molecular weight of 500 Dalton should be adhered to, before considering its further development for topical therapy or transcutaneous vaccination in man.

Acknowledgement

We wish to thank Ramzi Tubbeh of Novartis Pharma (Basel) for his help in collecting data for the list of most commonly used drugs in dermatology.

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County Clerk

From: Joe DiNardo <jmjdinardo@aol.com>
Sent: Sunday, November 26, 2017 6:47 AM
To: IEM Committee; County Clerk
Cc: cadowns@haereticus-lab.org
Subject: Dermatology Paper - Oxybenzone Review 2 of 4
Attachments: 11 Fishers Contact Dermatitis 2.pdf; 12 Gonzalez et al Absorption_of_BP3.pdf; 13 Danovaro et al Sunscreen Cause Coral Bleaching.pdf; 14 Hawaii Ban SB 1150.docx; 15 Tsui_et_al._2014b.pdf

Aloha Chair and Maui County Council,

Mahalo Nui Loa for inviting us to provide testimony before your committee. It was an honor and privilege.

We are submitting the attached studies to you, as requested by the Infrastructure and Environmental Management committee after our presentations on Oxybenzone and Octinoxate's effects on marine life and human health. After reviewing the attached studies, we are confident that you will all feel comfortable with your vote to support the legislation to ban the sale of SPF Sunscreen products containing Oxybenzone and/or Octinoxate.

Mahalo,

Craig Downs – Executive Director – Haereticus Environmental Laboratory

Joe DiNardo – Retired Personal Care Industry Toxicologist & Formulator

Notes:

- Because of the size of the files there will be several Emails sent per topic; all will be numbered appropriately.

- The first Email on the topic will contain the main article (Dermatology Paper – Oxybenzone Review, Oxybenzone HEL Monograph or Octinoxate HEL Monograph) and the references used to support the main article will be included.

- Chemical names used in the attached research papers may vary – please feel free to ask us to clarify any concerns you may have associated with terminology:

1) Oxybenzone = Benzophenone-3 (BP-3) and metabolites maybe noted as Benzophenone-1 and 4-Methylbenzophenone

2) Octinoxate = Ethylhexyl Methoxycinnamate (EHMC) = Octyl Methoxycinnamate (OMC)

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gan alone, whereas cross-photoallergic reactions may occur between both Phenergan and Thorazine (41).

Sulfanilamide

This antibacterial agent can apparently also produce both phototoxic and photoallergic reactions (42). The phototoxic reactions are also produced by systemic administration of the drug. Window glass usually protects the individual from such phototoxic reactions. However, the topical application of sulfanilamide preparations may engender photoallergic contact sensitivity. Fortunately, topical sulfanilamide preparations are not commonly used at this time. Other topically applied sulfonamide compounds, such as sulfacetamide, sulfathiazole, and sulfadiazine are apparently not photosensitizers. Hypoglycemic sulfonamides such as chloropropamide (Diabinese) and tolbutamide (Orinase), thiazide diuretics, such as chlorothiazide (Diuril) and hydrochlorothiazide (Esidrix, HydroDIURIL, and Oretic), and quinethazone (Hydromox), however, may produce phototoxic, photoallergic, and cross-photosensitization reactions when administered systemically (43).

SUNSCREENING AGENTS

Sunscreen agents have become the most common causative substances of photoallergic contact dermatitis in the U.S (7). These chemicals are also able to cause "regular" allergic contact dermatitis without UV light exposure. A list of the most commonly used sunscreening agents is given in Table 23.6. Many sunscreen lotions contain two or more active ingredients to provide a broader spectrum of photoprotection. In addition, many cosmetic products and moisturizing creams incorporate a sunscreening agent.

PARA-AMINOBENZOIC ACID (PABA) AND ITS ESTERS

These chemicals are among the earliest sunscreening agents and are still fairly commonly used. They are primarily effective at blocking UVB light. Some persons report a transient stinging or burning reaction upon application, especially to sensitive skin areas. This is much more common than a true allergy but may lead the patient to to believe that he is "allergic" to the chemicals. A number of sunscreen lotions are mistakenly marketed as being "hypoallergenic" because they contain no PABA.

DeLeo et al. (7) found 3 of 187 patients with allergic contact dermatitis to PABA or octyldimethyl PABA (PABA-0), 3 with photoallergic contact dermtitis to PABA, and 2 with combined photoallergic contact dermatitis and allergic contact dermatitis, almost 4% of their patients tested and 22% of the 37 positives with any positive reaction. They also reported 3 of 11 patients tested with pentil-dimethyl PABA had a reaction of either allergic contact dermatitis, photoallergic contact dermatitis, or both (7). In contrast, 54 suspected photosensitive cases were tested by Lenique et al. (44) in France with no reaction to PABA, and only 1 to PABA-0. However, seven were positive to oxybenzone (see below).

Many patients with positive patch tests to PABA may react due to cross-sensitivity between other para-amino substances such as para-phenylenediamine (PPDA) or benzocaine. Theeuwes et al. (45) showed that these reactions may not be clinically relevant. Fifty-four of 74 patients with positive reactions to a sunscreening agent (not photoreaction) had no history of problems from cosmetics or sunscreens. All 54 were positive to either PABA or PABA-0. Also, 46 of 328 patients reactive to PPDA and 51 of 180 benzocaine allergic patients were positive to PABA or PABA-0.

OXYBENZONE

Oxybenzone also known as benzophenone-3 and Eusolex 4360 is the most frequently utilized benzophenone in sunscreens. It is also the most common sunscreen agent to cause photoallergic contact dermtitis. Of the 54 patients treated by Lenique et al., 7 (13%) reacted to oxybenzone. There were three cases of pure photoallergic contact dermatitis, two cases of allergic contact dermatitis, and two cases with both.

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Percutaneous absorption of the sunscreen benzophenone-3 after repeated whole-body applications, with and without ultraviolet irradiation

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Conflicts of interest:

None declared.

Summary

Background Benzophenone-3 (BZ-3; 2-hydroxy-4-methoxybenzophenone, oxybenzone) is commonly used to absorb ultraviolet (UV) radiation. BZ-3 penetrates the skin and can be found in the urine. The amount varies between 0·4% and 2%. This seems to be the main metabolic pathway in rats.

Objectives To investigate the total amount of BZ-3 excreted in the urine after repeated topical whole-body applications of a sunscreen and to see if UV radiation has any effect on the amount excreted.

Methods Twenty-five volunteers applied a commercially available sunscreen containing 4% BZ-3 morning and night for 5 days. Their urine was measured during those 5 days and during a further 5 days after the last application. They were divided into groups A (unirradiated) and B. Group B received UV radiation according to skin type: UVA between 400 and 707 J cm^{-2}, and UVB between 0·46 and 2·0 J cm^{-2}. BZ-3 in urine was analysed with a high-performance liquid chromatography method.

Results The volunteers excreted 1·2–8·7% (mean 3·7%) of the total amount of BZ-3 applied. There was no significant difference between the two groups ($P < 0·99$, t-test).

Conclusions We show that a large amount of BZ-3 is absorbed. BZ-3 is accumulated in the body as the volunteers excreted BZ-3 5 days after the last application.

Sunscreens have been used for many years to protect against the adverse effects of ultraviolet (UV) radiation such as photoageing, erythema, and possibly skin cancer. It is mainly UVB that is responsible for erythema and sunburn. The first commercial sunscreens appeared in the 1920s.[1] Initially sunscreens were designed to protect against erythema but, during the last decade, sunscreens have also been used for protection against photoageing, photosensitivity, skin cancer and damage from free radicals.[2,3] Sunscreens are often applied to large areas of the body. It is recommended to apply them frequently and to reapply them after contact with water, and hence large amounts of sunscreens are used. Sunscreens are sometimes incorporated in everyday products, e.g. moisturizers and hair products; thus consumers use sunscreens without being aware of it. Systemic absorption is a factor to consider, as the use of sunscreens is widespread. Benzophenone-3 (BZ-3; 2-hydroxy-4-methoxybenzophenone, oxybenzone) is a popular component in sunscreens as it blocks both UVA and UVB. Previous studies of the *in vivo* absorption show that BZ-3 is absorbed by the skin and excreted in the urine. The amount varies between 0·4% and 2%.[4–6] In our previous study we showed that BZ-3 can be found in the urine up to 48 h after one single application of sunscreen.[7] The aim of the present study was to investigate the systemic absorption after several applications of sunscreen and to measure the excretion of BZ-3 in urine.[8] We also wanted to investigate whether there was a difference in the absorption depending on exposure to UV radiation.

Materials and methods

Twenty-five volunteers (16 women and nine men; mean age 27 years, range 22–42) participated in the study. They were instructed to use no medications and to avoid sunbathing. They were randomly divided into two groups, A and B. Height and weight were measured. Their body surface area (BSA) was calculated with the DuBois formula: $BSA = 0·007184 \times [\text{height (cm)}]^{0·725} \times [\text{weight (kg)}]^{0·425}$. The sunscreen used was a commercially available sunscreen, sun protection factor 14, containing 4% BZ-3. The sunscreen also contained the active compounds ethylhexyl methoxycinnamate

(8%) and butyl methoxydibenzoylmethane (2%), and the rest of the ingredients were isopropyl myristate, C12–15 alkyl benzoate, acrylates/C10–30, alkyl acrylate cross-polymer, stearic acid, glycerine, methyl paraben, propyl paraben, hydroxypropyl methyl cellulose and NaOH.

Before the first application of sunscreen each volunteer gave a urine sample to confirm that no BZ-3 was present in the urine prior to this investigation. Each volunteer received 2 mg cm^{-2} of sunscreen according to his or her BSA. The sunscreen was distributed in plastic containers, one for each application. The amount of sunscreen per application varied among the participants from 26 g to 47 g. The total amount of BZ-3 varied between 10·4 g and 18·8 g. BZ-3 was measured in the urine. One volunteer was excluded because the written instructions were not followed accurately.

Group A volunteers were instructed to apply the sunscreen evenly over the entire body, with the exception of the scalp and genital area, morning and night for 5 days, a total of 10 times. They were allowed one shower per day, before the second application. During the 5 days the sunscreen was applied, all urine was collected, the volume measured and 10 mL from each sample saved and stored at −70 °C. Hence, each volunteer produced a different number of urine samples. They collected the urine for the 5 days they applied sunscreen, and after the last application they continued to collect urine for a further 5 days, making a total of 10 days. The time of day, number and volume for each urine sample were recorded.

Group B volunteers were given the same instructions as group A, but they also received UV irradiation. The time for the irradiation varied between 09.00 and 15.00 h. For UVA irradiation, a Dermalight Ultra A1, equipped with six light tubes, Dr Hönle 200 W (Martinsreid, Germany), was used. The doses given were: day 1, 60 J cm^{-2}; day 2, 80 J cm^{-2}; day 3, 100 J cm^{-2}; day 4, 100 J cm^{-2}; day 5, 100 J cm^{-2}. The 60 J cm^{-2} irradiation took 34 min, 17 min on each side of the body. If erythema occurred, the UVA dose given was the same as on the previous day. The total dose of UVA varied among the participants, between 400 and 707 J cm^{-2}. The doses correspond to approximately 3 full days outdoors during summertime in Sweden, according to data verified by the Swedish Meteorological and Hydrological Institute.

For UVB irradiation, an Esshå Corona IV, equipped with 28 light tubes, Philips UVB TL 40 W/12 (Eindhoven, the Netherlands), was used. The volunteers received UVB irradiation according to Fitzpatrick skin type, and to avoid severe erythema we followed a standardized schedule for patients with psoriasis used at the Sahlgrenska University Hospital. The participants belonged to Fitzpatrick groups I–III. The dose is approximately 195 mJ cm^{-2} for 30 s, and the total dose of UVB varied among the participants from 0·46 to 2·0 J cm^{-2}. According to skin type the participants received a start dose of UVB, and if no adverse effects occurred the schedule was followed with increasing doses according to Table 1. The UV radiation was measured with a Sola-Hazard spectroradiometer provided by the Swedish Radiation Protection Authority.

Table 1 Ultraviolet B dose schedule used in this study

Skin type	Start dose	Dose increase up to 5 min	Dose increase 5–15 min
I	15 s	10 s	
II	30 s	15 s	30 s
III	45 s	20–30 s	60 s

BZ-3 was analysed by using high-performance liquid chromatography with UV detection.[9,10] The method was also further developed to increase the sensitivity (Gonzalez *et al.*, unpublished). Each urine sample from each individual was analysed separately, the concentration was calculated, corrected for total volume and subsequently summarized to give the 24 h excretion. Only 10 mL of urine was stored, but as participants measured the whole volume each time a sample was produced the amount of BZ-3 in the total volume could be calculated. Each urine sample was analysed for conjugated and nonconjugated BZ-3. The study was performed at the Department of Dermatology, Sahlgrenska University Hospital, and approved by the Local Ethics Committee.

Results

There was a large variation in the total amount of BZ-3 excreted in the urine. Even after compensating for the differences in BSA and thus the amounts applied, the variation was substantial. In relative terms, the total amount of urinary excreted BZ-3 during the 10-day period ranged from 1·2% to 8·7% of the total amount applied (Fig. 1).

UV exposure did not significantly affect the urinary excretion of BZ-3. There was no significant difference between the two groups ($P < 0{\cdot}99$; differences were compared by Student's t-test).

BZ-3 is conjugated with glucuronic acid, probably in the liver, to make it water-soluble. For this study we measured

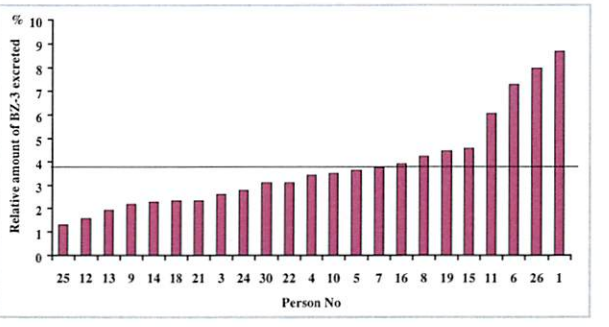

Fig 1. Urinary excretion of conjugated and nonconjugated benzophenone-3 (BZ-3) after 10 days varies among the subjects, and ranges between 1·2% and 8·7% of the total amount applied. The mean value of 3·7% is shown as a horizontal line. The nonconjugated part of BZ-3 was between 0·1% and 0·7% of the total amount of BZ-3 excreted.

