

INVESTIGATIVE REPORT

Skin Pigmentation Kinetics After UVB Exposure

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There have been few previous studies of the kinetics of pigmentation following ultraviolet B (UVB) exposure, and these have included only fair-skinned persons. The current study investigated pigmentation increase to steady state and fading in 12 Scandinavians and 12 Indians/Pakistanis. Over a period of 3 weeks the subjects were UV-irradiated 6 times on the right side of the back and 12 times on the left side using a Solar Simulator and narrowband UVB with equal sub-Minimal Melanogenesis Doses (individually predetermined). Pigmentation was measured from skin remittance at 555 nm and 660 nm (allowing correction for erythema). The absolute pigmentation increase was independent of pre-exposure pigmentation, therefore the percentage pigmentation increase was higher in fair-skinned volunteers. The UV dose to minimal pigmentation was higher in darker-skinned persons for single and multiple UV exposures for both UV sources. Going from a single exposure to 6 and 12 exposures, the required dose to minimal pigmentation was reduced by factors of 2 and 3, respectively, for both UV sources, thus reducing the risk of sunburn, but the cumulative dose increased 3- and 4-fold, respectively. This result was independent of skin type and pre-exposure pigmentation. Fading took 4–5 months and was not related to frequency of UV exposure or to ethnic origin. Key words: pigmentation; ultraviolet radiation; UVB; fading.

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Ultraviolet B (UVB) phototherapy of skin diseases such as psoriasis and atopic dermatitis always consists of repeated exposures, as is the case for sun exposure. However, few studies have investigated the pigmentation increase after multiple UV exposures, and these studies have been mainly in fair-skinned persons (1–5).

The aim of this study was to determine pigmentation increase to steady state after multiple UVB exposures and time to fading of this pigmentation in volunteers with a wide variation in constitutive pigmentation. Furthermore, the doses¹ required to give minimal

pigmentation after single and multiple exposures to 2 different UV sources were determined.

MATERIALS AND METHODS

Subjects

The study was approved by the local ethics committee (KF 11-057/00) at Bispebjerg Hospital, Copenhagen, Denmark. UV exposures were performed from the end of November to January 2001. Three volunteers were UV-exposed in March and agreed not to expose their back to sunlight until their last measurement at the beginning of July. In order to include volunteers with a wide variation in constitutive pigmentation, we selected both Scandinavian and Indian/Pakistani subjects. A total of 24 healthy volunteers, 15 women and 9 men (mean age 25 years, age range 20–33 years) were enrolled in the study after giving informed consent. Skin diseases, sunbathing or exposure to artificial tanning 3 months prior to entering the study excluded participation, as did sun-sensitizing medication. Volunteers were instructed to avoid sunbathing and use of tanning beds during the study and 4 months after the last UV exposure. Skin type distribution is shown in Table I (6). The measured baseline skin pigmentation on the back ranged from 13% to 60% (7).

Study design

When erythema is considered, the dosing unit is normally Minimal Erythema Dose (MED), expressed as number of SEDs (8). As we were considering pigmentation we chose to use Minimal Melanogenesis Dose² (MMD). The individual MMD was predetermined clinically 7 days after a single UV exposure to 6 different UV doses with 25% increments and used as our dosing unit during the multiple UV exposures.

Multiple UV exposures. The right and left side of the back was subdivided into 2 areas. On each side one area was allocated to irradiation with narrow-band UVB (nUVB) (TL01) (Philips, Rosendaal, Holland), the other area to UV irradiation with a Solar Simulator (Solar Light Co., Philadelphia, PA, USA) by randomization to avoid possible anatomical differences in UV sensitivity. The right side of the back was exposed on 2 consecutive days per week and the left side was exposed on 4 consecutive days per week during 3 weeks; a total of 6 and 12 UV exposures, respectively. For each of the 4 areas, 4 squares measuring 2×2 cm, each representing one UV dose (0.8, 0.6, 0.4 and 0.2 MMD) were arranged as openings in a UV impermeable

¹One standard erythema dose (SED) is defined as the UV dose that elicits just perceptible erythema in the most sensitive people in a group of very sun-sensitive, but otherwise healthy, individuals. One SED has the physical dimension of 100 J/m² using the Commission International de l'Eclairage (CIE) erythema action spectrum (normalized at 298 nm).

²The UV dose to elicit just perceptible tanning 7 days after a single UV exposure.

