

## Chapter 1

# Sun, Skin and Cancer Prevention

Skin cancer is more common than any other type of cancer. The estimated age-standardized incidence rates of cutaneous melanoma in several countries are reported in Figure 1. It has been estimated with reasonable certainty that 106 000 melanomas of the skin were diagnosed worldwide in 1990 (Parkin *et al.*, 1999) (Table 1). Less certainly, it was estimated that at least 2 750 000 non-melanocytic cancers (basal- and squamous-cell carcinomas) of the skin were diagnosed in 1985, representing more than 30% of all newly diagnosed cancers (Armstrong & Kricger, 1995). While non-melanocytic skin cancers are usually considered to be of little concern, they are the reason for many hospital admissions each year in white populations and for higher direct health care costs than any other cancer in Australia (Mathers *et al.*, 1998). The incidence rates of melanoma and non-melanocytic skin cancers have been rising steadily for some decades in many white populations (Armstrong & Kricger, 1994; Gray *et al.*, 1997; Staples *et al.*, 1998).

While the contribution of sunlight is difficult to estimate with any certainty, it probably causes the majority of melanomas of the skin worldwide. In predominantly white populations, it is estimated to cause approximately 80–90% of such cancers (Armstrong & Kricger, 1993). The proportion of non-melanocytic skin cancers caused by sunlight has not been estimated but

is probably about the same as that of melanoma. Given the apparently overwhelming importance of solar radiation as a cause of skin cancer (IARC, 1992), public health programmes aimed at preventing skin cancer focus almost totally on protection from sunlight. These programmes usually incorporate a range of strategies, including dissemination of knowledge about the intensity of sunlight in the local environment (as measured, for example, by the solar ultraviolet index (WHO *et al.*, 1995), staying out of direct sunlight during times when the ambient intensity is high, wearing a hat and clothing on unprotected skin when in direct sunlight, and using broad-spectrum, water-resistant sunscreens that protect uncovered skin from direct sunlight.

The first use of sunscreens was reported in 1928 (Shaath, 1997a). While sunscreens have been portrayed as a 'last resort' in sun protection (Marks, 1996), they have become increasingly popular, particularly during outdoor recreation in which as little clothing is worn as possible, such as at the seaside (Koh *et al.*, 1997; Robinson *et al.*, 1997a). Sunscreens were first developed to protect against sunburn and were designed to filter out the burning rays of sunlight (ultraviolet B, UVB; 280–315 nm). More recently, because of evidence that longer wavelengths of sunlight (ultraviolet A, UVA; 315–400 nm) participate in the sunburn reaction and can cause skin cancer in animals, and

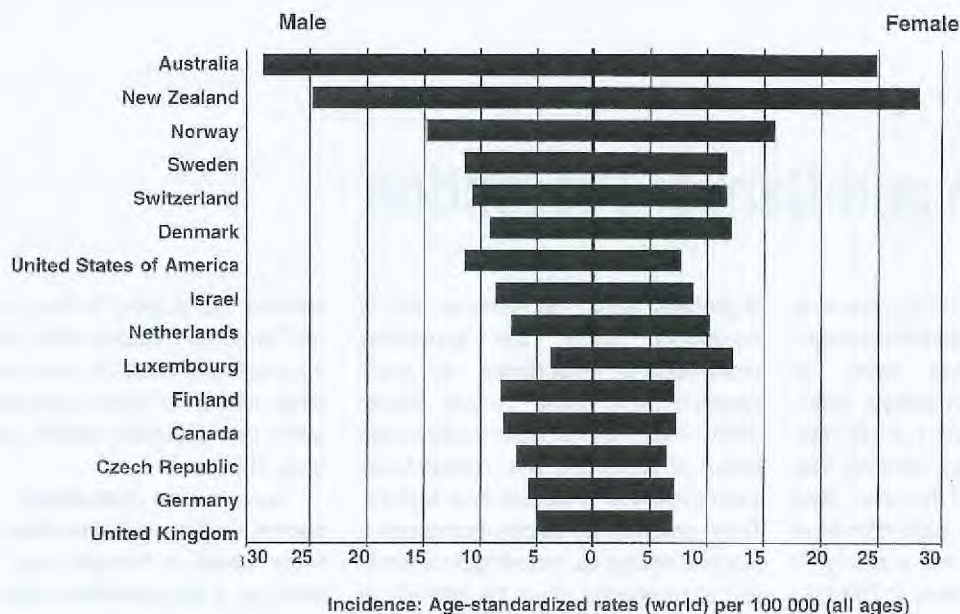
concern that staying in the sun longer with protection against UVB increases exposure to UVA, UVA absorbers have been added to most sunscreens to widen their absorption spectra (Gasparro *et al.*, 1998).

Sunscreens undoubtedly protect against sunburn, because they are routinely tested in humans and can be assigned a sun protection factor (SPF) which reflects their ability to prevent sunburn. Whether they can prevent skin cancer is the subject of this volume of handbooks.

### Sun and skin cancer in humans

An association between non-melanocytic skin cancer and exposure to the sun appears to have first been suggested in 1894; it was not until about 1952 that it was argued that exposure to the sun also causes melanoma (Armstrong *et al.*, 1997). Exposure to the sun causes the three major types of skin cancer: basal-cell carcinoma, squamous-cell carcinoma and melanoma, although the evidence does not permit identification of the causative part of the solar spectrum. Lip cancer may also be caused by solar exposure (IARC, 1992). Exposure to the sun may also cause some other, rarer skin cancers and, possibly, an internal cancer, non-Hodgkin lymphoma (IARC, 1992; English *et al.*, 1997; Iscovich *et al.*, 1998; McGregor *et al.*, 1999; Miller & Rabkin, 1999).

What is the evidence that skin cancer is caused by exposure to the sun?



**Figure 1** Estimated age-standardized incidence rates of malignant melanoma of the skin around 1990 in the 15 countries with the highest rates

**Table 1. Proportional distribution of new cases of malignant cutaneous melanoma of the skin by region for persons of all ages, 1990**

Region	Distribution of cases (%)	
	Males	Females
Africa	6	7
Asia	8	10
Central and South America	7	9
Europe	47	34
North America	25	33
Oceania	7	7

The risk for skin cancers at all sites increases with proximity to the equator in people with white skin in Australia and the USA, countries that cover a wide range of latitudes. At the individual level, the risk is greater for people who have lived much of their lives at low latitudes or in sunnier climates than people who have lived in such areas little or not at

all. Migrants to countries where there is heavy solar exposure, such as Australia and Israel, from countries where there is little solar exposure, such as northern and western Europe, have lower risks for skin cancer than people born in the countries with heavy exposure. Furthermore, the risk is greater the younger a person is when he or she migrates to a

country with heavy solar exposure. People with black skin, which is comparatively insensitive to sunburn, have a much lower risk for skin cancer than do people with white skin, and among white-skinned people the risk of those with fairer skin is higher than that of people with darker skin (Fig. 2). Few studies have been conducted of patterns among people with other skin types, such as Asians. People who tan easily and rarely burn are less likely to get skin cancer than people who sunburn easily and tan with difficulty. The highest density of occurrence, per unit of surface area, of the common types of skin cancer is on skin that is usually exposed to the sun (the head and neck), and the lowest density is on skin that is rarely if ever exposed (the buttocks) (English *et al.*, 1997).

These lines of evidence are all indirect, in that they do not relate



**Figure 2** Different skin types

people's actual exposure to the sun to their risk for skin cancer. Studies that have attempted to do this have generally had less persuasive results. Such studies are not easy to perform, because people have difficulty in recalling details of their exposure to the sun accurately. Nevertheless, a number of studies have shown relationships between recalled exposure to the sun and the occurrence of skin cancer at the time or subsequently. A particularly consistent relationship has been found between heavy recreational exposure to the sun and individual risk for melanoma (IARC, 1992; Elwood & Jopson 1997). Establishment of a relationship between total exposure to the sun and melanoma has been more elusive, and some studies have suggested that people with heavy occupational exposure to the sun have a lower risk for melanoma than people with little such exposure (Elwood & Jopson, 1997). A high frequency of sunburn has been shown to increase the risks for all major types of skin cancer, and people who have benign conditions associated with heavy exposure to the sun, such as solar keratoses ('sun

spots'), also have a high risk for skin cancer (English *et al.*, 1997).

