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# UVA-induced tumours in pigmented hairless mice and the carcinogenic risks of tanning with UVA

**H. van Weelden[1], S. C. J. van der Putte[2], J. Toonstra[1], and J. C. van der Leun[1]**

[1] Institute of Dermatology and [2] Institute of Pathology, State University Utrecht, Utrecht, The Netherlands

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**Summary. An animal experiment is presented in which two groups of pigmented hairless mice were exposed daily to suberythemal doses of UVA to study tumourigenesis. The aim of the study was to estimate the carcinogenic risks of tanning by UVA. The pigmented hairless mice, Skh-hr2, were separated by selective breeding into two groups, the "browns" and the "blacks". Both groups were exposed daily to UVA from fluorescent UVA lamps (Philips TL40W/09) purified by rigorously filtering out the shorter wavelengths. No acute actinic damage was observed after any exposure. However, in most UVA exposed animals, especially in the blacks, a marked scratching preceded the development of tumours. Hyperkeratosis was also observed. All animals developed tumours. Histopathologically at least 60% of the tumours were squamous cell carcinomas. Depositions of melanophages were observed, but no melanomas. It is beyond any doubt that UVA is carcinogenic in laboratory animals. The present state of knowledge justifies no preference for tanning with UVA over tanning with UVB.**

**Key words:** UVA-radiation – Tanning – Carcinogenesis

In recent years the potential of UVA for causing non-melanoma skin cancers has become more and more important, not only because people expose themselves to the sun, but also to high doses of UVA radiation from artificial tanning equipment [10]. Therefore, an estimation of the possible carcinogenic risks of tanning by UVA is of importance.

In a previous experiment albino hairless mice were exposed to UVA to establish its carcinogenic potential [15, 16]. However, people expose themselves to UV radiation with the aim of achieving a tan. Experiments for estimating risks are, therefore, better performed on mice capable of tanning. For that reason the present experiment on UVA carcinogenesis was performed on pigmented hairless mice. Experiments on these mice not only offer the opportunity of investigating the induction of non-melanoma skin cancers, but might also result in melanomas. From the latitudinal gradient in malignant melanomas it is not possible to determine whether UVB or UVA is the causative factor [8, 9, 11, 13] but it might be possible to induce melanomas with UVA rather than with UVB, which was tried unsuccessfully in previous studies. At the same time, the experiment offers the possibility of comparing albino and pigmented mice with respect to carcinogenesis by UVA.

## Materials and methods

### *Mice*

The experiments were performed on pigmented hairless mice. The mice, males and females, entered the experiments at the age of about six weeks. The mice were kept individually separated in cages which were subdivided into 12 compartments; they had free access to "mouse chow" and tap water. The mice were subjected to dorsal exposures of UV radiation from sources situated above the cages.

Two groups of mice, "blacks" and "browns", were exposed daily to UVA. These black and brown mice were attained by selective breeding for colour from the original stock Skh-hr2, generously supplied by The Skin and Cancer Hospital, Philadelphia, USA. Neither the black nor brown pigmentation in the mice, which was most obvious in ears and tails, was very marked. It slightly increased by the regular exposures. Eight blacks and 16 browns were subjected to the UVA regimen. The relevant genetics of the different strains are given in Table 1. Included are the genetics of the albino hairless mice, Skh-hr1, used in the previous experiments on UVA carcinogenesis [3, 16].

### *Radiation source*

The UVA source was a bank of Philips TL40W/09 fluorescent UVA tubes. A specially selected 10-mm-thick glass filter was used to filter out all UVB rigorously. The filter also absorbed much of the shorter

*Offprint requests to:* H. van Weelden, Department of Dermatology, University Hospital Utrecht, P.O. Box 85500, NL-3508 GA Utrecht, The Netherlands