Table I. Average ultraviolet (UV)-dose (SED*) to minimal pigmentation for each skin type group after a single exposure to Solar Simulator or narrow band UVB (nUVB). The total number of volunteers with the different skin types is given together with the Scandinavian/Indian-Pakistani ratio

Skin type	Total (n)	Pre-exposure pigmentation Mean pigmentation % Range (min. – max.)	UV-dose (SED) to minimal pigmentation	
			Solar	nUVB
II	5 (5/0)	22.9 (13–32)	5.3	5.2
III	6 (5/1)	26.7 (22–36)	7.5	6.6
IV	4 (2/2)	31.2 (22–42)	9.6	8.3
V	9 (0/9)	46.3 (35–60)	10.5	8.9

*1 SED corresponds to 1490 mJ/cm² (Solar) and 181 mJ/cm² (nUVB).
SED: standard erythema dose.

mask, 8 on each side of the back. In a previous project, with large steps between doses, we found that steady-state pigmentation was not reached after daily UV exposures during 5 days (9) and in a pilot study with UV-exposures during 4 weeks it took 3 weeks to reach steady-state pigmentation.

To avoid/minimize the number of burning events, only sub-melanogenic UV doses: 0.8, 0.6, 0.4 and 0.2 MMD were used. UV exposure was interrupted in a specific area if the visual erythema grade was +++ (bright red erythema with oedema raised above the adjacent non-irradiated skin) or if there was an excessive burning sensation irrespective of the erythema grade. Data derived from these specific UV doses were excluded from analysis.

Evaluation of the pigmentation. Skin pigmentation was measured by reflectance spectroscopy (UV-Optimize 555, Matic, Naerum, Denmark) (7) with peak wavelengths at 555 nm and 660 nm. The reflectance of these wavelengths is a direct measure of melanin in the skin (10). The methodology is more closely described elsewhere (11). Reflectance spectroscopy can be used to quantify both pigmentation and erythema response to UV (10, 12). We expected the highest pigmentation to occur one week after the last UV exposure (13).

Before the first UV exposure the baseline skin pigmentation was measured. Tanning was assessed both visually and instrumentally and erythema evaluated visually before irradiation in every exposed spot at every visit, and 1 week, 2 and 4 months after the last UV exposure. Skin pigmentation was evaluated clinically as no pigmentation (the same as in the surroundings) or + for just perceptible pigmentation.

Pigmentation was evaluated as the *absolute* and *percentage* increase after 6 and 12 UV exposures and was related to the pre-exposure pigmentation on the back and to Fitzpatrick skin type.

The minimum level of pigmentation was defined as the lowest measured value on day 1 or 2. The maximum pigmentation was set as the highest value observed in each test spot.

Pigmentation after a single high UV dose was still present after 10–12 weeks (13), so fading was evaluated 2 and 4 months after the last UV exposure. Finally, time to total fading, in days, was evaluated in relation to the total number of UV exposures and ethnic origin.

Time to total fading was determined as the day when the pigmentation value was equal to pre-exposure pigmentation. This was determined by a linear regression model based on 2 or 3 points (pigmentation 1 week, 2 months and 4 months after the last UV exposure, respectively). When fading was completed at the 4 months control, the linear regression was based only on the 1 week and 2 month measurements after exposure.

Fading data were excluded from analysis if:

- Pigmentation at the 2-month visit was higher than one week after the last UV exposure, or if pigmentation at the 4-month visit was higher than 2 months after the last UV exposure, thus taking into account volunteers who, despite the instructions, might have been sun-exposed during the fading period.
- Pigmentation values 2 or 4 months after the last UV exposure were lower than the initial pigmentation, indicating that these volunteers had been sun-exposed less than 3 months prior to entering the study.

Dosimetry and UV radiation sources

The emission spectra and intensity of the radiation sources were measured before the start and regularly during the study using a spectroradiometer calibrated by the manufacturer (Sola Tell, Sola-Hazard, 4D Controls Ltd, Redruth, UK). The erythema potential of the UV sources was measured in SED, as proposed at the 12th International Congress on Photobiology in 1996 (8, 14) and accepted by the CIE (15). One SED has the physical dimension of 100 J/m² using the CIE action spectrum (normalized at 298 nm) (16).

The nUVB consisted of a bank of 6 Philips fluorescent tubes (TL01). The nUVB emits 81% in the UVB range and 19% in the UVA range, with peak emission at 311 nm (1 SED corresponds to 181 mJ/cm²). During irradiation the distance from the skin to the tubes was 40 cm.

