Chapter 5

Cancer-preventive effects of sunscreens

Human studies

Many epidemiological studies have been conducted to assess the relationship between exposure to the sun and the risks for cutaneous melanoma and non-melanocytic skin cancer. Some of these investigations have also involved obtaining information on sunscreen use. Since use of sunscreens was not the primary question addressed in most studies, the information collected about use is often not optimal, so that it is often not known exactly when the agent was used, what quantities were used, the type of sunscreen or the frequency of use. Additional important considerations that must be kept in mind when interpreting the results of observational (cohort and case–control) studies of the relationship between use of sunscreens and skin cancer are outlined below.

First, there are problems of confounding. Sunscreens are most commonly used by people whose skin is sensitive to the sun (e.g. burn easily), expose their skin to the sun and do not protect their skin in some other way. These people are also those at highest risk of developing skin cancer. Thus, the relationship between use of sunscreens and skin cancer is confounded by sensitivity to the sun, exposure to the sun and lack of use of other protection against the sun. To deal with this confounding effectively, accurate measurements of sensitivity, lifetime exposure and other sun protection behaviour are required. Accurate measurements of sun sensitivity are difficult to obtain. There may also be confounding between sunscreen use and a past history of skin cancer or of a benign sun-related skin lesion, which indicate an increased risk for skin cancer, if use of sunscreens was recommended at the time these lesions were diagnosed or treated.

Second, the characteristics of sunscreens and sunscreen use that make them potentially efficacious are rarely adequately measured, either because of poor study design or poor recall. If sunscreens are efficacious, their efficacy almost certainly depends on a high SPF rating and proper use. Proper use includes application some time before engaging in outdoor activities, applying sufficient sunscreen to obtain the protection implied by the SPF value and re-application periodically during outdoor activities. If these are not documented, potentially efficacious use will be diluted by non-efficacious use, and a protective effect, if present, may be missed.

In the light of these and other considerations, it will be important in evaluating observational studies of sunscreens that the information shown in the box be available, in addition to that generally needed to assess the quality of a study.

Most of the evidence about the value of sunscreens for cancer prevention has

Information required for evaluation of epidemiological studies on sunscreens

- the period of study (earlier studies are less likely to cover experience with potentially efficacious sunscreens);
- distinction of potentially efficacious sunscreen use from use of related products (e.g. 'suntan lotions') that are unlikely to be efficacious;
- adequate measurement of sunscreen use, including, ideally, when use began and ended, frequency of use, amount usually used, the SPF of the sunscreen usually used and exposed sites normally protected;
- measurement of cutaneous sensitivity to the sun and adequate control for this variable in the analysis;
- measurement of patterns of sun exposure throughout life and control for this variable in the analysis;
- measurement of use of other protective measures against the sun and control for this variable in the analysis;
- measurement of past history of skin cancer or benign sun-related skin lesions and control for this variable in the analysis.

come from cohort and case–control studies. Relatively few randomized trials have been conducted to assess the use of sunscreens for preventing cancer, because it is commonly believed that skin cancers develop only after long-term exposure to UVR. In addition, cutaneous melanoma, the most serious type of skin cancer, is less common than other skin cancers. Precursor lesions lend themselves better to the randomized trial design (see p. 80) because they have a short latency; however, a recent study of the use of sunscreens in the prevention of squamous-cell carcinomas (Green *et al.*, 1999a) indicates that the problem of latency in studying cancer may not be insurmountable.

Cutaneous melanoma

No randomized trials or cohort studies have been reported on use of sunscreens and the risk for cutaneous melanoma (Fig. 25).

Case–control studies

Fifteen case–control studies have been conducted to examine the association between use of sunscreens and the risk for cutaneous melanoma (Table 17).

Klepp and Magnus (1979) assessed the use of 'sun lotion or oil' among 78 hospitalized patients with cutaneous melanoma and 131 controls who were being treated at the same institution in Norway (Norwegian Radium Hospital) for Hodgkin disease, non-Hodgkin lymphoma, testicular cancer or bone or soft-tissue sarcoma. The case and control cancers were diagnosed between 1 January 1974 and 1 May 1975, and the patients completed a written questionnaire [response rates not reported]. Patients who reported using such compounds 'sometimes, quite often or almost always' had a higher risk for melanoma (relative risk [RR], 2.3; 95% confidence interval [CI], 1.3–4.1) than those who had almost never used them. The elevated risk was seen only for males (RR, 2.8; 95% CI, 1.2–6.7) and not for females (RR, 1.0; 95% CI, 0.42–2.5). [A potential weakness of this study is that the term 'sun lotion or oil' was used rather than 'sunscreen', and this may have been interpreted as referring to compounds meant to promote tanning, such as 'tanning oils', or to moisturizing lotions used while in the sun or to sunscreens.]

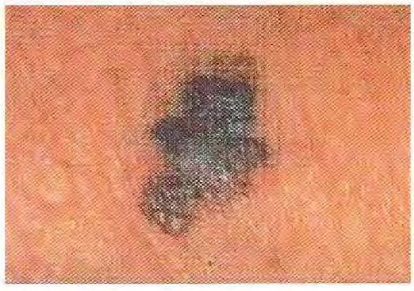

Figure 25 Melanoma of the trunk

Graham *et al.* (1985) conducted a case-control study of cutaneous melanoma in Buffalo, New York, USA, among 404 patients seen sequentially between 1974 and 1980. The controls were 521 patients with other cancers (gastrointestinal tract, respiratory, breast and reproductive neoplasms and Hodgkin disease and non-Hodgkin lymphoma) seen at the same institute. All interviews were conducted by nurses face-to-face [participation rates not reported]. The main purpose of the study was to evaluate the relationship between exposure to the sun and risk for melanoma, but the subjects were also asked about their use of 'suntan lotion' and of 'sunscreening lotion'. An elevated risk for cutaneous melanoma was seen among men who reported having used suntan lotion (RR, 1.7; 95% CI, 1.1–2.7) or sunscreen lotion (RR, 2.2; 95% CI, 1.2–4.1). No increased risk was detected with use of these products among women. [The strengths of the study include its large size and use of a specific question about sunscreen preparations; the potential weakness is the lack of data on sun sensitivity and on the duration and frequency of use of sunscreens.]

Herzfeld *et al.* (1993) conducted a case–control study of cutaneous melanoma of the trunk among men in upstate New York, USA, in order to determine the cause of the rapidly increasing incidence of tumours at this site. All 394 newly diagnosed cases ascertained between 1 January 1977 and 31 December 1979 were eligible for the study, and 324 participated in a telephone interview, although 38% of these interviews were with other respondents, usually the subject's wife. The overall participation rate was 82%. The major focus of the investigation was outdoor recreational activity, freckling, hair colour, sensitivity to the sun and use of 'suntan lotion'. Control subjects were selected by random-digit dialling, in which the area code and first three digits of the telephone exchange were matched with those of patients, and limited to white male respondents over the age of 18. The response rate among controls was estimated to be 65%. Before adjustment for host factors and exposure to the sun, patients who 'always' used suntan lotion were shown to have an increased risk for melanoma (RR, 2.6; 95% CI, 1.4–4.7) by comparison with men who used them less often or not at all. In a logistic regression analysis, however, sun lotion use was not a significant factor in risk for melanoma, although actual risk ratios are not given. The authors interpreted the elevated crude odds ratio as being due to the use of suntan lotions by sun-sensitive men at higher risk for melanoma. The authors also cautioned that 'sun lotion' may refer not only to sunscreens but also to tanning oils, as the two were not differentiated in the questionnaire. [The weaknesses of the study are use of the term 'suntan lotion' as the sole definition of exposure and use of respondents other than the patients, which makes assessment of sun sensitivity and sun exposure uncertain. Measurements of risk after adjustment for phenotype and exposure to sunlight were not given.]

Table 17. Case–control studies of sunscreen use and risk for cutaneous melanoma

Population Place/date	Type of cases/ controls	No. cases/ controls	Exposure	RR[a] (95% CI)	Comments	Reference
Norway 1974–75	Hospital cases Other cancer controls	78 cases 131 controls	Sometimes, often or almost always use sun lotion/oil	M 2.8[b] (1.2–6.7) F 1.0[b] (0.42–2.5) T 2.3[b] (1.3–4.1)	Elevated risks among males only. Sunscreens not differentiated from 'sun lotions'.	Klepp & Magnus (1979)
USA 1974–80	Hospital cases Other cancer controls	404 cases 521 controls	Used sunscreening Used suntan lotion	M.2.2[b] (1.2–4.1) M 1.7 (1.1–2.7) F 'No added risk'	Elevated risks among males only	Graham *et al.* (1985)
USA 1977–79	Population cases and controls	324 male trunk melanoma cases 415 controls	Always used 'suntan lotion'	2.6[b] (1.4–4.7) Not significant after control for 'tendency to sunburn and water sports'	'Suntan lotions' and 'sunscreens' not differentiated in questionnaire	Herzfeld *et al.* (1993)
Sweden 1978–83	Hospital cases Population controls	523 cases 505 controls	Often used sun protection agents	1.8[b] (1.2–2.7)		Beitner *et al.* (1990)
Canada 1979–81	Population cases and controls	369 trunk and lower limb melanomas 369 controls	Used sunscreen almost always	1.1 (0.75–1.6)	Highest risk in those using sunscreen 'only for first few hours' RR, 1.62 (1.04–2.52)	Elwood & Gallagher (1999)
Australia 1980–81	Population cases and controls	507 cases 507 controls	Used sunscreens ≤ 10 years	1.1 (0.71–1.6)		Holman *et al.* (1986)
USA 1981–86	Population cases and controls	452 cases 930 controls	Always used sunscreens	All cutaneous melanoma 0.62[b] (0.49–0.83) Superficial spreading melanoma (SSM) 0.43 (CI not available)	Study involved only women aged 25–59 at diagnosis. CI estimated. RR for SSM adjusted for host factors and sun exposure	Holly *et al.* (1995)
Denmark 1982–85	Population cases and controls	474 cases 926 controls	Always used sunscreens	1.1[b] (0.8–1.5)		Osterlind *et al.* (1988)
Australia 1987–94	Population cases Controls from same school	50 cases 156 controls All children < 15	Always used sunscreens	2.2 (0.4–12) on holidays 0.7 (0.1–6.0) at school		Whiteman *et al.* (1997)
Sweden 1988–90	Population cases and controls	400 cases 640 controls	Almost always used sunscreens	Trunk 1.4 (0.6–3.2) Other sites 2.0 (1.1–3.7)	No information on duration of use	Westerdahl *et al.* (1995)
Spain 1989–93	Hospital cases Hospital visitors	105 cases 138 controls	Always used sunscreens	0.2 (0.04–0.79)		Rodenas *et al.* (1996)

Table 17 (contd.)

Population Place/date	Type of cases/ control	No. cases/ controls	Exposure	RR[a] (95% CI)	Comments	Reference
Spain 1990–94	Hospital cases and controls	116 cases 235 controls	Used sunscreen	0.48 (0.34–0.71)	Inadequate description of measurement of sunscreen use	Espinoza Arranz *et al.* (1999)
Europe 1991–92	Hospital cases Neighbourhood controls	418 controls 438 controls	Ever use psoralen sunscreens Ever use sunscreens	2.3 (1.3–4.0) 1.5 (1.1–2.1) M 1.8 (1.1–2.7) F 1.3 (0.87–2.0)	Highest risk for sun-sensitive subjects using sunscreens to tan: RR, 3.7 (1.0–7.6)	Autier *et al.* (1995, 1997b)
Austria, 1993–94	Hospital cases and controls	193 cases 319 controls	Often used sunscreen	3.5 (1.8–6.6)		Wolf *et al.* (1998)
Sweden 1995–97	Population cases and controls	571 cases 913 controls	Always used sunscreen Used sunscreens to spend more time sunbathing	1.8 (1.1–2.9) 8.7 (1.0–76)		Westerdahl *et al.* (2000)

[a] Relative risk estimates adjusted for phenotype and sun-related factors where possible

[b] Crude relative risk ratio only available

Beitner *et al.* (1990) evaluated the roles of solar exposure and pigmentation in cutaneous melanoma and also examined sunscreen use among 525 patients with melanoma who had been referred to the Department of Oncology at the Karolinska Hospital in Stockholm, Sweden. This patient sample represented 64% of all newly diagnosed cases of cutaneous melanoma in the population of Stockholm County between February 1978 and December 1983. Patients with melanoma were compared with 521 controls matched for age and sex who were selected from the population registry of Stockholm County. The reported response rates were 99.6% among cases and 96.2% among controls, leaving 523 case and 505 control responses available for analysis. Data were collected from a postal questionnaire which included questions on sensitivity to the sun, eye and hair colouring, frequency of sunbathing, erythema and use of 'sun protection agents'. After control for age, sex and hair colouring, subjects who reported using protective agents 'often or very often' had an increased risk for cutaneous melanoma (RR, 1.8; 95% CI, 1.2–2.7) when compared with those who reported never having used these agents. The authors noted that the elevated risk for melanoma among patients who used sunscreens might be due to the fact that such use allows extended exposure to the sun. [The lack of specificity of the term 'sun protection agents' and the lack of specific categories of frequency of use are a weakness in this study.]

The Western Canada Melanoma Study was a case–control investigation undertaken to determine the relationship between phenotypic factors, history of tanning and sunburn, exposure to sunlight and risk for cutaneous melanoma in the four western provinces of Canada. All newly diagnosed cases among people aged 20–79 ascertained between 1 April 1979 and 31 March 1981 in the cancer registries of Alberta, British Columbia, Manitoba and Saskatchewan were approached for a face-to-face interview (Elwood *et al.*, 1984). For each case, one control was selected from the subscriber lists of the provincial medical services plan and frequency matched by sex, age (5-year age group) and province of residence. The rates of participation were 83% among cases and 59% among controls. Analysis of a subset of cases of melanoma on intermittently sun-exposed sites (trunk, lower limbs) and their controls (369 pairs) provided information about use of sunscreens on these sites during outdoor activity (Elwood & Gallagher, 1999). The risk of people who reported using sunscreen 'almost

always' was very similar to that of people reporting using sunscreens 'sometimes' (RR, 1.1; 95% CI, 0.75–1.6). Those who reported use 'only in the first few hours' of solar exposure had an increased risk for cutaneous melanoma (RR, 1.6; 95% CI, 1.0–2.5) after adjustment for hair, eye and skin colouring, propensity to burn and exposure to the sun. [The potential weaknesses of this study are that the information on sunscreens is relevant only to sites intermittently exposed to sunlight and the categorization of frequency of use of sunscreens is nonspecific.]

