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REMARKS ON BIOLOGIC EVALUATION OF PROTECTION FACTOR FOR SUN PRODUCTS

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SUMMARY

Skin and Sun Radiations. Filtering Power Measurement. The USA (FDA) and the German (DIN) methods: - Type of UV radiation source; - Quantity of product applied; - Radiation time increase on different skin areas; - SPF calculation; - Standard Reference Product; - MED Evaluation Time; - Type of Skin; - Washing-Away Resistance; - Variations of the Australian Standards Association. Cycloglucanic or cyclodextrinic complexing. Evaluation of anti-UV.A screening.

Skin and Sun Radiations

Excessive skin exposure to sun radiations may acutely cause inflammation, while chronically leading to skin ageing and cancer.[1]

From a biologic point of view, the most active part of the solar electromagnetic spectrum lies between 280 and 700 nm . UV.C radiations, namely those with wave length below 290 nm, are filtered through the stratosphere.[2]

UV.C radiations are used for their germicidal action.[3]

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[1]**UV.B** negative effects are the following: sunburn; DNA damages; early skin ageing, skin cancer; immunosuppression; photodynamic reactions; photodermatitis; eye inflammations. **UV.A** negative effects are the following: collagen damage; loss of skin elasticity; immunodepression; photodynamic reactions; photodermatitis; eye inflammation. Individuals who, as children or adolescents, have once or several times suffered from sunburns with blisters have double probability to develop **malignant melanoma**. Among these individuals, the risk for those with a light skin is three times higher [R.S. Stern, M.C. Weinstein, S.G. Baker: *Risk reduction for non melanoma skin cancer with childhood sunscreen use*. Arch. Dermatol. 122:537-545 (1986)].

The regular use of **sunscreens** reduces the incidence of non melanoma skin cancer by only 70%, with a 78% value when sunscreens are regularly used since childhood.

The results thought to be obtained from oral administration of b-**carotene** do not exactly meet the expectations. As a matter of fact, a-carotene and not b-carotene, has a ten times higher antiproliferative effect (H. Nishino, J. Takayasu, A. Iwashima, M. Murakoshi, J. Imanishi, 1988). Moreover, of the **three different carotenes** which in nature are always mixed together, namely a-, b- and c-carotene, a-carotene is the only optically active one with different cyclic ends: one is an a-ionone, the other a b-ionone. Therefore, in order to obtain the same effect as a, b-carotene should be taken in great quantities, which would even stain the skin. Moreover, empirical oral administration of natural substances, namely those containing the three carotenes, is only potentially effective. Therefore b-carotene is no guarantee today.

[2]These radiations are more exactly filtered by the **ozone** layer. Therefore, should the excessive use of chlorofluorocarbonates lead to the loss of this protective layer, UV.C radiations will have to be taken into account.

[3]Quite often, observations based on few experimental data were for a long time considered to be sufficient to correctly define the course of a phenomenon. This was also the case of **UV.B and UV.C radiations induced erythemas** which, on the basis of studies conducted many years ago, were thought to have a different course. Namely the UV.C induced erythema was thought to have its onset, reaching its maximum intensity and disappearing more rapidly than when induced by UV.B. This assumption is based on data reports resulting from limited studies which do not specify whether the compared UV.B and UV.C erythemas had the same intensity. Since a different course of these two types of erythema is likely to involve also a different pathogenetic mechanism, with a particular role played by prostaglandins as inflammation mediators, Farr and coworkers correctly decided to review the problem under experimental conditions permitting a more exact lesion degree quantification (1988). They conducted their research on 8 adult volunteers (4 males and 4 females) by using their central back skin. The radiation equipment employed was supplying 30 W/m^2 between 200 and 290 nm (namely within the UV.C spectrum, with 96% emission at 254 nm wavelength) and 80 W/m^2 between 290 and 320 nm (namely within the UV.B spectrum). At both sides of the median dorsal line, 6 areas were identified with a 20 mm diameter each, 5 of which underwent increasing UV.B radiation doses for factors 1, 3 or increasing UV.C radiations for factor 2, with an initial minimum amount of 0.6-1.2 kJ/m^2 UV.B and 0.08-0.25 kJ/m^2 UV.C radiation dose respectively. The erythema degree was assessed after 4-8-12-24-36 and 48 hours after radiation exposure with a suitable reflectance instrument to evaluate the quantity of red and green light reflected by the skin. This