The paradoxical findings that melanoma is more common among people working indoors than those working outdoors and, more recently, that heavy occupational exposure to the sun is associated with a lower risk for melanoma than light exposure led to the suggestion that the pattern as well as the intensity of exposure to the sun influences the risk for melanoma (Holman *et al.*, 1980). It was also suggested that the risk increases with increasing intermittency of exposure. This suggestion is strongly supported by evidence that increasing recreational exposure to the sun increases the risk for melanoma. The relationship may also be true for basal-cell carcinoma (Krickler *et al.*, 1995) but probably not for squamous-cell carcinoma, the risk for which appears to depend only on the total accumulated amount of exposure to the sun (English *et al.*, 1998a).

Does reducing exposure to the sun reduce the risk for skin cancer? Evidence that it does is quite limited. A number of white populations are now experiencing falling incidence and mortality rates of melanoma, particularly among young people, and it has been argued that these trends are due to greater protection from the sun over the past 20 years or so (Giles & Thursfield, 1996). In addition, at least one population has shown a similar trend for basal-cell carcinoma, but not squamous-cell carcinoma (Staples *et al.*, 1998). There have also been reports of downward trends in the incidence of melanoma in areas where local initiatives have been made to reduce the population's exposure to the sun (Cristofolini *et al.*, 1993; MacKie *et al.*, 1997). In a randomized, controlled trial of the effects of isotretinoin on the risk for further basal-cell carcinomas over 36

months, the risk of people who had reduced their solar exposure was lower than that of those who had not (Robinson & Rademaker, 1992). Reduction of recent exposure to the sun may also reduce the risk for squamous-cell carcinoma, as shown in a randomized, controlled trial of four to five years' use of sunscreens (Green *et al.*, 1999a,b).

It may be difficult to demonstrate that reducing exposure to the sun reduces the risk for skin cancer, since studies of migrant populations suggest that the lifetime risk is strongly determined by exposure to the sun during the first 15 or so years of life (English *et al.*, 1997; 1998b). There is some evidence, however, that exposure to the sun later in life also influences the risk for skin cancer (Robinson, 1987; Zanetti *et al.*, 1996; Armstrong, 1997; English *et al.*, 1998a).

What evidence do we have that ultraviolet radiation (UVR), the component of the sun's rays that is attenuated by sunscreens, is that which causes skin cancer? In humans, exposure to UVB produces a range of chemical changes in DNA, consisting most commonly of intra-strand cross-links between adjacent pyrimidine bases (IARC, 1992). These cross-links, if not repaired, can produce mutations, which might in turn lead to cancer development. This form of DNA damage can also produce 'signature mutations' in DNA, CC to TT transitions (in which two adjacent cytidine bases are mutated to two adjacent thymidine bases) or C to T transitions at dipyrimidine sites — the definitive indicator of carcinogenesis by UVR. These signature mutations have been found in the tumour suppressor *p53* gene in normal skin cells (and are probably present in other genes as well), and their presence has been correlated with the extent of exposure of the body

site from which the skin was taken (Ouhittit *et al.*, 1997). They have also been found quite frequently in the *p53* gene in basal- and squamous-cell carcinomas of the skin, whereas they are rare in *p53* gene mutation patterns of other types of cancer (IARC, 1992). Mutation of the *p53* gene is probably an important step in the development of these skin cancers (Ziegler *et al.*, 1996). The estimated density of CC to TT transitions in the *p53* gene in normal skin was shown to predict the risk for basal-cell carcinoma in one study, although the density did not correlate with estimates of individual exposure to the sun (Ouhittit *et al.*, 1998). Signature UVR-associated mutations have also been found in cyclin-dependent kinase (*p16*) genes in a primary melanoma cell line. While these signature mutations have been found in melanoma cell lines, only one was found in 26 samples from

human tumours (Pollock *et al.*, 1995; Healy *et al.*, 1996; Pollock *et al.*, 1996; Xu *et al.*, 2000).

Additional evidence that UVR, specifically, causes skin cancer is provided by the observation that people with the rare genetic syndrome xeroderma pigmentosum have a very high risk for skin cancer (IARC, 1992). With regard to the general population, there is conflicting evidence about the relationship between the capacity for excision repair of DNA and the risk for basal-cell carcinoma (Hall *et al.*, 1994; Wei *et al.*, 1995; D'Errico *et al.*, 1999; Xu *et al.*, 2000).

### Solar radiation of concern and its attenuation

#### Solar ultraviolet radiation

The spectrum of extraterrestrial solar radiation approximates to a black body at a temperature of about 5800 K. Of this, about 9% is UVR ( $\lambda < 400$  nm). Sunlight

consists of visible light in the spectrum from 400 nm (violet) to 700 nm (red), infrared radiation ( $> 700$  nm) and UVR. UVR has been subdivided by the International Commission for Illumination into UVA (315–400 nm), which is sometimes called 'black light', UVB (280–315 nm) and UVC (100–280 nm). The quality (spectrum) and quantity (intensity) of sunlight are modified during its passage through the atmosphere. The principal interactions in the stratosphere (~10–50 km above sea level) are absorption by ozone and scattering by interaction with molecules such as  $N_2$  and  $O_2$  (Fig. 3). In the troposphere (~0–10 km above sea level), absorption by pollutants such as ozone,  $NO_2$  and  $SO_2$  and scattering by particulates such as soot and clouds are the main attenuating processes. At ground level, UVR comprises about 5% of solar energy (Madronich, 1993).

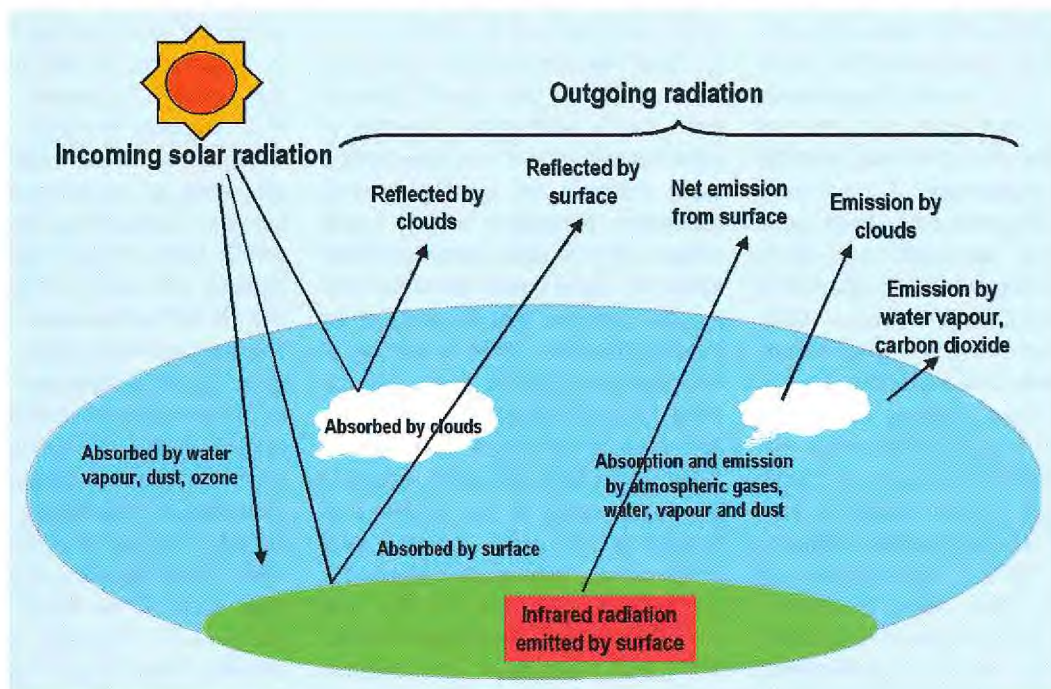


Figure 3 Interactions of solar radiation in the atmosphere

Both the quality and quantity of terrestrial UVR vary with the elevation of the sun above the horizon, or solar altitude. (The complementary angle between the sun and the local vertical is termed the 'solar zenith angle'.)