The Solar Simulator is equipped with a filtered xenon arc lamp that emits 8.7% in the UVB range and 91.3% in the UVA range (1 SED corresponds to 1490 mJ/cm²). The Solar Simulator conducted UV irradiation through 4 individually adjustable liquid light-guides ending 1 mm from the skin and irradiating a 1 cm diameter spot. The 4 doses used were calculated separately for each UV source by using the erythema effectiveness spectrum of the lamp and the conversion factor for 1 SED (Table I).

Statistics

The results were compared as follows: (i) dose in relation to pigmentation/percentage increase/absolute increase; (ii) days to total fading by linear regression analysis. Linear regression analysis and covariance analysis were performed to test whether the differences between the slopes and the differences between the intercepts were significant (Fig. 1). The absolute pigmentation increase after 6 and 12 UV exposures was normally distributed and therefore compared using a paired *t*-test. Most of the data were not normally distributed and were therefore compared using non-parametric tests. Days to reach total fading for Scandinavian and Indian/Pakistani subjects were compared using an unpaired non-parametric test (Mann-Whitney *U* test). Days to reach total fading after 6 UV exposures vs. 12 UV exposures were compared using Wilcoxon signed-rank test.

A normal distribution could not be assumed for skin type in relation to percentage increase in pigmentation due to few data. Therefore non-parametric ANOVA tests (Kruskal-Wallis test) were performed.

To test if there was a constant ratio between UV dose to minimal pigmentation after one UV exposure and multiple UV exposures for all skin type groups, non-parametric ANOVA tests (Kruskal-Wallis test) were performed. The UV sources were compared by Wilcoxon matched-pairs test. *p*-values less than 0.05 were considered to be significant.

RESULTS

By using equal individually predetermined MMD instead of the usual equal MED, different parameters

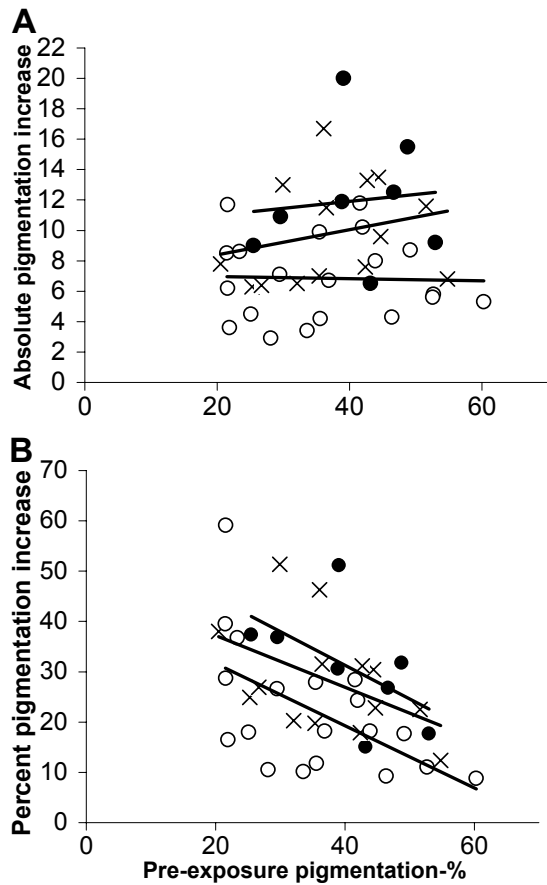


Fig. 1. (A) Absolute pigmentation increase after 12 ultraviolet (UV) exposures to narrow band UVB (nUVB) associated with pre-exposure pigmentation on the back. Equations for the linear regression lines for the 3 doses: (●) 0.8 MMD: $y = 0.046x + 10.1$, $R^2 = 0.01$ ($p = 0.81$), $n = 8$ (×) 0.6 MMD: $y = 0.082x + 6.8$, $R^2 = 0.05$ ($p = 0.41$), $n = 14$ (○) 0.4 MMD: $y = -0.0068x + 7.1$, $R^2 = 0.001$ ($p = 0.90$), $n = 20$ (B) Percentage pigmentation increase after 12 UV exposures to nUVB associated with pre-exposure pigmentation on the back. Equations for the linear regression lines for the 3 doses: (●) 0.8 MMD: $y = -0.67x + 58.1$, $R^2 = 0.30$ ($p = 0.16$), $n = 8$ (×) 0.6 MMD: $y = -0.52x + 47.6$, $R^2 = 0.22$ ($p = 0.09$), $n = 14$ (○) 0.4 MMD: $y = -0.62x + 44.0$, $R^2 = 0.32$ ($p = 0.01$), $n = 20$ MMD: Minimal Melanogenesis Dose

related to pigmentation may be determined more precisely.