Holman *et al.* (1986) conducted a large case–control study to examine the relationship between phenotype, sunlight and cutaneous melanoma in Western Australia in the early 1980s. All cases of this cancer diagnosed in people under the age of 80 in the accessible regions of Western Australia between 1 January 1980 and 5 November 1981 were eligible for inclusion in the study. A total of 507 patients were interviewed about outdoor recreational activities and occupational exposure to the sun. Information was also collected on skin reactions to sunlight, skin and hair colouring, freckling and the number of raised naevi on the arms for each subject. A total of 507 control subjects randomly selected from the Commonwealth Electoral Roll and public school system and matched to the cases by sex and 5-year age group were interviewed with the same standardized questionnaire as used for the cases. Of those approached for interview, 90% of cases and 69% of controls completed the questionnaire. Frequency and duration of sunscreen use were evaluated. People who had used sunscreens for less than 10 years did not have a reduced risk for cutaneous melanoma (RR, 1.1; 95% CI, 0.71–1.6), nor was any reduction seen for those who had used the compounds for 10–15 years (RR, 1.2; 95% CI, 0.78–1.7), after control for pigmentary traits and sensitivity to the sun. Frequency of use likewise did not appear to be related to risk, as people who had used sunscreens during more than one-half of episodes of exposure had a relative risk of 1.1 (95% CI, 0.76–1.6) when compared with those who had 'never or hardly ever' used sunscreens. In the absence of control for fair pigmentary traits and sun sensitivity, a positive relationship was seen between use of sunscreens and the risk for cutaneous melanoma. The authors noted that this underlined the importance of good assessment of phenotype in evaluating the protective value of sunscreens against skin cancers. In addition, the authors point out that the lack of a protective effect of sunscreens in this study might be due to the fact that effective sunscreens were not available in Australia at the time when most of the subjects were in their teens and early 20s, the period of life when the protection afforded by sunscreens might be most valuable. [The strengths of the study include the control for sensitivity to the sun and the availability of information on the frequency and duration of use of sunscreens. A weakness of the study is the use of several nonspecific categories of exposure to sunscreens.]

Holly *et al.* (1995) studied factors associated with cutaneous melanoma in northern California, USA. Women in one of the five counties in the San Francisco Bay area in whom cutaneous melanoma was diagnosed between 1 January 1981 and 31 December 1986 and were aged 25–59 at diagnosis were included. Although the primary aim of the investigation was to evaluate the effect of oral contraceptive use and pregnancy-related factors on the risk for this disease, detailed information was also collected on exposure to sunlight, phenotypic factors and sunscreen use 5 years before diagnosis. The controls were residents of the same geographic areas as the patients and were identified by random-digit dialling.The response rates were 79% for patients and 77% for controls. Women who reported using sunscreens 'almost always' had a lower risk for cutaneous melanoma than those who reported that they never used these agents [RR, 0.62; 95% CI, 0.47–0.83]. [These results were not controlled for phenotype or exposure to sunlight.] When the risk for superficial spreading melanoma (the commonest type of melanoma) was assessed after control for sun sensitivity and sunburn history before the age of 12, the risk of women who 'almost always' used sunscreens was lower [RR, 0.43; $p < 0.001$, CI not reported] than that of women who never used them. The authors concluded that use of sunscreens was strongly protective against melanoma, after adjustment for sensitivity to the sun, past history of sunburn and other host factors. [This study is unusual in showing the highest levels of risk for melanoma among women with the least solar exposure, after control for sun sensitivity. A potential weakness of the study is the lack of specific categories of frequency of use of sunscreens.]

Osterlind *et al.* (1988) evaluated the relationship between solar exposure and phenotype and the risk for melanoma, excluding lentigo maligna melanoma, in 474 patients in eastern Denmark aged 20–79 whose cancers were diagnosed between 1 October 1982 and 31 March 1985. All of the patients completed a face-to-face interview in their homes to assess occupational and recreational exposure to the sun, including holidays on the Mediterranean Sea, history of sunburn, sensitivity to the sun and use of sunscreens. The controls were selected from the population register of residents of the same area, and a total of 926 were matched to the cases by sex and 5-year age group and interviewed. The participation rates were 92% for cases and 82% for controls. In comparison with the incidence of melanoma among people who had never used sunscreens, a small, nonsignificant increase in risk (RR, 1.3; 95% CI, 0.9–1.7) was seen for people who had used them for less than

10 years or for more than 10 years (RR, 1.2; 95% CI, 0.9–1.5). Frequency of use was not associated with the risk for melanoma (RR, 1.1; 95% CI, 0.8–1.5) among people who always used them when compared with those who had never or hardly ever used them. Although the study did not find a protective effect of sunscreens, the authors cautioned that effective sunscreens were not available to the patients when they were young. [The strengths of the study include good control for sun sensitivity, high participation rates and the availability of information on duration and frequency of use of sunscreens.]

Whiteman *et al.* (1997) conducted a case–control study in Australia to evaluate the risk factors for melanoma in young people in whom cutaneous melanoma was diagnosed when they were less than 15 years old during the period 1987–94. The cases were ascertained through the Queensland Cancer Registry. Of 61 eligible patients, 50 completed an interview, and the parents of a further two deceased patients completed surrogate interviews. The exposures of interest were sunlight, history of sunburn, family history of melanoma and characteristics of sun sensitivity. Participants were also asked about use of sunscreens while on holidays and while at school. Data were collected on facial freckling and naevus density at the age of 5. After control for tanning ability, freckling (Fig. 26) and number of naevi, patients who had 'always' used sunscreens while on holiday had a nonsignificant elevated risk (RR, 2.2; 95% CI, 0.4–12) for cutaneous melanoma when compared with those not using sunscreen. Use of sunscreens while at school was associated with a non-significant reduced risk (RR, 0.7; 95% CI, 0.1–6.0). [Because this was a relatively small study (only 11 patients reported 'always' using sunscreens on holiday and only two reported using them at school), the relative risk estimates have wide confidence intervals. A weakness of this study is the nonspecific categorization of the frequency of use of sunscreens.]

Westerdahl *et al.* (1995) conducted a case–control study of melanoma in southern Sweden, in which they reported the effects of sunscreen use. A total of 454 cases of melanoma diagnosed between 1 July 1988 and 30 June 1990 among residents of the Southern Sweden Health Care Region aged 15–75 were ascertained through the regional cancer registry. Of these, 400 completed and returned a postal questionnaire. The 400 cases were compared with 640 healthy controls selected at random from the National Population Registry and matched to cases by age (within 1 year), sex and parish of residence. The response rates for cases and controls were 88% and 70%, respectively. Data were collected on exposure to sunlight, constitutional factors, freckling, naevi and use of sunscreens. When compared with people who never used sunscreens, those who used them 'almost always' had a relative risk for melanoma of 1.8 (95% CI, 1.1–2.8) after adjustment for history of sunburns, history of frequent sunbathing during the summer, number of raised naevi, freckling and hair colour. Similar risk ratios were seen for men and women. Evaluation of risk by use before the age of 15, at 15–19 and > 19 years showed elevated odds ratios at each age similar to those of people 'always using' sunscreens. The risks for trunk melanomas were similar to those for melanomas of the extremities and head and neck (RR, 1.4; 95% CI, 0.6–3.2 and RR, 2.0; 95% CI, 1.1–3.7, respectively) after adjustment for sunburns, frequent sunbathing, freckling and naevi. [A weakness of the study is the nonspecific measure of frequency of sunscreen use.]

A study of melanoma was conducted by Rodenas *et al.* (1996) in Andalusia, Spain. All patients in this Mediterranean population with cutaneous melanoma diagnosed during 1989–93 and who had been referred to the Dermatology Centre at the University of Granada Hospital were ascertained, and 105 of these agreed to participate in the study. Visitors to patients in wards other than dermatology were recruited as controls, and 138 agreed to take part in the study. The response rates were 80% for cases and 69% for controls. Exposure to sunlight, skin sensitivity to sunlight, medical history, use of sunscreens and personal and family history of cutaneous diseases were recorded at a personal interview, and each subject was examined by a dermatologist, at which time naevus density, freckling and skin and hair colour were assessed. Only 6% of the controls but 36% of the patients had sun-sensitive skin. People who reported 'always' using sunscreens had a decreased risk for cutaneous melanoma (RR, 0.20; 95% CI, 0.04–0.79) after adjustment for age, skin colouring, sun sensitivity, naevi, and recreational and occupational exposure to sunlight. [It is uncertain whether use of sunscreens by the control subjects was typical of that of

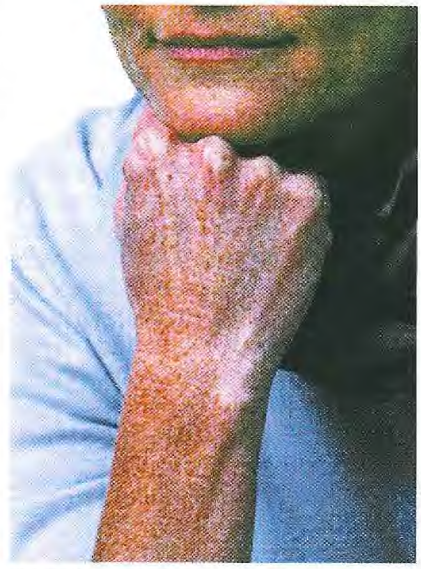

Figure 26 Woman with freckled complexion

the Spanish population, and the overall prevalence of sunscreen use in this study was low.]

A relatively small hospital-based case–control study of melanoma carried out in Madrid, Spain, included 116 patients with melanoma referred between January 1990 and January 1994 and 235 control patients admitted to the same hospital because of emergencies unrelated to cancer or skin disease (Espinosa Arranz et al., 1999). Data on exposure to the sun and use of sun-protection agents, including sunscreens, were collected by personal interview; sensitivity to the sun was recorded, and freckles, naevi and other actinic lesions were counted during a physical examination. Use of sunscreens, represented as a simple 'yes' or 'no' dichotomy, appeared to protect against melanoma. The relative risk of persons with 'no' use with reference to 'yes' use was 2.1 (95% CI, 1.4–2.9) after adjustment for sensitivity of the skin to the sun and number of naevi. The risk for melanoma was strongly related to the sensitivity of the skin to the sun, with a relative risk of 20 for those who always burned and never tanned with reference to those who always tanned and never burned (p for trend, < 0.001). Of the control subjects, 4.6% always burned and never tanned, and 48% always tanned and never burned. [The main weakness of this study is the lack of an adequate description of how sunscreen use was measured. It is uncertain what 'yes' and 'no' referred to exactly, because the question asked is not given. Use of sunscreens by the emergency department patients chosen as controls may not have been typical of that of the general population.]

Autier *et al.* (1995) conducted a case–control study of melanoma in five collaborating referral centres in Belgium, France and Germany. All cases diagnosed in white patients in the centres between 1991 and 1992 were eligible, and these patients were invited to participate in the study; face-to-face interviews were conducted in the patients' homes. Of the 456 eligible patients, 418 (91.7%) participated. Neighborhood controls were selected within the municipality of residence and frequency matched to cases by broad age ranges (20–39, 40–59, ≥ 60) and by sex. The rate of participation among controls was 78%. The focus of the study was exposure to solar and artificial UVR, and data were collected on recreational and occupational exposure and on host factors and phenotype. Sunscreen use was assessed from the answers to questions about the use of agents containing tanning accelerators such as psoralens and use of non-psoralen-containing sunscreens. Subjects who had ever used psoralen-containing sunscreens had an increased risk for cutaneous melanoma after control for age, sex, hair colouring and number of weeks spent each year in sunny destinations (RR, 2.3; 95% CI, 1.3–4.0), and the risk was found particularly among people who reported no history of sunburn. Use of psoralen-containing sunscreens was relatively uncommon. People who had ever used non-psoralen-containing sunscreens also had an increased risk after adjustment for the same factors (RR, 1.5; 95% CI, 1.1–2.1) when compared with subjects who had never used these agents. Use of sunscreens appeared to beassociated with an increased risk among subjects with either light or dark hair. Similarly, both sun-sensitive and sun-insensitive individuals showed an increased risk with use of sunscreens. Use of sunscreens tended to be associated with a higher risk for melanoma among people who sunbathed than in those who did not. The highest risk among sunscreen users was that of subjects with no history of sunburn after the age of 14. Use of clothing rather than sunscreen appeared to be protective. The authors suggested that the increase in risk associated with sunscreen use is due to the fact that their use allows greater duration of exposure to UVR and particularly UVA. [A potential weakness of this study is the categorization of sunscreen use into 'ever' and 'never'.]

The study of Wolf *et al.* (1998) was designed to evaluate the association between phenotype, exposure to sunlight, use of sunscreens and the risk for cutaneous melanoma. The cases were those of 193 Austrians in whom cutaneous melanoma was diagnosed between June 1993 and July 1994 and who were treated at the Department of Dermatology at the University of Graz. The controls were 319 patients with no history of skin cancer who were treated at the same university clinic during the same period. Each case and control patient completed a questionnaire designed to elicit information on occupational and recreational exposure to the sun, history of sunburns and use of sunscreens [response rates not reported]. Data were also collected on eye, hair and skin colouring, sun sensitivity and freckling and other factors. [It is not clear whether the data were collected by postal or telephone questionnaire or at a face-to-face interview.] After adjustment for skin colouring, sunbathing and history of sunburn, patients who reported having 'often used' sunscreens had a significantly increased risk for melanoma (RR, 3.5; 95% CI, 1.8–6.6) when compared with those who never used such agents. The investigators concluded that use of sunscreens does not prevent melanoma. [Potential weaknesses of the study include lack of information on response rates for cases and controls and on the way in which the questionnaire was administered and the use of patients with other dermatological conditions as controls. The information on frequency and duration of sunscreen use was non-specific.]

