INTERNATIONAL STANDARD

ISO 24444

First edition
2010-11-15

Cosmetics — Sun protection test methods — *In vivo* determination of the sun protection factor (SPF)

Cosmétiques — Méthodes d'essai de protection solaire — Détermination in vivo du facteur de protection solaire (FPS)

Reference number
ISO 24444:2010(E)

ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

Published in Switzerland

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Foreword

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International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 24444 was prepared by Technical Committee ISO/TC 217, *Cosmetics*.

Introduction

The level of sun protection provided by sunscreen products has traditionally been estimated using the sun protection factor or SPF test, which uses the erythemal response of the skin to ultraviolet (UV) radiation. The SPF is a ratio calculated from the energies required to induce a minimum erythemal response with and without sunscreen product applied to the skin of human volunteers. It uses ultraviolet radiation usually from an artificial source.

Different standard methods are available and described in the technical report ISO/TR 26369[4].

These standards are similar by some parameters but different by others. Differences can lead to discrepancy of results. Harmonization is therefore necessary to get the same SPF value for a single product whatever the country in which it is tested.

Cosmetics — Sun protection test methods — *In vivo* determination of the sun protection factor (SPF)

1 Scope

This International Standard specifies a method for the *in vivo* determination of the sun protection factor (SPF) of sunscreen products. This International standard is applicable to products that contain any component able to absorb, reflect or scatter ultraviolet (UV) rays and which are intended to be placed in contact with human skin.

It provides a basis for the evaluation of sunscreen products for the protection of human skin against erythema induced by solar ultraviolet rays.

2 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

2.1
ultraviolet radiation
UVR
electromagnetic radiation in the range of 290 nm to 400 nm

2.1.1
ultraviolet B
UVB
electromagnetic radiation in the range of 290 nm to 320 nm

2.1.2
ultraviolet A
UVA
electromagnetic radiation in the range of 320 nm to 400 nm

NOTE UVA II = 320 nm to 340 nm; UVA I = 340 nm to 400 nm.

2.2
erythema
reddening of the skin caused by UV radiation

2.3
sunscreen products
products containing any component able to absorb, reflect or scatter UV rays, which are intended to be placed in contact with human skin

2.4
minimal erythemal dose
MED
lowest dose of ultraviolet radiation (UVR) that produces the first perceptible unambiguous erythema with defined borders appearing over most of the field of UV exposure, 16 h to 24 h after UV exposure

2.4.1
MEDu
MED on unprotected skin

2.4.2
MEDp
MED on product protected skin

2.5
individual sun protection factor
SPFi
ratio of the minimal erythemal dose on product protected skin (MEDp) to the minimal erythemal dose on unprotected skin (MEDu) of the same subject:

$$SPFi = \frac{MED(\text{protected skin})}{MED(\text{unprotected skin})} = \frac{MEDp}{MEDu}$$

NOTE SPFi is expressed to one decimal place (see 7.1).

2.6
sun protection factor of a product
SPF
arithmetic mean of all valid individual SPFi values obtained from all subjects in the test

NOTE SPF is expressed to one decimal place (see 7.2).

2.7
test area
back between the scapula line and the waist

2.8
test site
site where a product is applied or the site used for the determination of the unprotected MED

2.9
exposure sub-sites
skin exposed spots

2.10
individual typology angle
ITA°
value characterizing the skin colour of the subject

3 General principle

The SPF test method is a laboratory method that utilizes a xenon arc lamp solar simulator (or equivalent) of defined and known output to determine the protection provided by sunscreen products on human skin against erythema induced by solar ultraviolet rays.

The test is restricted to the area of the back of selected human subjects.

A section of each subject's skin is exposed to ultraviolet light without any protection and another (different) section is exposed after application of the sunscreen product under test. One further section is exposed after application of an SPF reference sunscreen formulation which is used for validation of the procedure.

To determine the sun protection factor, incremental series of delayed erythemal responses are induced on a number of small sub-sites on the skin. These responses are visually assessed for presence of redness 16 h to 24 h after UV radiation, by the judgment of a competent evaluator.