The solar altitude depends on the time of day, the day of the year and geographical location (latitude and longitude). On a summer's day, UVB comprises approximately 3.5% of terrestrial UVR, and UVA the remaining 96.5%; UVC is blocked by the stratospheric ozone layer and does not reach the earth's surface. Since UVB is much more effective than UVA at causing biological damage (Figure 4), solar UVB contributes about 80% towards sunburn, and solar UVA contributes the remaining 20% (Figure 5). The spectrum of terrestrial sunlight measured at Melbourne, Australia (latitude 38°S) at noon in mid-summer (solar altitude, 75°) is shown in Figure 4.

Normally, less than 10% of sunlight is reflected from most ground surfaces. The main exceptions are gypsum sand, which reflects about 15–30%, and snow, which can reflect up to 90%. Contrary to popular belief, calm water reflects only about 5% of incident UVR, although up to 20% is reflected from choppy water (Diffey, 1998). Since UVR passes easily through water, swimming in either the sea or open-air pools offers little protection against sunburn. Furthermore, if sunscreens that are not water-resistant have been applied, they will wash off rapidly (Stokes & Diffey, 1999a) and increase the risk for sunburn if users believe they are protected and extend their time in the water accordingly.

Several artificial sources of UVR have been developed, including incandescent sources, gas discharge lamps, arc lamps, fluorescent lamps, metal halide lamps and electrodeless lamps

(IARC, 1992; WHO, 1994). These sources differ in the power consumption, rare gas and phosphor used, type of metal or metal halide incorporated, composition of the housing and pressure within the lamp (Council on Scientific Affairs, 1989). Depending on the filters used, they can provide either unfiltered UVR or simulated sunlight.

## Sunscreens

### Absorption by sunscreens

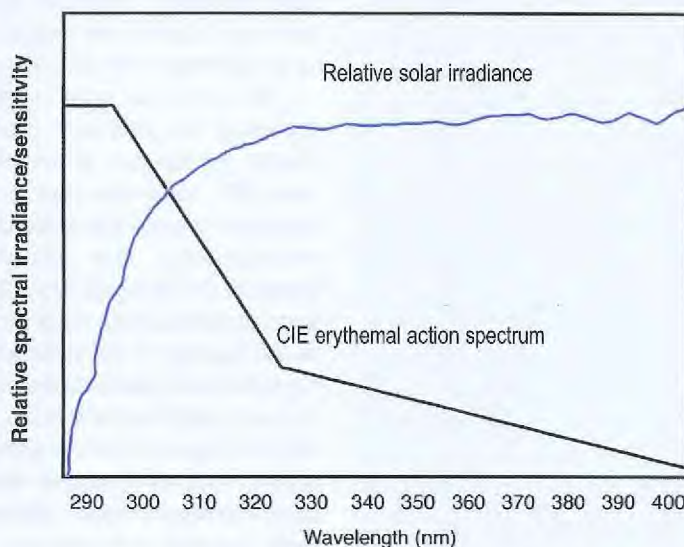
Topical sunscreens applied to the skin act by absorbing and/or scattering incident UVR. The shape of the absorption spectrum is the fundamental attribute of a topical sunscreen. It is expressed as the extinction coefficient, which is a measure of the degree to which the sunscreen absorbs individual wavelengths across the terrestrial UVR spectrum (290–400 nm). Absorbance is the product of the extinction coefficient, the concentration of the active ingredient and the effective thickness of application. The monochromatic protection factor,  $mPF(\lambda)$ , at wavelength  $\lambda$  is related to the absorbance  $[A(\lambda)]$  as follows:

$$mPF(\lambda) = 10^{A(\lambda)}$$

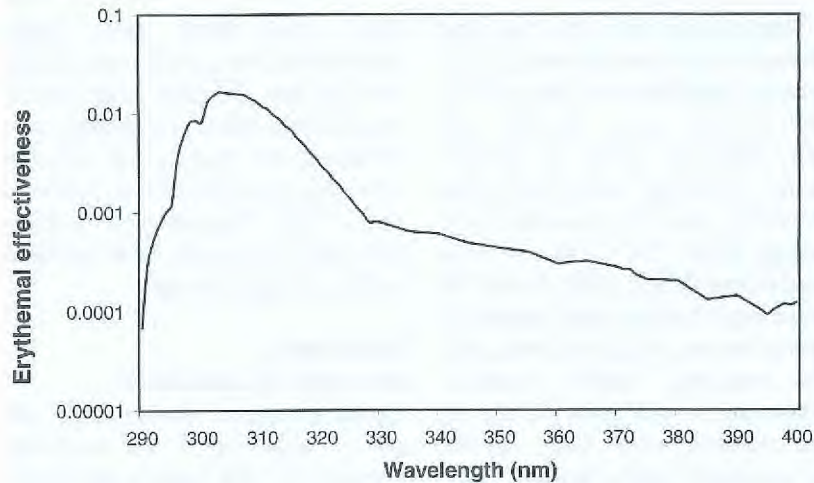
The monochromatic protection factors of a typical, modern, broad-spectrum sunscreen product are shown in Figure 6.

### Sun protection factor

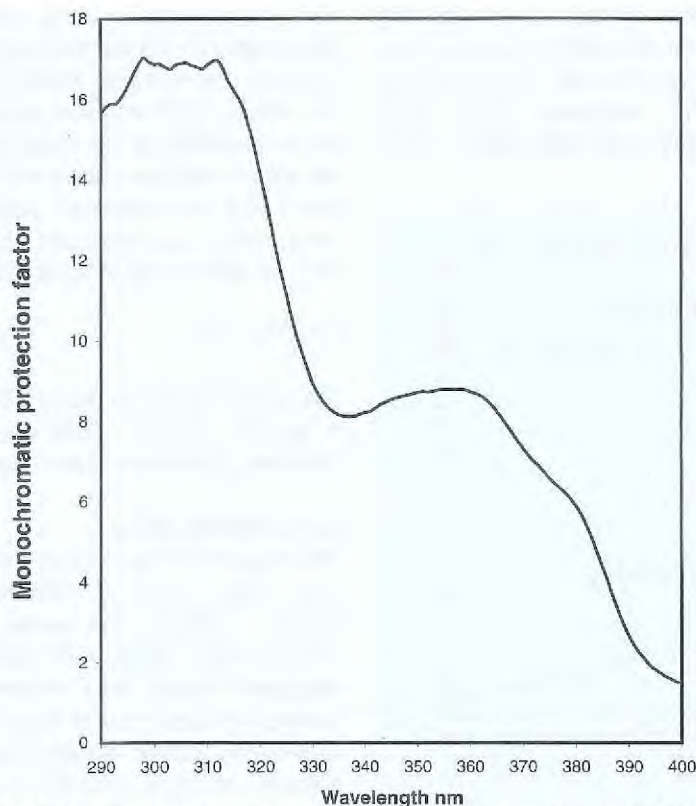
The concept of a sunscreen effectiveness index (ratio) is attributed to Schulze (1956a,b). The specific term 'sun protection factor' (SPF) and the associated method were proposed by Greiter (1974, 1981). Use of the SPF was subsequently adopted by many regulatory authorities and by the cosmetics and pharmaceutical industries. The SPF is defined as the ratio of the least amount of ultraviolet energy required to produce minimal



**Figure 4** Relative spectral irradiance of ambient midday summer sunshine in Melbourne, Australia (38°S) and the action spectrum of the International Commission for Illumination (CIE) for minimal erythema in human skin



**Figure 5** Erythral effectiveness spectrum of sunlight. This curve is the product of the relative solar irradiance and the erythral action spectrum of the International Commission for Illumination (see Figure 4) at each wavelength.



**Figure 6** Monochromatic protection factors of a broad-spectrum sunscreen (SPF 15) with butyl methoxydibenzoylmethane, methylbenzylidene camphor, octyl salicylate and titanium dioxide as active ingredients

erythema (reddening of the skin) on sunscreen-protected skin to the amount of energy required to produce the same erythema on unprotected skin (Food & Drug Administration, 1978, 1993, 1998, 1999). It is popularly interpreted as how much longer skin covered with sunscreen takes to burn compared with unprotected skin (Health Education Authority, 1996). Internationally agreed procedures (Food & Drug Administration, 1978; COLIPA, 1994) define protected skin as that to which a 2 mg/cm<sup>2</sup> layer of sunscreen has been applied.