Westerdahl *et al.* (2000) studied the association between sunscreen use and risk for melanoma in a population-based case–control study of 571 patients aged

16–80 in whom cutaneous melanoma was diagnosed between 1995 and 1997, and 913 healthy controls. The 674 eligible cases were identified in the Regional Tumour Registry of the South Swedish Health Care Region. For each case, two healthy controls matched by sex, age and parish were selected by random sampling from the National Population Registry of residents of the same Region. Eligible cases and controls were sent a comprehensive questionnaire, and 584 patients (86%) and 1028 controls (76%) completed it. After exclusion of 13 cases with no matched control and 115 controls with no matched case, the final sample comprised 571 patients (84% of eligible cases) and 913 controls (68% of selected controls). The questionnaire elicited information on medical history, medicaments, constitutional factors, educational level, UVR exposure, smoking habits and alcohol use. Detailed information was collected on sunscreen use (any use, use the first time in the sun each year, regular use, SPF of the sunscreen used, sunburns, age at first and last use, reason for using sunscreens), UVR exposure (sunbathing habits, holidays in sunny places, sunburns, use of sunbeds, outdoor employment, residence in a sunny climate) and constitutional factors such as skin phototype, hair and eye colour, naevi and freckles. The median SPF of the sunscreens used by patients and controls was 6 (range, 2–25). A significantly increased risk for melanoma was found for regular use ('always') of sunscreens (RR, 1.8; 95% CI, 1.1–2.9), after adjustment for hair colour, history of sunburns and frequency and duration of sunbathing. The risk for melanoma was significantly increased among subjects who reported using sunscreens with a SPF < 10 (RR, 2.9; 95% CI, 1.2–20), when compared with people who did not use sunscreens, and for subjects who had not experienced sunburn while using sunscreens (RR, 1.9; 95% CI, 1.0–3.7). The risk was even higher for subjects who reported using sunscreens in order to be able to spend more time sunbathing (RR, 8.7; 95% CI, 1.0–76), and, in an analysis by subsite, was significantly increased only for melanoma of the trunk (RR, 2.5; 95% CI, 1.2–5.2).

Squamous-cell carcinoma

Randomized trials

Green *et al.* (1999a) evaluated the use of sunscreens in the prevention of squamous-cell carcinoma of the skin in the Nambour Skin Cancer Prevention Trial (Table 18; Fig. 27). A total of 1850 residents aged 20–69 in the town of Nambour, Queensland, Australia, were invited to participate in a randomized trial of the value of daily application of SPF-16 sunscreen and use of 30-mg β-carotene supplements in the prevention of skin cancer. A total of 1647 eligible subjects attended the baseline survey for assessment of cancer risk factors, and a dermatologist conducted a full skin examination of each person in 1992. All clinically diagnosed skin cancers detected on initial examination were then removed. A total of 1621 of the 1647 subjects subsequently agreed to be randomized to one of four study groups: sunscreen and β-carotene, sunscreen and placebo, no sunscreen and β-carotene and no sunscreen and placebo. Participants randomized to sunscreen were instructed to apply the agent to their head and neck, arms and hands every morning, and re-application was recommended after heavy sweating, bathing and long solar exposure. Those randomized to no sunscreen were instructed to continue their usual use of sunscreens. The code that identified the group of each subject was known only to the principal investigator and to those who packaged the β-carotene tablets for distribution. None of these individuals had any contact with the study subjects. Participants attended a clinic every 3 months to assess their compliance with the study protocol and to receive new sunscreen, β-carotene or placebo. The weight of sunscreen returned to the study centre at 3-month intervals was noted, and a random subgroup of sunscreen users kept 7-day diaries on three occasions to record the frequency of sunscreen application and sun exposure. At follow-up clinics held in 1994 and 1996, the subjects were again examined by dermatologists, and all skin cancers diagnosed and removed were examined histopathologically by a single pathologist. The subjects reported any lesions that had been removed in the intervals between the clinics, and study personnel obtained the relevant clinical reports and pathology reviews. Reported skin cancers were counted only when verified from medical records. Skin cancers diagnosed within 1 year of the start of the trial were not counted as they were considered to represent latent disease at baseline. In 1996, after 4.5 years of follow-up, 1383 trial subjects remained in the study, and 789 new skin cancers had been diagnosed in 256 study subjects. Since lesions diagnosed in 1992 were not included for the reasons noted above, the analysis was limited to 758 new lesions diagnosed in 250 subjects after 1993. No protective effect was found against squamous-cell carcinoma in subjects randomized to β-carotene (RR, 1.2; 95% CI, 0.89–1.4). The relationship with sunscreen use was analysed for all subjects, regardless of β-carotene use, as no interaction was seen between the two interventions, but concentrated only on skin cancers that occurred on body sites where sunscreen

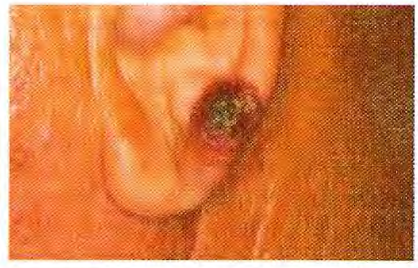

Figure 27 Squamous-cell carcinoma of the ear

Table 18. Randomized trial in Nambour, Australia, of risk for non-melanocytic skin cancer among 1383 subjects randomized to daily sunscreen use or no sunscreen

Tumour	No. of cases	Exposure	Rate ratio (95% CI)
Squamous-cell carcinoma	Sunscreen arm: 28 tumours in 22 subjects Non-sunscreen arm: 46 tumours in 25 subjects	Daily sunscreen application to head, neck, arms and hands	*SCC lesions* RR, 0.61 (0.46–0.81) *SCC participants* RR, 0.88 (0.50–1.6)
Basal-cell carcinoma	Sunscreen arm: 153 tumours in 65 subjects Non-sunscreen arm: 146 tumours in 63 subjects	Daily sunscreen application to head, neck, arms and hands	*BCC lesions* RR, 1.0 (0.82–1.3) *BCC participants* RR, 1.03 (0.73–1.5)

From Green *et al.* (1999a)

had been applied (head and neck, arms and hands). A total of 28 new squamous-cell carcinomas were detected in the group given sunscreen and 46 in those not given sunscreen (RR, 0.61; 95% CI, 0.46–0.81), a statistically significant difference. These lesions were seen in 22 participants given sunscreen and 25 not given sunscreen (RR, 0.88; 95% CI, 0.50–1.6). The authors concluded that sunscreen use could be of significant benefit in protecting against squamous-cell carcinoma. They noted that because no placebo sunscreen was used, the comparison group was less than ideal, reducing the ability of the study to detect an effect of daily sunscreen application. [The strengths of this study are that it is large and prospective and included good intermediate assessment of sunscreen use and solar exposure.]

A supplementary report by Green *et al.* (1999b) noted that the solar exposure of people given sunscreen did not differ from that of people who did not receive sunscreen. This observation was made in a randomly selected sample of 175 participants who wore UVR-sensitive polysulfone strips on 4 separate days, 2 in the summer and 2 in the winter. In addition, the prevalence of sunburn was lower among those receiving sunscreen than among those not receiving it. These findings suggest that the reduction in the incidence of squamous-cell carcinoma seen in the group given sunscreen was probably due to attentuation of the UVR by the sunscreen rather than to alterations in sun-related behaviour. The finding also suggests that use of high-SPF sunscreens by an older population in day-to-day activities may not result in longer exposure to the sun.

Cohort studies

Grodstein *et al.* (1995) examined the factors involved in squamous-cell carcinoma in a cohort of 107 900 female nurses, 197 of whom had had a histologically confirmed diagnosis of squamous-cell carcinoma. Once those with lesions on the anus, vulva and vagina had been excluded, 191 remained for analysis. The analysis showed that use of sunscreens over a 2-year period by women who spent 8 h or more per week in the sun was not protective by comparison with no use of such agents (RR, 1.1; 95% CI, 0.83–1.7). The authors noted that long-term use might produce different results.

Case–control studies

Table 19 summarizes the results of studies of non-melanocytic skin cancer in relation to use of sunscreens.

Pogoda and Preston-Martin (1996) completed a population-based case–control study among women in Los Angeles County, USA, to evaluate whether the use of lip coverings lowered the risk for lip cancer. A total of 74 women age 25–74 in whom lip cancer was diagnosed between 1978 and 1985 were interviewed, as were 105 female controls identified by random-digit dialling who were frequency matched to cases by decade of birth. The response rates of patients and controls were 57% and 66%, respectively. The rate was low because the prolonged retrospective case-finding period meant that 13% were deceased and a further 13% could not be located by the study personnel. Major items of interest on the etiologic questionnaire were the effects of complexion, sunlight exposure, and use of lip coverings on risk. After adjustment for complexion, history of skin cancer and cigarette smoking, women with low mean sun exposure who applied lip covering more than once per day had a slightly lower risk than similarly exposed women who did not use it or applied it only once per day (estimated RR, 0.77 [95% CI, 0.24–2.5]). Women heavily exposed to the sun appeared to have had greater protection from more than one application per day than similarly exposed women who did not use lip coverings

Table 19. Case–control studies of sunscreen use and non-melanocytic skin cancer

Place/date	Type of cases/ control	No. cases/ controls	Exposure	RR[a] (95% CI)	Comments	Reference
California, USA 1978–85	Lip cancer cases and population controls	74 cases 105 controls	Lip covering > 1/day High UVR conditions	Estimated RR, 0.41 (95% CI not available)	Lip covering likely to be coloured lipstick in most cases	Pogoda & Preston-Martin (1996)
			Lip covering > 1/day Low UVR conditions	Estimated RR, 0.77 (0.24–2.5)		
Australia 1987–88	Basal-cell carcinoma cases and controls from population cohort	226 cases 102 controls	Use of SPF > 10 sunscreen half the time or more in the 10 years before diagnosis			Kricker *et al.* (1995)
			1–9 years	1.8 (1.1–2.9)		
			≥ 10 years	1.1 (0.69–1.7)		
			Use of SPF > 10 sunscreen half the time or more 11–30 years before diagnosis			
			1–9 years	1.2 (0.69–2.1)		
			≥ 10 years	0.72 (0.40–1.3)		
Australia 1987–94	Squamous-cell carcinoma cases and controls from population cohort	132 cases 1031 controls	Use of SPF > 10 sunscreen			English *et al.* (1998a)
			Age 8–14	0.61 (0.08–4.7)		
			Age 15–19	1.9 (0.82–4.4)		
			Age 20–24	0.99 (0.44–2.2)		
Spain 1990–92	Hospital cases of non-melanocytic cancer and hospital controls	260 cases 552 controls	Use of solar protective creams	Males 0.6 (0.3–1.1) Females 0.7 (0.4–1.4)	Cases of basal-cell and squamous-cell carcinoma combined in analysis	Suarez-Varela *et al.* (1996)

Relative rate of naevi ≥ 2 mm in children in highest quartile of sunscreen use adjusted for sun exposure index, sex, study

[a] Relative risk estimates for phenotype and sun-related factors where possible

(estimated RR, 0.41; 95% CI not available). [The relevance of the results of this study are difficult to assess, as much of the lip covering worn by the women is likely to have been cosmetic coloured lipstick rather than sunscreen. Although lipstick is not a chemical sunscreen, the study does suggest that attenuation of UVR can be effective in preventing lip cancer.]

The relationship between exposure to the sun and squamous-cell carcinoma was addressed in a study in Geraldton, Western Australia, to evaluate whether the timing of exposure to sunlight was important (English *et al.*, 1998a). The cases included both prevalent cases from 1987 and new squamous-cell carcinomas diagnosed up to 1994. Thus, 132 patients and 1031 controls, some of whom had basal-cell carcinoma, were available for analysis. Use of sunscreens with SPF-10 or more was examined in three age groups, 8–14, 15–19 and 20–24. Subjects who reported use of sunscreens at age 8–14 appeared to have a slightly reduced risk for squamous-cell carcinoma (RR, 0.61; 95% CI, 0.08–4.7), although this was not statistically significant. Those who had used sunscreens at the age of 15–19 had a relative risk of 1.9 (95% CI, 0.82–4.4), and those who had used sunscreens at 20–24 had a risk of 0.99 (95% CI, 0.44–2.2) by comparison with subjects who had not used them. Although the small sample size

limits the power of this study, overall, no strong protective effect of sunscreens was evident. [The strengths of this study include the complete examination of each subject by a dermatologist, close annual follow-up for new lesions and good assessment of exposure to the sun. The weaknesses include the relatively nonspecific description of exposure to sunscreens.]

Suarez-Varela *et al.* (1996) conducted a case–control study in Valencia, Spain, to evaluate protective measures against non-melanocytic skin cancer in a Mediterranean population. The study population comprised 260 cases of skin cancer treated at La Fe University Hospital between 1990 and 1992, and 552 controls recruited from among other patients at the same institute and from an old-age centre within the hospital's area of coverage. The controls were frequency matched to cases by sex and age (± 5 years). Use of 'solar protective creams' appeared to be associated with a protective effect among both men (RR, 0.6; 95% CI, 0.3–1.1) and women (RR, 0.7; 95% CI, 0.4–1.4). The authors noted that few of the subjects used sunscreens and consequently the power of the study is low. [The authors did not analyse the data by histological type of skin cancer, and separate risk estimates were not available for squamous-cell and basal-cell carcinoma. In addition, the sun exposure and sunscreen use of persons in an old-age centre may not be typical of those of the Spanish population.]

Basal-cell carcinoma

Randomized trials

In the study described above, Green *et al.* (1999a) also evaluated the role of sunscreen in the prevention of basal-cell carcinom (Fig. 28; Table 18). No protective effect against this tumour was found in persons randomized to β-carotene (RR, 0.89; 95% CI, 0.64–1.1), and no significant effect of daily sunscreen use was seen. Thus, 153 new tumours were found in the group receiving sunscreen and 146 in the group not given sunscreen (RR, 1.0; 95% CI, 0.82–1.3). The tumours occurred in 65 persons randomized to daily sunscreen use and 63 randomized to no sunscreen (RR, 1.0; 95% CI, 0.73–1.5).

Cohort study

In an analysis of 771 cases of basal-cell carcinoma in a cohort of 73 366 nurses, Hunter *et al.* (1990) demonstrated that those who usually used sunscreens when outdoors during the summer had an elevated risk for this tumour when compared with those who did not use these agents (RR, 1.4; 95% CI, 1.2–1.7). Sunscreen use was analysed only among study participants who had spent 8 h per week or more outdoors. The authors noted that the relative risk declined after adjustment for hair colour, childhood sensitivity to the sun and history of sunburn and suggested that the continued presence of an elevated risk was probably due to further, unmeasured confounding.