The minimal erythemal dose (MED) for unprotected skin (MEDu) and the MED obtained after application of a sunscreen product (i.e. the MED for product protected skin, MEDp) shall be determined on the same subject on the same day. An individual sun protection factor (SPFi) for each subject tested is calculated as the ratio of individual MED on product protected skin divided by the individual MED on unprotected skin i.e. MEDp/MEDu.

The sun protection factor for the product (SPF) is the arithmetic mean of all valid SPFi results from each subject in the test.

4 Test subjects

4.1 Selection of the test subjects

4.1.1 General

For subject inclusion and non inclusion criteria, refer to Annex A.

4.1.2 Skin phototype of the test subjects

Test subjects included in the SPF test shall be only phototypes I, II or III according to Fitzpatrick[7] or shall have an ITA° value > 28° by colorimetric methods (see Annexes A and E) and be untanned on the test area. An SPF test should not contain subjects who are all of the same phototype.

A competent scientist or technician should examine each subject to ensure that there is no condition which might put the subject at risk and that the outcome of the test cannot be compromised by adverse skin conditions such as sun damage, pigmentation marks and previous history of abnormal response to the sun (see Annex A).

4.1.3 Age restriction

Test subjects below the age of consent or older than 70 y shall not be included in the SPF test panel.

4.1.4 Frequency of participation in tests

Since a sufficient interval after a previous test is needed in order to allow for reversal of skin tanning resulting from that previous test, a test site that has been exposed to UV should not be used in a subsequent test before two months have elapsed and the site is clear.

4.1.5 Ethics and consent

All testing shall be done in accordance with the Declaration of Helsinki[8] and National Regulations regarding human studies, if any.

Informed, written (signature) consent shall be obtained from all test subjects.

4.2 Number of test subjects

The minimum number of valid SPFi results shall be 10 and the maximum number of valid SPFi results shall be 20. In order to achieve between 10 and 20 valid results, a maximum of five individual invalid results may be excluded from the calculation of the mean SPF. Consequently the actual number of test subjects used will fall between a minimum of 10 and a maximum of 25 subjects (i.e. a maximum of 20 valid results plus 5 rejected invalid results).

Results may only be declared invalid and excluded from the calculation of the mean SPF according to 6.7.4 or because of non-compliance with the related protocol.

In order to determine the number of test subjects, the 95 % confidence interval (95 % CI) on the mean SPF shall be taken into account. A minimum of 10 subjects shall be tested. The test shall be considered valid for the first 10 subjects if the resulting range of the 95 % CI of the mean SPF is within ±17 % of the mean SPF. If it is not within ±17 % of the mean SPF, the number of subjects shall be increased stepwise from the minimum number of 10 until the 95 % CI statistical criterion is met (up to a maximum of 20 valid results from a maximum of 25 subjects tested). If the statistical criterion has not been met after 20 valid results from a maximum of 25 subjects, then the test shall be rejected. For details on statistical definitions, sequential procedure and calculations, refer to Annex D.

4.3 Test area

The back is the chosen anatomical region for the test area. The individual product test sites and the unprotected test site shall be delineated within the region between the scapula line and the waist.

Skeletal protrusions and extreme areas of curvature should be avoided.

5 Apparatus and materials

5.1 Source of ultraviolet radiation

5.1.1 General

The artificial light source used shall comply with the source spectral specifications as described in 5.1.2 and Annex B. A xenon arc solar simulator with appropriate filters is recommended.

5.1.2 Quality of ultraviolet radiation

5.1.2.1 The solar UV simulator shall emit a continuous spectrum with no gaps or extreme peaks of emission in the UV region. The output from the solar UV simulator shall be stable, uniform across the whole output beam (particularly important for a single large-beam) and suitably filtered to create a spectral quality that complies with the required acceptance limits (see Table B.1).

5.1.2.2 To ensure that appropriate amounts of UVA radiation are included in the spectrum of the solar UV simulator, the total radiometric proportion of the UVA II (320 nm to 340 nm) irradiance of the simulator shall $\geqslant$ 20 % of the total UV (290 nm to 400 nm) irradiance. Additionally, the UVA I region (340 nm to 400 nm) irradiance shall be $\geqslant$ 60 % of the total UV irradiance.