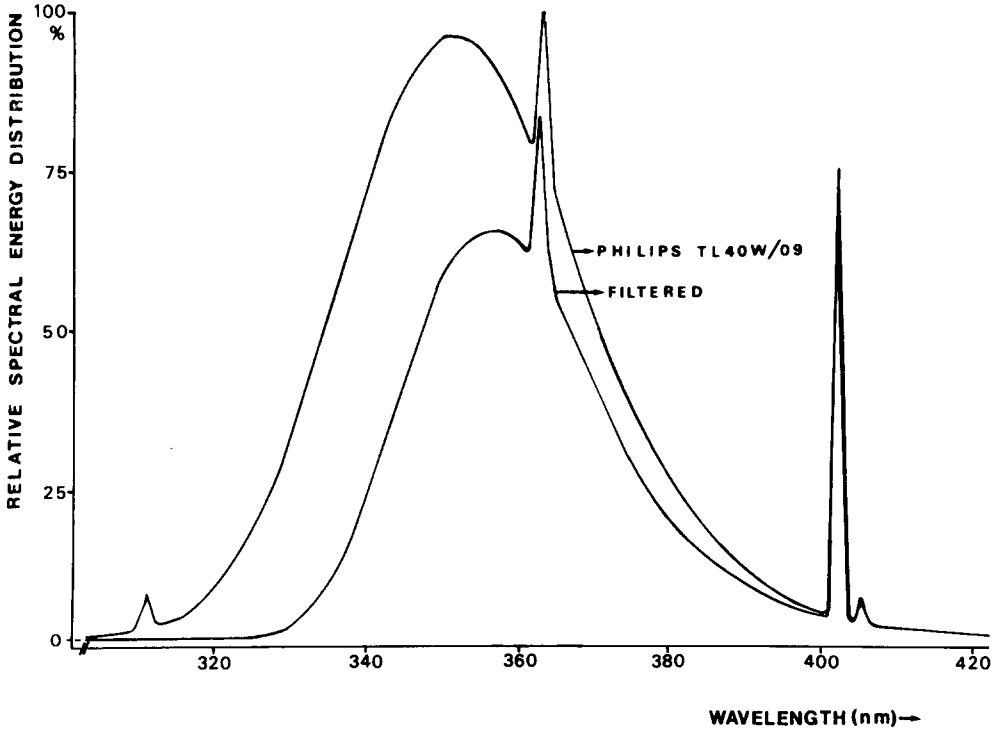


**Fig. 1.** Relative spectral energy distribution of a Philips TL40W/09 UVA light source, before and after filtering

**Table 1.** Relevant genetics of the hairless mice

| Designation | Relevant genetics[a] | |
|---|---|---|
| Skh-hr1 | hr/hr | c/c |
| Skh-hr2 | hr/hr | +/a, +/b, +/c |
| "brown" | hr/hr | a/a, b/b, C/C |
| "black" | hr/hr | a/a, B/B, C/C |

[a] Genotype symbols: hr, hairless; a, non agouti; b, brown; c, albino; +, non specific; B, non brown; C, non albino

wavelength UVA (Fig. 1). The experimental set-up used in our department for animal experiments on carcinogenesis has been described in more detail previously [2, 15].

## *Dosimetry*

The actual dose at the level of the animals was lower than the dose at the top of the cage where the light measurements were taken routinely. This reduction was not only caused by the distance from the top of the cage, but also by the construction of the cages and the geometry of the various experimental arrangements [15]. Therefore, only the actual doses at the level of the animals will be mentioned.

The mice received a daily dose of 22 J/cm$^2$ measured over the full spectrum for 7 days per week. The exposure time was 12 h per day. The output of the light source over the full spectral range was measured with a Kipp calibrated thermopile type E11 (Kipp, Delft, The Netherlands). The possible residual daily dose of UVB during the UVA exposures in our set-up was certainly lower than $10^{-7}$ J/cm$^2$ [16]. The UVA irradiance was checked regularly with the UVA detector device of Waldmann (Waldmann AG, Schwenningen, FRG). During the 12 h of UV exposure, the room was illuminated by yellow fluorescent lamps without any emission in the UV region (Philips TL40W/16); during the remaining 12 h the mice were kept in the dark [2]. This was done to simulate a normal day and night rhythm.

## *Observations*

All skin reactions were assessed regularly. The tumour locations were mapped and recorded for each animal separately. With each check-up date, newly appeared tumours and changes in tumour diameter and height were recorded. Records were also made of newly damaged skin, caused by scratching, and of hyperkeratosis. When an animal had developed tumours larger than 4 mm in diameter it was taken out of the experiment.