Case–control study

Kricker *et al.* (1995) conducted a case–control study of basal-cell carcinoma in Geraldton, Western Australia (Table 19). A cohort of 4103 subjects aged 40–64 were recruited and given a physical examination, and those 226 in whom a basal-cell carcinoma had been diagnosed at the examination in 1987 or in the previous year constituted the cases for the analysis. Controls were selected from among members of the same cohort who did not have a basal-cell carcinoma (although several had had a squamous-cell carcinoma). A total of 1021 controls matched to cases in three strata were chosen: women, men aged 40–54 and men aged 55–64. Age appeared to have little effect on the incidence or prevalence of basal-cell carcinoma among women in this cohort. Subjects who had used sunscreens one-half the time or more while in the sun during the 1–9 years prior to diagnosis had a higher relative risk for basal-cell carcinoma than those who had never used sunscreens or had used them less than half the time (RR, 1.8; 95% CI, 1.1–2.9) during the same period. The risk persisted after adjustment for sex, age, ability to tan and site of the lesion. No change in relative risk was found for those who had applied sunscreens more than half the time throughout the decade preceding diagnosis (RR, 1.1; 95% CI, 0.69–1.7) by comparison with those who had not used them or had used them less than half the time. Relatively few subjects had used sunscreens in the period 11–30 years before diagnosis. The risk of those who had used sunscreens for 1–9 years during this period was similar to that of those who had not used them or had used them less than half the time (RR, 1.2; 95% CI, 0.69–2.1). People who had used them for 10 or more years in the interval 11–30 years before diagnosis had a RR of 0.72 (95% CI, 0.40–1.3). The authors concluded that there was little evidence that use of sunscreen protects against basal-cell carcinoma. They noted that the elevated risk of those who had used sunscreens for 1–9 years in the 10 years before diagnosis was probably artefactual and was due to the fact that people identified as being at higher risk in the years before diagnosis may have been advised to use sunscreens. [The strengths of this study include the complete examination of each subject by a dermatologist, close annual follow-up for new lesions

Figure 28 Basal-cell carcinoma

and good assessment of exposure to the sun. The weaknesses include the relatively nonspecific description of exposure to sunscreens.]

Precursor lesions

Melanocytic naevi

Naevus counts are the strongest individual predictors of risk for cutaneous melanoma (Fig. 29) (Holman & Armstrong, 1984; Holly *et al.*, 1987; Grob *et al.*, 1990) and are likely to be the precursors of many melanomas (Skender-Kalnenas *et al.*, 1995). Therefore, a number of investigations have been conducted to explore the causes of acquired melanocytic naevi. The results have demonstrated a positive relationship between exposure to sunlight and naevus density (Pope *et al.*, 1992; Harrison *et al.*, 1994; Kelly *et al.*, 1994). Several of these studies have also addressed the question of whether sunscreen use can modify the risk for acquiring melanocytic naevi (Table 20). Most such studies have been carried out in children, as most neonates are born with no naevi and develop their highest naevus density by adolescence. The maximal density may be reached at an earlier age in areas with a great deal of sunlight, such as Australia (Gallagher *et al.*, 1990; English & Armstrong, 1994a,b; Kelly *et al.*, 1994).

Randomized trials: One randomized trial has been conducted to evaluate whether use of sunscreens can reduce the development of naevi in children (Gallagher *et al.*, 2000). The study was conducted in six elementary schools in Vancouver, Canada, in which 696 children in grades 1 and 4 (ages 6–7 and 9–10, respectively) were ascertained, and 458 (66%) were enrolled in the trial. The naevi on the children were counted at enrolment, and each child was randomized to receive sunscreen (SPF 30, broad spectrum) or no sunscreen but allowed to continue usual use. Both groups were followed for 3 years, during which time their sun exposure was assessed. Of the children who were recruited, 86% completed the trial, when their naevi were counted again. Analysis of the data for white children showed a modest reduction in the median number of new naevi (the outcome measure) among those randomized to sunscreen use by comparison with those receiving no sunscreen (median counts, 24 and 28; $p < 0.05$). Further modelling of the data demonstrated an interaction between freckling and the intervention, suggesting that sunscreen use was more effective in preventing new naevi in children who freckled than in those who did not. Measures of exposure to sunlight showed little difference between the two groups, indicating that the differences in counts of new naevi in the two groups were not due to differences in exposure.

A further trial to evaluate use of sunscreens in preventing naevi in children is under way in Australia (Milne *et al.*, 1999a,b).

Cohort studies: In an unusual cohort study, with retrospective assessment of exposure to sunshine and sunscreen use and prospective recording of changes in naevus counts, Luther *et al.* (1996) examined the risk factors for the development of naevi in a cohort of 866 German children. The children were examined in 1988, and 377 underwent a second physical examination in 1993. The number of naevi more than 2 mm in diameter was counted at each examination on all body sites except the scalp, and the counts in 1988 were subtracted from those in 1993 to obtain the outcome measure, the number of new naevi. Freckling, sun sensitivity, hair and skin colouring, exposure to the sun during holidays, history of sunburn and use of sunscreens were assessed from responses to the questionnaire. After elimination of the records of 20 children with the darkest skin, data on the 357 remaining subjects (41%) showed relationships between high naevus count, sun sensitivity and days of intense exposure to the sun. In a univariate analysis, regular use of sunscreen was associated with an increased risk for having a large number of new naevi (RR, 1.8; 95% CI, 1.0–3.3) by comparison with children who had never used sunscreens. The final logistic regression model of factors accounting for the development of new naevi did not contain sunscreen use. The authors noted that children who had used sunscreens tended to have greater cumulative exposure to the sun than those who had not used them, although no data were presented to quantify this statement. [The strengths of this study are the large number of children involved and the reliability of the naevus counts. The potential weaknesses are the low subject retention over the 5-year period and the retrospective assessment of sunscreen use and sun exposure.]

Cross-sectional studies: Three cross-sectional studies have been conducted to evaluate the relationship between acquired naevi and sunscreen use among children (Table 20), and two have been conducted among adults.

Pope *et al.* (1992) recruited 2140 British schoolchildren aged 4–11 to study the relationship between pigmentation characteristics, sun sensitivity, freckling, sunburn history, sun exposure and the prevalence of naevi. The children either attended one of 10 primary schools in the West Midlands or were selected from the patient lists of five general practitioners in the same geographical area. The

Figure 29 Dysplastic naevus of the trunk

Table 20. Studies of sunscreen use and acquired melanocytic naevi among children

Place/date	Type of study	No. of subjects	Exposure	End-point	RR[a] (95% CI)	Comments	Reference
West Midlands, Englands (dates not reported)	Cross-sectional	1130 girls and 1010 boys aged 4–11	Always or often used sunscreens	Naevi, any size; ≥ 2 mm	Not reported	Significantly higher naevus count ($p < 0.001$) in children using sunscreen	Pope *et al.* (1992)
Townsville, Australia (dates not reported)	Cross-sectional	506 children aged 1–6	Use of summer sunscreens	Naevi, any size; ≥ 2 mm	Not reported	Use of sunscreen 'not associated with naevus number or density'	Harrison *et al.* (1994)
Bochum, Germany 1988–93	5-year cohort study	357 children aged 1–6 at start	Regular use of sunscreens	Naevi ≥ 1 mm; ≥ 2 mm	1.8 (1.0–3.3)	Unvariate result only. Not statistically significant in multivariate model	Luther *et al.* (1996)
Vancouver, Canada 1993–96	Randomized trial	309 children aged 6–10 at start	Use of SPF 30 sunscreen when in the sun > 30 min	Naevi, any size	Not reported	Significantly fewer new naevi in children using suncreen	Gallagher *et al.* (2000)
Belgium, France, Germany, Italy 1995–97	Cross-sectional	631 children aged 6–7		Naevi ≥ 2 mm	Trunk, 1.7 (1.1–2.6) Head and neck, 1.5 (0.86–2.5)		Autier *et al.* (1998)

[a] Relative to naevi ≥ 2 mm

participation rates varied among the schools from 38% to 66%. The children were examined by a nurse who was trained to identify naevi and to differentiate them from freckles. Naevi of any size, those 2 mm or more and those 5 mm or more in diameter were enumerated on all body sites except those covered by the child's underpants and the scalp. Skin, hair and eye colour were recorded, and sun exposure and use of sunscreens were assessed from answers to a questionnaire. Analysis of the data on sunscreens was not presented in detail; however, the authors noted that "children who often or always use a sunscreen in strong sunlight had more moles than those who never or sometimes use a sunscreen ($p < 0.001$)". [The strength of the association between use of sunscreens and number of naevi and whether this association was adjusted for sun sensitivity and sun exposure could not be determined because of incomplete reporting of the study.]

Harrison *et al.* (1994) studied sun exposure and the prevalence of naevi in a sample of children aged 1–6 in a cross-sectional study in Townsville, northern Australia. The mothers of the children were identified from the records of maternity wards in the two local hospitals and from lists of the mothers of children who had participated in previous studies of naevi. A total of 707 children were invited to participate in the study. After exclusion of non-respondents, those who had left the area and a few with two or more non-European grandparents, 506 children remained (72%). The naevi of the children were enumerated according to an IARC protocol (English *et al.*, 1990), and the degree of freckling on the face and shoulders was estimated. Hair and eye colour were noted, and skin reflectance was assessed with a spectrocolorimeter. Sun exposure before examination, sun sensitivity, sunburn

history and use of sunscreens were determined for each child from the answers to a questionnaire completed by the parents. No quantitative data on the relationship between sunscreen use and the prevalence of naevi is presented in the paper; however, the authors stated that 'use of summer sunscreen significantly reduced the number of sunburns (p = 0.022) but was not associated with annual sun exposure or with naevus number or density'. [The strength of the association between use of sunscreens and number of naevi and whether this association was adjusted for sun sensitivity and sun exposure are uncertain because of incomplete reporting of the study.]

Autier *et al.* (1998) conducted the most complete cross-sectional study to date on the relationship between sunscreen use and the prevalence of naevi. The investigation was conducted among elementary school children in Belgium, Germany, France and Italy. A total of 1234 parents of children aged 6–7 were approached by letter to participate in the study, and 682 agreed. Of these, 51 were eliminated from the study because the child was not of 'Caucasian' origin, the child's skin examination could not be completed or the parents could not be reached for interview, leaving 631 children (51%). Whole-body skin examinations (with the exception of the buttocks, genital area and scalp) were conducted on each child by a trained physician, who enumerated naevi 2 mm or greater in diameter. The degree of freckling on the face, arms and shoulders was also noted. Naevi were counted by the IARC protocol (English *et al.*, 1990). Parents were interviewed in their homes by trained, non-medical, female interviewers about each child's sun exposure, sun sensitivity, sunburn history, clothing preferences and sunscreen use. Particular attention was paid to assessing holiday sun exposure by collecting data on the month of each holiday, its duration, place and latitude, whether it had been sunny during the vacation, and the child's clothing and sunscreen use during the holiday. The frequency of sunscreen use was evaluated in categories ranging from 'never' to 'always' using such agents during each holiday. Questions were also posed about the sun exposure and sunscreen use of each child during recreational pursuits apart from holidays. Total sunscreen use on the head and neck and the trunk—sites representing constantly and intermittently exposed body sites—was evaluated. A direct relationship was seen between the prevalence of naevi and use of sunscreen on both the head and neck (RR for highest quartile of use, 1.5; 95% CI, 0.86–2.5) and the trunk (RR for highest quartile of use, 1.7; 95% CI, 1.1–2.6). The association persisted after adjustment for sun exposure, sex, study area, eye colour and sun sensitivity. A significant exposure–response gradient of naevus count with increasing sunscreen use was reported. The authors noted that since the relationship persisted after control for potential confounders, it was probably due to the fact that children who used sunscreens could remain in the sun longer than those who did not use these agents.

In a study conducted in Belgium, France and Germany in 1991 and 1992 of 438 controls selected for a case–control study of melanoma, the use of sunscreen was associated with a higher density of pigmented lesions of the skin (Autier *et al.*, 1995). The naevus count on both arms of control subjects increased significantly from those with no sunscreen use to those who had ever used non-psoralen-containing sunscreens, with a rate ratio of 1.3 (95% CI, 1.2–1.4) after adjustment for age, sex, hair colour, number of holiday weeks spent each year in sunny resorts and sunbathing during 'the hottest hours of the day'. The increase in naevus count was greater for subjects who had ever used psoralen-containing sunscreens (RR, 2.1; 95% CI, 1.8–2.4); the adjusted rate ratio between non-psoralen-containing sunscreen users and psoralen-containing sunscreen users was 1.6 (95% CI, 1.4–1.8).

The other study that addressed the issue of sunscreen use and the prevalence of naevi in adults is that of Dennis *et al.* (1996), which was conducted in Washington State, USA. The results were presented in such a way that they did not provide any information on the association between sunscreen use specifically and number of naevi, and the study was not considered further.

Actinic (solar) keratoses

Actinic keratoses are a risk factor for basal-cell carcinoma (Fig. 30) and a precursor lesion for squamous-cell carcinoma (Marks *et al.*, 1988). They are known to be related to solar exposure and, like basal-cell and squamous-cell carcinoma, are more common in individuals with light skin and hair colouring, a propensity to freckle and sun-sensitive skin (Vitasa *et al.*, 1990). The rate of transformation of actinic keratoses to squamous-cell carcinomas is low, however, and many of these lesions appear to regress spontaneously, particularly in the absence of exposure (Marks *et al.*, 1986). As they are known to be potential precursors of squamous-cell carcinoma, these lesions have been used as intermediate end-points in recent studies of the use of sunscreens in preventing squamous-cell carcinoma. The relationship has been assessed in several randomized trials and one cross-sectional study (Table 21).