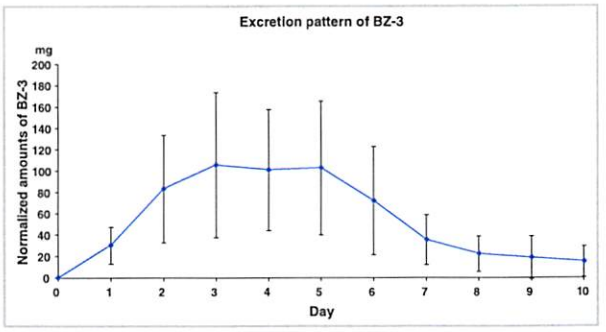

Fig 2. Excretion pattern of benzophenone-3 (BZ-3) during 5 days of sunscreen application and the subsequent 5 days. Values are normalized with respect to body area. The normalization method used was: [amount of BZ-3 excreted (mg) ÷ amount of BZ-3 applied (mg)] × 15. Results are shown as mean ± SD.

both conjugated and nonconjugated BZ-3 and found that the nonconjugated form was present to a minor but considerable extent. As much as 0·1–0·7% of the total amount applied during the course of the study was excreted in a nonmetabolized form.

The second phase of the study, during which no sunscreen lotion was applied, indicates a substantial accumulation of BZ-3 (Fig. 2). On the fifth day after the last application the participants excreted 5–15 mg of BZ-3, which represents a significant amount (Fig. 2).

Discussion

This study has investigated the amount of BZ-3 excreted in urine after several whole-body applications of a sunscreen containing BZ-3. As the recommended amount of sunscreen is 2 mg cm^{-2} and people have different skin areas, we wanted each individual to have the appropriate amount of sunscreen. The mean value of BZ-3 found in urine was 3·7%, which is higher than in previously described studies.[4,5] This study presents evidence that repeated applications of BZ-3 increase the concentration of BZ-3 found in urine. These findings may be of special concern when it comes to sunscreens containing BZ-3 used by younger children. Children under the age of 1 year have a greater ratio of BSA to body weight; hence the absorption might be even greater for them. There are other studies investigating BZ-3, e.g. Janjua *et al.*[6] have investigated the systemic absorption of three active sunscreens, BZ-3, octyl methoxycinnamate and 3-(4-methyl-benzylidene) camphor: they focused on their effects on the levels of endogenous reproductive hormones and on whether the active ingredients could be found in plasma and urine, but did not intend to estimate the amount absorbed, in contrast to our study. The possible oestrogenic effect of BZ-3 has been discussed; several studies have dealt with this problem but the results are inconclusive.[6,11,12]

Sarveiya *et al.* have also studied the penetration of common sunscreen agents.[5] In their study they found that up to approximately 1% of the applied dose of BZ-3 was found in urine. However, the area of application of sunscreen was approximately 864 cm^{-2}, and they concluded that one could apply to at least double that area in a beach sunbathing situation, and thus the total systemic absorption of BZ-3 could be higher in practice. In our study we have shown that repeated topical applications over large areas of the body give a higher total amount of systemic absorption.

Our study has measured BZ-3 exclusively in urine, but this may be an underestimate of the skin penetration, as some BZ-3 may be tissue-bound and excretion via other routes was not measured. In addition, sunscreen can be absorbed by clothes. We also wanted to investigate whether UV irradiation had any effect on the absorption of BZ-3, as sunscreens are designed to be used under those circumstances. The UV radiation doses were chosen to simulate 5 days outdoors in Sweden during summertime. The doses were adjusted to prevent harmful effects to the volunteers, and hence the UV radiation doses are not as high as may be encountered in a real-life situation.

There seems to be no effect of UV radiation on the uptake of BZ-3; there was no statistically significant difference in excretion between the groups. This finding may depend on factors such as time of irradiation: the volunteers received the UV irradiation at lunchtime, whereas the sunscreen was applied night and morning, several hours earlier. Some BZ-3 might then already have penetrated the skin. If the sunscreen had been applied more shortly before irradiation, there might have been a significant difference and factors such as degradation of BZ-3 on the skin surface due to irradiation would have been more directly studied. However, in earlier studies, BZ-3 has proven to be reasonably photostable; other sunscreens have proven to be unstable.[13] Temperature can also play a role in absorption with UV filters, but the study by Clarys *et al.* showed no such relation.[14] Other compounds such as dihydrotestosterone showed a positive relation between temperature and skin penetration.[15]

Most of the BZ-3 excreted in urine was in the conjugated form; this is consistent with previous studies showing that BZ-3 undergoes extensive conjugation in the body. Strassburg *et al.*[16] have studied the developmental aspects of human hepatic drug glucuronidation in young children and adults. They found that the hepatic glucuronidation activity in children aged 13–24 months was lower than in adults for several drugs. The differential regulation of some subtypes of uridine 5′-diphosphate-glucuronosyltransferase (UGT) expression extends beyond 2 years of age and the development of hepatic UGT is significant for the prevention of adverse drug effects.[16] The authors did not particularly study BZ-3, but as BZ-3 undergoes extensive glucuronidation in the body their results are likely to be relevant for BZ-3 as well. It is still important not to use sunscreens with BZ-3 on young children. Okereke *et al.* found BZ-3 in liver, spleen, heart, kidney and testes in rats.[8]

The individual differences in excretion of BZ-3 were quite large, and this may be due to differences in enzyme activity. First, the activity of glucuronidation may be an explanation;

secondly, Okereke *et al.* have also shown that cytochrome P-450 metabolizes BZ-3 to 2,4-dihydroxybenzophenone.[8] Another factor that might influence the uptake is the condition of the skin: if the skin is dry, more of the lotion and also more BZ-3 might be absorbed.

The present study also emphasizes the fact that the volunteers excreted BZ-3 in urine many days after the last application, as is expected with a lipid-soluble substance. In this study, none of the 24 volunteers reached their reference value 5 days after the last application; all of them still excreted BZ-3, although in small amounts. In our previous study, four of 10 volunteers excreted BZ-3 in urine 48 h after one single application.[7]

Protection against the harmful effects of UV radiation is important, but we have to be certain that the sunscreens used are safe. Clothes are the best choice for protection, and sunscreens should be used as a complement.

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Sunscreens Cause Coral Bleaching by Promoting Viral Infections

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BACKGROUND: Coral bleaching (i.e., the release of coral symbiotic zooxanthellae) has negative impacts on biodiversity and functioning of reef ecosystems and their production of goods and services. This increasing world-wide phenomenon is associated with temperature anomalies, high irradiance, pollution, and bacterial diseases. Recently, it has been demonstrated that personal care products, including sunscreens, have an impact on aquatic organisms similar to that of other contaminants.

OBJECTIVES: Our goal was to evaluate the potential impact of sunscreen ingredients on hard corals and their symbiotic algae.

METHODS: *In situ* and laboratory experiments were conducted in several tropical regions (the Atlantic, Indian, and Pacific Oceans, and the Red Sea) by supplementing coral branches with aliquots of sunscreens and common ultraviolet filters contained in sunscreen formula. Zooxanthellae were checked for viral infection by epifluorescence and transmission electron microscopy analyses.

RESULTS: Sunscreens cause the rapid and complete bleaching of hard corals, even at extremely low concentrations. The effect of sunscreens is due to organic ultraviolet filters, which are able to induce the lytic viral cycle in symbiotic zooxanthellae with latent infections.

CONCLUSIONS: We conclude that sunscreens, by promoting viral infection, potentially play an important role in coral bleaching in areas prone to high levels of recreational use by humans.

KEY WORDS: bleaching, corals, sunscreens, UV filters, viruses. *Environ Health Perspect* 116:441–447 (2008). doi:10.1289/ehp.10966 available via *http://dx.doi.org/* [Online 3 January 2008]

Coral reefs are among the most biologically productive and diverse ecosystems in the world, representing hot spots of marine biodiversity, and directly sustaining half a billion people (Moberg and Folke 1999; Wilkinson 2004). Approximately 60% of coral reefs are currently threatened by several natural and anthropogenic impacts (Hughes et al. 2003; Pandolfi et al. 2003). Over the last 20 years, massive coral bleaching (i.e., loss of symbiotic zooxanthellae hosted within scleractinian corals) has increased dramatically, both in frequency and spatial extent (Hoegh-Guldberg 1999; Hughes et al. 2003; Knowlton 2001). This phenomenon has been associated with positive temperature anomalies, excess ultraviolet (UV) radiation or altered available photosynthetic radiation, and presence of bacterial pathogens and pollutants (Brown et al. 2000; Bruno et al. 2007; Douglas 2003; Glynn 1996; Jones 2004).

Production and consumption of personal care and cosmetic sun products are increasing worldwide, reaching unexpected levels, with potentially important consequences on environmental contamination. The release of these products is also linked with the rapid expansion of tourism in marine coastal areas (Wilkinson 2004). Chemical compounds contained in sunscreens and other personal care products have been demonstrated to reach detectable levels in both fresh and seawater systems (Daughton and Ternes 1999; Giokas et al. 2007). These compounds are expected to be potentially harmful for the environment; hence, the use of sunscreen products is now banned in a few popular tourist destinations, for example, in marine ecoparks in Mexico, and in some semi-enclosed transitional systems (Xcaret 2007; Xel-ha 2007). Because sunscreens are lipophilic, their UV filters can bioaccumulate in aquatic animals (Giokas et al. 2007) and cause effects similar to those reported for other xenobiotic compounds (Balmer et al. 2005; Daughton and Ternes 1999). Paraben preservatives and some UV absorbers contained in sunscreens have estrogenic activity (Daughton and Ternes 1999; Schlumpf et al. 2004). In addition it has been demonstrated that several sunscreen agents may undergo photodegradation, resulting in the transformation of these agents into toxic by-products (Giokas et al. 2007, and literature therein).

Recently, it has also been demonstrated that sunscreens have an impact on marine bacterioplankton (Danovaro and Corinaldesi 2003), but there is no scientific evidence for their impact on coral reefs.

To evaluate the potential impact of sunscreen ingredients on hard corals and their symbiotic algae, we conducted several independent *in situ* studies with the addition of different concentrations of sunscreens to different species of *Acropora* (one of the most common hard-coral genus), *Stylophora pistillata,* and *Millepora complanata.* These studies were performed from 2003 to 2007 in different areas of the world, including the Celebes Sea (Pacific Ocean), the Caribbean Sea (Atlantic Ocean), and the Andaman Sea and the Red Sea (Indian Ocean).

Materials and Methods

Study areas and experimental design. In situ experiments were conducted in four coral reef areas: Siladen, Celebes Sea (Indonesia, Pacific Ocean); Akumal, Caribbean Sea (Mexico, Atlantic Ocean); Phuket, Andaman Sea (Thailand, Indian Ocean), and Ras Mohammed, Red Sea (Egypt, Indian Ocean). Nubbins of *Acropora* spp. (~ 3–6 cm) were collected, washed with virus-free seawater filtered onto 0.02-μm membranes (Anotop syringe filters; Whatman, Springfield Mill, UK), immersed in polyethylene Whirl-pack bags (Nasco, Fort Atkinson, WI, USA) filled with 2 L virus-free seawater, and incubated *in situ.* Additional experiments were also performed with other hard coral genera: *S. pistillata* and *M. complanata.* Replicate sets containing nubbins from different colonies (n = 3, including more than 300 polyps each) were supplemented with aliquots of sunscreens (at final quantities of 10, 33, 50, and 100 μL/L seawater) and compared with untreated systems (used as controls). Corals were incubated at the same depth of donor colonies at *in situ* temperature (Table 1). During two experiments conducted in the Red Sea and in the Andaman Sea, we tested the effects on coral bleaching of the same chemical filters and preservatives contained in the sunscreen formula of different brands (Tables 1 and 2). Subsamples (50 mL) of seawater surrounding coral nubbins were collected at 12-hr intervals and fixed in 3% glutaraldehyde for subsequent

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analyses (i.e., zooxanthellae counts and transmission electron microscopy, TEM). Additional seawater samples were immediately processed without any preservation for viruslike particles counts. At the end of the experiments, samples of coral tissue were fixed in 3% glutaraldehyde and stored at 4°C for zooxanthellae count and TEM.

Quantification of bleaching. To quantify the levels of coral bleaching (Siebeck et al. 2006), we performed a colorimetric analysis on digital photographs of corals taken at the beginning of the experiments and after various times of treatment with sunscreen and organic UV filters. Photographs were taken under identical illumination with a Canon PowerShot A620 digital camera (Canon Inc., Tokyo, Japan) with a scale meter on the background. The photographs were successively analyzed with a photo-editing software for color composition [cyan, magenta, yellow, black (CMYK)]. Levels of bleaching were measured as the difference between the coral's color at the beginning of the experiments and after treatments. Variations in the percentage of the different color components (CMYK) were analyzed with one-way analysis of variance (ANOVA; Table 3). To rank the bleaching effect due to the different ingredients tested, we obtained Bray–Curtis similarity matrix and multidimensional scaling analysis of the shifts in CMYK color composition of treated corals using Primer 5.0 software (Primer-E Ltd., Plymouth, UK). Bleaching rates were measured as the dissimilarity percentage in CMYK color composition between treated and control corals using the SIMPER tool of Primer 5.0 software (Primer-E Ltd).