The SPF of sunscreens applies strictly to human skin exposed *in vivo* to a simulated source of sunlight achieved by defined optical filtering of xenon arc lamps. Determination of SPFs by phototesting *in vivo* is subject to increasing variability with increasing SPF. This is illustrated in Table 2, which shows the results of a series of inter-laboratory tests performed by seven major European sunscreen manufacturers (Ferguson, 1997). It can be seen that even under the same laboratory conditions there is a threefold variation in the measured SPF for the high-factor (SPF 20–25) sunscreen.

The numerical value of the SPF appearing on sunscreen products is usually not identical to the measured mean SPF, since other factors, such as regulatory requirements and commercial considerations, also influence the choice of the declared SPF. The measured protection factor depends strongly on the topology of the surface to which the sunscreen is applied. Determinations *in vivo* in experimental animals, such as hairless mice, or *in vitro* in artificial substrates, such as Transpore tape, may result in protection factors different from those obtained in human skin (Diffey, 1989a). Furthermore, the strong dependence of the efficacy of sunscreens on wavelength means that the spectral

**Table 2. Measured SPF of four products tested in seven laboratories by the COLIPA SPF test method**

Nominal SPF	Measured SPF	
	Mean	Range
4	4.2	3.9–4.6
15	12.7	11–14
15	15.5	14–18
20–25	22.4	11–34

From Ferguson (1997)

SPF, sun protection factor; COLIPA, European Cosmetic Toiletry and Perfumery Association

emission of the UVR source will influence the measured protection factor (Wilkinson, 1998). This is particularly important when fluorescent UVB sunlamps (e.g. Philips TL12) are used as the source (Farr & Diffey, 1985). For high-SPF products (> 30), the SPFs determined with a solar simulator will be higher than those expected in sunlight because of the relatively small amount of UVA in xenon arc solar simulators (Stokes & Diffey, 1997a; Wilkinson, 1998).

In 1990, the labelled SPFs on most commercially available sunscreen products were < 10, but by 2000 there was a trend for higher factors, most manufacturers offering products with factors of 15–20 and, not uncommonly, products claiming a factor of 50 or higher.

It is important to know if protection from erythema results in comparable levels of protection from photobiological end-points that are thought to be important in photocarcinogenesis. These include epidermal DNA photodamage and mutation as well as immune suppression. In theory, an agent that gives protection from erythema without giving comparable levels of protection from these end-points could enhance the risk for skin cancer.

As the SPF of a sunscreen is a measure of protection from erythema in

human skin that is determined with solar-simulated UVR (see above), valid comparisons of the SPF with the degree of protection against other biological end-points, such as immune suppression, can be made only when these have been determined in human skin with a solar-simulated UVR source and the standard sunscreen application density of 2 mg/cm<sup>2</sup>. Comparison of SPFs with protection from important end-points other than erythema induced by other sources of UVR (such as UVB fluorescent lamps) is not valid, because protection factors depend on the spectral emission of the UVR source.

Although ideally all studies of sunscreens should be done with solar-simulated UVR, there may be situations in which this is not feasible or appropriate, for example, in studies in animals that require the use of selected wavebands. Furthermore, studies of dose-response relationships for the same biological end-points should be done with and without sunscreen. When studies are conducted with sources that do not simulate sunlight, it is important to compare the level of protection from the end-point in question with protection from erythema or other markers of inflammation, such as mouse skin oedema.

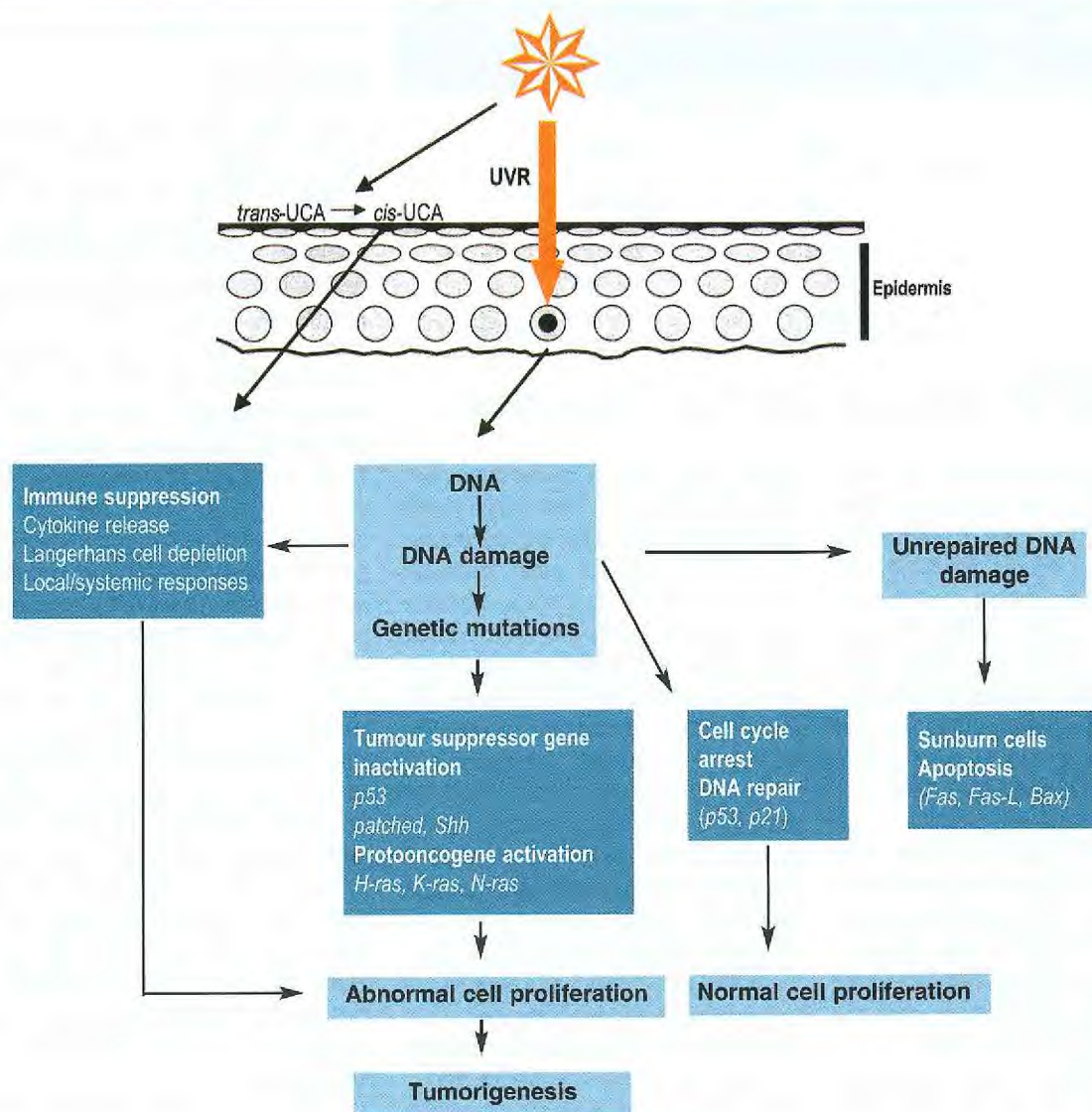
## Interaction of solar radiation with biomolecules

### Chromophores

Since solar UVC does not reach the earth's surface, the radiation wavelengths of interest are in the UVA and UVB regions. Changes following the excitation induced by absorption of solar energy in molecules known as chromophores may generate a biological effect either directly or by secondary reactions. Chromophores are endogenous biomolecules, such as DNA, or exogenous molecules, such as the active molecules of sunscreens. They absorb energy from the different wavelengths with differing efficiencies, and this pattern of response is defined as the absorption spectrum characteristic of the particular chromophore. Genetic effects such as mutation (Brash *et al.*, 1991) implicate DNA as a major chromophore. In particular, induction of skin cancer by UVB involves damage to DNA, which then leads to a cascade of events including cell cycle arrest, DNA repair, mutation and transformation (Fig. 7). Both UVB and UVA have been reported (Morlière *et al.*, 1991; Punnonen *et al.*, 1991; Vile *et al.*, 1994) to cause lipid peroxidation at biologically relevant fluences in the membranes of human fibroblasts and keratinocytes.