Yet, most studies were devoted to UV.B radiations (290-320 nm) because of their photobiologic effects: cell mitosis inhibition, vitamin D synthesis, carcinogenic processes.[4]

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method is employed to obtain an "erythema index" which is proportional to skin blood content. The sixth radiation-free area was used as control. Radiation induced vasodilation increase was calculated by subtracting from the values obtained at several time intervals the erythema index value recorded at zero time. A 0.05 increase corresponds to a minimum erythema producing dose, namely a uniform clear-cut edge rash in the radiation exposed area, whereas a 0.3 increase corresponds to marked erythema. Finally, to better identify the erythema course in each subject, data obtained under different radiation dosages at each observation time have been added together thus obtaining a dose independent index. The results have shown that at both wave lengths, namely with both UV.B and UV.C, the erythema appears 4 hours after radiation exposure with its peak between 8 and 24 hours. Moreover, although peak time varies considerably from person to person, in each of them the peak for both radiation types seemed to coincide. Obviously enough, UV.B and UV.C radiation induced inflammation duration depended on radiation dose and, when the erythema index increase at 12 hours was lower than 0.1, the erythema subsided within 48 hours. Finally, by comparing the course of UV.B and UV.C induced erythemas, significant differences were observed only in three out of eight subjects examined. Major differences were observed between individual subjects regarding erythema course, especially with reference to the time interval from exposure to inflammation peak. Yet, in most of them, UV.B and UV.C radiations were observed to lead to an alteration sequence which was substantially similar with respect to time-dependence. This would suggest that in both radiation types the same action mechanism is at the origin of inflammation. Furthermore, the results obtained would indicate that previous reports about a more rapid onset and shorter course of UV.C radiation induced erythemas should be considered as questionable and even likely to be wrong, since they were based on the comparison of radiation doses causing different intensity and duration reactions.

[4]**The mechanism and consequences of UV radiations and DNA interaction** are well known. Today there is general agreement on the role played by these radiations as a trigger of carcinogenic processes of the cutaneous tissue. Less understood is whether and by which mechanism UV radiations promote **skin carcinogenesis**. This process can be experimentally promoted by topical application of phorbol esters which cause inflammation and increase the proliferative activity. These processes are mediated by structural and functional alterations of **membrane phospholipids**, by phospholipase activation and arachidonic acid metabolites production. Therefore it was interesting to verify whether UV radiations have effects analogous to phorbol esters, particularly by taking into account that both agents give origin to oxygen free radicals and therefore to oxydation stress. Being choline containing phospholipids (phosphatidylcholine, lysophosphatidylcholine and sphingomyelin) among the most important components of cell membrane lipid structure in the human skin, De Leo and coworkers (1988) have recently studied UV effects (290-380 nm) on choline metabolism in "in vitro" cultivated human keratinocytes. The study was conducted on **human skin culture cells** obtained with normal methods from breast skin taken during surgeries. When, 10-14 days after seeding, the cells were almost merging together, they were incubated for 24 hours with ^{3}H-choline and then exposed to different UV.B radiation dosages. Cultures were then maintained at 37°C for variable periods of time. Afterwards, the ^{3}H-choline content in the culture medium was assessed by liquid phase scintillation. Moreover, choline containing metabolites were identified through paper chromatography, their origin was determined, and their amount in the acid-precipitable and acid-soluble fractions of radiations exposed cells assessed while the ^{3}H labelled cellular phospholipids were separated by means of HPLC. First of all, the Authors observed that over a 24 hours period, keratinocytes take in from 40 to 60% of ^{3}H-choline present in the culture medium and that in non radiation-exposed cells only 1.5-3% of ^{3}H-choline employed for phospholipids synthesis is released into the culture medium over the following 4 hours period. Treatment with 400-2,400 J/m^{2} UV.B radiation doses, over the same period of time, resulted in a dose-dependent increase in the quantity of ^{3}H-choline yielded to the medium and that, at its maximum level, it was from 2 to 4 times greater than in control cultures. By measuring the time-dependence of ^{3}H-choline yield by keratinocytes, they observed it already after 15 minutes UV.B radiation exposure while lasting for 26 hours and reaching a 12 times greater value than in controls. Since several ^{3}H-choline containing metabolites could be hydrolyzed by membrane phospholipids under the effect of various lipases, they were identified by their conjugation with known standard values on paper chromatography. If compared to observation results in control culture medium, it was evident that glycerophosphorylcholine showed the highest increase. Keeping in mind that in mammal cells choline is not only present in membrane phospholipids but also in watersoluble metabolites intracellular pool, De Leo and coworkers have conducted their studies to establish the origin of ^{3}H-choline released in the culture medium. Their studies pointed out that cell membrane associated radioactivity (precipitable with trichloroacetic acid) would diminish after UV.B radiation exposure, whereas the intracellular pool radioactivity (trichloroacetic acid soluble) would not. This would indicate that ^{3}H-choline released into the culture medium under the effect of UV.B radiations, derives from membrane phospholipid demolition, and it cannot be ascribed to ^{3}H-choline spilling out from the watersoluble pool, caused by cell membrane permeabilization by UV.B radiations. Finally UV.B effect on membrane phospholipids was examined through HPLC separation and by measuring the quantity of ^{3}H-labelled ones by means of appropriate standard methods. Whereas there was 82% phosphatidylcholine and lysophosphatidylcholine in control cells and 15% sphingomyelin, radiation exposed cells showed a clear reduction of the first two substances, thus indicating that the medium released ^{3}H-choline was mainly originating from this class of phospholipids. **The quantity of phospholipids present in human skin cells was already known to differ**