Analysis of zooxanthellae. Zooxanthellae were extracted from coral nubbins using a jet of artificial seawater with a WaterPick (Braun, Germany) and centrifuged (4,000 × *g*, for 10 min) to separate the algae from the host tissue. Replicate suspensions (200–500 µL) of zooxanthellae extracted from coral tissue and those released during the experiment were filtered through 2.0-µm polycarbonate filters and mounted on glass slides. Zooxanthellae were counted under a Zeiss Axioplan epifluorescence microscope (Carl Zeiss Inc., Jena, Germany; ×400 and ×1,000), and the number of cells was normalized to nubbins' area. Based on the autofluorescence and gross cell structure, zooxanthellae released or extracted from nubbins were classified as *a*) healthy (H, brown/bright yellow color, intact zooxanthellae); *b*) pale (P, pale yellow color, vacuolated, partially degraded zooxanthellae); transparent (T, lacking pigmentations, mostly empty zooxanthellae; Mise and Hidaka 2003). Cell integrity was also examined by TEM (see below).

Standard sunscreen UV filters for the experiments. The UV filters ethylhexylmethoxycinnamate (OMC), octocrylene (OCT), benzophenone-3 (BZ), ethylhexylsalicylate (EHS), and the solvent propylene glycol (PG) (Table 2) were purchased from Sigma-Aldrich Co. (Milan, Italy); 4-*tert*-butyl-4-methoxydibenzoylmethane was obtained in the form of Eusolex 9020 from Merck (Darmstadt, Germany). 4-Methylbenzylidene camphor was synthesized according to Saito et al. (2004). Specifically, a mixture of *d*-camphor (10 mmol), *p*-tolualdehyde (12 mmol), and potassium *t*-butoxide (15 mmol) was refluxed in *t*-butyl alcohol (12 mL) for 5 hr. The reaction course was monitored by thin-layer chromatography using cyclohexane–ethyl acetate 8:2 as the eluant. The reaction mixture was neutralized with 5% HCl and extracted with ethyl acetate (10 mL × 3); the combined organic extracts were washed with saturated NaCl solution and dried over Na_2SO_4. Evaporation of the solvent and column chromatography of the crude residue on silica gel

Table 1. Experiments on hard-coral species treated with different sunscreens and sunscreen ingredients.

Ocean	Reef area	Reef water temperature (°C)	Treatments	Sun protecting factor	Quantity [µl/L (%)][a]	Species	No. of experimental sets	Bleaching initiation (hr)	Bleaching rate [hr (%)][b]	Zooxanthellae released (%)
Pacific	Celebes Sea, Indonesia	28, 30[c]	Sunscreen brand 1	15	100	*Acropora divaricata*	6	ND	24 (81, 95)	ND
			Sunscreen brand 1	15	10	*A.divaricata*	6	ND	36 (ND)	ND
			Nutrients		100[d]	*A. divaricata*	6	No bleaching	No bleaching	ND
			Controls			*A. divaricata*	6	No bleaching	No bleaching	ND
Atlantic	Caribbean Sea, Mexico	28	Sunscreen brand 2	8	10	*Acropora cervicornis*	3	18	36 (84)	87
			Controls			*A. cervicornis*	3	No bleaching	No bleaching	3
			Sunscreen brand 2	8	10	*Millepora complanata*	3	24	36 (35)	10
			Controls			*M. complanata*	3	No bleaching	No bleaching	2
Indian	Red Sea, Egypt	24	Sunscreen brand 1	8	33	*Acropora* sp.	3	24	48 (81)	44
			Sunscreen brand 1	15	33	*Acropora* sp.	3	24	48 (89)	30
			Controls			*Acropora* sp.	3	No bleaching	No bleaching	1
			Sunscreen brand 1	15	33	*Stylophora pistillata*	3	nd	48 (65)	ND
			Controls			*S. pistillata*	3	No bleaching	No bleaching	ND
			BMDBM		33 (2)	*Acropora* sp.	3	No bleaching	No bleaching	13
			MBC		33 (3)	*Acropora* sp.	3	24	48 (63)	10
			OCT		33 (6)	*Acropora* sp.	3	No bleaching	No bleaching	3
			EHS		33 (5)	*Acropora* sp.	3	No bleaching	No bleaching	3
			OMC		33 (6)	*Acropora* sp.	3	2	24 (91)	86
			BZ		33 (6)	*Acropora* sp.	3	24	48 (86)	83
			BP		33 (0.5)	*Acropora* sp.	3	24	48 (84)	90
			PG (solvent)		33	*Acropora* sp.	3	No bleaching	No bleaching	16
Indian	Andaman Sea, Thailand	25[e]	Sunscreen brand 3	8	50	*Acropora pulchra, Acropora aspera, Acropora intermedia, Acropora sp.*	15	24	48–62 (74–88)	88–95
			Controls			*A. pulchra, A. aspera, A. intermedia, Acropora sp.*	15	No bleaching	No bleaching	1–2
			MBC		50 (3)	*A. pulchra*	3	48	62 (95)	95
			OMC		50 (6)	*A. pulchra*	3	48	96 (91)	90
			BZ		50 (6)	*A. pulchra*	3	48	96 (93)	84
			BP		50 (0.5)	*A. pulchra*	3	48	96 (90)	79

Abbreviations: BMDBM, 4-*tert*-butyl-4-methoxydibenzoylmethane; BP, butyl paraben; BZ, benzophenone-3; EHS, ethylhexylsalicylate; MBC, 4-methylbenzylidene camphor; ND, not detected; OCT, octocrylene; OMC, ethylhexylmethoxycinnamate; PG, propylene glycol.

[a]Percentage concentrations of the filters allowed in sunscreen formulations in both American and European markets. [b]Bleaching rates measured as percentage chromatic dissimilarity with the coral used as a control (CMYK) at different experiment times (hr). [c]Temperature in outdoor aquarium. [d]Concentrations of nutrients relative to added sunscreen are calculated on the ratio of organic carbon to total nitrogen and phosphorous (wt:wt) of 31:2:1. [e]Local temperature during the experiment was below average season values.

eluting with cyclohexane–ethyl acetate 8:2 gave 4-methylbenzylidene camphor as a white solid which was crystallized from hexane (70% yield). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.8 (s, 3H), 0.99 (s, 3H), 1.03 (s, 3H), 1.48–1.60 (m, 2H), 1.70–1.85 (m, 1H), 2.12–2.20 (m, 1H), 2.37 (s, 3H), 3.10 (d, 1H, J = 4.1 Hz), 7.19 (d, 2H, J = 8.0 Hz). 7.21 (s, 1H), 7.38 (d, 2H, J = 8.0 Hz) ppm. The preservative BP (butyl paraben) was obtained through esterification of 4-hydroxybenzoic acid with butyl alcohol: 20 mmol 4-hydroxybenzoic acid was dissolved in 25 mL butyl alcohol in the presence of a catalytic amount of *p*-toluensulfonic acid (~ 2 mmol) and refluxed for 7 hr. The reaction mixture was washed with $NaHCO_3$ 0.5 M and extracted with diethyl ether (25 mL × 3). The organic layer was dried over Na_2SO_4 and the solvent evaporated under reduced pressure. Butyl paraben was obtained with a 75% yield. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.97 (t, 3H, J = 7.1 Hz), 1.38–1.65 (m, 2H), 1.70–1.76 (m, sH), 4.30 (t, 2H, J = 6.5 Hz), 6.89 (d, 2H, J = 8.88 Hz), 7.95 (d, 2H, J = 8.8 Hz) ppm. The amounts of UV filters and preservatives used in the sunscreen addition experiments were calculated on the basis of the percentage concentrations of the respective filters allowed in sunscreen formulations in both American and European markets. Hence, concentrations below the more restricted limits imposed by American regulations were used: BMDBM (2%), BZ (6%), OMC (6%), OCT (6%), EHS (5%), MBC (3%), BP (0.5%).

Quantification of sunscreen release in seawater. To estimate the amount of UV filters and preservatives released from sunscreen formulae, 2 mg sunscreen/cm^2 [dose recommended by the U.S. Food and Drug Administration (FDA); Poiger et al. 2004] was applied to the hands of two volunteers. The hands were then immersed in 2 L of 0.45-μm filtered seawater at 24°C for 20 min. Hands without sunscreen applications were used as controls. All experiments were repeated 3 times. The percentage of sunscreen released into the seawater was estimated by high performance liquid chromatography (HPLC) analyses on the sunscreen and seawater samples.

Some investigators suggest that the sunscreen dose recommended by the U.S. FDA is much lower than the amount actually used by tourists (Giokas et al. 2007, and literature therein); thus, the quantity of sunscreen released during a usual bath could be far higher than that estimated in this study.

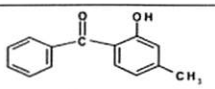
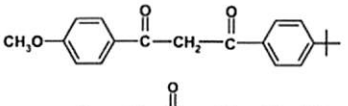
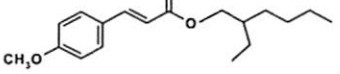
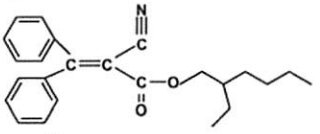
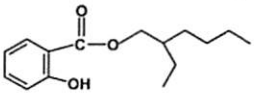
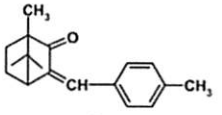
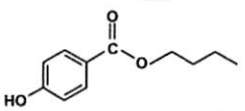
HPLC analysis of sunscreens. UV filters were extracted from 1 L seawater obtained from the sunscreen release experiment by solid-phase extraction (SPE) (C_{18} Bakerbound

Table 3. Shifts in the percentage contribution of the different coral color components [cyan, magenta, yellow, black (CMYK)] that occurred during the experiments (addition of sunscreen and sunscreen ingredients).

Treatments	Coral color shift[a]				Bleaching	Significance[b]
	C	M	Y	K		
Control	0	2	3	0	NV	NS
Sunscreen	19	25	17	33	Visible	****
BMDBM	6	22	12	33	NV	**
BZ	6	24	7	43	NV	**
OMC	13	37	23	53	Visible	***
OCT	7	23	18	39	NV	**
EHS	6	20	7	38	NV	NS
MBC	8	17	5	37	NV	**
BP	9	32	33	29	Visible	***

Abbreviations: NS, none of the four variables is significant; NV, nonvisible bleaching. For acroynm definitions under "Treatment," see Table 1.
[a]Shift estimated as the average of 20 measurement points of the four colorimetric variables (CMYK). [b]Significance ($p < 0.05$) of each variable calculated by ANOVA; number of asterisks indicate the number of significant variables.

Table 2. Physicochemical properties of the UV filters.

Chemical name (INCI name)	Key[a]	Chemical structure	Molecular weight (g/mol)	Water solubility (mg L^{-1}) at 25°C	Log K_{ow}[b]	λ_{max}
2-Hydroxyl-4-methoxybenzophenone (*benzophenone-3*)	BZ		228.25	68.56	3.52	286
4-*tert*-Butyl-4′-Methoxydibenzoyl methane (*butyl methoxydibenzoylmethane*)	BMDBM		310.39	1.52	2.41	355
2-Ethylhexyl-4-methoxycinnamate (*ethylhexylmethoxycinnamate*)	OMC		290.41	0.15	5.80	305
2-Ethylhexyl 2-cyano-3,3-diphenylacrylate (*octocrylene*)	OCT		361.49	1.3	6.88	303
2-Ethylhexyl salicylate (*ethylhexyl salicylate*)	EHS		250.37	NA	6.02	305
3-(4′-Methylbenzylidene) camphor (*4-methylbenzylidene camphor*)	MBC		240.35	0.57	5.47	300
Butyl *p*-hydroxybenzoate[c] (*butylparaben*)	BP		194.23	207	3.57	253

Abbreviations: INCI, International Nomenclature for Cosmetic Ingredients; NA, not available.
[a]Key abbreviations adopted in this paper. For acronym definitions," see Table 1. [b]Octanol/water partition coefficient. [c]This is a preservative, not a UV filter.

SPE column, 500 mg/6 mL; J.T. Baker, Phillipsburg, NJ, USA). Before extraction an internal standard, butyl-cinnamate (BC, Sigma-Aldrich Co.) was added to the seawater sample. The SPE column was conditioned with 10% methanol, and the sample was passed through the column at approximately 20 mL/min. The ingredients were recovered from the column using 1 mL acetonitrile. Analyses were performed on an HPLC apparatus consisting of a Varian RP-C18 column (5 µm, 250 × 4.60 mm), a 20-µL injection loop, a Varian Pro Star solvent delivery module, a Varian Star 5.0 Workstation and Varian 9050 variable wavelength UV-VIS detector (Varian Inc., Palo Alto, CA, USA). The analytes injected into the chromatograph eluted in 18 min (1 mL/min) using a linear gradient starting from solution A (methanol:acetonitrile:water:acetic acid, 55:20:24:1, vol/vol) and ending with solution B (methanol:acetonitrile:water:acetic acid, 55:40:4:1, vol/vol). UV detection was carried out at λ = 255 nm for BP and λ = 300 nm for MBC, OMC and BC. Chromatograms were analyzed with the Varian Interactive Graphics Program.