As the wavelength increases through the UVB and UVA regions, damage to proteins becomes increasingly important because of the absorptive properties of aromatic amino acids relative to nucleic acids. In addition, many proteins (which include the antioxidant enzymes catalases and peroxidases) contain haem groups, thus making the proteins UVA chromophores and potentially photosensitizers.

Photoimmunological effects implicate *trans*-urocanic acid, DNA photodamage



**Figure 7** DNA and *trans*-urocanic acid (UCA) are chromophores implicated in the induction of non-melanoma skin cancer by ultraviolet radiation (UVR). Absorption of UVR by the chromophores DNA and *trans*-UCA initiates the process of non-melanoma skin carcinogenesis, involving at least two distinct pathways. One is the action of UVR on keratinocyte (neoplastic) transformation; the other is the action on the host's immune system. These two pathways interact or converge to cause skin cancer.

and cytokines (Kripke *et al.*, 1992; Noonan & De Fabo, 1992; Ullrich, 1995; Nishigori *et al.*, 1996; Kibitel *et al.*, 1998; Petit-Frère *et al.*, 1998). Melanin, the major pigment in the skin, is also considered important in human photoprotection.

As solar radiation is composed of many wavelengths, their effects may interact.

The skin is a complex, many-layered organ, and the radiation spectrum that impinges on its surface is not the same as that which reaches the lower layers

(Fig. 8). The consequence of this interaction is that the action spectrum or wavelength dependence of a specific biological end-point, as measured in the skin, is unlikely to match exactly the absorption spectrum of a chromophore.



### The lesions

The major photoproducts formed in DNA by direct absorption can be detected in human skin *in vivo* (Freeman *et al.*, 1986; Young *et al.*, 1998a,b; Bykov *et al.*, 1999). These include cyclobutane pyrimidine dimers (TT > TC > CT >> CC) and pyrimidine (6–4) pyrimidone photoproducts (Fig. 9) (Cadet & Vigny, 1990; Cadet *et al.*, 1997). Specific lesions such as TT and TC dimers and 6–4 photoproducts have been described in humans *in vivo* (Bykov *et al.*, 1998), and such lesions constitute 70–80% and 20–30% of the total UVC-induced damage, respectively (Mitchell, 1988; Sage, 1993). The thymine-containing pyrimidine dimer is also the commonest lesion induced by UVB (Mitchell *et al.*, 1992). Thymine glycols (Hariharan & Cerutti, 1977; Mitchell *et al.*, 1991), pyrimidine hydrates (Fisher & Johns, 1976), purine or purine–pyrimidine moieties (Gallagher & Duker, 1989),

DNA single-strand breaks and DNA–protein cross-links are all present but at much lower frequencies than pyrimidine dimers.

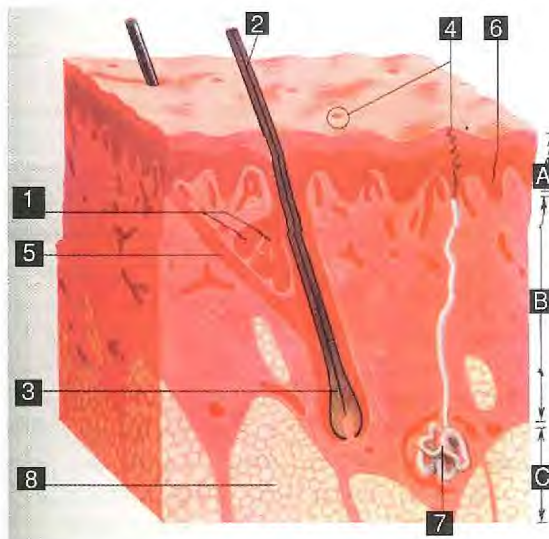
UVA induces direct damage, but less efficiently than it does indirect damage (Tyrrell, 1973; Freeman *et al.*, 1989). Techniques have been developed to measure specific types of oxidative damage, in particular 8-hydroxy-2'-deoxyguanosine (8-OHdG), which is one of many such lesions. UVA induces significant levels of 8-OHdG in mammalian cells (Kvam & Tyrrell, 1997a; Zhang *et al.*, 1997), although the action spectra demonstrate that there are various wavelength dependences for this induction (Kielbassa *et al.*, 1997; Kvam & Tyrrell, 1997b). Although the measurements were not made in the same way, the difference between a response peaking with UVA (Kvam & Tyrrell, 1997b) and one peaking with near-visible radiation (Kielbassa *et al.*,

1997) may be due to a difference in the chromophore profile of the two cell types—human skin fibroblasts and Chinese hamster cells—used in these studies. In an investigation of oxidative DNA damage induced by several types of broad-spectrum sources, Douki *et al.* (1999) concluded that 8-OHdG-induced damage was not involved in cell death and was unlikely to be involved in mutagenesis. This conclusion was based on the spectrum of UVA-induced mutations, which proved to be characteristic of changes at dipyrimidine sites rather than oxidized guanines. Analysis of UVA-induced damage in mouse skin tumours led to a similar conclusion (van Kranen *et al.*, 1997). Until a more detailed picture of the spectrum of oxidative DNA damage emerges, however, no final conclusion can be drawn.

### Modulation of gene expression

Alterations in gene expression at both the transcriptional and the translational levels occur in response to UVR, the effect being dependent on cell type and the intensity and wavelength of radiation used (Tyrrell, 1996a,b). Cell signalling pathways activated after exposure to UVR include those involving jun and p38MAP kinases and, in some cases, ERK kinases. The activation states of various transcription factors (AP-1, NFκβ) and p53 stability change specifically in response to short-wavelength UVR. Increased production of stress proteins is seen after exposure of epidermal and dermal cells to UVR.

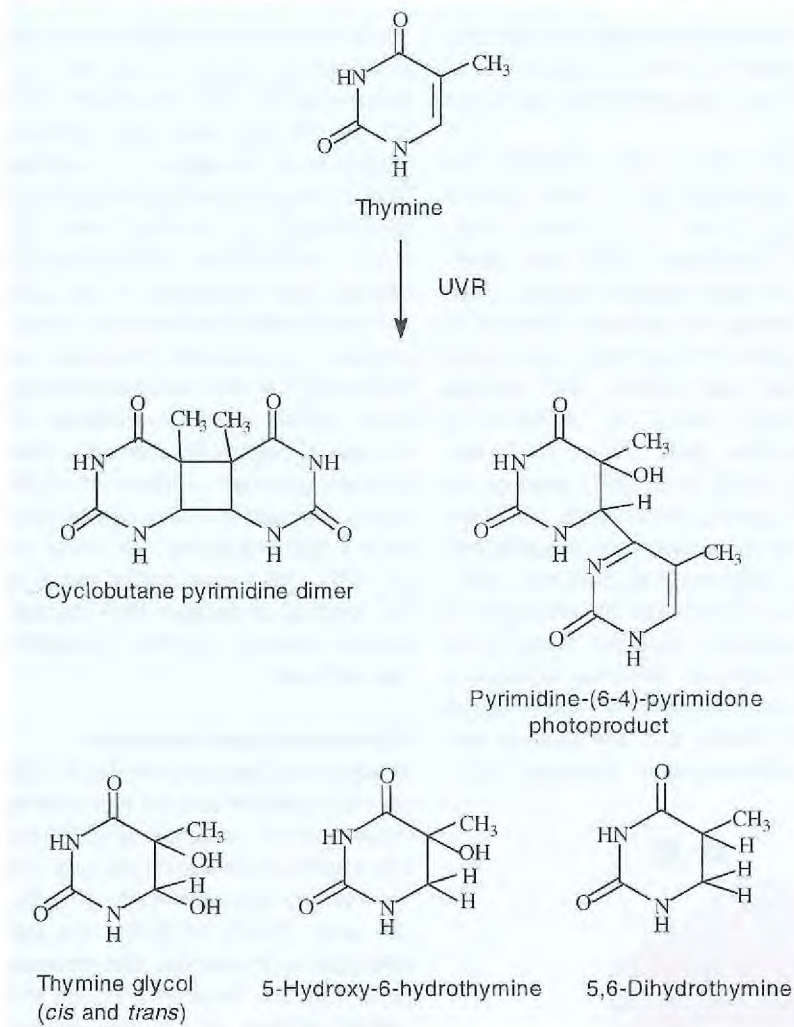
Many of the studies of changes in gene expression have involved use of high, usually lethal levels of irradiation, although physiologically relevant doses were used in some studies in cells and human skin (e.g. Fisher *et al.*, 1996). Low levels of UVA and UVB can activate cytokine production in strains



**Figure 8** Section of the skin showing the three different layers:

(A) epidermis (B) dermis and (C) hypodermis.