UV.A radiations (320-400 nm) are responsible for *physiologic* tan (different from the *reactive*, post erythematous tan caused by UV.B) and for most phototoxic and photoallergenic reactions.
The visible light is essential circadian cycles, pigment darkening and the induction of some photodynamic effects.
The penetration of sun radiations is directly proportional to their wavelength. It depends on skin layer thickness, on the quantity of melanin and other pigments and on vascularization degree.
Just to give an example, only about 40% of UV.B radiations get through the corneous layer, whereas radiations with greater wavelength have more penetrating effects.
Sunscreens are compounds which, once applied on the skin, protect it from the dangerous UV radiation effects.
They can absorb and/or reflect only UV.B or both UV.B and UV.A radiations. They are not only employed to reduce the acute burning action of sun light, but also to prevent chronic alterations like actinic elastosis, solar keratosis and carcinogenic processes.

according to cell differentiation degree. Upon progress of the keratinization process, such a quantity decreases with a specific purpose: as a matter of fact, the membrane phospholipids transformation into neutral lipids, catalyzed by **epidermal lipases**, ensures one of the most important skin functions, namely the production of a **lipid barrier** defending us from the external environment and reducing transepidermal waterloss. More recently, phospholipids have also been found to be at the origin of two important **mediator** classes: a first class represented by **arachidonic acid metabolites** (prostaglandins and leukotrienes) and a second one by **phosphatidylcholine metabolites, platelets activating factor and lysolecithine**. These are active factors producing edema, erythema, leukocyte chemiotaxis and keratinocytes proliferation alterations, while regulating melanocytes and Langerhans cells functions. In the latter work, De Leo and coworkers have demonstrated that **UV.B radiations do not only stimulate arachidonic acid release from phospholipids in the keratinocytes as well as the formation of its metabolites, but they are also triggering the release of another membrane phospholipids component: glycerophosphorylcholine.** This implies that two enzymes are concurrently activated: *phospholipase A* and *lysophospholipase*, both present in the human skin.
In conclusion, UV.B radiations in human keratinocytes, in addition to the already described DNA-damaging effects, clearly provoke phospholipase activation as well, which, by degrading membrane phospholipids, generate metabolites analogous to those normally formed after application of conventional chemical inducers of skin carcinogenic process. Therefore, UV.B radiations are likely not only to trigger but also to promote this process.

The effectiveness of a UV filter can be assessed by physical methods, namely by measuring its extinction coefficient at different wavelengths.