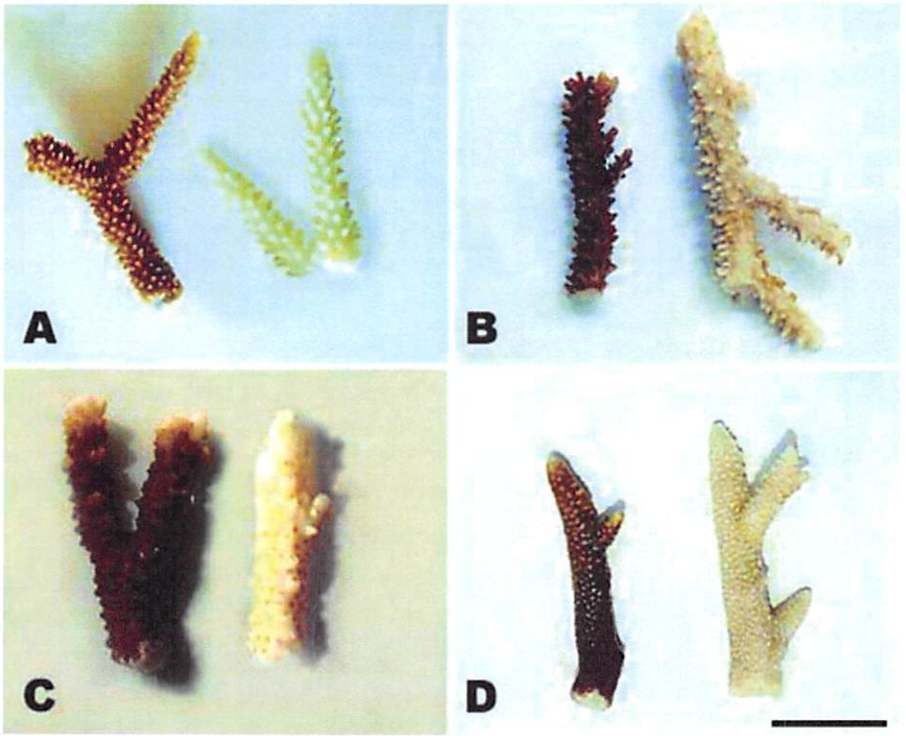


Figure 1. Impact of sunscreen addition on nubbins of *Acropora*. Untreated (brown) and treated (bleached) nubbins of (*A*) *Acropora cervicornis* (Caribbean Sea, Mexico); (*B*) *Acropora divaricata* (Celebes Sea, Indonesia); (*C*) *Acropora* sp. (Red Sea, Egypt); and (*D*) *Acropora intermedia* (Andaman Sea, Thailand). Images were taken within 62 hr of the start of sunscreen incubations. Scale bar = 2 cm.

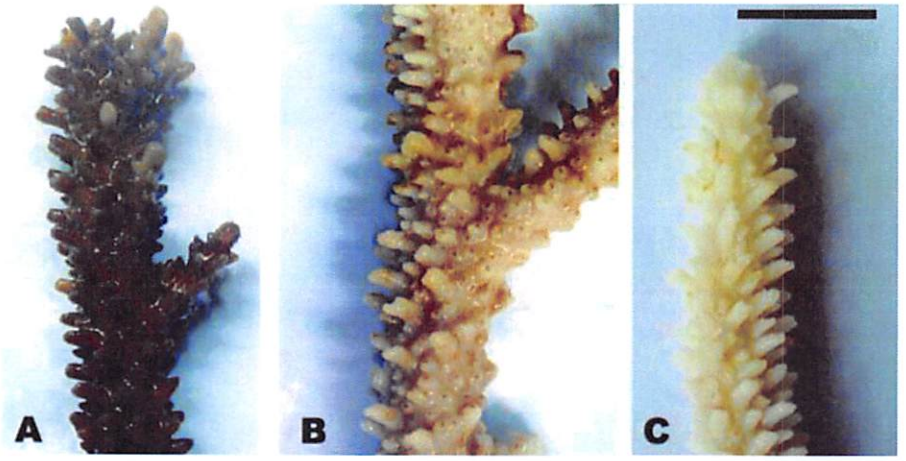


Figure 2. Effect of 100-µL sunscreens on *Acropora divaricata* nubbins after 24-hr incubation at various temperatures. (*A*) control; (*B*) nubbins incubated at 28°C; and (*C*) nubbins incubated at 30°C. Scale bar = 1 cm.

Viral counts and infection of zooxanthellae and TEM analysis. Water samples for viral counts were processed immediately without any fixative with SYBR green and SYBR Gold staining (Shibata et al. 2006). Immediately after collection, subsamples (200 µL) of seawater surrounding coral nubbins were diluted 1:10 in prefiltered MilliQ, filtered through a 0.02-µm pore-size Anodisc filter (25-mm diameter, Al_2O_3; Whatman) and immediately stained with 20 µL SYBR Green I and SYBR Gold (stock solution diluted 1:20 and 1:5,000 respectively; Invitrogen, Carlsbad, CA, USA). Filters were incubated in the dark for 15 min and mounted on glass slides with a drop of 50% phosphate buffer (6.7 mM, pH 7.8) and 50% glycerol containing 0.25% ascorbic acid (Shibata et al. 2006; Helton et al. 2006; Wen et al. 2004). Slides were stored at 20°C until analysis. Counts were obtained by epifluorescence microscopy (magnification, ×1,000; Zeiss Axioplan) by examining at least 10 fields, that is, at least 200 cells or particles per replicate.

TEM analyses were conducted on decalcified corals (2% vol/vol formic acid, 4°C, 8 days. *Acropora* tissue and pellets of zooxanthellae released during the experiment were post-fixed in 1% osmium tetroxide (Sigma-Aldrich Co.), dehydrated through an increasing acetone series (25%, 50%, 75%, 100%) and embedded in an Epon–Araldite mixture (Multilab Supplies, Fetcham, UK). Ultrathin resin sections (50–70 nm) were cut with a Reichert Ultracut E microtome (Reichert, Wien, Austria). Before analysis, sections were stained with saturated uranyl acetate and 1% lead citrate and collected on 200-mesh copper/rhodium grids (Multilab Supplies).

Estimates of release of sunscreen in reef areas. The global release of sunscreens in areas harboring coral reefs can be roughly estimated from their average daily use and the number of tourists. An average dose application of 2 mg/cm^2 of sunscreen (dose suggested by the U.S. FDA) for a full body surface of 1.0 m^2 results in an average usage of 20 g per application (Poiger et al. 2004). We consider a conservative measure of two daily applications per tourist traveling on a 5-day average tourist package, and a rough estimate of 78 million of tourists per year in areas hosting reefs [10% of world tourists registered in 2004; United Nations World Trade Organization (UNWTO) 2007]. Based on this calculation and on annual production of UV filters, between 16,000 and 25,000 tons of sunscreens are expected to be used in tropical countries. According to our experiment, it is estimated that at least 25% of the amount applied is washed off during swimming and bathing, accounting for a potential release of 4,000–6,000 tons/year in reef areas. Because 90% of tourists are expected to be concentrated in approximately 10% of the total reef areas,

we estimated that up to 10% of the world reefs is potentially threatened by sunscreen-induced coral bleaching.

Results and Discussion

Coral bleaching caused by sunscreens and UV filters. In all replicates and at all sampling sites, sunscreen addition even in very low quantities (i.e., 10 µL/L) resulted in the release of large amounts of coral mucous (composed of zooxanthellae and coral tissue) within 18–48 hr, and complete bleaching of hard corals within 96 hr (Figure 1; Table 1). Different sunscreen brands, protective factors, and concentrations were compared, and all treatments caused bleaching of hard corals, although the rates of bleaching were faster when larger quantities were used (Table 1). Untreated nubbins (coral branches of 3–6 cm) used as controls did not show any change during the entire duration of the experiments (Table 1). Bleaching was faster in systems subjected to higher temperature, suggesting synergistic effects with this variable (Table 1; Figure 2). TEM and epifluorescence microscopy analyses revealed a loss of photosynthetic pigments and membrane integrity in the zooxanthellae released from treated corals (30–98% of zooxanthellae released from *Acropora* nubbins were partially or totally damaged, appearing pale and transparent), whereas zooxanthellae membranes from untreated corals were intact (37–100% of the zooxanthellae released showed a defined shape and red fluorescing color; Figures 3 and 4). All these results indicate that sunscreens have a rapid effect on hard corals and cause bleaching by damaging the symbiotic zooxanthellae.

We tested sunscreen (10 µL/L) containing concentrations of UV filters higher than those reported in most natural environments. At the same time, the coral response to sunscreen exposure was not dose dependent, as the same effects were observed at low and high sunscreen concentrations. Therefore, we hypothesize that UV filters can have potentially negative impacts even at concentrations lower than those used in the present study.

Sunscreens typically comprise up to 20 or more chemical compounds. To identify the organic UV filters or preservatives possibly responsible for coral bleaching, seven compounds typically present in sunscreens were selected (Table 2), and additional experiments were carried out in which each single ingredient was tested on *Acropora* spp. Among the ingredients tested, butylparaben, ethylhexylmethoxycinnamate, benzophenone-3 and 4-methylbenzylidene camphor caused complete bleaching even at very low concentrations (parabens account for 0.5% of sunscreen ingredients). Conversely, all other compounds tested (i.e., octocrylene, ethylhexylsalicylate, and 4-*tert*-butyl-4-methoxydibenzoylmethane) and the solvent propylene glycol, which is also present in sunscreen formulations, had a minor effect or no effects when compared with controls (Table 1). These results suggest that sunscreens containing parabens, cinnamates, benzophenones, and camphor derivatives can contribute to hard-coral bleaching if released into natural systems.

Amounts of sunscreen released into tropical environments and their impacts. Sunscreen product sales exceed half a billion dollars (Shaath and Shaath 2005), and it is estimated that 10,000 tons of UV filters are produced annually for the global market. According to official data of the UNWTO, it can be estimated that 10% of sunscreens produced are used in tropical areas with coral reefs (Wilkinson 2004). We estimated that, on average, about 25% of the sunscreen ingredients applied to skin are released in the water over the course of a 20-min submersion. According to these estimates, we believe that up to 10% of the world's coral reefs would be threatened by sunscreen-induced coral bleaching.

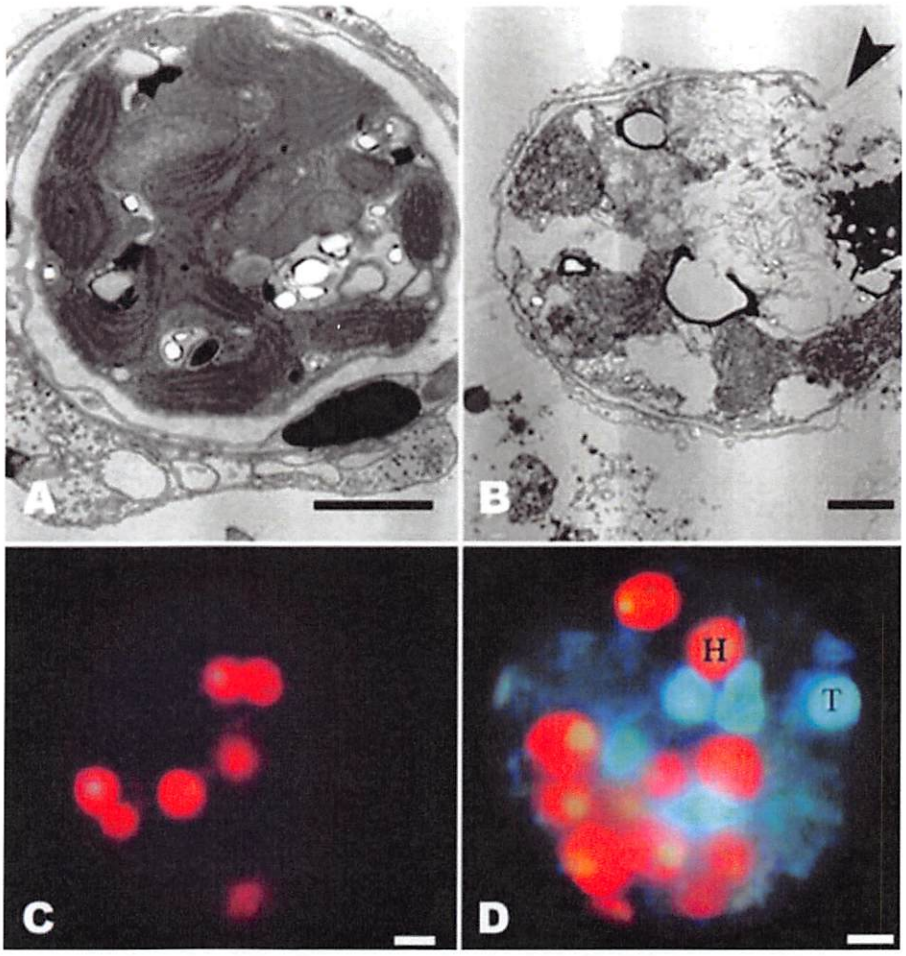


Figure 3. Zooxanthellae release from hard corals in control and sunscreen addition samples. (*A*) TEM images of healthy zooxanthellae (intact cell structure and membrane) in control untreated *Acropora* nubbin, and (*B*) zooxanthellae damaged by sunscreen treatment: cells appear swollen and vacuolated, without chloroplasts and double the size of the controls; the thylakoids are unpacked and dispersed inside the cells, and cell-membrane integrity is lost (arrowhead). (*C*) Autofluorescence images showing healthy (red) zooxanthellae in control sample and (*B*) some healthy (H) and damaged and partially damaged (T, transparent and pale) zooxanthellae released after sunscreen treatment. Scale bars = 2 µm (*A, B*) and 5 µm (*C, D*).

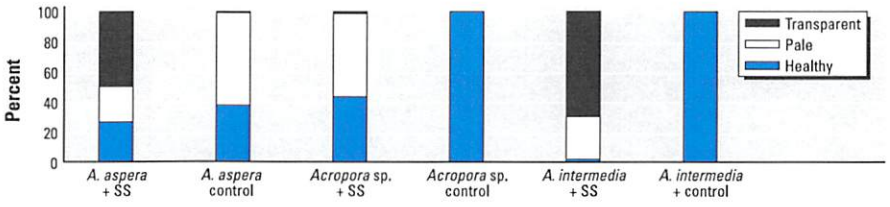


Figure 4. Epifluorescence microscopy analysis of the level of damage in zooxanthellae released after sunscreen (SS) addition.

The impact of sunscreens would be expected to be crucial in atolls and coastal coral reefs with low water renewal and strong tourist vocation. Our results provide strong scientific evidence of the potential impact of these products in tropical habitats and represent a pointer for outlining specific regulations for protecting coral reefs.