## *Histopathology*

After termination of the experiments the animals were sacrificed, and samples of the larger tumours were excised and sectioned for histopathological examination. The samples were fixed for 24 h in Lillies AAF (formaldehyde 10 parts, glacial acetic acid 5 parts, absolute ethyl alcohol 85 parts), routinely processed and then embedded in paraffin. Five-micrometer-thick sections stained with haematoxylin-eosin were examined.

## Results

All mice, blacks and browns, exposed daily to suberythemal doses of UVA, developed tumours. In a previous experiment on UVA carcinogenesis performed with the albino hairless mice, Skh-hr1, we noticed clinical differences in the reactions of the skin in the mice exposed to UVA compared with the reactions in those exposed to UVB. On the mice exposed to suberythemal doses of UVB the first tumours usually appeared on apparently normal skin and scratching did not start before several tumours had developed. However, in the mice exposed to UVA marked damage caused by scratching was observed well before the appearance of the tumours. The scratching without any preceding effect such as redness was prob-

**Table 2.** Induction of scratching in albino and pigmented hairless mice

| Type of mouse | ln $t_{scr} \pm$ SD | $t_{scr}$ (days) |
|---|---|---|
| albino | $5.18 \pm 0.21$ | 177 |
| "brown" | $5.70 \pm 0.23$ | 300 |
| "black" | $5.01 \pm 0.26$ | 150 |

$t_{scr}$: Median induction time for scratching
SD: Standard deviation of the log normal distribution

**Table 3.** Induction of hyperkeratosis in albino and pigmented hairless mice

| Type of mouse | ln $t_{hyp} \pm$ SD | $t_{hyp}$ (days) |
|---|---|---|
| albino | $5.38 \pm 0.24$ | 217 |
| "brown" | $6.17 \pm 0.22$ | 478 |
| "black" | $5.61 \pm 0.20$ | 273 |

$t_{hyp}$: Median induction time for hyperkeratosis
SD: Standard deviation of the log normal distribution

ably provoked by itching and/or irritation. This phenomenon of scratching without any preceding visible effect was seen in both the blacks and browns exposed to UVA [14], but blacks were much more vulnerable in this respect than the browns. When the skin of all blacks was already markedly damaged, there was hardly any damage to the skin of the browns although they were exposed to the same UV regimen and housed in the same type of cage.

In blacks the median induction time for scratching was 150 days, which was even shorter than in the albinos used in the previous experiment with the same irradiation conditions [16]. The median induction time for scratching in all three types of mice was calculated and is presented in Table 2. In all animals the median induction time for scratching was at least a few months. To call this an acute effect would be incorrect. Most of the damaged areas healed after a while, leaving scar tissue and also, in the pigmented mice, hyperpigmentation.

The induction of hyperkeratoses in all mice exposed to UVA was another remarkable phenomenon; it was