Figure 30 Actinic keratosis of the scalp

Table 21. Studies of sunscreen use and actinic keratoses

Place/date	Type of study	No. subjects	Randomization or exposure	End-point	RR[a] (95% CI)	Comments	Reference
Lubbock, Texas USA 1987–90	Randomized trial	50 persons with clinically diagnosed actinic keratoses	SPF-29 sunscreen daily vs base cream (placebo)	Average annual rate of actinic keratosis formation	Sunscreen, 21 Placebo, 28 36% reduction, $p = 0.001$	All subjects warned against solar exposure and encouraged to use hats and other mechanical protection measures	Naylor *et al.* (1995)
Cardiff, Wales, United Kingdom, 1988–92	Cross-sectional	560 men and women aged ≥ 60	Normally used sunscreens	Prevalent actinic keratoses or squamous-cell carcinomas	Sunscreens vs placebo, 0.56 (0.34–0.82) RR adjusted for age not given	Use of sunscreen protective in univariate analysis; in multivariate analyses, effect largely accounted for by confounding with age	Harvey *et al.* (1996a,b)
Maryborough, Australia 1991–92	Randomized trial	431 persons aged ≥ 40 with 1–30 actinic keratoses	SPF-17 sunscreen daily vs base cream (placebo)	Prevalent actinic keratoses on head, neck, hands and forearms No. of new actinic keratoses No. of remissions	Placebo vs sunscreen, 1.5 (0.81–2.2) Sunscreen vs placebo, 0.62 (0.54–0.71) Sunscreen vs placebo, 1.5 (1.3–1.8)	All participants also told to avoid midday sun and wear hats	Thompson *et al.* (1993)

[a] Risk ratio

Randomized trials: Naylor *et al.* (1995) conducted a randomized controlled trial between December 1987 and December 1990 to test the hypothesis that sunscreen use can reduce the appearance of new actinic keratoses. Fifty-three individuals with a history of prior actinic keratoses or a non-melanocytic skin cancer who had sought treatment at a university or Veterans Affairs dermatology practice in Lubbock, Texas, USA, were recruited for the trial. All of the subjects lived close enough to the clinic for follow-up visits every 3 months. The volunteers were examined, and then all actinic keratoses were removed with liquid nitrogen and non-melanocytic skin cancers were excised surgically. A detailed history of sun exposure was taken, and the subjects were randomized to receive either a broad-spectrum SPF-29 sunscreen (containing ethylhexy methoxycinnamate, benzophenone-3 and ethylhexyl salicylate) or a placebo of identical appearance containing the sunscreen base cream but without the active ingredients. The subjects were warned against overexposure to sunlight and were encouraged to use hats. Use of sunscreens other than that provided in the study was discouraged. The subjects were instructed to apply the sunscreen to all sun-exposed body sites and not to change their usual activity patterns. Participants in both groups were seen at 1 month, 3 months and every 3 months afterwards for 2 years. At each visit, new lesions identified clinically as actinic keratoses were noted and removed

with liquid nitrogen, and new non-melanocytic skin cancers were removed surgically. Only lesions on sunscreen-treated areas were counted, and several actinic keratoses that appeared on bald areas of the scalp were not included. Of the 53 subjects originally enrolled in the trial, 50 visited the clinic at least once at or after 3 months, and 37 came for their final visit at 2 years. Three subjects dropped out of the study before the first 3-month visit and were excluded from the analysis. The 13 subjects who did not report for the 2-year follow-up were not included in the analysis but did not have a different outcome from those who completed the trial. The outcome of the trial was determined by comparing the annual rates of formation of actinic keratoses in the two groups. Participants given sunscreen had an average of 13.6 (SD 18.5) new actinic keratoses per year, while those given placebo had 27.9 per year (SD 31.8). The distribution of risk factors between the two groups differed, however, and when these were taken into consideration in a Poisson regression model, an expected value of 21.1 new actinic keratoses per year was estimated for the group given sunscreen on the basis of the experience of the placebo group. Thus, the annual rate of new actinic keratoses was estimated to be 36% lower for people who received sunscreen than for those who did not (p = 0.001). Too few non-melanocytic skin cancers were diagnosed during the 2-year trial for analysis.

Thompson *et al.* (1993) conducted a randomized trial to determine whether daily use of high-SPF sunscreens could attenuate the development of new actinic keratoses in subjects who already had at least one. The trial was conducted in Maryborough, southern Australia, among 588 subjects aged 40 or more, each of whom had 1–30 actinic keratoses and who were recruited in September 1991. An examination was conducted to note all actinic keratoses on a body site map, and the subjects were randomized into one of two groups, with stratification to equalize the sex distribution and sun sensitivity. One group received an SPF-17 broad-spectrum sunscreen containing ethylhexyl methoxycinnamate and butyl methoxydibenzoylmethane, with instructions to apply 1.5 ml to the skin of the head and neck and the same amount to the forearms and hands every morning. They were told to re-apply the sunscreen during the day if necessary. Each subject kept a diary, recording the time of day they applied the sunscreen. Those in the other group received a placebo cream containing the same sunscreen base but without the active ingredients. The colour and consistency of the placebo were identical to those of the sunscreen. All subjects were instructed not to rely solely on the sunscreen but to avoid the sun in the middle of the day and to wear hats and appropriate clothing in order to moderate their solar exposure. Three follow-up examinations were made in the 7 months after recruitment, the season in southern Australia when there is the most sunlight. At each examination, total actinic keratoses, remissions and new actinic keratoses were noted, the diaries were examined, and the bottles of sunscreen were weighed. By March 1992, 431 subjects (73%) had completed the trial and were re-examined by the same physician who had seen them at the start. All actinic keratoses were recorded on a new body site map so that the physician would be unaware which lesions had been present at the start of the study. The groups were compared for the overall prevalence of actinic keratoses on the head and neck, arms and hands, the number of new actinic keratoses arising during the study and the number of actinic keratoses that regressed during the study. Subjects given the placebo had a greater increase in the mean number of actinic keratoses during the course of the study (1.0 ± 0.3 SE) than those given sunscreen (0.6 ± 0.3 SE; RR, 1.5; 95% CI, 0.81–2.2). Fewer new actinic keratoses appeared during the course of the study among people given the sunscreen than those given placebo (mean, 1.6 versus 2.3 lesions per subject; RR, 0.62; 95% CI, 0.54–0.71). After control for sex and sun sensitivity, the likelihood of remission of actinic keratoses present at the start of the study was greater for people given sunscreen than for those given placebo (25% versus 18% of initial lesions regressing; RR, 1.5; 95% CI, 1.3–1.8). [This was a short-term investigation, and many subjects did not complete the protocol.]

Cross-sectional studies: Harvey *et al.* (1996a,b) completed a cross-sectional study of factors associated with actinic keratoses in an older (age 60 or more) population in Wales, United Kingdom. A random sample of 1034 men and women over the age of 60 and living in the county of South Glamorgan were sent letters of invitation to participate in a study of skin cancer. Of those invited, 560 (54%) were seen in their homes by a research registrar in dermatology (Harvey *et al.*, 1996a), when a detailed questionnaire was completed on risk factors for non-melanocytic skin cancer and actinic keratoses, including cumulative sun exposure and sun sensitivity. The skin of the head, neck, arms (to the shoulder), legs (below the knee) and feet was examined. Polaroid photos and 35-mm slides were made of any suspected skin cancer or actinic keratosis. The physicians of any patients with a suspected non-melanocytic skin cancer were contacted directly, while those subjects with actinic keratoses were reassured that no immediate treatment was necessary and that the lesion would be reassessed at the next visit. A second visit was made 1–2 years later, and the details of treatment for any lesion removed between visits were recorded. New lesions were also noted and photographed. At the conclusion of the

study, the slides of all lesions were reviewed by three consultant dermatologists, and the majority view was accepted as correct for each lesion. Of the 154 actinic keratoses diagnosed by the registrar, 135 were confirmed by the panel. The panel also appears to have added a further two lesions to this total, giving 137 actinic keratoses. Univariate analysis showed that subjects who had used sunscreen had a reduced risk for a prevalent actinic keratosis, but a multivariate analysis indicated that the inverse relationship was accounted for by the age of the subjects, as those who were older had a greater probability of having a prevalent actinic keratosis and were also less likely to have used sunscreens.

Intermediary end-points

DNA damage

The ability of sunscreens to prevent the formation of UVR-induced lesions in human skin DNA has been evaluated in a few studies. (Fig. 31)

Untanned gluteal skin sites from five healthy volunteers were treated with 2 mg/cm^2 of sunscreen (7.5% ethylhexyl methoxycinnamate and 4.5% benzophenone-3, SPF 15) or the vehicle and then exposed to solar-simulated UVR at doses up to 10 MEDs (Freeman *et al.*, 1988). Biopsy samples were obtained within 3 min of the end of the exposure, and DNA was extracted from epidermis. After cleavage by *Micrococcus luteus* UV endonuclease, which recognizes pyrimidine dimers in DNA, an alkaline agarose gel electrophoresis method was used to quantify the number of lesions. After exposure to an equivalent dose of UVR, the number of pyrimidine dimers was 32 per 10^7 bases in untreated skin and 0.8 per 10^7 bases in sunscreen-treated skin.

van Praag *et al.* (1993) evaluated the effect of a sunscreen (SPF 10) in biopsy samples obtained from the UVB-exposed, sunscreen- or vehicle-treated right buttock and from the UVB-exposed, untreated left buttock of 10 volunteers. Cyclobutyl thymine dimers were assayed in skin sections by immunofluorescence microscopy with a monoclonal antibody. A single dose of UVB resulted in significant dimer-specific nuclear fluorescence, which was abolished by pretreatment with sunscreen, indicating that the sunscreen offered good protection against UVB-induced DNA damage.

Seven male and seven female volunteers with sun-sensitive skin were irradiated with UVB (unfiltered Waldmann F 85/100 W-UV21 tubes) at a dose of 0.15, 0.15, 0.37, 0.92 or 2 kJ/m^2 over areas of the lower back measuring 2 x 3 cm. The last four areas were covered with 2 µl/cm^2 of a sunscreen of SPF 10 before irradiation (Bykov *et al.*, 1998b). Biopsy samples from each irradiated site and from one unirradiated area were taken less than 15 min after irradiation and rapidly frozen. DNA was extracted, and UVB-induced photoproducts were measured by a post-labelling HPLC technique. Cyclobutane dimers were formed in unprotected skin at a rate of about 2.5 photoproducts per 10^6 nucleotides and were about five times more abundant than 6–4 photoproducts. The sunscreen reduced the rate of adduct formation to about 1/20th of the value seen in unprotected skin. There was a large difference in individual response to UVB and no correlation between the photoproduct levels in unprotected and protected skin. Thus, the effective dose of solar irradiance to DNA may be highly individual, and protection against erythema by sunscreens is unrelated to protection against DNA damage.

Young *et al.* (2000) assessed the ability of two sunscreens with an identical SPF of 4 but with different spectral absorption profiles to inhibit photodamage in human epidermis *in situ* in eight volunteers with sun-sensitive skin (types I and II). One formulation contained ethylhexyl methoxycinnamate, a UVB absorber (λ_{max} = 308 nm), while the other contained terephthalylidene dicamphor sulfonic acid (λ_{max} = 345). The sunscreen-treated sites were exposed to 4 MEDs of solar-simulated radiation (1 MED = 20 kJ/m^2 full solar-simulated UVR spectrum), whereas control and

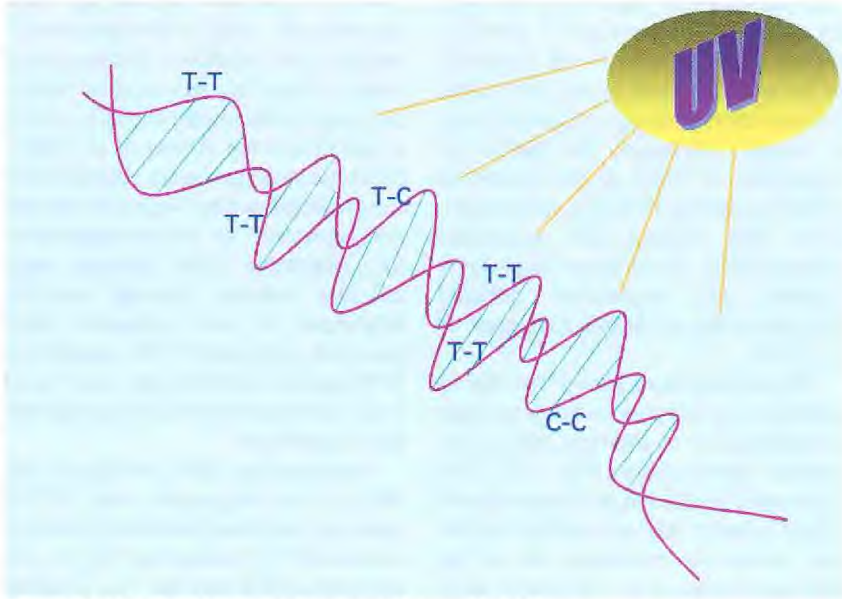

Figure 31 UV-induced cyclobutane pyrimidine dimers in DNA

vehicle-treated sites were exposed to 1 MED. Biopsies were performed immediately after UV irradiation, and the sections were analysed for thymidine dimers and 6–4 photoproducts by monoclonal antibody immunohistochemistry and image analysis. Four MEDs of solar-simulated radiation administered to skin protected by each sunscreen resulted in a comparable number of DNA lesions to that induced by 1 MED of solar-simulated radiation on unprotected skin. The authors concluded that the DNA protection factors of these sunscreens were similar to their SPFs and noted that the lack of difference between the sunscreens suggests that the action spectra for erythema and DNA photodamage are similar.

p53 *expression*

The protein TP53 plays an important role in the cellular response to DNA damage. After exposure to genotoxic agents such as ionizing radiation and UVR, wild-type TP53 accumulates and becomes immunohistochemically detectable. In human skin, UVR induces accumulation of TP53 in the epidermis. This response is rapid and transient: it is detectable as early as 2 h after irradiation, peaking at 24 h and persisting for several days (Hall *et al.*, 1993). After UV irradiation of usually unexposed skin of healthy volunteers, the pattern of expression of TP53 in the epidermis differed according to the UV wavelength: while UVA induced *p53* expression predominantly in the basal layer, UVB induced *p53* expression diffusely throughout the epidermis (Campbell *et al.*, 1993).