Pigmentation increase

No tanning appeared after multiple doses of the lowest dose, 0.2 MMD.

After 0.4, 0.6 and, especially, 0.8 MMD several volunteers (1, 17, 44, respectively) developed excessive erythema, mainly due to nUVB. Twenty-three volunteers did not develop pigmentation, mainly in the 0.4 MMD group (10 Scandinavians, 8 Indians), leading to exclusions. That leaves us with 95 measurements of absolute and percentage increase in pigmentation after 12 UV exposures (2 UV sources and 3 doses) and 89 measurements after 6 UV exposures.

Absolute increase in pigmentation in relation to pre-exposure pigmentation and dose

The absolute increase in pigmentation was constant for equal MMD of both UV sources, thus independent of pre-exposure pigmentation (nUVB, 12 exposures, Fig. 1A). Hence, after multiple UV exposures all the volunteers obtained the same absolute increase in pigmentation after exposure to the same MMD dose (0.8, 0.6 and 0.4 MMD). This proves that the MMD at the pre-test (single UV exposure) was determined correctly.

There was a significant linear relationship between doses and absolute increase in pigmentation (both UV sources combined) ($p = 0.036$); the higher the dose the higher the absolute increase in pigmentation (Table II).

Percentage increase in pigmentation in relation to pre-exposure pigmentation and dose

There was a negative linear relationship between percentage increase in pigmentation and pre-exposure pigmentation, which means that the less pigmented the higher percentage increase in pigmentation, Table III (nUVB, 12 UV exposures, Fig. 1B). When comparing linear regression lines for the different MMD doses the intercepts were significantly different ($p = 0.021$, Fig. 1B), therefore the percentage increase in pigmentation after 12 UV exposures to nUVB was dose-dependent, whereas slopes and intercepts after 6 UV exposures were not significantly different. The 3 curves can therefore be considered as one.

For Solar Simulator, the negative linear relationship between percentage increase in pigmentation and pre-exposure pigmentation was significant after both 6 and 12 UV exposures (Table III). When comparing linear

Table II. Pigmentation increase in relation to the number of UV exposures, UV dose and pre-exposure pigmentation

Number of exposures	MMD	n*	Mean pre-exposure pigmentation-%†	Absolute pigmentation increase	Percent pigmentation increase†
				(pigmentation-%)	
6	0.8	26	37.2	7.6	23.5
	0.6	36	31.7	6.9	27.9
	0.4	27	32.3	6.9	27.9
12	0.8	21	37.1	9.1	25.9
	0.6	33	35.4	9.0	28.2
	0.4	41	33.3	7.2	24.9

*Number of measurements that could be analysed. Ideally it should have been 48 (24 volunteers, each irradiated by 2 UV sources), but due to the excessive redness in a high number of volunteers, which led to exclusion from further exposure, the number of data was reduced.

†The mean values differ due to this difference in number of volunteers. MMD: minimal melanogenesis dose

Table III. Association between percentage pigment increase and pre-exposure pigmentation. Linear regression was calculated between percentage pigment increase and pre-exposure pigmentation for each MMD dose and each of the UV sources at 6 and 12 exposures, respectively. *p*-values and *r*² values of the individual regression lines are shown. Equation lines for nUVB, 12 exposures, see Fig. 1B.

MMD	Solar Simulator						nUVB					
	6 exposures			12 exposures			6 exposures			12 exposures		
	<i>n</i>	<i>r</i> ²	<i>p</i>	<i>n</i>	<i>r</i> ²	<i>p</i>	<i>n</i>	<i>r</i> ²	<i>p</i>	<i>n</i>	<i>r</i> ²	<i>p</i>
0.8	15	0.29	0.04	13	0.35	0.033	11	0.48	0.02	8	0.30	0.16*
0.6	16	0.34	0.02	19	0.29	0.017	20	0.29	0.01	14	0.22	0.09*
0.4	12	0.53	0.007	21	0.45	0.0009	15	0.45	0.01	20	0.32	0.01

*Not significant.

nUVB: narrowband UVB; UV: ultraviolet; MMD: minimal melanogenesis dose.

regression lines for the MMD doses, there was no significant difference between slopes or intercepts, which means that the percentage increase in pigmentation after Solar Simulator was *independent of dose*. For the combined UV sources, the percentage increase in pigmentation showed a trend to be *dose-dependent*; higher doses gave larger increases in pigmentation (Table II). When data from each of the UV sources were tested separately, the correlation was significant only for nUVB after 12 UV exposures. The mean pre-exposure pigmentation shown in Table II appears to be higher for 0.8 MMD compared with the lower doses, but the background for this is the high number of excluded persons specifically for the 0.8 MMD group.