Effect of sunscreen ingredients on viral infections. Previous studies have demonstrated that sunscreens can significantly enhance viral production in seawater by inducing the lytic cycle in prokaryotes with lysogenic infection (equivalent to the latent infection of eukaryotes; Danovaro and Corinaldesi 2003). Here, we demonstrate that a similar phenomenon occurs also in hard corals. After the addition of sunscreens, viral abundance in seawater surrounding coral branches increased significantly, reaching values greater by a factor of 15 than in controls (Figure 5A). Because, prior to any treatment, the hard corals were carefully washed with and incubated in virus-free seawater, we conclude that the viruses encountered were released from the corals or their symbionts. Moreover, addition of organic nutrients without UV filters or preservatives did not result in coral bleaching or in a significant increase in the number of viruses in the ambient seawater (Figure 5A). Hard-coral bleaching and the increase in viral abundance in seawater were also seen after coral treatment with mitomycin C, an antibiotic commonly used to induce the lytic cycle in latent viral infections (Figure 5B). TEM analysis of sunscreen-treated corals showed the presence of virus-like particles (VLPs) around and inside the zooxanthellae. The VLPs were round-hycosahedral in shape and 50–130 nm in size (Figure 6). No viruses were encountered either inside or outside the zooxanthellae in control samples. All these results indicate that sunscreens caused coral bleaching by inducing the lytic cycle in symbiotic zooxanthellae with latent viral infections.

Causative agents (mostly bacteria and fungi; Rosenberg et al. 2007) have been isolated and characterized for only 6 of more than 20 coral diseases described in natural environments. To date, viruses have been found in cells of about 50 algal species, representing nearly all major algal classes. This suggests that viruses have a significant role in algal ecology (Brussard 2004). There are, however, only a few studies on viruses infecting zooxanthellae: viruses were encountered in heat-shocked or UV-treated zooxanthellae of *Pavona danai*, *Acropora formosa*, and *S. pistillata*, suggesting the presence of latent viral infections (Davy et al. 2006; Lohr et al. 2007). All our samples from different areas of the world showed viral lytic cycles after treatment with sunscreens and other inducing factors. The results of the present study and these data from the literature indicate that latent infections are common in symbiotic zooxanthellae.

Viruses have a key role in population dynamics and in community composition and diversity of marine bacterioplankton and phytoplankton (Brussard 2004; Suttle 2005) Viruses also contribute significantly to horizontal gene transfer, and can influence the pathways of energy and material flow in aquatic ecosystems, with important implications for global biogeochemical cycles (Fuhrman 1999). The results presented here provide new insights into the functional and ecological role of aquatic viruses and indicate that induction of the lytic cycle in zooxanthellae with latent infection represents an important factor contributing to coral bleaching.

Recent studies have reported that pesticides, hydrocarbons, and other contaminants can cause coral bleaching (Brown 2000; Douglas 2003). We suggest that these factors, which also have the potential to induce the viral lytic cycle in microorganisms or algae with latent infections (Cochran et al. 1998; Danovaro and Corinaldesi 2003; Davy et al. 2006; Jang and Paul 1996) could act synergistically with suncare products, thereby increasing the frequency and extent of coral bleaching.

Our results indicate that sunscreens promoting lytic cycle in viruses can cause coral bleaching. Because human use of tropical ecosystems and coral reef areas is progressively increasing, we predict that the impact of sunscreens on coral bleaching will grow considerably in the future on a global scale. Actions are therefore needed to stimulate the research and utilization of UV filters that do not threaten the survival of these endangered tropical ecosystems.

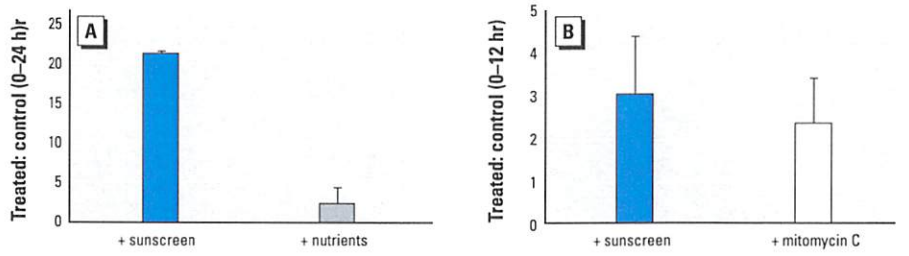


Figure 5. Viral enrichment factors of ambient seawater (as the ratios of viral density in treated and control samples) after the addition of sunscreen, nutrient, and mitomycin C. (*A*) Viral enrichment factor of ambient seawater within 24 hr after sunscreen and organic nutrients addition. (*B*) Viral enrichment factor of ambient seawater within 12 hr after sunscreen and mitomycin C addition. Organic nutrients (lipids, proteins, and carbohydrates) were added at concentrations equivalent to those contained in sunscreens according to Danovaro and Corinaldesi (2003). Values are ± SE.

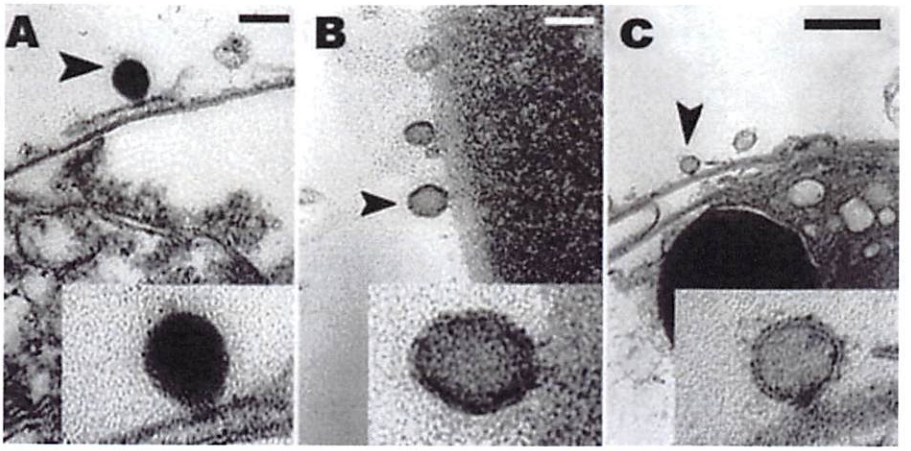


Figure 6. TEM images of viruslike particles (VLPs) associated with zooxanthellae released from nubbins after sunscreen treatment. (*A*, *B*) VLPs attached to zooxanthellae membranes. (*C*) Viruses attached to outer part of zooxanthellae with visible tail penetrating cell membrane. Scale bars = 100 nm (*A*, *B*); 200 nm (*C*). Arrowheads indicate sections magnified in insets.

Correction

In Table 2, the log K_{ow} value for 2-ethylhexyl salicylate has been corrected from "NA" in the original version published online to "6.02."

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Hawaii - **Ban On Many Sunscreen Products Likely To Pass In Hawaii Senate**

The Democratic Party, Office of Hawaiian Affairs and others are urging the House to follow suit, but the measure faces opposition there.
By Nathan Eagle / March 2, 2017

The Hawaii Senate is on track to pass a bill banning the use of sunscreens that contain oxybenzone, which studies have shown harms coral reefs.
But with mounting opposition from convenience store owners and personal care product companies, the legislation's fate in the House remains uncertain.
Nearly 9 million tourists who visit Hawaii each year, along with the state's 1.4 million residents, would have to start paying closer attention to the labels on their sunscreen containers if Senate Bill 1150 becomes law. It would prohibit the use of sunscreens or cosmetics that contain oxybenzone while on a beach or in the ocean.
On Thursday, it cleared the Judiciary and Labor Committee, chaired by Sen. Gil Keith-Agaran,

Scientists have conducted studies that show products containing the chemical — the active ingredient in many sunscreens — contribute to the destruction of coral reefs and weaken their ability to mitigate the effects of climate change.
People would instead have to switch to sunscreens containing zinc oxide or other mineral blockers that still protect against the sun's cancer-causing UVA and UVB rays but aren't known to hurt corals.
Supporters of the ban also suggest wearing rash guards or other lightweight long-sleeved clothing as an alternative sun block.
The bill's next stop is a vote before the full Senate, where it's expected to pass.
It could be a different story in the House, where Rep. Angus McKelvey, chairs of the Consumer Protection and Commerce Committee, has so far blocked oxybenzone-related bills from advancing.
The House Energy and Environmental Protection Committee, chaired by Rep. Chris Lee, passed two oxybenzone-related bills, one banning the sale of products containing the chemical and another requiring any advertisements or displays for sunscreens with oxybenzone to include a warning about how its use in nearshore waters poses serious hazards to coral and reef health.
The ad-warning bill was amended last month in the Ocean, Marine Resources and Hawaiian Affairs Committee, chaired by Rep. Kaniela Ing, losing the part about the ad warning and instead just banning the sale of products containing oxybenzone.
But both bills have been pending for weeks waiting for a hearing in McKelvey's committee, their last hurdle before a vote by the full House.

If the House bills languish, and signs indicate they will, the last vehicle to address oxybenzone will rest in SB 1150, which could cross over to the House as early as next week. The legislative session ends May 4.
There's been strong opposition from the Hawaii Food Industry Association, a trade group that represents some 200 companies, including ABC Stores and KTA Super Stores.
"The combination of reduced choice and less effective products could have the dangerous consequence of individuals using less protective sunscreens or worse no longer using sunscreen, thereby causing more skin damage and potentially increasing skin cancer rates," the association wrote in testimony on the legislation.
The Consumer Healthcare Products Association, based in Washington, D.C., raised similar concerns.
Supporters include the Office of Hawaiian Affairs, the Democratic Party of Hawaii, environmental nonprofits, ocean-tourism groups and state agencies.
OHA noted in its testimony that economic studies in 2002 and 2003 found an overall revenue contribution of $800 million from Hawaii's coral reefs and coastal resources, with an added recreational, amenity, fishery, biodiversity and educational value of $364 million per year.

"While our ocean waters clearly hold cultural, spiritual, and biological significance beyond any monetary value, these economic analyses clearly reflect the critical nature of our marine environment to our islands," OHA's Beneficiary Advocacy and Empowerment Committee wrote in its testimony.

Rick Gaffney of the Hawaii Fishing and Boating Association, based on the Big Island, also submitted written testimony in support.
"Products containing these deleterious chemicals should not be allowed in Hawaii's nearshore waters, especially as they are already suffering from the impacts of global warming, runoff and overfishing," he said. "A bill of this nature is essential to better protecting the reefs of Hawaii so that they in return can protect and feed us, and continue to serve as one of the primary attractions for our tourist industry."
The state departments of Health and Land and Natural Resources expressed support for the bill's intent, but also raised concerns.
DLNR is worried about how it would enforce the ban. And the Department of Health wants to make sure there are sufficient alternatives, such as sunscreens with zinc oxide.
Democratic Party of Hawaii Chair Tim Vandeveer and others have prodded McKelvey to hear the bills in the House.
"The Democratic Party of Hawaii, of which you are a member, has identified these bills as priorities for this legislative session," Vandeveer wrote in a letter to McKelvey. "We understand you may have some reservations about the implementation of these bills. That is the conversation that should be had at the hearing and not behind closed doors."

With no hearings scheduled as of Thursday, the bills in the House are dead this session due to the Legislature's Friday deadline to have all bills passed out of committees and ready for final votes before the full House or Senate.
Still, amendments can be made to the bills that do survive when they cross over to the other chamber, which will schedule another round of committee hearings.
Meanwhile, some businesses have voluntarily stopped carrying sunscreens that contain oxybenzone, including the concession at Hanauma Bay, the most popular snorkeling site in Hawaii.
Craig Downs of Haereticus Environmental Laboratory in Virginia has been a leading scientist researching why corals have been dying, even in protected marine areas like Hanauma Bay.
"It's pretty horrifying," Downs said in June at the International Coral Reef Symposium in Honolulu.
Seawater testing discovered concentrations of oxybenzone — which is found in over 3,500 sunscreen products — exceeded the minimum toxicity level of 62 parts per trillion (equivalent to a drop of water in 6.5 Olympic-sized swimming pools) by 12 times in Hawaii. The oxybenzone causes the coral to bleach at temperatures several degrees cooler and inhibits its ability to reproduce.
Worldwide, scientists estimate 8,000 to 16,000 tons of sunscreen enter coral reefs each year.
Downs, a University of Hawaii graduate, has been testifying in support of the oxybenzone-related bills.

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Occurrence, distribution and ecological risk assessment of multiple classes of UV filters in surface waters from different countries

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ABSTRACT

Organic UV filters are common ingredients of personal care products (PCPs), but little is known about their distribution in and potential impacts to the marine environment. This study reports the occurrence and risk assessment of twelve widely used organic UV filters in surface water collected in eight cities in four countries (China, the United States, Japan, and Thailand) and the North American Arctic. The number of compounds detected, Hong Kong (12), Tokyo (9), Bangkok (9), New York (8), Los Angeles (8), Arctic (6), Shantou (5) and Chaozhou (5), generally increased with population density. Median concentrations of all detectable UV filters were <250 ng/L. The presence of these compounds in the Arctic is likely due to a combination of inadequate wastewater treatment and long-range oceanic transport. Principal component analysis (PCA) and two-way analysis of variance (ANOVA) were conducted to explore spatiotemporal patterns and difference in organic UV filter levels in Hong Kong. In general, spatial patterns varied with sampling month and all compounds showed higher concentrations in the wet season except benzophenone-4 (BP-4). Probabilistic risk assessment showed that 4-methylbenzylidene camphor (4-MBC) posed greater risk to algae, while benzophenone-3 (BP-3) and ethylhexyl methoxycinnamate (EHMC) were more likely to pose a risk to fishes and also posed high risk of bleaching in hard corals in aquatic recreational areas in Hong Kong. This study is the first to report the occurrence of organic UV filters in the Arctic and provides a wider assessment of their potential negative impacts in the marine environment.

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1. Introduction

Organic ultraviolet (UV) filters are widely used as UV radiation-absorbing substances in personal care products (PCPs) to protect human skin from the negative effects of sunlight as well as in materials and paints to prevent product photodegradation. Authorized contents of organic UV filters in PCPs vary according to regulations in the countries/regions of their manufacture, where they may comprise up to 20% of product mass (Chisvert and Salvador, 2007). Owing to their large annual production quantities and widespread usage, particularly because of greater awareness of skin cancer risks in recent decades, organic UV filters can enter the aquatic environment (i) indirectly from wastewater treatment plants (WWTPs) after entering sewage systems following bathing or from industrial discharge due to incomplete removal as well as surface runoff and (ii) directly from recreational activities (e.g. swimming) (Giokas et al., 2007).