5.1.2.3 The source spectral specification is described in terms of cumulative erythemal effectiveness by successive wavelength bands from $<$ 290 nm up to 400 nm. The erythemal effectiveness of each wavelength band is expressed as a percentage of the total erythemal effectiveness from $<$ 290 nm to 400 nm, or as the percentage relative cumulative erythemal effectiveness (% RCEE). The definition and calculation of % RCEE values is described in Annex B and the acceptance limits are given in Table B.1.

5.1.3 Total irradiance (UV, visible and near infrared rays)

If total irradiance is strong, an excessive feeling of heat or pain may be induced in the irradiated skin of subjects. Therefore, total irradiance shall not exceed 1 600 W/m^2. When total irradiance is $<$ 1 600 W/m^2, it shall still be confirmed, prior to conducting an SPF test, that the irradiance to be used (UV, visible and near-infrared rays) will not induce an excessive feeling of heat in the skin.

5.1.4 Uniformity of beam

5.1.4.1 When a large-beam UV source is used to simultaneously expose several sub-sites (i.e. at least two sub-sites) within an irradiation series by varying the exposure time, the intensity of the beam shall be as uniform as possible. The minimum beam irradiance, at any sub-site, shall be no more than 10 % lower than

the maximum beam irradiance at any sub-site. If the variation exceeds 10 %, then appropriate compensation for different irradiance should be made in the exposure time on each sub-site.

5.1.4.2 For a small beam UV source, which exposes sub-sites individually, the erythema generated following exposure shall be as uniform as possible. An uneven erythema in unprotected skin (such as a half-moon shape) indicates that the irradiance is not uniform and the delivery system shall be corrected.

5.1.5 Maintenance and monitoring the UV solar simulator output

5.1.5.1 Radiometry

Before UV exposure of each test site, the UV irradiance should be measured and recorded with a radiometer calibrated against a spectroradiometric measurement of the solar simulator output.

5.1.5.2 Spectroradiometry

It is recommended that a complete spectroradiometric check (UVA and UVB) of output spectrum and intensity be made by the laboratory at least once every 18 months or after 3 000 h of lamp running time and after changing any significant physical (optical) component of the solar simulator. This periodical inspection should be conducted by a competent and suitably qualified person.

The simple use of specific filters is not in itself adequate assurance that the UV output is of the correct quality. Detailed instructions for ensuring correct lamp output are given in Annex B.

5.2 Reference sunscreen formulations

5.2.1 General

The method is controlled by the use of one of three reference sunscreen formulations to verify the test procedure. Therefore one of the prescribed reference formulations shall be measured on the same day as products are tested. Whether a low or high SPF reference formulation is to be used depends on the expected SPF of the test products.

5.2.2 Expected SPF < SPF 20

Any one of the following reference sunscreen formulations shall be used: P2, P3 or P7.

If a high SPF reference formulation is used, there is no necessity to also include the low SPF reference formulation in the test even though there may be low SPF test products.

5.2.3 Expected SPF ⩾ SPF 20

One of the following reference sunscreen formulations shall be used: P2 or P3.

If a high SPF reference formulation is used, there is no necessity to also include the low SPF reference formulation in the test even though there may be low SPF test products.

5.2.4 Acceptance SPF limits for the reference sunscreen formulations

Acceptance SPF ranges for the reference sunscreens are shown in Annex C. If the mean SPF obtained in any test does not fall within the acceptance limits of the reference values then the entire test (i.e. all test products) shall be rejected. If the 95 % confidence interval on the mean SPF for the reference sunscreen falls outside a range defined by the mean reference sunscreen SPF ±17 %, then the entire test (i.e. all test products) shall be rejected.

5.2.5 Formulae and preparation of the reference sunscreen formulations

The formulae details and manufacturing instructions for the reference formulations are given in Annex C.

6 Procedure

6.1 Main steps

— delineation of test sites on the back of the subject;

— weighing of the product;

— application of the product;

— waiting period before UV exposure;

— UV exposure;

— MED assessment;

— calculations.

6.2 Test conditions

Product application, UV exposures and MED assessment should be carried out in stable conditions, with the room temperature maintained between (22 ± 4) °C.

6.3 Position of the test subjects

All steps in the procedure shall be performed in the same position: an upright, seated or prone position.

Powder should be tested in the prone position to prevent the samples from falling off the surface.