(1) sebaceous gland; (2) hair; (3) hair root; (4) sudoriparous gland pore; (5) hair erector muscle; (6) papillae dermis; (7) sudoriparous gland and its excretor channel; (8) adipose tissue



**Figure 9** Chemical structures of major thymine photoproducts

cultured from primary fibroblasts and certain established lines (Ullrich, 1995). Physiological doses of solar-simulated UVR and UVB induce cytokines in human skin *in vivo* (Skov *et al.*, 1998; Barr *et al.*, 1999).

Exposure to UVA at physiologically relevant doses increases the expression of collagenase, intercellular adhesion molecule 1, CL100 phosphatase and haem oxygenase 1 in cultured human skin fibroblasts. Up-regulation of *c-fos*

and *c-jun* (components of the AP-1 complex) is also observed in response to UVA (Bose *et al.*, 1999; Soriani *et al.*, 2000). A growth stimulatory response may be detected after UVB irradiation, specifically including alterations in the status of STAT 1 and related kinases (Aragane *et al.*, 1997). Both UVA and UVB can activate nitric oxide synthase in a human cell strain cultured from primary keratinocytes (Romero-Graillet *et al.*, 1997). Formylated indolocarba-

zoles may act as Ah-receptor agonists, and UVB may activate CYP 1A (a cytochrome P450 subtype) in various human cell types (Wei *et al.*, 1999).

Certain patterns of gene expression elucidated in cultured cells, such as increases in p53, collagenase and ornithine decarboxylase, may be demonstrated in irradiated skin in both human and rodent models (Tyrrell, 1996a,b). Expression of the AP-1 components is increased in biopsy samples of human skin after irradiation *in vivo* (Fisher *et al.*, 1996).

### **Endogenous cellular defence mechanisms**

#### **Repair of DNA damage**

One way in which the incidence of photolesions can be reduced is by repair of cellular DNA damage. The major mechanism for reducing the incidence of photolesions is excision repair (Lindahl & Wood, 1999). The importance of these mechanisms for the repair of UVB-induced damage is demonstrated most elegantly by the increased susceptibility of patients with xeroderma pigmentosum to solar-induced skin cancer (Arlett & Lehmann, 1996; Bootsma *et al.*, 1998). The majority of these patients have defects in the progressive steps of nucleotide excision repair and fall into seven genetically distinct complementation groups, A–G. Approximately 20% of these patients (variants) do not have defects in the excision repair genes but have defects in DNA polymerase  $\eta$  (Masutani *et al.*, 1999), which is responsible for translesion synthesis, a component of post-replication repair.

#### **Antioxidant defence**

Small antioxidant molecules are crucial in protecting human skin against UVR, particularly at the longer wavelengths

(Tyrrell *et al.*, 1991; Fuchs & Packer, 1999). In cultured human skin fibroblasts, glutathione depletion leads to strong sensitization to mutations caused by UVB (302–313 nm), UVA (334–365 nm) and near-visible (405 nm) radiation (Tyrrell & Pidoux, 1986, 1988), and there is a direct correlation between cellular glutathione content and the degree of photosensitization. Glutathione is depleted by exposure of human skin to UVA (Connor & Wheeler, 1987).

All the major antioxidant enzymes are present in skin, but their role in protecting the cells against oxidative damage induced by UVA has not been fully elucidated. Likewise the role of other endogenous antioxidant molecules such as ascorbate, carotenoids and  $\alpha$ -tocopherol in protection against UVR-induced damage in humans requires further investigation.

### Effects of solar radiation other than cancer

#### *Erythema (sunburn)*

Erythema is the most readily clinically apparent reaction to exposure to the sun (Fig. 10). It appears after 3–4 h and intensifies for 12–24 h, resulting in vasodilatation and an increased volume of blood in the dermis. UVB, particularly the shorter wavelengths, is most efficient in causing erythema. UVA can also cause erythema but at much higher doses (Gange & Parrish, 1983); however, as UVA represents the majority of sunlight, it contributes to about 15–20% of sunburn.

The clinically observed minimal erythematous dose (MED) is defined as the minimal amount of energy required to produce a qualifying erythematous response, usually after 24 h. The erythematous responses that qualify can be either just perceptible reddening or uniform redness with clearly demarcated borders, depending on the criterion adopted by

the observer. The former end-point (just perceptible) is more reliable than the latter (Quinn *et al.*, 1994; Lock-Anderson & Wulf, 1996). The MED depends on factors such as phenotype (e.g. skin complexion and hair colour), anatomical site and previous exposure, the amount of melanin in the epidermis at the time of irradiation and the intensity of the radiation. Individuals who burn easily and tan slightly reach their MED value after about 20 min of unprotected exposure to midday sun in midsummer in temperate latitudes. Five MEDs (100 min for such an individual) produce a painful burn. Ten MEDs lead to oedema, vesiculation and the formation of bullae.

Individual susceptibility to solar radiation depends on skin complexion (pigmentation of unexposed skin), hair colour and eye colour, which define the phenotype of the individual. The population can be grouped broadly into three levels of risk for burning in response to solar exposure: very sensitive, moderately sensitive and less sensitive. Very sensitive individuals burn easily and have difficulty tanning. Moderately sensitive individuals burn initially but then tan. Less sensitive individuals rarely or never burn and always tan. The Fitzpatrick six-point scale for human skin type defined the burning and tanning response of individuals to UVR. Generally, individuals with skin types I and II are very sensitive, types III and IV are moderately sensitive, and V and VI are less sensitive to UVR (Weinstock, 1992). The predisposition to burn correlates with the risk for developing skin cancer (IARC, 1992).

The typical histological changes associated with sunburn include slight epidermal spongiosis, increased nuclear diameter and nucleolar size of keratinocytes, alterations of Langerhans cells, induction of sunburn cells, hyperkeratosis,

acanthosis and migration of inflammatory cells in the exposed areas. Depending on the amount of the skin surface involved, severe sunburn can cause systemic symptoms including fever, nausea, vomiting, severe headache and even shock.

#### *Pigmentation (suntanning)*

Individuals have varying degrees of basic melanization of the skin. Fair-skinned individuals have limited melanin, which may be nested predominantly in freckles. Those with deeply pigmented skin tend to have a uniform distribution of melanin over the surface. The tanning response depends on the biological distribution of melanocytes. Tanning is the facultative increase in epidermal melanin pigmentation above the constitutional baseline level in response to UVR. Immediate pigment darkening, a transient greyish-brown change in skin colour due to oxidation of existing melanin, is induced by UVA and some visible wavelengths. It begins during exposure and persists, depending on the duration and intensity of exposure, but does not involve the production of new melanin. Tanning, the production of melanin, begins 48–72 h after UVR exposure, peaks at 7–10 days, may persist for several weeks to months, and is the result of increased production of melanin. Tanning

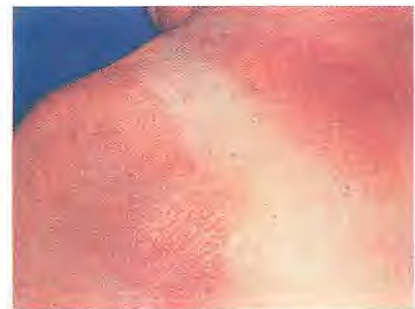


Figure 10 Shoulder of a man overexposed to the sun

may follow exposure to either UVB or UVA, but larger doses of UVA are required to give the same degree of tan as can be obtained with UVB.