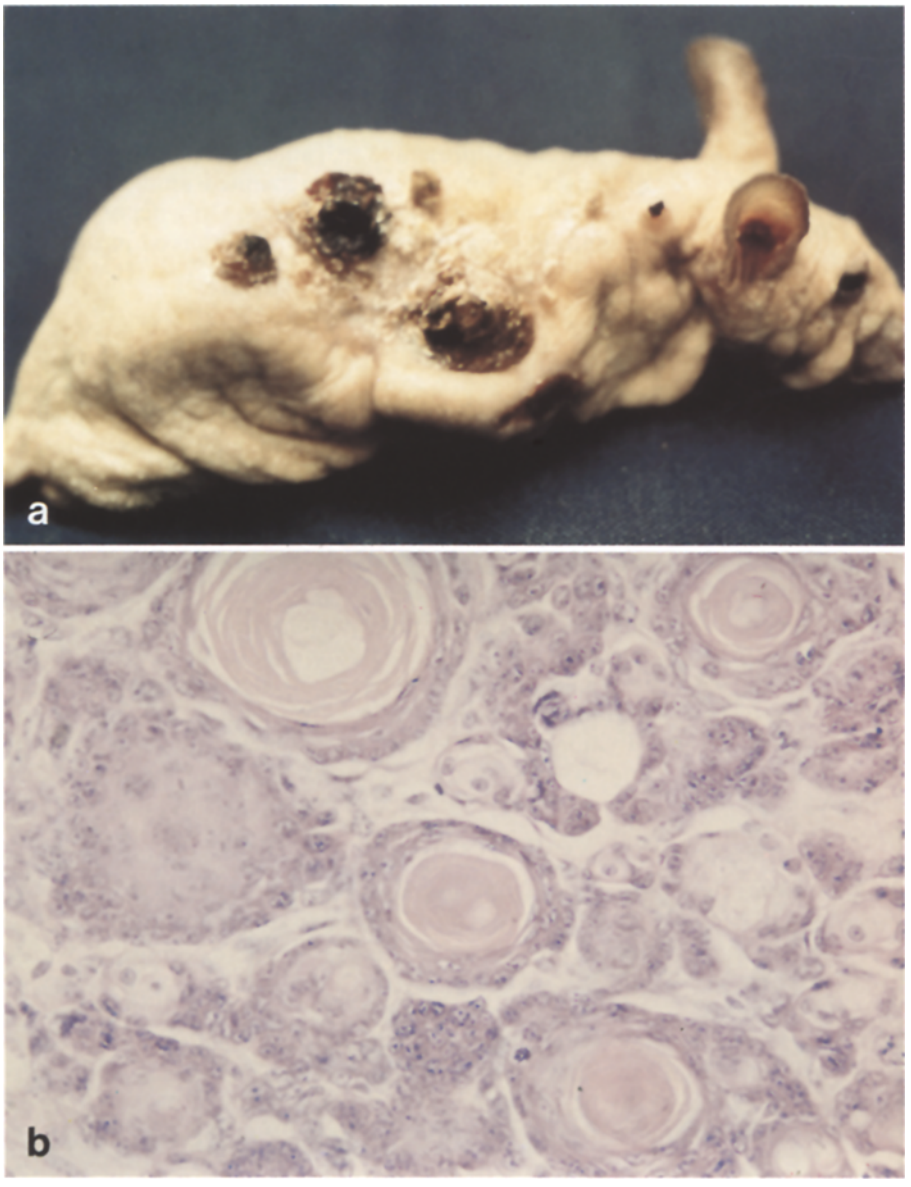


**Fig. 2. a** Scratching, hyperkeratosis and tumours induced by UVA **b** Squamous cell carcinoma induced by UVA

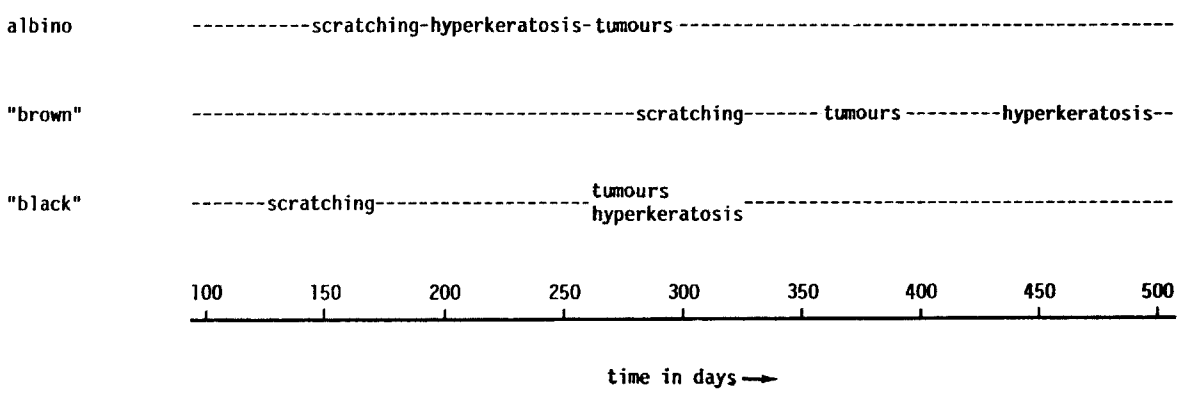


**Fig. 3.** Sequence of UVA-induced skin reactions (srcatching, hyperkeratosis, tumours) in albino and pigmented hairless mice