6.4 Procedure for product application

6.4.1 General

The amount of product applied and the uniformity of spreading on the test sites affect the magnitude and variability of the test results. It is therefore very important to follow the recommendations set out in 6.4.2 to 6.4.5.

6.4.2 Test sites and product application

6.4.2.1 The test sites intended for UV exposure shall be free from blemishes and have an even colour tone.

6.4.2.2 The minimum total area for a test site for product application shall be 30 cm^2 and the maximum shall be 60 cm^2.

6.4.2.3 The positions of the test products and reference sunscreen test sites shall be distributed randomly on the backs of subjects over the whole test group in order to reduce error arising from anatomical differences in skin. The unprotected test site used to determine MEDu should be randomized as one of the test sites across the test area and across subjects.

6.4.2.4 There shall be a minimum distance of 1 cm between the borders of adjacent test sites.

6.4.2.5 Before product application, the test area may be cleaned by using a dry cotton pad or equivalent.

6.4.2.6 The test sites shall be delineated by a method which does not interfere with the test or harm the subject e.g. skin marker and/or a template made from non-absorbent material.

6.4.3 Amount of product applied

6.4.3.1 The amount of test product and reference sunscreen formulation applied to the skin before spreading shall be (2,00 ± 0,05) mg/cm^2.

6.4.3.2 The balance used to weigh the products should be capable of weighing to the nearest 0,000 1 g, i.e. to the nearest 0,1 mg.

6.4.3.3 All products should be homogeneous and should be shaken if necessary, before weighing, to ensure uniform dispersion.

6.4.3.4 When handling the product during weighing or before application to the skin, take appropriate measures to prevent evaporative loss of the volatile components. It is important that the total quantity of weighed product is transferred to the product application site.

6.4.3.5 The amount of product to be applied is weighed in a syringe or in another device such as a watch glass. A method of weighing by loss is strongly recommended.

6.4.4 Mode of delivery

6.4.4.1 General

The use of a finger cot is optional but is recommended. When employed, a new finger cot shall be used for each new application of product and should not be pre-saturated with the test product. When a naked finger is used, the finger should be cleaned between product applications.

6.4.4.2 Liquid type products (e.g. lotions, liquids, milks, creams, sprays and sticks)

6.4.4.2.1 To aid uniform coverage, droplets (approximately 15 per 30 cm^2, 30 per 60 cm^2) of the product should be deposited within the test site using a syringe/pipette, then spread over the whole test site using light pressure.

6.4.4.2.2 Spreading time should be in the range of (35 ± 15) s depending on the surface and ease of spreading of the product.

6.4.4.3 Powders

6.4.4.3.1 In the case of powder products, aliquots of powder should be transferred to the skin in a grid-like manner, using a spatula or finger.

6.4.4.3.2 The accumulated powder is tapped and then spread over the whole test site using a finger with or without a finger cot. Alternatively, the tip of a pre-loaded cosmetic applicator puff may be used instead of a finger. In this case, it is important to verify that (2,00 ± 0,05) mg/cm^2 of test powder product remains on the skin after spreading, by weighing the powder remaining on the tip of the applicator puff.

6.4.4.3.3 Purified water or another suitable solvent that has no UV protection properties may be applied on the skin before the powder application to help the sample adhere to the application site.

NOTE Powders present a unique form of cosmetic product. The modified method for these, described above, takes into account the need to present a reproducible application on the skin.

6.4.5 Drying time between application and UV exposure

Exposure of the test site to the sequence of UV doses shall start 15 min to 30 min after the application of the product(s). Any extraneous exposure of the test sites to UV light (artificial or natural) shall be avoided during this period and for a period of 24 h after exposure.

6.5 Procedure for UV exposure

6.5.1 Exposure sub-sites or skin exposed spots

6.5.1.1 Where a template is used to demarcate the exposure sub-sites (e.g. large-beam UV solar simulator), the template should be of non-absorbent material.

6.5.1.2 The minimum area of each exposure sub-site is 0,5 cm^2.

6.5.1.3 The minimum distance between borders of each exposure sub-site (spots) shall be at least 0,8 cm.

6.5.1.4 The distance between any exposure sub-site and any edge of the test site shall be at least 1 cm.

6.5.1.5 The minimum number of exposure sub-sites used shall be five for unprotected MED (MEDu) and five for protected MED (MEDp).