### **Immune suppression**

Studies on UVR-induced suppression of the immune system have been reviewed (Ullrich *et al.*, 1999). UVR induces local immune suppression, defined as an inability to induce contact hypersensitivity through locally UVR-irradiated skin, or systemic immune suppression at a skin site distant from that which was irradiated. The effects of UVR that contribute to such immune suppression include depletion from the skin of Langerhans cells, epidermal dendritic antigen-presenting cells which pick up antigen and transport it to local lymph nodes where they activate specific T lymphocytes. UVR also disrupts production of cytokines by various cells in the skin, creating an environment which is not conducive to activation of immunity. Upon exposure to UVR, urocanic acid is isomerized from the *trans* to the *cis* form (see Fig. 7), which is immune suppressive. Application of antigen to the skin under these conditions activates suppressive rather than protective immunity.

### **Photoageing**

The changes seen in human skin with age are really due to a combination of ageing of the skin *per se* and ageing of the skin due to exposure to sunlight (photoageing). Ageing skin in doubly covered areas such as the buttocks is characterized primarily by atrophy (Gilchrest, 1996). This results in a thinner, more transparent skin, increasing prominence of the underlying vasculature and loss of elasticity. While there are relatively few changes in the stratum corneum, the epidermis thins and the rete ridges are effaced, reflected histologically by flattening of the undulations of the dermo-epidermal junction. The dermis also thins with age, resulting in more fragile skin (Fig. 11).

Changes considered to be signs of photoageing include wrinkling, mottled pigmentation, telangiectasia and epidermal thickening (Pearse *et al.*, 1987; Montagna *et al.*, 1989; Gilchrest & Yaar, 1992; Gilchrest, 1996). The dermal changes include the deposition of large quantities of abnormal, thickened, elastic fibres, a decrease in mature collagen, changed production of proteoglycan, chronic inflammation and damage to the microcirculation (Kligman, 1969, 1979; Mera *et al.*, 1987).

### **Photodermatoses**

Up to 20% of the fair-skinned adult populations in Sweden and the USA has been reported to experience symptoms of polymorphic light eruption, a sun-sensitivity disorder that manifests as itching papules on sun-exposed skin. (Morison & Stern, 1982; Ros & Wennersten, 1986). Even though some sufferers from this condition may require medical care, most cases are mild and tend to resolve with further exposure to the sun, in a so-called 'hardening' phenomenon. Other, less common but more severe photodermatoses include solar urticaria and chronic actinic dermatitis. There are no indications that sufferers from photodermatoses are more prone to skin cancers than the general population.

### **Melanocytic naevi**

Naevi (moles) are focal collections of non-dendritic melanocytes (naevocytes), usually found at the junction of the epidermis and dermis (junctional naevi) or at various depths in the dermis (compound or dermal naevi) (Fig. 12). Some naevi show a clinical resemblance to melanoma and may in addition be histologically atypical (Piepkorn *et al.*, 1994). Common acquired naevi arise after birth, and their ultimate density is related to a family history of naevi and increases with exposure to the sun (Harrison *et al.*, 1994). Acute exposure to the sun is implicated in the development of naevi in children. The number of naevi increases with age through adolescence and with a history of exposure to the sun and sunburn (Gallagher *et al.*, 1990; Harrison *et al.*, 1994). Naevi occur more frequently on sun-exposed areas, and there is strong evidence that the number of naevi on exposed areas increases with total cumulative exposure to the sun during childhood and adolescence (Holman & Armstrong, 1984; Kelly *et al.*, 1994). Children with



**Figure 11** Severe signs of photoageing with mottled pigmentation and stellate pseudoscars indicating epidermal and dermal damage



**Figure 12** Intradermal naevus on scalp

light skin who tend to burn rather than tan have more naevi at all ages. Many cutaneous melanomas arise in acquired naevi (Sagebiel, 1993; Skender-Kalnenas *et al.*, 1995). Most acquired naevi are clonal, while most melanocytes in areas without naevi are not (Robinson *et al.*, 1998a). The number of common naevi or the number of atypical naevi in any given individual constitutes the best predictor of individual risk for melanoma (Holman & Armstrong, 1984; Holly *et al.*, 1987; Grob *et al.*, 1990; see Boyle *et al.*, 1995, for review).

### **Actinic (solar) keratosis**

Actinic keratoses are proliferations of epidermal keratinocytes. They are associated with total and occupational exposure to the sun (Marks *et al.*, 1983; Goodman *et al.*, 1984; Vitasa *et al.*, 1990), with phenotypic indicators of cutaneous sensitivity to the sun (Vitasa *et al.*, 1990) and with other indicators of solar damage (Holman *et al.*, 1984; Green, 1991). Some of these lesions develop into squamous-cell carcinomas (Marks *et al.*, 1988).

### **Carcinogenicity of ultraviolet radiation in animals**

The carcinogenicity of UVR in experimental animals (including studies on solar-simulated UVR, UVB and UVA) were reviewed in detail (IARC, 1992).

The first proof of the carcinogenicity of UVR was provided by Findlay (1928) in experiments in which he induced skin tumours in mice by repeated daily exposure. Subsequently, Roffo (1934) showed that sunlight induced skin cancer in rats and that this carcinogenic action was blocked by glass, which filters out UVB. The finding of the carcinogenic effectiveness of UVB concords with its genotoxicity, which is considerably greater than that of UVA (IARC, 1992).

Studies in experimental animals conducted in the course of the twentieth century have yielded much information on the induction by UVR of skin tumours. Most of these studies investigated fibrosarcomas and squamous-cell carcinomas in mice and melanomas in opossum and fish (hybrids of the genus *Xiphophorus*). The commonest sun-related skin cancer in humans, basal-cell carcinoma, is, however, hardly ever observed in such experiments, underlining the need for a suitable animal model for each type of human skin tumour. The suitability of an animal model depends on the fidelity with which it reproduces the biology and pathology of the human tumour and on the genes that are involved in its development. The mouse model for the induction of squamous-cell carcinoma with long-term exposure to UVR is now well established: UVR-induced mutations in the *p53* tumour suppressor gene appear to play a role in both human (Brash *et al.*, 1991) and murine tumours (Kress *et al.*, 1992; Kanjilal *et al.*, 1993; Dumaz *et al.*, 1997). The validity of the opossum and fish models for melanoma is still being debated, but the genes and oncogenic pathways involved in hereditary melanoma in fish resemble those in humans (Kazianis *et al.*, 1998; Wittbrodt *et al.*, 1989), notably the *INK4a/p16* locus and the *RTK-RAS* pathway.

Species differences such as the absence of photolyase activity in human melanocytes will, however, complicate attempts to extrapolate the results of carcinogenicity studies in these animal models to humans. In humans, mutations in the *PTCH* gene (part of the *sonic hedgehog* pathway) were found to be involved in basal-cell carcinomas, and in heterozygous *Ptch* knock-out mice the development of basal-cell carcinomas was enhanced by UVR (Aszterbaum *et al.*, 1999). Thus, there is a robust model for the induction of squamous-cell carcinoma by UVR, and transgenic mouse models for basal-cell carcinoma and melanoma are emerging, which will facilitate further investigation of relevant genetic changes and an assessment of the protective effect of sunscreens against these tumour types.

### **Protection by a sunscreen depends on dose, time and wavelength**

Although experiments with animals may elucidate and allow quantification of the role of some of these factors, the number of variables in such experiments must be limited, and they must be standardized in certain well-controlled ways in order to provide reproducible, comparable data. Specification of the animals, the UVR sources, the exposure regimen, the UVR dosimetry and tumour evaluation are of the utmost importance. Unfortunately, many studies on the carcinogenicity of UVR do not fulfill these requirements and are, therefore, of limited use, especially for quantitative analyses such as are needed for the evaluation of protective effects of sunscreens.