Two studies have shown that topical application of sunscreens decreases the overexpression of wild-type *p53* in epidermal keratinocytes (Fig. 32). The expression of *p53* and of its major downstream effector *p21* was studied in normal, previously unexposed skin of the buttocks (Pontén *et al.*, 1995) from eight volunteers of each sex aged 28–68 years, six with skin type II or III and two with type V skin. An area of 16 cm^2 on each buttock was irradiated with 2 MEDs of UVB of broad-band UVB–UVA (UVA SUN 3000) 15 min after application of 0.2 ml/cm^2 of a broad-spectrum SPF-15 sunscreen containing ethylhexy methoxycinnamate and benzophenone-3. A 3-mm punch skin biopsy sample was obtained from both treated and untreated areas before and 4, 24, 48 and 120 h after irradiation. The expression of *p53* was found to have been induced in epidermal cells 4 h after irradiation, peaked at 24–48 h and returned to nearly normal levels by 120 h. The expression of *p21* mirrored that of *p53* but disappeared at a slower rate. In addition, *p21* was induced in the papillary dermis and to a lesser extent in the reticular dermis, without concomitant expression of *p53*. In all subjects, the *p53* reaction was either absent or very weak in the sunscreen-treated areas. *p21* expression showed a pattern similar to that in unirradiated skin, indicating that it was not affected by UVR when a sunscreen had been applied.

The effect of topical application of a SPF-15 sunscreen containing benzophenone-3, butyl methoxydibenzoylmethane and ethylhexyl methoxycinnamate on chronically sun-exposed human skin was examined after exposure during a normal summer (Berne *et al.*, 1998). Skin biopsy samples were obtained from sun-protected and sun-exposed skin and were compared for immunohistochemically detectable TP53. Although large individual variation, possibly reflecting differences in sun exposure, were observed, a significant 33% reduction in TP53-positive keratinocytes was found in sun-protected skin as compared with sun-exposed skin.

Krekels *et al.* (1997) investigated the ability of two sunscreens (one SPF 10 containing ethylhexyl methoxycinnamate and one SPF 20 containing TiO_2) to protect against DNA damage. The products were applied at 2 mg/cm^2 to 25 volunteers with skin types I–III, who were then exposed for 1 or 1.5 h to natural sunshine. An increase in *p53* expression was found immunohistochemically in punch biopsy samples of unprotected skin from all subjects 24 h after exposure, although erythema was seen in only 19 of the 25 volunteers. Both sunscreens significantly reduced the fraction of cells containing TP53 in basal and suprabasal epidermal layers and the amount of TP53. The authors concluded that *p53* expression is a sensitive indicator of sun-induced dermal damage.

Krekels *et al.* (2000) investigated the DNA-protective qualities of three sunscreens with SPFs of 8, 30 and 40 applied at 2 mg/cm^2 to 12 volunteers with skin types I–III. An immunohistochemical study was conducted of the induction of expression of TP53 protein in skin biopsy samples taken 24 h after exposure to 1 MED (unprotected areas) or 3 MED (protected areas) of solar-simulated radiation (exact dose not specified). The high-SPF sunscreens provided protection against both erythema and TP53 induction, whereas the SPF-8 sunscreen protected against erythema but not against TP53 expression after exposure to UVR.

Seité *et al.* (2000a) used expression of *p53* as the end-point for evaluating the protection provided by two sunscreens with the same SPF of 7 but with different UVA protection factors: one contained 7% octocrylene and 3% butyl methoxydibenzoylmethane and had a UVA protection factor of 7, and the other contained 3.8% ethylhexyl methoxycinnamate and 7.5% ZnO in the same oil-in-water vehicle and had a UVA protection factor of 3. Human skin biopsy samples were exposed eight times to 5 MEDs of solar-simulated radiation. Both sunscreens gave only partial protection against *p53* overexpression, but significantly fewer TP53-positive cells were found in areas covered with the sunscreen with the higher UVA

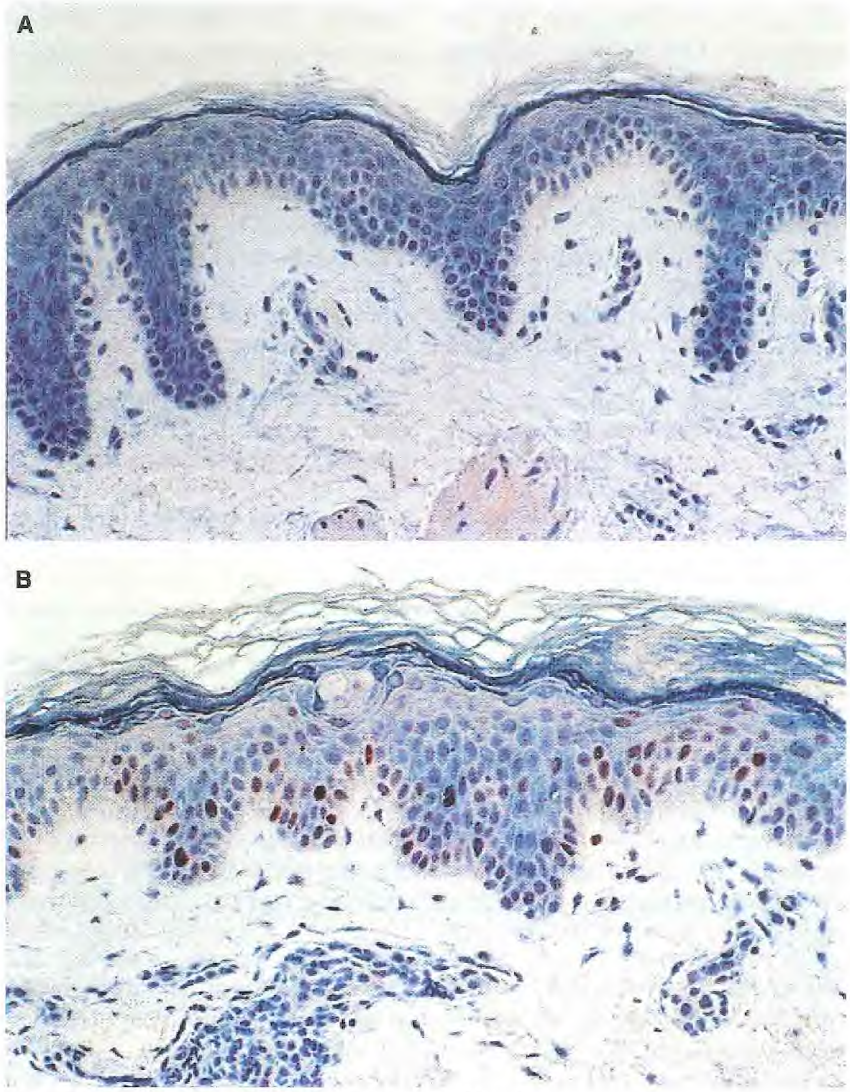

Figure 32 Accumulation of TP3 protein in human epidermis
A, Unexposed skin shows no immunostaining against *p53* protein; B, in sunscreen (low SPF)-protected skin, positive staining for TP53 is indicated by the dense, dark, nuclear colouration seen in both basal and suprabasal epidermal cells.

protection factor. The difference in protection by the two sunscreens was shown to be due to the difference in UVA absorption in a study in six volunteers in which *p53* overexpression was induced in their epidermis by eight exposures to 125 or 250 kJ/m^2 of UVA1 or UVA.

Sunburn cells

Sunburn cells are identified in conventionally stained epidermal biopsy samples as keratinocytes with a dense, pyknotic nucleus and a homogeneously eosinophilic cytoplasm. They represent keratinocytes that have sustained UVR-induced damage and are undergoing apoptosis (Fig. 33). As sunburn cells can be produced by sub-erythemal doses of UVR (Grove & Kaidbey, 1980), formation of sunburn cells in the epidermis is a quantifiable indicator of acute damage by UVR.

Sixteen healthy volunteers with skin types I–III were exposed to a 15-MED dose of UVR from a xenon arc solar simulator on areas of the middle of the back protected by application of 2 mg/cm^2 of a SPF-15 or SPF-30 sunscreen (Kaidbey, 1990). An unprotected area of normal skin received a dose of 1 MED and served as a control site. The SPF-30 sunscreen prevented the formation of sunburn cells in biopsy specimens more efficiently than the SPF-15 formulation, the decimal logarithm of the number of sunburn cells in 10 microscope fields being 1.1 ± 0.41 in the control area, 0.69 ± 0.41 in the area protected by the SPF-15 sunscreen and 0.16 ± 0.53 in that protected by the SPF-30 sunscreen ($p < 0.001$).

Immune suppression

UVR-induced immune suppression and its modulation by topical sunscreen application have been studied in humans, with emphasis on contact hypersensitivity, delayed-type hypersensitivity, density of Langerhans cells, release of immune modulatory molecules such as interleukin-10 or urocanic acid, natural killer cell activity and stimulation of allogeneic lymphocytes by epidermal cells.

Dinitrochlorobenzene (DNCB) is a potent contact sensitizer to which spontaneous sensitization is rarely encountered in human populations and which has been widely used for evaluating immune capacity in patients with a variety of diseases. Typically, sensitization to DNCB is induced by applying a small patch of filter paper containing 30–50 µg of the compound in acetone solution to skin in a Finn chamber and removing the patch after 48 h. The sensitization induced is tested

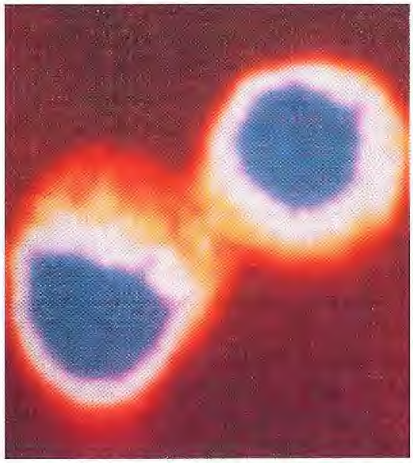

Figure 33 Apoptotic (blue) keratinocytes as a consequence of exposure to UVB

2 weeks after the first contact with DNCB by application of challenge patches containing concentrations of DNCB usually ranging from 3.125 to 12.5 µg. The challenge patches are removed after 48 h, and the contact hypersensitivity reactions are assessed 24 h later.

Whitmore and Morison (1995) used a commercially available SPF-29 sunscreen containing ethylhexyl methoxycinnamate, benzophenone-3 and ethylhexyl salicylate to prevent UVB-induced suppression of the induction of contact hypersensitivity to DNCB. Seventeen healthy volunteers aged 21–48 with skin type II or III were exposed to three MEDs of UVB from unfiltered fluorescent tubes on 3 consecutive days on one 16-cm^2 site on the buttock (1 MED = 1.5 kJ/m^2), with or without application of the sunscreen [amount not specified] before irradiation. One day after the last exposure, 30 µg of DNCB were applied to the irradiated site, and 2 weeks later a forearm was given a challenge dose of 3.125, 6.25, 8.8 or 12.5 µg of DNCB. A control group of nine volunteers aged 21–45 with skin type II or III underwent immunization and challenge with DNCB but were not exposed to UVB. Both the development of primary allergic dermatitis at the sensitization site and the reaction at the elicitation sites were scored clinically. The proportion of subjects who developed a primary allergic response to DNCB at the sensitization site was reduced from 5/9 to 0/10 in the UVB-treated group (p = 0.1, Fisher's exact test), while 7/7 subjects given sunscreen plus UVB developed a primary allergic response to DNCB (p = 0.00005). The group exposed to UVB had a reduced response rate at the challenge site to all challenge doses of DNCB, except for the highest dose (12.5 µg), when compared with a control group with no exposure to UVB ($p \leq 0.008$) and the group given sunscreen plus UVB ($p \leq 0.02$). The response rates to the DNCB challenge doses did not differ between the group given no UVB and that given sunscreen plus UVB.

Irritant and contact hypersensitivity responses to DNCB were studied in 160 male volunteers aged 18–60 (mean, 28 ± 9.3) with skin types II and III, who were randomly allocated to groups of 20 subjects each (Serré *et al.*, 1997). The men received an application of 2 mg/cm^2 of a broad-spectrum SPF-15 sunscreen containing 9% octocrylene, 3% butyl methoxydibenzoylmethane, 0.7% terephthalylidene dicamphor sulfonic acid and 0.3% phenylbenzimidazole sulfonic acid (UVA protection factor, 9) over a 4-cm^2 area on one buttock 20 min before a single exposure to 3 MEDs of solar-simulated radiation (1 MED = 210 ± 49 kJ/m^2). They were sensitized by application of DNCB on the irradiated skin 3 days later, and challenged 14 days later with DNCB at a dose of 3.125, 6.25, 8.8 or 12.5 µg. The reactions were read at 48 h and quantified as increases in skin thickness. Subjects in the seven control groups received either the sunscreen but no UVB, sunscreen with or without UVB but no DNCB sensitization, DNCB sensitization with or without prior UVB, UVB but no DNCB sensitization or no UVB and no DNCB sensitization. All groups were challenged with DNCB. In subjects sensitized with DNCB, the elicitation response was linear and dose-dependent. Exposure to UVB resulted in significantly decreased responses to all doses of DNCB (p = 0.009, 0.008 and 0.004 for the doses of 6.25, 8.8 and 12.5 µg, respectively), and the percentage of positive responses to DNCB dropped from 95% to 50% (p = 0.003). Neither sunscreen nor UVB influenced the irritative response to DNCB, and prior application of the sunscreen did not modify the percentage of positive responses to DNCB (90%). Pretreatment with the sunscreen maintained a high immunization rate (85%) among volunteers exposed to UVB and restored the contact hyper-sensitivity responses to the three higher challenge doses of DNCB. Hence, an erythemal exposure to UVR significantly impairs the afferent arm of the contact hypersensitivity reaction, and the application of a high-SPF sunscreen can prevent the UV-induced suppression of induction of contact hypersensitivity.

Hayag *et al.* (1997) confirmed that sunscreens *per se* do not interfere with contact hypersensitivity and that an SPF-30 sunscreen (7.5% ethylhexyl methoxy-cinnamate, 10% octocrylene, 5% menthyl anthranilate) applied before UVB irradiation partially prevents induction of suppression of contact hypersensitivity by DNCB. This study also showed that application of a high-SPF sunscreen before UVB irradiation prevents the decrease in density of epidermal Langerhans cells at the irradiated site that usually follows exposure to UVB.