As a result of their extensive application and continuous release into aquatic systems, organic UV filters are regarded as pseudo-persistent environmental contaminants, and their ubiquity has raised concerns about their potential environmental impacts (Giokas et al., 2007). They have been found in various environmental samples including surface water, wastewater and sediment (e.g. Tsui et al., 2014; Kameda et al., 2011) generally at ng/L to sub-ug/L levels for aqueous matrices and sub-ng/g levels for solid matrices. However, only a few studies have reported the occurrence of UV filters in the marine environment, and only a limited number of globally authorized compounds have been investigated; for example, benzophenone-3 and -4 (BP-3 and BP-4), ethylhexyl methoxycinnamate (EHMC) and octocrylene (OC) were detected in surface waters in some European countries and Japan (Tashiro and Kameda, 2013; Tovar-Sánchez et al., 2013; Rodil et al., 2008).

Many organic UV filters have high lipophilicity, with octanol-water partition coefficients (log K_{ow}) values generally greater than 3. They have been detected in various aquatic organisms such as brown trout (*Salmo trutta fario*) up to 1800 ng/g (4-methylbenzylidene camphor, 4-MBC) and 2400 ng/g (OC) lipid weight (lw) in Swiss rivers (Buser et al., 2006) and in marine mussels (*Mytilus edulis*) up to 256 ng/g (EHMC) and 7112 ng/g (OC) dry weight (dw) along the French Atlantic and Mediterranean coasts (Bachelot et al., 2012). Moreover, Fent et al. (2010b) suggested food chain accumulation of EHMC, reporting its concentrations in fish and cormorants (*Phalacrocorax* sp.) from six Swiss rivers up to 337 and 701 ng/g lw, respectively. Accumulation of these compounds in organisms is a concern because organic UV filters and their metabolites have been shown to interfere with endocrine function by acting as environmental estrogens both *in vitro* and *in vivo* (Schlumpf et al., 2001; Kunz and Fent, 2006). Moreover, they have been shown to induce bleaching in corals by promoting viral infections (Danovaro et al., 2008).

Data on the occurrence of organic UV filters in fresh surface waters are available for several developed countries (e.g. Kameda et al., 2011; Fent et al., 2010b), but relevant information is lacking for the marine environment in countries outside of Europe or Japan for certain uniformly approved and widely consumed UV filters (e.g. butyl methoxydibenzoylmethane (BMDM) and homosalate (HMS)). Moreover, previous studies have reported the occurrence of UV filters at beaches, but little information is known about coastal waters. In contrast to other organic contaminants (e.g. perfluoroalkyl substances (PFAS) and pharmaceuticals) which have been studied in detail (Richardson and Ternes, 2014), information on the occurrence, distribution, transport pathways and risks of organic UV filters in the aquatic environment is lacking. Therefore, it is of crucial importance to study the environmental distribution and concentrations of these emerging contaminants in order to evaluate their ecological risks.

In light of these considerations, the objectives of this study were to (i) determine the concentrations and spatial occurrence of twelve commonly consumed UV filters, including benzophenone-1, -3, -4 and -8 (BP-1, -3, -4 and -8), ethylhexyl salicylate (EHS), isoamyl p-methoxycinnamate (IAMC), octyl dimethyl-p-aminobenzoic acid (ODPABA), BMDM, EHMC, HMS, 4-MBC and OC in surface water samples collected from different countries including China (Hong Kong, Shantou and Chaozhou), the United States (New York City and Los Angeles), Japan (Tokyo Bay), Thailand (Bangkok) and the Arctic region, as well as their seasonal variation in Hong Kong over the course of one year; and (ii) conduct an ecological risk assessment by using the measured environmental concentrations and available toxicity data.

2. Materials and methods

2.1. Chemicals and materials

Information on chemical standards and preparation of standard solutions can be found in the Supplementary material. Standard purities were all ≥97%. Detailed information on the targeted UV filters is shown in Table A1.

2.2. Sampling

Surface water samples were collected from eight locations (Hong Kong, $n = 60$; Tokyo, $n = 8$; New York, $n = 6$; Los Angeles, $n = 4$; Shantou, $n = 4$; Chaozhou, $n = 3$; Bangkok, $n = 2$) and the Arctic ($n = 14$) from 2012 to 2013 using plastic or stainless steel buckets or glass bottles which were pre-cleaned by rinsing (in sequence) with methanol, Milli-Q water, and water from the specific location. All samples were marine surface water samples except those collected from Bangkok which were freshwater samples. Most of the selected cities are metropolitan areas featuring both commercial and industrial development. Temporal and spatial samples were collected in Hong Kong in both the wet and dry seasons; spatial samples were collected from Tokyo Bay, Los Angeles, New York City and the Arctic, while only a single location was sampled in Bangkok. Detailed information on the sampling locations is shown in Supplementary material Table A2 and Figs A1–5.

Surface water samples were collected from 20 points in Hong Kong in August 2012, February and June 2013; June and August samples represented the wet season, while the February samples represented the dry season. The sampled

points were expected to reflect WWTPs, beaches and aquatic recreational activities (further details are given in the Supplementary material). The Tokyo samples were collected in Tokyo Bay, while the New York samples were collected in Jamaica Bay, Upper New York Bay and the East River near WWTP discharge points. In Los Angeles, Shantou and Chaozhou, surface water samples were collected at beaches and near WWTP discharge points while only river water receiving municipal wastewater was collected in Bangkok. Arctic samples were collected in the Arctic Ocean and Chukchi Sea between 65 and 75 °N.

Water samples were stored in glass bottles pre-rinsed with Milli-Q water and methanol. All glass bottles were wrapped with aluminum foil to avoid contamination and photodegradation of the target compounds. Samples were stored in the dark at 4 °C prior to analysis.

2.3. Analytical procedures

Chemical analysis of the 12 target compounds was modified from a previously reported method (Tsui et al., 2014). Briefly, the analytical procedures consisted of addition of 5% (w/v) Na_2EDTA to each sample, solid phase extraction (SPE) with Bond Elut C18 cartridges, elution by 3 × 4 mL of 50:50 v/v methanol: ethyl acetate (MeOH: EA), concentration under nitrogen flow to less than 0.5 mL, reconstitution to 0.5 mL by MeOH and analysis by high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry (HPLC-ESI-MS/MS). Detailed analytical procedures and optimal parameters of HPLC-MS/MS for quantification are shown in the Supplementary material.

2.4. Method validation

Details on method validation are given in Table A4 in the Supplementary material. Recoveries ranged from 63% to 106%, while the relative standard deviations (RSD) of target compounds ranged from 1.5% to 7.9%. The results presented in this study were not corrected by recoveries. The method limit of detection (MLOD) was defined as three times the standard deviation of procedural blank peak areas plus their mean value and then corrected by a matrix-induced interference factor which was the slope difference (ratio) of two calibration curves separately constructed in methanol and in water sample extracts (Leung et al., 2012). MLODs ranged from 0.03 to 1.38 ng/L. Field and procedural blanks were analyzed for each sampling trip and for each batch of samples in the laboratory by using Milli-Q water. All of the target compounds were below MLODs in both field and procedural blanks.

2.5. Statistical analyses

Normality tests (Kolmogorov–Smirnov) were performed before statistical analyses. Parametric Pearson correlation analysis was used for the examination of significant correlations among concentrations of different UV filters in surface water from different sampling cities/regions. Log_{10}-transformed values were used to perform the Pearson correlations in all locations except Hong Kong, for which principal component analysis (PCA) and permutational analysis of variance (PERMANOVA) were conducted to explore spatio-temporal patterns in organic UV filter levels because of the larger sample size for this city. Two-way analysis of variance (ANOVA) and *post hoc* Student-Newman-Keuls (SNK) tests were carried out to test spatiotemporal differences in compound concentrations in Hong Kong samples. Samples with concentrations < LOD were treated as zero in the analysis. The significance level was set at $\alpha = 0.05$. Univariate statistical analyses were carried out using SigmaStat 3.5 (Systat Software Inc, Chicago, USA) or SPSS 17 (SPSS Inc.). Multivariate analyses were carried out using PRIMER 6 & PERMANOVA+ (PRIMER-E Ltd, Plymouth, UK).

2.6. Environmental risk assessment (ERA)

Hazard quotients (HQs) for individual UV filters were obtained by dividing measured environmental concentrations (MECs) obtained in this study by predicted-no-effect concentrations (PNECs) calculated by dividing the effect concentrations (ECs) by a standard assessment factor, 1000, to account for intra- (factor = 10) and inter-species variability (10) and chronic exposure conditions (10) (European Commission, 2003). Toxicity data were obtained from the literature focusing on aquatic organisms at different trophic levels including protozoa, algae, crustaceans, invertebrates and fishes. Because of the lack of toxicological literature on many organic UV filters, the risk assessment was only conducted for six compounds: BP-1, BP-3, BP-4, EHMC, 4-MBC and ODPABA.

Preliminary screening of the potential ecological risks of organic UV filters was carried out using the worst-case scenario, HQ_{worst}, in which the maximum MECs of each compound and minimum PNECs were applied in the hazard assessment (Table 1 and A5). The risk classification was based on risk ranking criteria in which HQ < 0.01: "Unlikely to pose risk"; 0.01 ≤ HQ < 0.1: "Low risk"; 0.1 ≤ HQ < 1: "Medium risk" and HQ ≥ 1: "High risk" (Hernando et al., 2006). Probabilistic risk assessment was conducted if the HQ_{worst} of UV filters exceeded 1 by plotting cumulative probability on a log scale. Risk probabilities (p) were calculated by substituting the log PNECs of each species in the linear equations for each sampled city, in which (100-p)% would be the percentage of samples containing concentrations of that compound exceeding the PNEC of a particular species and thus posing risk based on the assessed endpoint.

3. Results and discussion

3.1. Occurrence and composition of UV filters in surface waters

A total of 101 surface water samples collected from August 2012 to October 2013 were analyzed, and median concentrations in the samples ranged from <LOD to 230 ng/L (Table 1). The number of compounds detected was Hong Kong (12), Tokyo (9), Bangkok (9), New York, Los Angeles (8), Arctic (6), Shantou (5) and Chaozhou (5).

BP-3, EHMC and OC were detected in all cities and in the Arctic with detection frequencies ≥30% in each location (calculated by dividing the number of positive detections by

Table 1 – Concentrations (ng/L) and detection frequencies of 12 UV filters in surface water samples from different cities. Conc.: (median-maximum); DF: detection frequencies (calculated by dividing the number of positive detections by the total number of samples from each location); LOD: limit of detection.

Compounds	Hong Kong (n = 60)		Tokyo (n = 8)		New York (n = 6)		Los Angeles (n = 4)		Shantou (n = 4)		Chaozhou (n = 3)		Bangkok (n = 2)		Arctic (n = 14)	
	Conc.	DF	Conc.	DF	Conc.	DF	Conc.	DF	Conc.	DF	Conc.	DF	Conc.	DF	Conc.	DF
ODPABA	95–182	17	<LOD	0	<LOD	0	<LOD	0	<LOD	0	<LOD	0	<LOD	0	<LOD	0
4-MBC	173–379	12	<LOD	0	<LOD	0	<LOD	0	<LOD	0	<LOD	0	<LOD	0	<LOD	0
BMDM	24–721	97	78–104	100	70–87	100	67–109	100	53–100	75	<LOD	0	36–38	100	18–70	57
EHMC	89–4043	93	46–95	63	89–150	83	91–138	75	52–78	75	<LOD-79	33	88–95	100	25–66	71
IAMC	63–173	27	<LOD	0	<LOD	0	<LOD	0	<LOD	0	<LOD	0	<LOD	0	<LOD	0
OC	103–6812	100	87–108	75	117–128	83	145–377	100	75–107	75	36–102	67	153–205	100	26–31	43
BP-3	39–5429	95	24–86	100	23–178	100	227–601	100	55–188	100	37–49	100	86–116	100	17–33	71
EHS	61–1030	59	71–95	88	<LOD	0	53–120	50	<LOD	0	121–128	67	28–56	50	<LOD	0
BP-4	54–389	49	71–136	100	89–574	100	<LOD	0	<LOD	0	<LOD-49	33	80–95	100	<LOD	0
HMS	66–2812	76	65–110	100	91–114	67	142–270	100	<LOD	0	<LOD	0	29–59	50	<LOD	0
BP-1	82–135	76	52–95	63	<LOD-74	33	100–117	75	22–58	50	<LOD	0	127–166	100	2.5–5	36
BP-8	64–117	88	76–96	100	72–92	100	29–96	50	<LOD	0	<LOD	0	63–71	100	2–3.3	50

the total number of samples in each place), showing their widespread distribution in the marine environment. Among these three frequently detected UV filters, the highest median concentrations were found in Los Angeles (227 ng/L; BP-3), New York (100 ng/L; EHMC) and Bangkok (153 ng/L; OC), while the lowest median concentrations were found in the Arctic (16.6, 25.4 and 25.8 ng/L for BP-3, EHMC and OC, respectively). The concentrations of five compounds including BP-3, EHMC, EHS, HMS and OC exceeded 1000 ng/L in surface water samples collected on hot summer days with strong UV radiation in June and August 2013 at a popular beach in Hong Kong. Apart from recreational activities and surface runoff, the incomplete removal of organic UV filters in WWTPs is a major contributor to their ubiquitous occurrence in the environment; in Hong Kong, their environmental loading can reach 200 g/day (Tsui et al., 2014). BP-4, with median concentrations lower than 100 ng/L at all locations, was only detected in indirect sources (i.e. through WWTP discharge) because it is used primarily in PCPs such as hand washes/soaps, shower gels and shampoo rather than sunscreen products and it is poorly removed in WWTPs (Tsui et al., 2014). In contrast, 4-MBC (173–378 ng/L), IAMC (62.7–173 ng/L) and ODPABA (95.1–182 ng/L) were only detected at snorkeling hot spots and other recreational beaches in Hong Kong, indicating that recreational activities would be the main sources of these three compounds instead of wastewater effluent discharge. The low detection frequencies of these compounds in WWTP-influenced samples are likely due to their relatively lower use (used in less than 2% of commercially available PCPs in Swiss and British markets; Manová et al., 2013; Kerr, 2011) together with the stronger dilution effects of ocean currents in Victoria Harbour.