6.5.2 Provisional MEDu

Before starting the main test, it may be necessary to determine a provisional MEDu in order to centre the UV dose ranges for the exposures of MEDu and MEDp. A provisional MEDu is a pre-test in which the MEDu of a subject is determined prior to establishing the test MEDu. This is performed by applying a preliminary series of UV exposures up to one week before the test.

6.5.3 Estimated/anticipated MEDu

The MEDu can be estimated by colorimetric technique (ITA°) without UV exposure (Annex E) or predicted by an experienced technician (i.e. history of the subjects) (anticipated MEDu).

6.5.4 MEDu

For each subject, the unprotected MEDu shall be determined on the same day as the test product protected MEDp.

6.5.5 Incremental progression of UV dose

6.5.5.1 For the unprotected site, the range of UV doses applied shall be established using the subject's provisional MEDu, the estimated MEDu or the anticipated MEDu. A minimum of five sub-sites centered on or close to the provisional/estimated MEDu shall be exposed with incremental UV doses using a recommended geometric progression of 1,25 $\times$. Other geometric progressions of less than 1,25 $\times$ may be used (e.g. 1,2; 1,15; 1,12) but should be consistent throughout the test.

6.5.5.2 For the product protected sites, the UV doses delivered are defined by the expected MEDp, which is the multiple of the expected SPF of the test product and the provisional MEDu for the subject. A minimum of five sub-sites centered on or close to the expected MEDp shall be exposed with incremental UV doses using a recommended geometric progression of 1,25 $\times$. Other geometric progressions may be used (e.g. 1,2; 1,15; 1,12). A maximum geometric progression of 1,15 shall be used for expected SPF $>$ 25. Smaller geometric progressions (e.g. 1,12) may be used but shall also be consistent throughout the exposure sequence.

6.6 Product removal

After UV exposures, reference and test products may be gently removed, using an appropriate means.

6.7 Procedure for MED assessment

6.7.1 General

The minimal erythemal dose for unprotected skin (MEDu), that for test product protected skin (MEDp) and the MEDp for the reference sunscreen formulation, shall all be determined on the same day.

6.7.2 Time of assessment of MED

The MED shall be assessed when the erythemal response is optimal, i.e. 20 h ± 4 h after UV exposure (between 16 h and 24 h). During the time interval between UV exposure and MED assessment, the subject shall avoid any extra UV exposure (artificial UV light or sunlight) to the exposed area. Any additional exposure to the test area will invalidate the whole test.

6.7.3 MED assessment

6.7.3.1 The MED shall be assessed visually. The observer's eyesight should have been checked for normal colour vision. A yearly check of acuity of vision is recommended.

6.7.3.2 Visual assessment should be performed in sufficient and uniform illumination. At least 450 lux are recommended.

6.7.3.3 The determination of MEDs shall be carried out in a room with matt, neutral wall colours.

6.7.3.4 Erythemal responses shall be observed in a "blind" manner. The observers of erythemal responses on any subjects should not be the same persons as the ones who performed product application and exposure. The observers shall be not aware of the test design (randomization of test sites) on that subject.

6.7.4 Data rejection criteria

Test data are deemed invalid and shall be rejected under the following circumstances:

— the series of UV exposures on a subject fails to elicit an erythemal response on any sub-site, 20 h ± 4 h after exposure;

— erythemal responses within an exposure series are randomly absent 20 h ± 4 h after exposure;

— all sub-sites in the exposure series show an erythemal response 20 h ± 4 h after exposure.

When one of the above criteria applies to the exposure series on unprotected skin or to the reference sunscreen formulation exposure sites, then all data for all products on that subject are invalid and shall be rejected.

When one of the above rejection criteria applies to a test product treated exposure series, then all data for that test product on that subject are invalid and shall be rejected.

If invalid data (whether MEDu or MEDp) have to be rejected for any one product on more than five subjects, then the whole test for that product is invalid and shall be rejected.

If invalid data have to be rejected for the reference sunscreen on more than five subjects, then the whole test is invalid and shall be rejected.

Any additional exposure to the test area will invalidate the whole test.