Absolute and percentage increase in pigmentation in relation to skin type

Overall there was no correlation between absolute and percentage increase in pigmentation and skin type.

However, a significant correlation was found between percentage increase in pigmentation and skin type after 12 UV exposures to Solar Simulator 0.4 MMD (*p*=0.048) and after 6 UV exposures to nUVB 0.8 MMD (*p*=0.0397) (Kruskal-Wallis test).

UV dose to minimal pigmentation in relation to skin type and number of UV exposures

UV dose (SED) to minimal pigmentation after a single UV-exposure to Solar Simulator and nUVB is shown for each skin type group in Table I. It shows that the

UV dose to minimal pigmentation was higher in volunteers with higher pre-exposure pigmentation and skin type. This was also seen after *multiple* (6 and 12) UV exposures (Table IV). Thus the UV-dose to minimal pigmentation is higher in a dark-skinned person compared with a fair-skinned person after single and multiple UV exposures for the examined UV sources.

Table IV furthermore shows that the dose fractions for clinically evaluated minimal pigmentation (MMD) decrease with number of UV exposures. There was a constant ratio between MMD after one UV exposure and multiple UV exposures independent of skin type (*p*>0.25) and UV source (*p*>0.17). There is a reduction factor of 2 in dose fraction (Solar 1.9, nUVB 2.2) when giving 6 UV exposures compared with one UV exposure and a reduction factor of 3 (Solar 3.4, nUVB 3.2) when giving 12 UV exposures compared with one UV exposure (Table IV). There is a 3-fold increase in the cumulative dose to minimal pigmentation (Solar 3.3, nUVB 3.0) when giving 6 UV exposures compared with one UV exposure and a 4-fold increase (Solar 4.4, nUVB 4.2) when giving 12 UV exposures compared with one UV exposure. This means that the cumulative dose to minimal pigmentation is higher the more UV exposures given, even though the individual exposure doses are lower.

Fading

Forty-three measurements could be used to determine fading after 12 UV exposures, and 31 measurements could be used for 6 UV exposures.

Table IV. Individual UV dose in SED to minimal pigmentation 7 days after last UV exposure in skin type II–V for a total of 1, 6 and 12 exposures (cumulative dose)

Skin type	<i>n</i>	Number of UV exposures					
		Solar Simulator			nUVB		
		1 exposure	6 exposures	12 exposures	1 exposure	6 exposures	12 exposures
II	5	5.3 (5.3)	2.8 (17)	1.7 (20)	5.2 (5.2)	2.7 (16)	1.6 (19)
III	6	7.5 (7.5)	3.9 (23)	2.2 (26)	6.6 (6.6)	3.1 (19)	2.0 (24)
IV	4	9.6 (9.6)	4.6 (28)	3.4 (41)	8.3 (8.3)	4.2 (25)	2.9 (35)
V	9	10.5 (10.5)	5.7 (34)	4.0 (48)	8.9 (8.9)	4.7 (28)	3.6 (43)

nUVB: narrowband UVB; UV: ultraviolet; SED: standard erythema dose.

No significant difference in days to total fading was found between Scandinavian and Indian/Pakistani subjects, except for tanning obtained after 12 UV exposures to nUVB 0.6 MMD, in which case tanning faded faster in Scandinavian (median 104 days) than in the Indian/Pakistani volunteers (132 days) ($n=10$, $p=0.033$) and after 6 UV exposures to Solar Simulator 0.6 MMD, in which case tanning also faded faster in Scandinavian (median 101 days) than in the Indian/Pakistani volunteers (152 days) ($n=4$, $p<0.0001$).

The mean number of days to fading after 6 UV exposures to nUVB was 132 and after Solar Simulator 134. After 12 UV exposures to nUVB fading was reached after 137 days and after Solar Simulator 175 days. For nUVB 0.8 and 0.6 MMD there was no significant difference in days to reach total fading after 6 UV exposures compared with 12 UV exposures.