Three physical dimensions are of the essence to UVR carcinogenesis: the spectrum (wavelength) of UV irradiation, the radiant energy (dose), the exposure scheduling and the latency of the tumour (time). Knowledge about the

relationship between these physical aspects and tumour induction has been advanced greatly by experiments in animals. In the 1940s, Blum *et al.* (1941) conducted an elaborate series of experiments on skin tumours induced by long-term exposure to UVR in which they carefully determined the quantitative relationships between tumour induction and the schedule of exposure (Blum *et al.*, 1941; Blum, 1959). The quantitative relationships they found were very similar to those reported by Druckrey (1967) after long-term application of chemical carcinogens to the skin and to those found by Raabe *et al.* (1980) for induction of bone cancer by radium. They all found that  $D^r \cdot t_m = \text{constant}$ , where  $t_m$  is the median tumour latency period,  $r$  is a power constant which depends on the carcinogen ( $0 < r < 1$ ), and  $D$  is the average daily (monthly or yearly) dose. Thus, a two-fold higher daily dose does not shorten the tumour induction time by a factor of 1 but by a factor  $< 2$ , i.e. there is no direct reciprocity between dose fraction and induction time. This lack of reciprocity is fully understandable if tumour development is envisaged as a process of multiple rate-limiting steps (e.g. mutations), of which only some are directly dependent on the carcinogen. The lack of reciprocity may be further attributed to protective mechanisms that become more active as the daily dose is increased. The nature of the dose–response relationship must be taken into consideration carefully in assuming a protective effect of a sunscreen (see page 91): a reduction of the dose by a factor of 10 will delay tumour development by a factor of 2–4, and, because of the steep increase in the incidence over time, the lifetime risk may decrease by a factor of up to 10 000.

Protection from carcinogenesis must be determined from dose–response

relationships with and without sunscreen: the degree of protection should be assessed from the ratios of the UVR doses required to evoke identical tumour responses. As this has been done in only a few studies, the level of protection from carcinogenesis by sunscreens is generally unknown.

In these early experiments, exposure to UVR induced fibrosarcomas and carcinomas on the ears of haired animals (see page 91). In the 1960s, immune-competent hairless mice became available, which respond consistently to UVR with induction of squamous-cell carcinoma, with actinic keratoses or sessile-based papillomas as precursor lesions, similar to the lesions observed in chronically exposed human skin. This model was studied extensively at the former Skin and Cancer Hospital in Philadelphia, USA (from which the hairless strain 'SKH' originates), and at

the department of Dermatology of the University Hospital in Utrecht, the Netherlands (for a review, see de Gruijl & Forbes, 1995). The earlier results of Blum *et al.* (1941) were refined, and the dependence of the induction of squamous-cell carcinoma on wavelength (the 'action spectrum') was derived mathematically from accumulated data obtained with UVR sources of various spectral compositions (de Gruijl *et al.*, 1993). The result was called the 'SCUP-m' action spectrum (SCUP stands for Skin Cancer Utrecht–Philadelphia, and the 'm' for murine). From the SCUP-m, a SCUP-h action spectrum ('h' for human) could be estimated by correcting for differences in UVR transmission between human and murine epidermis (de Gruijl & van der Leuw, 1994), the differences being largest below 300 nm. The result is depicted in Figure 13, with the directly measured action spectrum

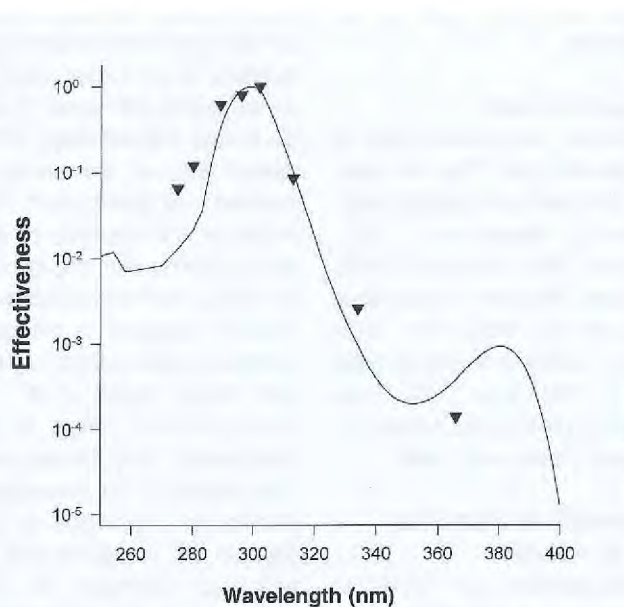


Figure 13 Comparison of the SCUP-h action spectrum (line; de Gruijl & van der Leuw, 1994) with the wavelength dependence of the dimers in induction of cyclobutane pyrimidine dimers in human skin (inverted triangles; Freeman *et al.*, 1989)

of UVR-induced DNA damage in human skin (Freeman *et al.*, 1989). The similarity of these two action spectra clearly indicates the importance of UVB-induced pyrimidine dimers in the formation of squamous-cell carcinoma. This in turn is fully in line with the nature of the mutations found in the *p53* gene (see previous section).

In view of the dominance of pyrimidine dimers in the genotoxicity of sunlight, one would expect the SCUP action spectrum to be generally valid for all types of UVR-induced skin cancer. This appears to be confirmed by the finding of gene point mutations (*p53* and *Ptch*) in murine (genetically modified) and human basal-cell carcinoma (Aszterbaum *et al.*, 1999). Surprisingly, the action spectrum for the induction of melanoma in certain hybrid fish (Setlow *et al.*, 1993) appears to be quite different from the SCUP-m action spectrum. The data on melanoma induction in opossum do not appear to confirm those in fish. UVB induces melanoma in opossum, and although UVA induces precursor lesions it does not appear to cause a conversion to malignancy (Ley, 1997).

The protective effect of sunscreens can be tested in the robust model of UVR-induced squamous-cell carcinomas. The action spectrum indicates that most protection would be provided by

passive shielding from UVB; however, as a significant portion of the carcinogenic dose of sunlight stems from the UVA band, good protection can be achieved only if a substantial amount of UVA is filtered out. Any deviation from the expected results of UVR filtering would be suspect and would need further investigation (see section 6.2 of the handbook on sunscreens). Because the available data refer to squamous-cell carcinoma, any protective effect of sunscreens against melanoma, or even basal-cell carcinoma, would differ importantly. A proper assessment of such protection must await good animal models for derivation of relevant action spectra for these types of skin cancer.

#### ***Experiments in transgenic mice and human skin***

As described earlier, great advances are being made in basic research on skin cancer by the use of transgenic mice. The natural proneness of mice to develop primarily squamous-cell carcinomas after exposure to UVR may be overcome by activation of the *Hedgehog* pathway (e.g. by introducing a defect in the *Ptch* gene), which will enhance the induction of basal-cell carcinomas (Aszterbaum *et al.*, 1999). Transgenic mice can also be used to introduce human proteins (such as H-ras) into the murine system and

thus test their specific responses (Chin *et al.*, 1997). Moreover, mice can be manipulated to accept human skin, which can then be tested freely. For example, immune-deficient RAG-1 mice have been used to host human skin grafts in which skin tumours were subsequently induced by long-term exposure to UVB in combination with application of a known promoter of skin tumours. One of the lesions in one of 48 grafts was a melanoma (Atillasoy *et al.*, 1998). The latter experiment is, of course, not a true in-vivo experiment, since human skin grafts lack the normal interaction with the rest of the body.

Although animal models for basal-cell carcinomas and melanomas are emerging, they are not yet well established. The effects of sunscreens can be tested most reliably in the model for squamous-cell carcinomas. A proper model for assessment of the protective effect against the most frequently fatal skin cancer, melanoma, is not yet available but clearly deserves to be high on the research agenda.

- **Skin cancer is more common than any other type of cancer**
- **It has been estimated that 106 000 melanomas of the skin were diagnosed worldwide in 1990.**
- **At least 2 750 000 non-melanocytic cancers of the skin were diagnosed in 1985**
- **Exposure to sun causes the three major types of skin cancer, basal-cell carcinoma, squamous-cell carcinoma and cutaneous melanoma.**
- **Sunscreens protect against sunburn**