Nickel is a frequent contact allergen in the general population: up to 15% of women and 5% of men develop allergic contact dermatitis when exposed to this metal. UVR suppresses the allergic response of these individuals to patch testing with nickel, and clinical improvement of nickel allergy occurs after whole-body irradiation. This model has been developed into a technique for

evaluating the immune protection afforded by sunscreens (Damian *et al.*, 1997). A group of 29 subjects, aged 19–58 years, with skin types I–V and confirmed allergy to nickel were irradiated on the mid-back with a sub-erythemal dose of UVB (975 ± 25 J/m^2) and UVA (12.3 kJ/m^2) daily for 5 consecutive days. A sunscreen or base lotion was applied at 2 mg/cm^2 15 min before irradiation and washed off after irradiation. Three sunscreens based on cinnamate with an identical UVB protection factor of 10 but differing in their UVA protection capacity were used. After the final irradiation, nickel patches were applied to each of four segments used to test the sunscreens or the base lotion; other patches were used to test unprotected irradiated skin, and control patches were placed on adjacent unirradiated skin. A placebo patch was also included in the test array. The patches were left in place for 48 h, and the cutaneous hypersensitivity response was assessed clinically and with an erythema meter 24 h later. When the reactions of unprotected irradiated skin to nickel were compared with those of unirradiated skin, there was, on average, immune suppression of 35% ($n = 16$; $p < 0.001$). Prior application of the cinnamate sunscreen reduced but did not prevent significant UVR-induced immune suppression (mean reduction, 18%; $p = 0.004$). In contrast, immune suppression was prevented by sunscreens containing either benzophenone or ZnO (reduction, 6.7% and 10%). None of the sunscreens or their base lotion had any effect in the absence of UVR. To determine whether UVR-induced suppression of the response to nickel is specific for cell-mediated immunity or reflects suppression of nonspecific inflammation, a further 16 subjects matched for age and skin type with the group allergic to nickel were patch-tested with a skin irritant, sodium lauryl sulfate, after application of a sunscreen and an irradiation protocol identical to that of the volunteers allergic to nickel. Neither UV irradiation nor sunscreens significantly affected sodium lauryl sulfate-induced erythema. The authors concluded that nickel patch testing is a valid means of assessing UVR-induced immune suppression in humans and its modulation by sunscreens. They also concluded that, even with sub-erythemal doses, immune protection was provided only by broad-spectrum sunscreens, suggesting that UVA plays an important role in immune suppression.

Using the same approach of nickel patch-testing on irradiated and unirradiated skin of volunteers with nickel allergy, the same group of authors proposed to define the 'minimal immune suppression dose' as that dose of UVR that reduces nickel contact hypersensitivity by 20% (Damian *et al.*, 1999). The authors concluded that the immune protection factor of a sunscreen can be evaluated by dividing the mean minimal immune suppression dose of sunscreen-treated skin by that of unprotected skin. The immune protection factor of a given sunscreen does not reflect its SPF (see p. 124).

The effects of two SPF-9 sunscreens with different absorption spectra on local and systemically induced immune suppression were evaluated with respect to the delayed-type hypersensitivity skin response after application at 2 mg/cm^2 (Moyal *et al.*, 1997; Moyal, 1998). The first sunscreen contained two UVB (9% octocrylene, 2% phenyl benzimidazole sulfonic acid) and two UVA filters (0.7% terephthalylidene dicamphor sulfonic acid, 2% butyl methoxydibenzoylmethane), thus covering the entire range of UVR, while the second contained only the same two UVB filters and covered essentially the UVB range. Volunteers were exposed to UVA plus UVB (total dose, 58 MEDs) or UVA only (3500 kJ/m^2 over 12 exposures). The delayed hypersensitivity response was measured 48 h after application of a Multitest antigen kit on an exposed and an unexposed area. Both local and systemic immune suppression was found in all UV-irradiated groups. The second sunscreen did not prevent local or systemic immune suppression induced by UVB plus UVA, while the first reduced local immune suppression and prevented the systemic effects.

The preventive effect of two sunscreens was also measured under conditions of real solar exposure (Moyal, 1998). The sunscreens had absorption spectra covering the entire UVR range but had different SPFs and UVA protection capacity (measured by persistent pigment darkening). The first sunscreen (SPF 15, UVA protection factor 6) did not provide immune protection, while the second (SPF 30, UVA protection factor 12) significantly prevented immune suppression. These studies demonstrate that immune protection can be obtained by use of sunscreens that cover the entire UVR spectrum and with high SPF and UVA protection factors.

Neale *et al.* (1997) showed that use of a sunscreen (8% ethylhexyl methoxycinnamate, 2.5% benzophenone-3, 1% butyl methoxydibenzoylmethane, SPF 15) reduced the density of Langerhans cells during current but not chronic solar exposure, with a trend to greater protection at higher levels of exposure.

Partial protection against Langerhans cell depletion was also demonstrated with sunscreens containing ethylhexyl methoxycinnamate (SPF 12) or ZnO (SPF 16) (Hochberg & Enk, 1999). In the same study, both sunscreens nearly totally inhibited UVB-induced interleukin (IL)-10 mRNA expression.

The *trans* to *cis* isomerization of urocanic acid in the epidermis is considered to play an important role in the mechanism of UVR-induced immune suppression. Krien and Moyal (1994) investigated the effects of applications of 2 mg/cm^2 of sunscreens on the UVR-induced *cis*-urocanic acid formation in groups of volunteers aged 19–48 who

were given single or multiple irradiations with either UVB (0–1.5 MEDs from a FS20 Westinghouse lamp with a peak emission at 313 nm) or UVA (100–300 kJ/m^2 from a Uvasun 5000 sunlamp, 335 nm, with a WG335 filter) or UVB plus UVA (1 MED on day 1, with a 25% increase each day until day 5 from a xenon arc lamp filtered with a WG305 filter). The sunscreens tested were two with SPFs of 3 and 4.5 and containing a UVB filter (3 and 5% ethylhexyl methoxycinnamate) and one with an SPF of 3 containing a broad-spectrum UVA filter (5% terephthalylidene dicamphor sulfonic acid). The rate of *cis*-urocanic acid formation was reduced by topical application of sunscreen; the reduction increased with increasing SPF, and broad-spectrum UVB plus UVA sunscreens were the most effective in reducing *cis*-urocanic acid formation in the stratum corneum.

Application of a broad-spectrum sunscreen lotion (8% ethylhexyl dimethyl PABA, 2% benzophenone-3, 2% butyl methoxydibenzoylmethane, SPF 15) did not protect against changes in natural killer (NK) cell activity induced by solarium lamps (Hersey *et al.*, 1987).

Another end-point for studying modulation of UVR-induced immune suppression is the mixed epidermal cell–lymphocyte reaction, in which epidermal cells are used to stimulate allogeneic lymphocytes *in vitro*, which is abrogated by UVR. van Praag *et al.* (1991) studied 32 patients with a variety of dermatoses (predominantly psoriasis) who were undergoing routine treatment with whole-body UVB irradiation (19 patients) or psoralen plus UVA therapy (13 patients). The interval between the last treatment and the start of the experiment was at least 1 year. The patients received treatment with UVR three times a week for 4 weeks, providing total doses of 72–392 kJ/m^2 of UVA and 5.8–34.6 kJ/m^2 of UVB. Immediately before each irradiation, one of two broad-spectrum sunscreens (SPF 6 and 15) or their vehicles were applied to the right forearm. The SPF-6 sunscreen contained butyl methoxybenzoylmethane, 4-methylbenzylidene camphor and phenylbenzimidazole sulfonic acid, whereas the SPF-15 sunscreen contained these three ingredients plus ethylhexyl dimethyl PABA. Ten healthy volunteers received local UVB irradiation of the forearm. Epidermal sheets were obtained from the forearms by the suction blister method 48 h after the last irradiation, and epidermal cells inactivated by 20 Gy were used to stimulate allogeneic peripheral blood cells from two volunteers. Neither the tested sunscreens nor their vehicles prevented the UVR-induced suppression of the alloactivating capacity of epidermal cells. [The authors made no attempt to characterize the populations of epidermal cells in these studies.]

In a further study by the same group (Hurks *et al.*, 1997), 40 healthy volunteers with skin phototypes II and III were exposed to 1–2 MED of UVB for 4 days. In this study, the mixed epidermal cell–lymphocyte reaction responses were significantly increased by UVR irradiation, and this enhancement was associated with an influx of CD36$^+$DR$^+$ macrophages into the irradiated skin. Application of the SPF-15 sunscreen used in the previous experiment, either directly onto the irradiated skin or onto a quartz slide to prevent penetration of the sunscreen into the stratum corneum, prevented the increased responses and the influx of CD36$^+$DR$^+$ cells.

[These conflicting results from the same group of investigators show that the ability of sunscreens to interfere with UVR-induced modulation of cell-mediated immune responses depends critically on the irradiation protocol and on the end-points measured, which may involve different mechanisms of UVR-induced immune modulation.]

Experimental systems

Since a wide variety of artificial sources of UVR and methods were used in the reported studies in experimental animals, criteria were drawn up to define studies that are relevant for examining protection against solar-simulated UVR (Fig. 34):

- The source of radiation should not include wavelengths outside the solar spectrum.
- The UVR dosimetry should be adequate.
- The amount of sunscreen applied should be quantified.
- Adequate control treatments should have been included.
- The experimental protocol should be consistent.

Studies in which sources of UVR were used which contain bands outside those that reach the earth's surface (e.g. UVC) are useful for proof of principle and for determining mechanisms of action, and the Working Group decided to summarize them in the text but not in the tables.

In most of the experimental studies, a single, arbitrary regimen of exposure to UVR was used to induce a biological response, such as skin cancer or immune suppression, and sunscreen was applied to investigate a possible protective effect. Although protection was observed in most studies, others showed 'no protection' (e.g. against immune suppression) and yet others showed 'total' or 'complete' protection (e.g. against carcinogenesis). Such absolute statements usually reflect limitations of study design rather than an

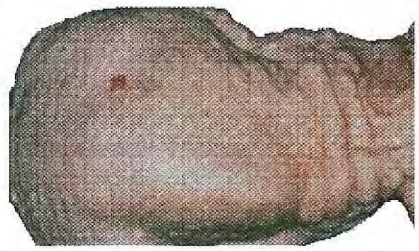

Figure 34 Solar elastosis induced in a hairless mouse after repeated exposure to solar-simulated UVR

adequate evaluation of protection against a UVR-induced biological effect. For instance, 'total protection' against photocarcinogenesis is commonly seen in a small group of animals over a limited period of observation and is often proved incorrect in an adequately expanded study. The main problem with most studies is that a dose–effect relationship is not determined, which would allow determination of any reduction in the effective exposure to UVR achieved by filtering through the sunscreen. Therefore, these studies do not allow quantification of a protection factor for photocarcinogenesis or another endpoint.

Cancer and preneoplastic lesions

Studies of the potential of sunscreens to protect against photocarcinogenesis in experimental animals, mainly hairless mice, (Fig. 35) have evolved in complexity, both with the creation of more sophisticated UVR absorbers and with the evidence for a contributory role of UVA in photocarcinogenesis. A reduction in photocarcinogenesis in skin exposed to UVR through a topical sunscreen has not been difficult to demonstrate. In most studies, mice were irradiated daily with UVR at doses lower than, or approximately equal to, 1 MED through a sunscreen that provided several-fold protection from erythema. The large increases in the SPF values of commercial sunscreens has increased the difference between the effective dose of UVR received by unprotected control mice and the sunscreen-treated groups in experimental testing for protection against photocarcinogenesis. Humans are increasingly likely to expose themselves to multiples of their MED on sunscreen-protected skin, with an unknown and untested concomitant effect on photocarcinogenesis.

In addition, as new active sunscreen ingredients have been developed, with absorption spectra spanning both the UVB and UVA wavebands, it has become more difficult to obtain comparative data on protection against carcinogenesis. It is all the more important that solar simulation, rather than unfiltered UVB radiation sources, be used, firstly to remove the environmentally irrelevant but carcinogenic UVC waveband, which may be relatively effectively absorbed by many UVB-absorbing sunscreen ingredients and thus confound measurements of the relevant protectivity, and secondly to account for variably efficient UVA absorbance by the sunscreen and the influence of the transmitted UVA wavelengths.

Of the active sunscreen ingredients that have been tested for anti-photocarcinogenic properties in animals, the simplest is the UVB absorber PABA; esterified derivatives of PABA (glyceryl and ethylhexyl dimethyl PABA) were developed later in response to the photosensitization reported by human PABA users (see p. 133 and Funk *et al.*, 1997). Another compound, ethylhexyl methoxycinnamate, remains one of the most popular UVB absorbers, and a number of other chemicals offer a broader absorption spectrum, covering a portion of the UVA waveband. Many contemporary sunscreens contain TiO_2 and/or ZnO, which are purported both to absorb and scatter UVR broadly. Experimental photocarcinogenesis has been induced by chronic exposure to UVR applied either as a constant daily dose or as periodically incremented daily doses, which can be done without burning the unprotected control animals because of the adaptive responses of the skin (i.e. epidermal hyperplasia and keratinization). Incremental exposures result in much larger cumulative doses of UVR, but whether the adaptive responses of sunscreen-irradiated skin are activated to the same degree as those of unprotected skin and whether they are involved in protection from photocarcinogenesis has not been examined.

Studies with radiation sources including UVC

In a landmark study, at a time when the sunburn spectrum was known to span 280–310 nm but the photocarcinogenesis action spectrum was still unknown, Knox *et al.* (1960) reported the first evidence for protection from UVR-induced carcinogenesis in experimental mice. The ears of Swiss albino mice were irradiated with a mercury arc lamp after application of 10% benzophenone-4 for five months. The sunscreen protected against tumour development, but the study was marred by the reported burning and necrosis of the unprotected ears and is not useful.

In later studies, more realistic UVR sources were used. Flindt-Hansen *et al.* (1990a) examined the effect of a 5%

Figure 35 Hairless mouse with multiple skin tumours

PABA solution in a vehicle of 70% ethanol, 5% glycerol and water applied to the dorsal skin immediately before irradiation in groups of 30 female hr/hr hairless mice aged 8–12 weeks. The animals were exposed on 5 days per week for 30 weeks to an incremental regime of unfiltered UVB radiation from a Philips TL40 W/12 lamp. The initial sub-erythemal exposure (1.55 kJ/m^2) was increased up to 8 weeks by a maximum factor of 2.3, and the cumulative dose at 30 weeks was 490 kJ/m^2. This resulted in a mean time to tumour onset of about 22 weeks for unprotected mice, and all had acquired tumours ≥ 1 mm in diameter by 30 weeks, with an average tumour yield of 4.5. In contrast, only 12% of the PABA-protected mice had tumours by 40 weeks, with an average tumour yield of 0.16. The yield of histologically identified squamous-cell carcinomas was reduced from 2.7 to 0.04. The vehicle alone did not affect tumour induction. The dorsal skin of the mice was excised and weighed at the end of the study to verify that PABA had reduced the total tumour weight per mouse; however, no difference was seen for PABA-irradiated mice and unirradiated controls. [The significantly increased skin weight in unprotected mice may have been due partly to chronic hyperplasia induced by UVB.] The overall rate of survival was approximately 93%, so that the mice did not die of their tumours during the study.