Generally, the occurrence of individual UV filters at each location (calculated by dividing the total concentration of each UV filter by the total concentration of UV filters at that location) was <30%, except for EHS in Chaozhou which was >40% (Fig. A6). The composition profiles of UV filters in surface waters from Hong Kong, Tokyo, New York, Los Angeles and Bangkok were similar while those in samples from Shantou, Chaozhou and the Arctic showed higher percentages of each detectable compound due to the relatively lower number of positive detections. BP-3, EHMC and OC were the dominant compounds detected in all samples. Hong Kong imports a wide variety of PCPs from several countries/regions and has no local regulations for organic UV filter content in products, and therefore more compounds from several chemical classes were detected in these samples. The number of compounds detected in Shantou and Chaozhou were the lowest among all sampled cities, likely because of their lower population densities (2655/km^2 and 849/km^2, respectively; Shantou and Chaozhou Government, 2010) and development level. Liao and Kannan (2014) reported overall geometric mean levels of BP-3 in PCPs purchased in China and the United States, which were 20.1 and 1200 ng/g, respectively. Moreover, the maximum authorized concentrations of some of the targeted compounds in China are lower than those in other countries (e.g. up to 20% of product mass can consist of EHMC in Japan, but only 10% of product mass is permitted in China) (MoH, 2007), indicating comparatively lower application of these UV filters in PCPs in China. Sediments with high organic

carbon content are an environmental sink for contaminants with high log K_{ow} and UV filters can also be detected in sediment (e.g. Kameda et al., 2011).

3.2. Distribution and source determination of UV filters in surface water

Correlation analyses were performed among individual UV filters from Tokyo, the Arctic and the United States for source determination (Table A6). Significant positive correlations ($p < 0.05$) were observed between BP-3 and BP-8 ($r = 0.879$) and BP-3 and EHMC ($r = 0.774$) in the Arctic samples. Higher detection frequencies of UV filters were found in samples collected near Alaska (<72°N) than in those from the open ocean (>72°N). The overall detection frequencies of BMDM, BP-3 and EHMC were >50%, while concentrations of all detectable compounds were <70 ng/L (Table 1). This is the first report of the occurrence and distribution of organic UV filters in the Arctic, for which there are two possible pathways: (i) oceanic transport via ocean currents or (ii) atmospheric transport; these pathways may be either long-range or short-range. Inadequate wastewater treatment facilities could result in the direct release of untreated or undertreated wastewater to the marine environment via oceanic currents (Gunnarsdóttir et al., 2013) and thus wastewater runoff could be one of the local contamination sources of UV filters as some of the sampling points are located offshore of Point Hope and Point Barrow in Alaska (population: 674 and 4212, respectively; United States Census, 2010), both of which employ sewage lagoons as the major wastewater treatment method (BUECI and URS Corporation, 2005). Some compounds such as BP-3 and OC have been reported to be highly photostable towards UV irradiation (half-lives >72 h; Rodil et al., 2009), and they may undergo long-range or local transport via oceanic currents. However, information about the environmental half-lives of organic UV filters is limited. Though some of these compounds have similar Henry's Law constants (ranging from 10^{-5} to 10^{-15} atm·m^3/mol, Table A1) as other organic contaminants known to undergo long-range atmospheric transport such as PFAS and endosulfan (Butt et al., 2010; Weber et al., 2010), there is currently not enough evidence to conclude that they partition into the gas phase as no studies have reported the occurrence of UV filters in air samples or wet or dry deposition, and their atmospheric half-lives are also unknown. More work should be conducted to investigate the fate on these compounds in order to understand their occurrence in aquatic environments.

In Tokyo Bay, significant positive correlations ($p < 0.05$) were observed between EHMC and OC ($r = 0.957$); EHS and HMS ($r = 0.795$) and BP-1 and BP-8 ($r = 0.885$), suggesting that these compounds likely share contamination sources such as wastewater effluents from urban and industrial areas in Tokyo. Moreover, Tokyo Bay receives fresh water from the Tama, Arakawa and Edo Rivers, which flow through densely populated areas with WWTPs at different treatment levels. All wastewater collected from public sewers in Japan is treated with secondary treatment, while 15% is further treated with tertiary methods, including sewage discharged to Tokyo Bay (Ueda and Benouahi, 2009). Chemicals used in PCPs (e.g. UV filters and synthetic musks) were detected in these rivers in previous studies (Kameda et al., 2011; Yamagishi et al., 1983). Kameda (2007) reported the occurrence of EHMC in sediment core samples collected in Tokyo Bay (1977–1997) showing the long history of the occurrence of this compound in the Bay, and the present study showed its wide distribution in surface water in Tokyo Bay as well.

Significant positive correlations were observed between BMDM and OC ($r = 0.971$), BP-3 and BP-4 ($r = 0.903$) as well as HMS and BP-8 ($r = 0.985$) in the samples from New York City, suggesting that they shared similar contamination sources (WWTP effluents). There are 14 WWTPs in New York City serving 7.8 million people (New York City Department of Environmental Protection), and wastewater is treated by secondary treatment and chlorination disinfection, neither of which completely remove organic UV filters from effluents (Tsui et al., 2014). In Los Angeles, the highest concentrations of UV filters were detected at a popular beach, Huntington Beach.

No significant correlations were observed among UV filters in surface water from the two Chinese cities, suggesting that UV filters in the marine environment in these locations have distinct contamination sources such as both municipal and industrial wastewater discharge.

3.3. Seasonal variation of UV filter concentrations in surface waters in Hong Kong

The occurrence of organic UV filters in the Hong Kong samples was investigated in greater depth using PCA. Significant seasonal and zone differences (all $p < 0.0001$, PERMANOVA) were observed. The sample patterns were considered in monthly chronological order without considering the sampling year, and monthly data are presented individually to illustrate spatial patterns (Fig. 1a–c). In all sampling months, the concentrations measured at the sampled points largely conformed to expectations about the major sources of organic UV filters in each zone. February is the end of the dry season, when water temperatures are colder (Table A7) and there is little marine or coastal recreational activity, and thus it was assumed that there would be no or small inputs of organic UV filters at sites representing direct sources and at beaches; these sites cluster together in the PCA (Fig. 1a, I and III) and apart from sites representing indirect sources (WWTPs; Fig. 1a, IV). In contrast, samples collected at the beginning of the wet summer season in June from indirect and direct sources showed less separation, likely due to marine recreational activities increasing with warmer temperatures (Fig. 1b, V and VII), while beach samples (Fig. 1b, VI) were dissimilar to both of these groups. The collected samples were most similar to one another in August, when temperatures were highest (Fig. 1c), and increased usage of organic UV filters at the sampled beaches (Fig. 1c, IX) was evident. Surface water samples collected from Point-16 and -17 were dissimilar to the other beach sampling locations, grouping apart from all other points in February (Fig. 1a, II) and grouping with samples representing direct sources in June and August (Fig. 1b, V and 1c, VIII, respectively). These two points were located on a small beach adjacent to a village community where snorkeling and diving are the major recreational activities, and where there may also be some local wastewater release by

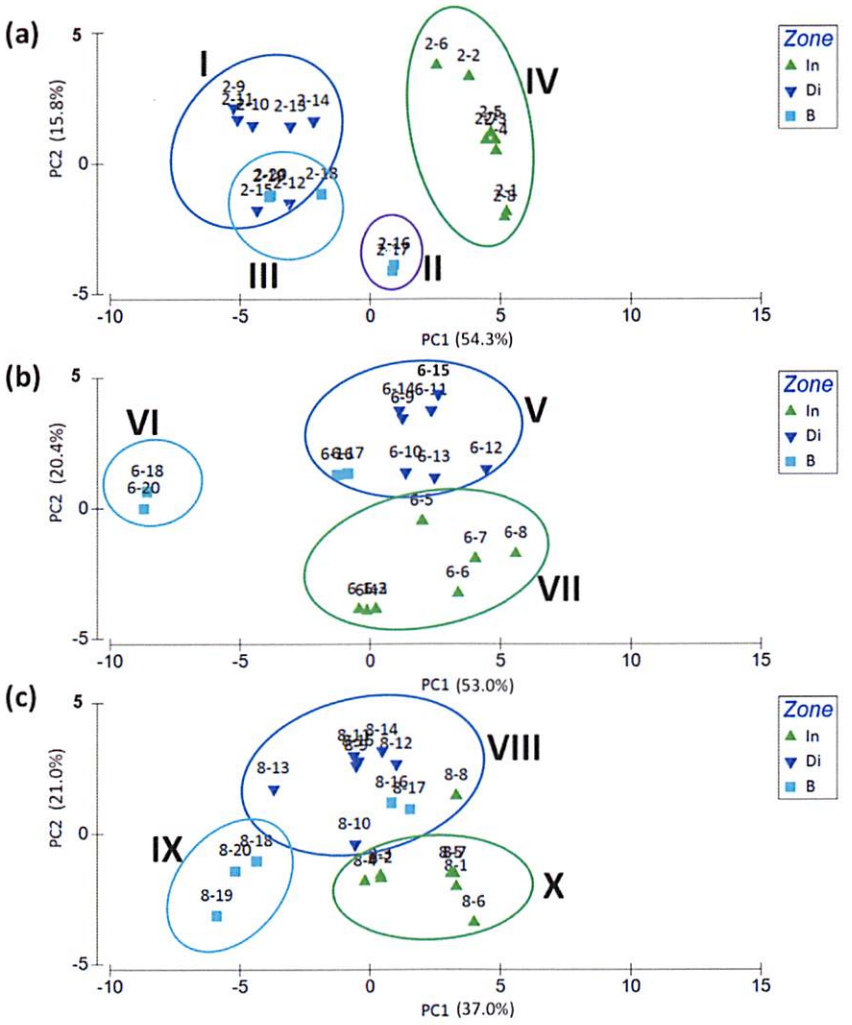

Fig. 1 – Principal Component Analysis (PCA) plots for surface water samples collected in (a) February 2013, (b) June 2013, and (c) August 2012 from Hong Kong (In: Indirect sources (WWTPs), Di: Direct sources (water sports & marine recreational activities), B: Beaches).

residents or tourists. These points therefore showed a distinct pattern compared to the other locations.

Concentrations of all the tested compounds varied according to season and/or sampling zone (ANOVA, Tables A8a & b). The results of *post hoc* SNK tests illustrated that all compounds showed significantly higher concentrations in the wet season in at least one sampling zone with the exception of BP-4, generally reflecting greater usage of PCPs containing organic UV filters during the sunnier and hotter wet season (Tables A7 and A8). BP-4 concentrations were greater in the dry season perhaps because it is increasingly used as a photodegradation retardant and shelf life extension ingredient in many types of PCPs (Hughes and Stone, 2007) that are not used seasonally. Moreover, it is the most hydrophilic of the target compounds (log K_{ow}: 0.89), and therefore this disparity may also be due to differences in precipitation in the dry and wet seasons. IAMC, 4-MBC and ODPABA were mainly detected in the wet season and at locations reflecting direct sources and beaches. This finding is consistent with our previous study of wastewater in Hong Kong which reported low detection frequencies of these compounds in effluent (Tsui et al., 2014).

The detected levels of EHMC showed no significant spatial differences in both seasons and its high detection frequency indicated continuous release of this compound to the marine environment throughout the year. On the other hand, the significantly higher concentration of BP-4 found in locations representing indirect sources in both seasons confirmed that WWTP effluent is a major source of this compound in the aquatic environment. However, the lack of information on product composition/formulations and usage by consumers in Hong Kong makes it difficult to understand how the occurrence patterns of organic UV filters in the environment are related to their use and release.

3.4. *Global comparison of UV filters in surface water*

3.4.1. *Marine environment*

To date, few studies have reported the occurrence of UV filters in the marine environment, and published reports have focused primarily on European countries and reported levels of a small number of compounds. One recent study reported that the maximum concentration of UV filters

detected at coral reef sites in Okinawa Island, Japan was 8.1 ng/L (OC) (Tashiro and Kameda, 2013), which was 28 times less than that detected in coral reef areas in Hong Kong (OC, 231 ng/L). A global comparison of median-to-maximum levels of the target compounds in the present study is shown in Fig. 2. This study is the first report of BMDM, BP-8 and IAMC in the marine environment. Maximum concentrations of BMDM were <200 ng/L in all sampled locations except Hong Kong (BMDM: 721 ng/L), while detection frequencies for BMDM and BP-8 were >60% and IAMC was only detected in Hong Kong. The maximum concentrations of OC, BP-3 and EHMC found in this study were 6810, 5420 and 4040 ng/L, respectively, which were comparable, 1.5 and 10 times higher than those detected in Norway and Spain (7300, 3300 and 390 ng/L, respectively; Langford and Thomas, 2008; Tarazona et al., 2010). The maximum concentration of BP-4 (574 ng/L) was found in a sampling point near the effluent discharge of a WWTP in Jamaica Bay in New York, but it was <LOD in all samples from beaches in this study; in contrast, BP-4 was detected at a Spanish beach at a maximum concentration of 138 ng/L (Rodil et al., 2008). 4-MBC is not permitted for use as a cosmetic ingredient in Japan and the United States, and thus it was not detected in seawater there, but it was detected in Europe and Hong Kong at maximum concentrations of 799 (Langford and Thomas, 2008) and 379 ng/L (present study), respectively.