Filtering Power Measurement

In this short paper we shall just give some information on how to measure the filtering power of a compound through biologic methods based on the results of "in vivo" studies on human or laboratory animal skin.
The methods employed measure the erythema-threshold rise. *Erythema-threshold* means the skin inflammatory reaction characterized by erythema, with or without edema, hardly noticeable yet clearly defined, which is obtained through consistent experimental conditions (same radiation source and incidence) and assessed by the same observer.
Under constant radiation intensity, the *erythema-threshold* and therefore the minimal erythematogenic dose (MED) is defined by radiation time. Radiation time corresponding to MED clearly depends on the type of skin and radiation source.
The *Sun Protection Factor* (*SPF*) of a solar filter is obtained by calculating the ratio between MED on sun protected and unprotected skin. In other words, it indicates the prolongation rate of the solar filter under examination with respect to the radiation time necessary to provoke the *erythema-threshold*. A more accurate *SPF* value is obtained by taking into account also the quantity of product applied per surface unit. This is obtained by multiplying *SPF* by the following ratio (ml of applied product/cm^2 of treated skin).

The *SPF* of a UV filter can be determined according to:
the USA method proposed by the OTC group of FDA,
- the German method proposed by the Deutches Institut für Normung (DIN).

In both cases, the method is the following: on the back of a volunteer a series of generally square surfaces of limited size arranged along horizontal lines is marked. The product under examination and a reference standard product are then applied on the surfaces of different lines. The remaining, untreated surface is the unprotected control skin. These areas then undergo radiation for different periods of time and, at a 16-24

hours interval, the different erythema degrees are recorded.
The ratio between the time necessary to obtain the *erythema-threshold* with the tested product (namely its MED) and the time necessary to induce the same degree of erythema on unprotected skin corresponds to the individual *SPF*.
Since this test is conducted on a population of individuals, it is more appropriate to define the effectiveness of a UV filter as average protection rate (Q).
The average protection rate:
Q (log)
is the average of individual protection rates (Qi). Therefore it is sufficient to know the number of protected areas in which no erythema has occurred (D_i)
Qi(log) = delta i / 2
Then, the average of the sum of these individual values
(delta/$_2$)
is converted by means of an appropriate table into the average protection rate, with the following mathematic formula:
Q=2 ^ delta/2
The compound has no effect if Q=1, it has screening properties if Q>1 and it is phototoxic if Q<1.
The USA (FDA) and the German (DIN) methods differ from each other on several points indicated here below:

Type of UV radiation source:
- the FDA method employs a filtered Xenon arc lamp producing a continuous emission spectrum without peaks in UV.B and UV.A regions;
- the DIN method employs an Osram Ultravitalux lamp (or a Philips MLU 300-WE) having a spectrum with two peaks at 312 and 365 nm which virtually does not produce UVA, apart from the second peak. There is no common agreement on the most suitable type of lamp when the *SPF* is less than 15. Conversely, when *SPF* is >15 the Xenon arc lamp should be used, since it produces a higher quantity of UVA.

Quantity of product applied:
FDA method: 2.0 mg/cm^2;
DIN method: 1.5±0.15 mg/cm^2.
The quantity of product applied corresponds to a filter of about 20lm and 15lm in the FDA and DIN method respectively.

Radiation time increase on different skin areas:
FDA method: 25%;
DIN method: 40%.

SPF calculation:
FDA method: it is based on arithmetic average;
DIN method: it is based on geometric average.

Standard Reference Product:
FDA method: 8% homosalate, *SPF*=4.1;
DIN method: 2.7% p-methoxy-2-ethylhexyl-cinnamate, *SPF*=3.7.

MED Evaluation Time:
FDA method: unprotected skin MED is measured 24 hours before evaluating the MED value of the skin protected with the tested product.

Type of Skin:
FDA method: 20 subjects with light skin who, after a 30-60 minute sun exposure after the winter season get easily or relatively easily sunburnt (Skin type I-III according to a classification distinguishing 6 types of skin with progressively decreasing sun sensitivity);
DIN method: 20 subjects.

Washing-Away Resistance:
Unlike the DIN method, the FDA standard requires product resistance examination after water immersion. Each immersion lasts 20 minutes followed by a 20 minute interval before radiation exposure.
If the product is still effective after two immersions, it can be defined as *water-resistant*. If it is still effective after four immersions, it can be declared as *waterproof*.