**Table 4.** Induction of tumours in albino and pigmented hairless mice

| Type of mouse | ln $t_m \pm$ SD | $t_m$ (days) |
|---|---|---|
| albino | $5.58 \pm 0.26$ | 265 |
| "brown" | $5.93 \pm 0.19$ | 375 |
| "black" | $5.59 \pm 0.26$ | 267 |

$t_m$: Median tumour induction time
SD: Standard deviation of the log normal distribution

**Table 5.** Tumours induced by UVA in albino and pigmented hairless mice

| Type of tumour | Percentage |
|---|---|
| Hyperplasia (e.g. papillomas and hyperkeratoses) | 20 |
| Squamous cell carcinoma | 60 |
| Carcinoma not certain (mild cellular and nuclear atypia) | 20 |
| Melanomas[a] | 0 |

usually not seen in mice exposed daily to suberythemal doses of UVB. Most of the hyperkeratotic lesions were rather flat. The median induction times for hyperkeratoses in all three types are presented in Table 3. In the albinos exposed to UVA the hyperkeratoses were mostly observed well before the tumours developed. In the blacks the median induction time for hyperkeratosis was not significantly different from the median induction time for tumours (Table 4). In the browns, however, the median induction time for hyperkeratosis was considerably longer than the median induction time for tumours.

The third clinical event was the appearance of tumours, eventually in all types of mice. About 60% of the first tumours observed in each individual animal were papillomas. In time, however, more and more verrucous tumours developed as well as keratoacanthoma- and cornucutaneum-like tumours (Fig. 2a). As well as developing in scar tissue, tumours also appeared on parts of the skin not affected by scratching. Also for tumour induction, the blacks turned out to be more susceptible than the browns. As can be seen from Table 4 there was no difference in susceptibility for tumour induction by UVA between the blacks and the albinos. Figure 3 illustrates the sequence of the skin reactions observed in all three types of mice, albino, black and brown.

Histopathological examination to characterize the tumours revealed a spectrum of abnormalities ranging from epidermal hyperplasia, through papilloma- or keratoacanthoma-like lesions, to partially verrucous and partially invasive squamous cell carcinomas, or deeply invasive poorly differentiated squamous cell carcinomas (Fig. 2b). Sharp delimitation of these categories was not possible, but by using cytological and architectural criteria most lesions could be grouped as either "hyperplastic" or "carcinomatous". The distribution of the tumours is presented in Table 5. The histopathology of excised lesions from the pigmented animals did not reveal any indication of melanomas. Only depositions of melanin in melanophages could be observed in tumours as well as in non-tumourous lesions.

## Discussion

It is well known that UVB is capable of inducing squamous cell carcinomas using all kinds of UVB sources and dose regimens [1, 2, 5]. That UVA is also carcinogenic is less well known, but has been demonstrated [6, 12, 16]. This is again confirmed by the present study where we used predominantly long wavelength UVA (Fig. 1). In our experiments we were able to induce tumours by this purified UVA in three strains of hairless mice, in albinos in a previous study [16] and in blacks and browns in the present study. Histologically at least 60% of the larger tumours induced by UVA appeared to be squamous cell carcinomas. There were pigmented tumours, but no melanomas were observed. The absence of melanomas in this experiment is no proof for the non-existence of a relationship between exposure to sunlight and the incidence of melanomas. The circumstantial evidence for such a relationship is too impressive to be ignored [8, 9, 11].

By plotting the prevalence of tumour-bearing mice versus the induction time (Fig. 4), it becomes obvious that the susceptibility for tumour induction by UVA in albinos is equal to the susceptibility in blacks. The browns are somewhat less susceptible. From these results one may conclude that, if pigmentation plays any role, other genetic properties are probably more decisive in determining carcinogenic susceptibility to UVA.