3.4.2. *Freshwater environment*

The organic UV filter concentrations reported for river surface water samples from Bangkok in the present study are the first reported in Southeast Asia. The maximum concentration of BP-4 detected in Bangkok was 95 ng/L, which was 1.5 and 9 times lower than that in rivers in the United Kingdom and Spain (Kasprzyk-Hordern et al., 2008; Rodil et al., 2008), suggesting lower consumption of BP-4 in Bangkok. The median concentration of EHMC (88 ng/L) in Bangkok falls between those measured in heavily and moderately polluted rivers in Japan (266 and 26 ng/L, respectively) receiving industrial and domestic wastewater discharge (Kameda et al., 2011). Only 23% of wastewater in Bangkok is treated in WWTPs using conventional secondary treatment with activated sludge, and thus a high percentage of untreated wastewater is discharged directly to rivers (Tsuzuki et al., 2009), which likely explains the high number of UV filters detected in these samples. Moreover, incomplete removal of UV filters by secondary treatment with activated sludge in WWTPs may also contribute to their high detection (Tsui et al., 2014).

3.5. *Ecological risk assessment*

A preliminary screening of the worst-case scenario was conducted to assess the potential hazards of UV filters to the aquatic environment (Table A9). In the worst case scenario, BP-1 and BP-4 posed low to medium risk to crustaceans based

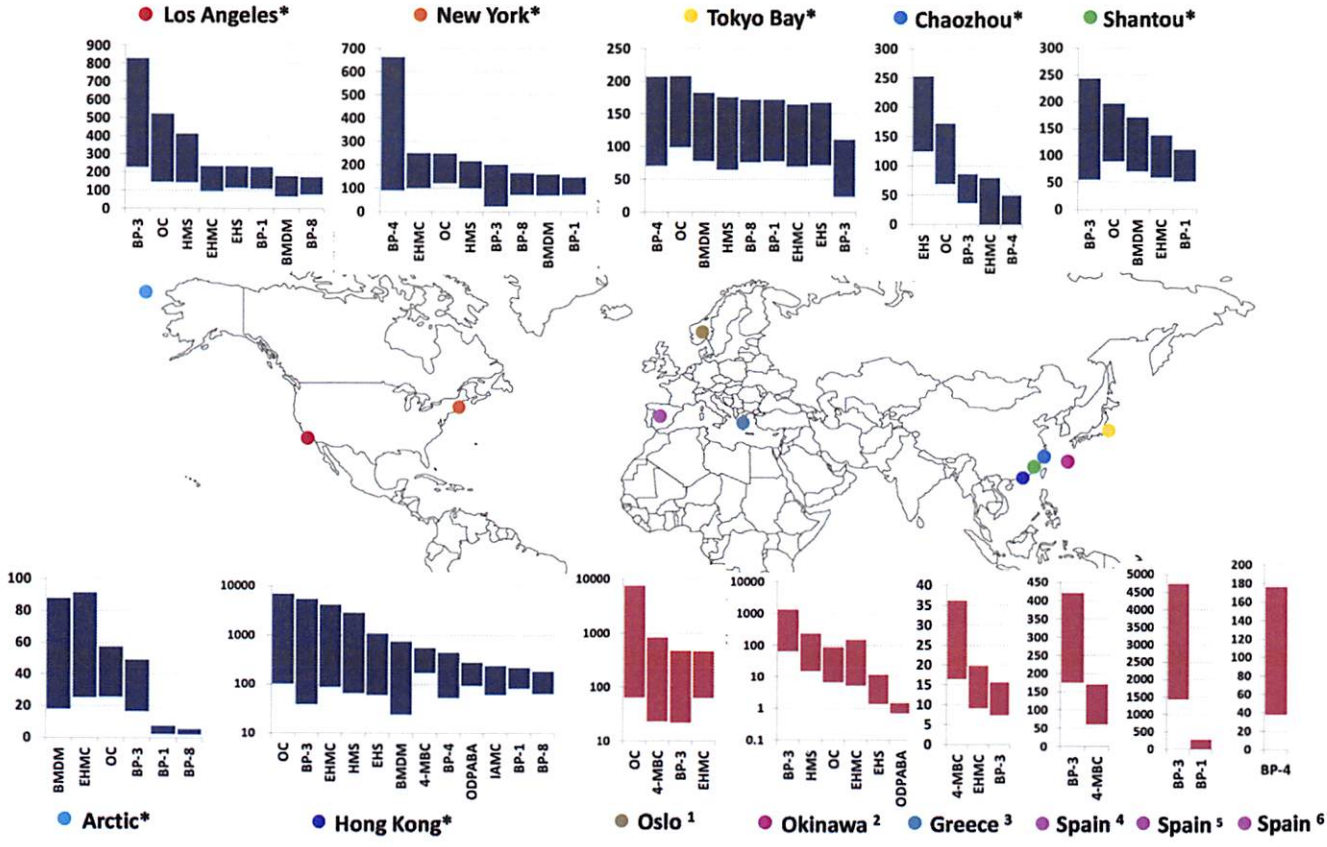

Fig. 2 – Global comparison of UV filters concentration (median-maximum, ng/L) in marine surface waters (*: this study; 1: Langford and Thomas, 2008; 2: Tashiro and Kameda, 2013; 3: Giokas et al., 2005; 4: Tovar-Sánchez et al., 2013; 5: Tarazona et al., 2010; 6: Rodil et al., 2008).

on the endpoints of immobilization and lethality in *Daphnia magna*, as well as in fish based on changes in the expression of endocrine genes in fathead minnow (*Pimephales promelas*) and zebrafish (*Danio rerio*) with HQ_{worst} ranging from 0.001 to 0.06 for BP-1 and 0.001 to 0.19 for BP-4 (Sieratowicz et al., 2011; Fent et al., 2010a; Zucchi et al., 2011). The medium risk posed by BP-4 for zebrafish occurred in Hong Kong and New York, with HQ_{worst} at 0.13 and 0.19, respectively. ODPABA posed medium risk (HQ_{worst}: 0.18) to invertebrates based on changes in endocrine-related genes in *Chironomus riparius* as the endpoint (Ozáez et al., 2013).

As some of the HQ_{worst} values for BP-3, EHMC and 4-MBC exceeded 1, probabilistic plots were constructed for their concentrations in surface water samples (Fig. 3a and b, A7). Because of the small number of sampling points in Bangkok, Shantou and Chaozhou, probabilistic risk assessment was not performed for these cities. As a distinct pattern was observed for the concentrations of UV filters in the samples collected at beaches in Hong Kong, these data were considered separately. Detailed information on regression coefficients is shown in Table A10. Multiple threshold values were available for freshwater fish, and all of these were used for the risk assessment to include a range of sensitivities among species (shown as thresholds F_1–F_4 on Fig. 3a and b). The probabilities of 4-MBC causing growth inhibition in algae (based on the inhibitory concentration-10% (IC_{10}) for *Desmodesmus*

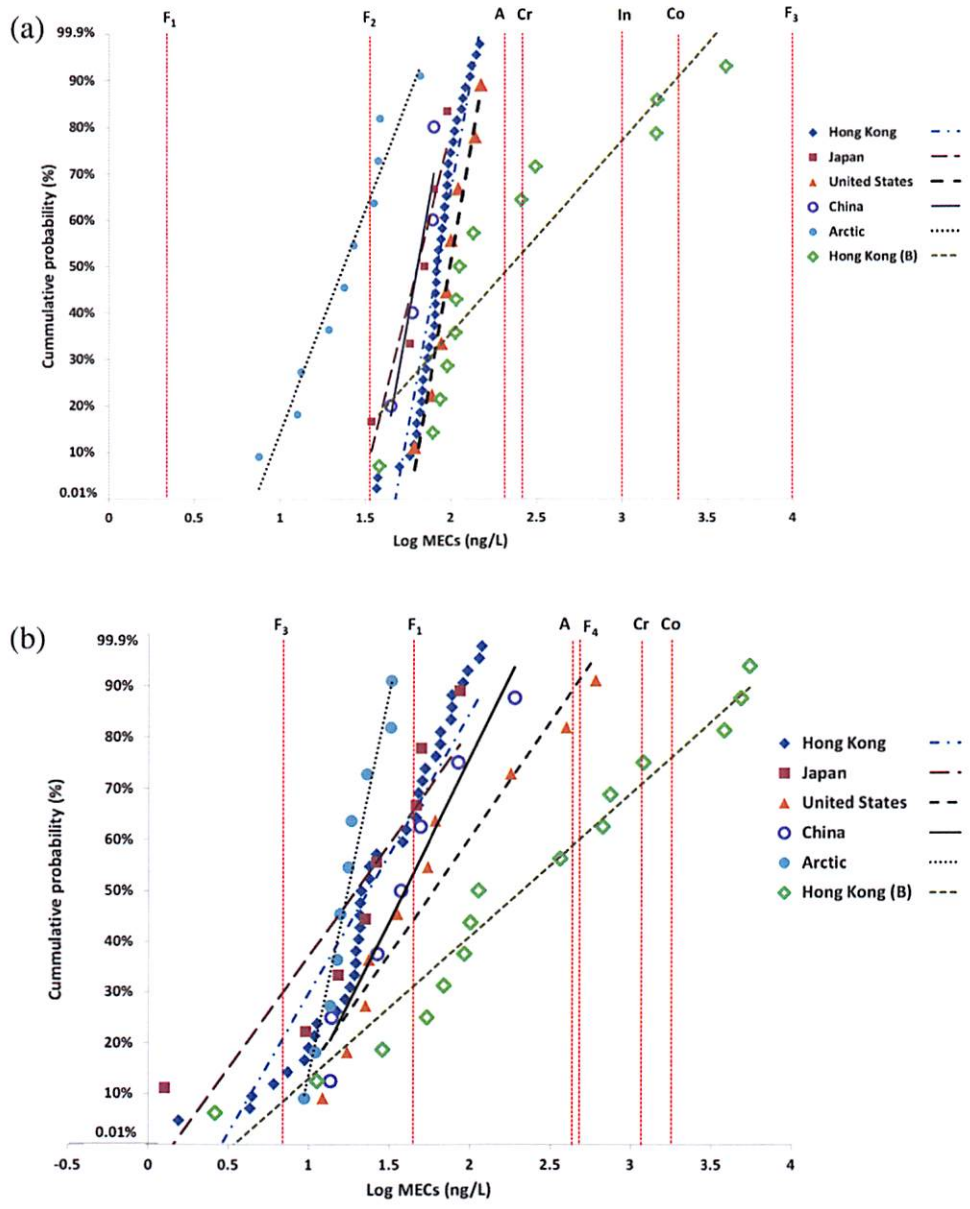

Fig. 3 – a. Probabilistic risk assessment of EHMC in marine surface waters from different locations. "F" thresholds are those derived from toxicity data for different fish species (F_1: Zebrafish; F_2: Fathead minnow; A: Algae; Cr: Crustacean; In: Insect; Co: Coral; F_3: Japanese medaka). Toxicity thresholds and endpoints are given in Table A5. 3b. Probabilistic risk assessment of BP-3 in marine surface water from different locations. "F" thresholds are those derived from toxicity data for different fish species (F_3: Japanese medaka; F_1: Zebrafish; A: Algae; F_4: Rainbow trout; Cr: Crustacean; Co: Coral). Toxicity thresholds and endpoints are given in Table A5.

subspicatus), altering endocrine genes in mosquito larvae (*C. riparius*) and inducing oxidative stress in a protozoan (*Tetrahymena thermophila*) were 34%, 68% and 99.9%, respectively (Gao et al., 2013; Ozáez et al., 2013; Sieratowicz et al., 2011). This result showed that organisms at lower trophic levels were more susceptible to 4-MBC. The probability of risk to fish based on transcriptional changes of endocrine genes in zebrafish was 99.9% for EHMC for all samples with positive detections in all locations, while that based on toxicity in fathead minnow was over 75% in all places except the Arctic, for which the risk was 31%. Moreover, EHMC posed high risk to both cladocerans and algae based on immobilization and growth inhibition as the endpoints (24 and 29%, respectively). For BP-3, the probability of effects on induction of vitellogenin in fish based on data from rainbow trout (*Oncorhynchus mykiss*), egg development in fish based on data from Japanese medaka (*Oryzias latipes*) and induction of oxidative stress in protozoans were over 34%, 50% and 99.9% in all locations, respectively (Coronado et al., 2008; Gao et al., 2013).

It should be noted that the majority of the toxicity values used for the assessment were derived from tests using freshwater organisms, as little information is available in the literature for marine species. The sensitivities of freshwater and saltwater species to different organic contaminants (e.g. pesticides, trace elements) are known to vary, and species sensitivity distributions have been used to understand whether freshwater datasets are protective enough; in some cases, saltwater species have been found to be less sensitive to contaminant effects than freshwater species, though the information available, both in terms of number of species and number of chemicals, is far from comprehensive (Wheeler et al., 2002). Because of the scarcity of information for marine species for UV filters, an inter-species safety factor of 10 was used in this study. Both BP-3 and EHMC posed 21% and 11% risk, respectively, of causing bleaching of hard corals (*Acropora* sp. and *A. pulchra*) at some beaches in Hong Kong located near snorkeling hotspots. It should be noted that these two compounds were detected widely and frequently at high concentrations at the majority of the sampled locations, and therefore their ecological risks and negative impacts should be investigated further.

4. Conclusions

Data on the international distribution and possible negative impacts of organic UV filters in the aquatic environment and the first report of their occurrence in the Arctic have been presented in this study. BP-3 and EHMC showed high detection frequencies at all sampled locations as well as high concentrations in recreational areas; probabilistic risk assessment indicated that these compounds posed various ecological risks to marine ecosystems, including causing coral bleaching and affecting reproduction in fish, though toxicity data for several compounds were not available. The pathways by which organic UV filters are transported to remote Arctic areas remain to be elucidated. These findings indicate that there is a need for greater understanding of the toxicities of these chemicals, both singly and in mixtures, and to consider the current extent of their use, particularly in potentially sensitive ecosystems such as coral reefs.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.watres.2014.09.013.

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