Variations of the Australian Standards Association
Within the FDA method there is also a variation approved by the Australian Standards Association in which the major change refers to the number of persons employed for the test, namely 10 instead of 20. Therefore, the standard error in the individual *SPF* average must not exceed ±10%, instead of ±5% as required by the FDA.
Because of all these differences, different values are obtained with these two meth-

ods, which result to be definitely better in the FDA procedure.
For example, **regarding the same W/O emulsion** containing 1.5% 2-ethylhexyl-4-dimethylaminobenzoate
- with the DIN method (FRG) a 5-6 *SPF* value is obtained;
- with the FDA method (USA) a 10-12 *SPF* value is obtained.

This difference creates confusion in the consumer as well as wrong interpretations about the screening power of any advertised product. It mainly derives from a different quantity of product applied and therefore a different film thickness on the skin.
As a matter of fact, if a 20lm film corresponds to a 10 *SPF*, theoretically a 15lm film of the same sunscreen shall have a 7.5 *SPF*.

Cycloglucanic or cyclodextrinic complexing

Obviously enough, the *SPF* value also depends on the carrier with which it is combined. The higher its skin adhesion and its capability to form a compact film, the better its final filtering result and, at equal dose, the higher its *SPF* value. Very good results are obtained with ointments and W/O creams [see non-sensitizing Seboside (Vevy codex 02.1310) based W/O creams]. Yet skin adherence and water-repellent properties can also be effectively obtained with PME (Vevy codex 03.0775) containing O/W emulsions, with the advantage, over W/O ones, of a better and more uniform filter adsorption - if lipid soluble - into the corneous layer. On page n.93 of the 5th issue of Lexicon Vevy Europe Skin Care Instant Reports, dated May, 1989, we mentioned a new release model. In a next issue of this monthly magazine we will discuss thoroughly complexing with 6-8 units of D-glycopyranose which will form cyclodextrins or cycloglucans. In view of the fact that at the end of 1986, 750 Patents cover many sectors (for instance: food, pharmaceutical, hygiene and cosmetic sectors) you may realize that our comparison between complexing and UV absorbers with other customary complexing agents is very important.

Evaluation of anti-UV.A screening

A final remark refers to the difficulty of evaluating a film protection property against UV.A radiations only. To determine the MED value very long exposure periods or extremely powerful sources would be necessary. Therefore, in order to avoid this obstacle, several expedients have been suggested. For example, the use of very UV.A sensitive individuals which is hardly feasible and ethically rejectable, or rather the use of photosensitizing substances, like psoralens.
A test on UV.A sensitized hairless mice has been devised for this purpose by means of intraperitoneal treatment with 8-methoxypsoralen.[5]

[5]**See Lexicon Vevy Europe Skin Care Instant Reports 1987, 2:2-28:**
5.1. Immunodepressive effects of ultraviolet radiations (UV and Langerhans cells. Immunologic reactivity. Corneal layer and UV-absorbing molecule. Skin production of a mediator).
5.2. Skin colour and ultraviolet radiation effect relation: quantitative aspects (Skin colour. Melanometry).
5.3. Studies on butylhydroxytoluene photoprotective action mechanism (Oral administration of antioxidants. Topical application of antioxidants. Problems with the following antioxidants: BHA, BHT, TBHQ, DTBHQ, NDGA, ethoxyquinine, dodecylgallate, propylgallate, monosulfiram, tocopherols).
[Lexicon Vevy Europe Skin Care Instant Reports 1985, 9:9-10, EDTA].
5.4 Megasol-complex selective filtering properties (UV.B + UV.A). (Sun light. Tanning effect. Selective filtering. Synergic effect of component association contained in Megasol-Complex. Photoselective action evaluation. Toxicologic study and non-enzymotoxic effect evaluation. In vitro release evaluation from excipients for topical use. Evaluation of transcutaneous non-absorption).
5.5 Experimental dermatological assessment of the protective action of Megasol-Complex against sun rash (*SPF experimental protocol: Megasol-Complex 5.4% ¢* SPF_{DIN} *= 8 corresponding to* SPF_{OTC} *= 15*)