DISCUSSION

It is debatable whether the few studies that have investigated pigmentation increase after multiple UV exposures have been able to determine a person's *tanning ability*, as steady-state pigmentation was not reached, due to the use of increasing UV doses (3) or too few UV exposures (1, 2, 4, 5). Since pigmentation from UVB is always preceded by erythema (2, 17) a study like ours with multiple UVB exposures will unavoidably have a high number of drop-outs due to excessive erythema. Pigmentation after multiple UVB exposures is therefore difficult to obtain, as multiple exposures to very small UVB doses will not induce erythema and thereby result in no pigmentation, as occurred in both the Scandinavian and the Indian/Pakistani subjects after exposure to the lowest doses (0.2 and 0.4 MMD). On the other hand, after a higher dose that induces erythema and thereby pigmentation, the erythema does not disappear from day to day, leading to excessive erythema. This dilemma explains why most of the Scandinavians, as expected, had to be excluded during the repetitive exposures to the highest dose 0.8 MMD. Future studies should investigate more doses in between 0.4 MMD and 0.8 MMD, e.g. 0.5 MMD and 0.7 MMD, in order to lower the number of extreme sunburns.

nUVB is more erythemogenic and melanogenic than Solar Simulator (18), therefore a lower UV dose is needed to produce minimal pigmentation than for Solar Simulator (Table I). Higher UV doses induce more erythema and result in more pigmentation, as for nUVB in this study (18).

With pigmentation as our endpoint, we could not have used equal UV doses (SED), especially not after multiple UV exposures, as this would have led to very large dose steps, which would make the study very imprecise. The choice of MMD was therefore done to make our study more precise for our purpose. Moreover, MMD

determined at a single UV exposure worked well when used at multiple UV exposures in volunteers with a wide variation in constitutive pigmentation.

It is difficult to calculate days to steady-state pigmentation, as pigmentation build-up does not follow a simple linear increase. If, however, we assume that the pigmentation build-up follows an S-curve, we find that there was no correlation between days to reach steady-state pigmentation and pre-exposure pigmentation or skin type (data not shown), which confirms that the use of MMD (determined after a single UV-exposure) works for repetitive doses. After 12 UV exposures, 4–6 more days were needed to reach steady-state pigmentation for nUVB compared with 6 UV exposures (data not shown). This could be explained by the fact that the absolute increase in pigmentation was higher after 12 UV exposures and therefore may require longer time.

However, the absolute increase in pigmentation was, in contrast to what was expected, only significantly higher after 12 than 6 UV exposures for nUVB 0.8 and 0.6 MMD, but not for Solar Simulator. For the latter we found that the pre-exposure pigmentation generally was higher in the test areas of 12 UV exposures compared with the pre-exposure pigmentation in the test areas of 6 UV exposures due to a higher number of burns leading to exclusion of fair-skinned persons. This could induce a bias towards higher pigmentation, as is also seen in Table II, as the initial pigmentation appears to be higher the higher the MMD. However, pigmentation values from 25.5% to 53% are present for 0.8 MMD and therefore the results remain valid. This difference in pigmentation at the start explains why no difference in absolute pigmentation is found for Solar Simulator. It is well known that the baseline pigmentation differs according to the location on the back, but the observed difference in pigmentation at the start of this study cannot be explained by the location of the exposed areas, as they were chosen by randomization.

During fading few data from Scandinavian subjects could be analysed. This gives a bias and, consequently, some uncertainty in the evaluation of the data. Fading was investigated by measuring only 3 times; at 1 week, 2 and 4 months after the last UV exposure. This is obviously a very approximate scale, but the overall fading time was 4–5 months and should thus be expected to have reached a base level by mid-winter in northern Europe.

In conclusion, tanning ability, expressed as absolute pigment increase, is independent of skin type and pre-exposure pigmentation and is linear with the used sub-MMDs; the higher the dose the higher the absolute increase in pigmentation. The UV dose to minimal pigmentation was higher in dark-skinned persons for both single and multiple UV exposures independent of UV source. From a single exposure to 6 and 12 exposures spread over 3 weeks, the required dose fraction to mi-

nimal pigmentation was lowered by a factor 2 and 3, respectively, for both UV sources, thus lowering the risk of sunburn, but the cumulative dose increases 3- and 4-fold, respectively. This was independent of skin type and pre-exposure pigmentation.

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