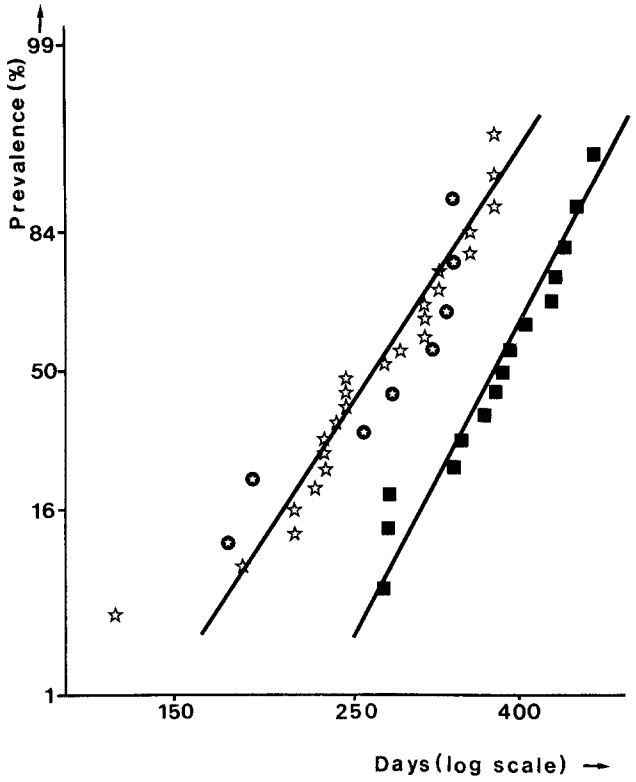


**Fig. 4.** Prevalence of tumour-bearing mice on a probability scale time in days on a log scale; ☆ albino; ○ "black"; ■ "brown"

De Gruijl et al. [2] determined a relationship between the daily dose of UVB, $D$, and the median tumour induction time, $t_m$. This relationship is:

$$t_m \approx D^{-0.6}$$

Using this relationship, van Weelden et al. [16] calculated the daily dose of UVB that would be necessary to induce tumours in albino mice with the same median tumour induction time as in the UVA experiment. The same median induction time of 265 days resulted from a daily dose of 22 J/cm$^2$ of UVA (cumulative dose 5.83 kJ/cm$^2$) and from a daily dose of 12 mJ/cm$^2$ of UVB (cumulative dose 3.18 J/cm$^2$). The cumulative dose of UVB possibly still present in our UVA experiments at day 265 was less than $2.65 \times 10^{-5}$ J/cm$^2$, which is smaller by a factor of 100000 than the dose of UVB necessary to induce tumours at this rate. This supports the conclusion that the tumours are not caused by residual UVB in the UVA spectrum.

One major drawback in our experiments with UVA was thought to be the prolonged daily exposure time. The low-intensity lamps used are stable and produce little heat, but in order to achieve the doses required we needed exposure times of 12 h, whereas the daily exposure times in the UVB experiments amounted to only 1.25 h. The question has been raised, as to whether this could be the reason for our success in inducing tumours by UVA alone. With the total daily dose held constant it has been shown that the carcinogenic efficiency of UVB indeed increases with decreasing dose rates [4]. In a study performed in our department [7], the influence of the dose rate on tumourigenesis was investigated. The effect of prolonged exposure times (1.25, 4 and 12 h) was studied in the same albino hairless mice, Skh-hr1, as were used in our study to compare the carcinogenic efficiencies of UVB and UVA [16]. A daily dose of UVB radiation administered in 4 and 12 h was more effective than the same daily dose given in 1.25 h. The enhancement in efficiency turned out to be significant but small, amounting to less than 20%. From this study it may be concluded that a prolonged daily exposure time does indeed enhance the carcinogenic effectiveness, but is certainly not decisive for the induction of tumours.

Taking all the evidence together, it is beyond any doubt that UVA is carcinogenic in laboratory animals, including albino as well as pigmented mice, and it is reasonable to assume that the same applies to humans, until there is evidence to the contrary. From a quantitative comparison of carcinogenesis by UVA and UVB in mice, van Weelden et al. [16] concluded: "the carcinogenic risks involved in tanning with UV-A and in tanning with UV-B are slightly in favour of UV-A, but in the same order of magnitude." This statement still holds, but only for non-melanoma skin cancers. No statement can be made with regard to melanoma. Some skin damage such as hyperkeratoses and scratching marks are worse with UVA. The present state of knowledge, therefore, justifies no preference for tanning with UVA over UVB.

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