This model was also used to examine the effect of intermittent sunscreen application in UVB-irradiated female hairless mice (Flindt-Hansen *et al.*, 1990b). The same 5% PABA sunscreen was applied during weeks 16–26, accounting for one-third of the 30-week application of UVB. The sunscreen significantly reduced both the incidence of tumour-bearing mice at 40 weeks, from 100% of the unprotected mice to 67%, and the average tumour yield from 4.3 to 1.8. The treatment also significantly reduced the average yield of squamous-cell carcinomas from 2.7 to 0.8 per mouse, and the proportion of the total tumour load that had progressed to squamous-cell carcinoma.

Using the same protocol on groups of 30 mice, Flindt-Hansen *et al.* (1989) examined the effect of PABA in which 40% photodegradation had been induced *in vitro* by exposure in 70% ethanol and 5% glycerol in water to 270 kJ/m^2 UVB from an unfiltered Philips TL40 W/12 light source. Although photoproducts were identified by both mass and UV spectroscopy, the photolysis induced only insignificant alterations in the absorption spectrum of the sample. The photodegraded PABA sunscreen did not induce tumours when applied alone for 30 weeks without UVB irradiation and was as effective as intact PABA in protecting against UVB-induced carcinogenesis (time to first tumour, number of tumours, number of squamous-cell carcinomas, weight of dorsal skin) when used as a daily sunscreen during 30 weeks of irradiation.

Kligman *et al.* (1980) performed the first comparative study of the tumour-protective activity of two sunscreens containing ethylhexyl PABA, at a concentration of 2% alone or at a concentration of 7% with 3% benzophenone-3, which provided SPFs of 2 and 15, respectively, as determined by standard methods on human skin. Groups of 20 albino (Skh-I) and 20 pigmented (Skh-II) hairless mice [sex and age not specified] were irradiated after application of either sunscreen, with a constant daily exposure of 1.85 kJ/m^2, from a bank of unfiltered UVB tubes (nine Westinghouse FS420 lamps) three times per week for 30 weeks. [The volume of sunscreen applied and the composition of the vehicle were not specified.] The unprotected mice, which received no topical treatment with the vehicle, developed large numbers of tumours, which caused the deaths of 75% of the Skh-I mice and 33% of the Skh-II mice at 40 weeks. These mortality rates obviated detailed analysis of the tumour load. The pigmented Skh-II mice were markedly more resistant to irradiation than the albino Skh-I mice, developed a tan, and showed an extended latent period for appearance of the first tumour (21 weeks compared with 19 weeks for Skh-I); 67% survived to week 40 compared with only 25% of Skh-I mice. Markedly fewer Skh-II mice produced tumours with a diameter > 4 mm (3/23 mice compared with 6/10 Skh-I mice), although the average tumour yield at 40 weeks was approximately 13 tumours per mouse for both strains. At 30 weeks, when the survival rate was still high (83% overall), all of the unprotected irradiated mice had tumours, whereas the SPF-2 sunscreen reduced the tumour incidence to 22% (50% at 40 weeks) and the tumour yield to less than 2.0 per Skh-I mouse and prevented the appearance of tumours in Skh-II mice. The Skh-II mice developed only slight hyperpigmentation. The SPF-15 sunscreen was more effective, and prevented both tumour appearance and hyperpigmentation within the time of the study.

As part of a larger study in which 5% of an iron chelator (2-furildioxime) was incorporated into the sunscreen, Bissett and McBride (1996) irradiated groups of five Skh:HR-1 hairless mice [sex and age not specified] 2 h after application of 0.1 mL of 5% ethylhexyl PABA in ethanol and propylene glycol. [This interval is somewhat lengthy.] The protection factor of the sunscreen was determined to be 7.2 in shaved guinea-pig skin exposed to a solar simulator, according to a standard method, 15–20 min after topical application. The mice received a daily dose of 0.3 kJ/m^2 (stated to be approximately 0.5 MED in mice) of unfiltered UVB three times per week for over 40 weeks from four Westinghouse FS40 lamps. The sunscreen increased the average time to tumour onset from 19 in mice given the vehicle plus UVR to 31

weeks, and reduced the average tumour yield [from approximately 13 to approximately 5.0 tumours per mouse]. The tumours were described as papillomas and squamous-cell carcinomas, but the numbers of each were not given. This larger study demonstrated synergistic protection from UVB-induced photocarcinogenesis by 2-furildioxime in combination with ethylhexyl PABA sunscreen, extending the average time to tumour onset to 77 weeks.

Recognition that UVA would probably have to be included in the radiation spectrum tested resulted in use of sources consisting of combinations of UVB and UVA fluorescent lamps. Wulf *et al.* (1982) exposed groups of 25 female *Hr*/*Hr* pigmented hairless mice, 10 weeks of age, to one UVB (Westinghouse FS40) and two black light lamps (Philips TL40 W/09) immediately after application of 0.1 mL per animal of one of two commercial sunscreen lotions labelled as SPF 5 and SPF 6. The animals were exposed to an incremental regime of UVR, with UVB and UVA intensities of 0.3 and 0.8 mW/cm^2, respectively, starting from 1 MED [1.8 kJ/m^2 UVB adjusted stepwise to 7.2 kJ/m^2] after 3.5 months and then remaining constant. The treatments were repeated on 4 days per week for 12 months [for a cumulative dose of 1250 kJ/m2 for UVB and 3200 kJ/m^2 for UVA]. The SPF-5 sunscreen contained glyceryl PABA and the SPF-6 contained ethylhexyl methoxycinnamate plus benzophenone-3, and therefore had greater UVA absorbance. No vehicle was used for control treatments. Observation continued until 18 months, by which time all of the unprotected UVR-exposed mice had died of skin tumours. Both sunscreens significantly delayed the average time to onset of tumours, from 205 days to 254 days for the SPF-5 product and to 284 days for the SPF-6 product, and the time of death from 86 days to 94 days for the SPF-5 product and to 128 days for the SPF-6 product. The latter therefore provided significantly greater protection against tumour growth than the SPF-5 product, although no difference was seen between the two sunscreens in the time to death after the appearance of progressive tumours. Some toxic effects on the skin were found in mice treated with the SPF-6 product (see p. 135).

Using similar UVR sources, Gallagher *et al.* (1984) irradiated groups of 10 female HRA/Skh-1 mice after application of two samples of ethylhexyl methoxycinnamate, one of which had previously been found to induce reverse mutation in bacteria [The carcinogenicity of ethylhexyl methoxycinnamate is discussed on p. 130]. The two unfiltered UVR sources were one comprising one UVB and one UVA lamp and another comprising one UVB and three UVA lamps. Irradiation was delivered in a stepwise incremental exposure regime starting with either a 0.33 MED ('sub-erythemal') or 1 MED ('erythemal') dose daily [reaching exposures equivalent to 0.9 and 2.8 MED, respectively, in the last week]. The cumulative dose of UVB at the sub-erythemal exposure was about 18 kJ/m^2 from both sources, and that of UVA was 56 kJ/m^2 with the first source and 127 kJ/m^2 with the second. The erythemal exposure was three times greater. Groups of 20–22 mice (8–12 weeks old for the first source and 20–28 weeks old for the second) were irradiated on 5 days per week for 10 weeks 30 min after topical application to the dorsum of 0.1 mL of 50% ethylhexyl methoxycinnamate in ethanol, and then observed until day 200. The control mice received no topical application. The normal mid-dorsal skinfold thickness was not affected by the age of the mice. The sub-erythemal dose from the first source unexpectedly produced tumours slightly faster than the erythemal doses [with average times to tumour onset of 107 days and 116 days, respectively], but the first sub-erythemal regime did not induce visible or histologically evident erythema at any time. The absence of epidermal hyperplasia was suggested to permit a greater effective dose of UVB to penetrate the skin. The erythemal dose from the second source resulted in faster tumour production than the sub-erythemal dose, which induced distinct epidermal hyperplasia histologically [with average times to tumour onset of 116 days and > 200 days, respectively]; only 40% of mice exposed to the sub-erythemal dose had tumours by day 200. More severe epidermal hyperplasia was seen histologically after the erythemal than after the sub-erythemal doses with both sources. The 50% preparation of ethylhexyl methoxycinnamate strongly protected against tumours induced by either source, and only 4/146 surviving mice acquired a persistent skin tumour after erythemal exposure. The rate of survival after UV irradiation alone was not given, but 91% of the mice exposed to ethylhexyl methoxycinnamate and UVR survived to day 200. At this time, in order to determine whether ethylhexyl methoxycinnamate had initiated any latent tumours, both irradiated and unirradiated ethylhexyl methoxycinnamate-treated mice were subjected to eight dorsal applications of 0.1 mL of 0.05% croton oil in acetone twice a week and were observed until day 300. Croton oil had no effect in 40 previously untreated control mice, significantly induced tumours on 3/16 mice that had previously received ethylhexyl methoxycinnamate alone and revealed latent tumours in 15–46% of the mice previously exposed to UVR after application of ethylhexyl methoxycinnamate.

Snyder and May (1975) gave groups of 5 or 10 hairless mice (Jackson), nine weeks of age [sex not stated], a single topical application of 0.5% 7,12-dimethylbenz[*a*]anthracene (DMBA) in acetone or acetone only [volume not stated] with a paintbrush as a thin coat, followed 4 weeks later by irradiation for 15 min on 3 days per week for 29 weeks with FS40 lamps [unknown number] emitting 3.0 × 10^3 W/cm^2. The cumulative dose was 240 kJ/cm^2. [If the daily exposures were

consistent, the mice received 2.8 kJ/m^2 per day.] This protocol was developed because DMBA treatment is known to accelerate the development of squamous-cell carcinomas after exposure to UVR. Some groups of mice were treated with a sunscreen containing 5% PABA [SPF and volume not stated] or with the sunscreen base only, which contained 55% ethanol and emollients, 60 min before irradiation. The mice were observed for a further 10 weeks. The mean time to tumour onset was 35 weeks after application of DMBA plus base and 37 weeks after UVR plus base, but the tumours appeared earlier (19 weeks) after DMBA plus base plus UVR. This study demonstrated a clear co-carcinogenic synergism between DMBA and subsequent UVR, and protection against the apparent promotion of DMBA-initiated tumours by irradiation through the PABA sunscreen; however, the poor survival rate precludes reasonable quantification of the protective effect of the sunscreen.

Studies with solar-simulated UVR

The studies described below are summarized in Table 22.

An extensive study (Forbes *et al.*, 1989) of the protective effect of ethylhexyl methoxycinnamate was conducted with groups of 12 male and 12 female Skh:Hr-1 mice, 8–10 weeks of age. A mutagenic sample of ethylhexyl methoxycinnamate (see p. 137) was also included. Ethylhexyl methoxy-cinnamate was applied in 0.1 mL acetone and ethanol (1:1) at a concentration of 7.5, 50, 75 or 500 mg/mL [0.75%, 5%, 7.5% and 50%] to the rump and saddle region, and a constant dose of 600 Robertson Berger units (equivalent to an exposure of approximately 1200 sub-erythemal doses per week) of solar-simulated UVR from a filtered xenon arc source was administered 30 min after the topical applications on 3 days per week for 8 weeks. The UVR was produced horizontally, and the target area of the mice was the skin of the hind flank rather than the mid-dorsum. The control group received the vehicle only. A regime of tumour promotion was begun 2 weeks after exposure to UVR, comprising application of 12-*O*-tetradecanoylphorbol 13-acetate (TPA) in acetone at a dose of 2 μg/mL to the dorsum three times weekly for 20 weeks. Control mice exposed to solar-simulated UVR and the sunscreen vehicle received acetone only, and one irradiated group that received ethylhexyl methoxycinnamate at a dose of 75 mg/ml was also treated with acetone only. The animals were observed for tumours until 57 weeks, at which time the survival rate was > 75% in all groups. In unprotected mice, tumours 1 mm or more in diameter began to appear from 12 weeks, and the prevalence reached 100% at 42 weeks. Treatment with TPA slightly but significantly reduced the mean time to tumour onset, from 27 to 24 weeks. Ethylhexyl methoxycinnamate delayed the appearance of tumours in a dose-dependent manner, so that the four doses resulted in progressive reductions in the maximum prevalence of 86%, 45%, 14% and 8.3%, respectively. The protective effect of the mutagenic sample of ethylhexyl methoxycinnamate was also dose-dependent, but to a lesser extent, as 50% of this sample was as effective as 7.5% of the first ethylhexyl methoxycinnamate sample. The reason for this difference was not apparent. [The SPF values of the two samples were not measured; a difference in the SPF might have accounted for the difference in the effective dose.]

In another study (Reeve *et al.*, 1985), fluorescent tubes (one UVB tube, six UVA tubes, cellulose acetate-filtered) were used as the source of solar-simulated UVR. In groups of 24–28 female hairless HRA/Skh-1 mice that received 0.1 mL of 50% ethylhexyl methoxycinnamate and 35% ethylhexyl PABA in ethanol on the dorsum 30 min before irradiation, both products protected against the effects of radiation up to 200 days after a 10-week regime of irradiation with stepwise increments of 20% per week on 5 days per week, beginning with 1 MED. The final daily dose after adaptation was 2.8 times the initial MED. The cumulative doses were stated to be 15.4 J of UVB and 294 J of UVA [which can be calculated to be 30.8 kJ/m^2 of UVB and 592.8 kJ/m^2 of UVA]. When the mice were given promotion treatment with croton oil (8 × 0.1 mL of 0.1% croton oil in acetone over 4 weeks) from day 200, latent tumours were revealed, with a 16.5% incidence in irradiated mice given ethylhexyl PABA and 39% in those given ethylhexyl methoxycinnamate by day 300. Control groups did not receive topical applications of the vehicle. Thus, even in the absence of overt tumour growth on sunscreen-protected skin, tumours were initiated and were sensitive to exogenous promotion. The difference in induction of these tumours was accounted for by the unequal photoprotection afforded by the two UVB absorbers at the tested concentrations.

In the two studies described below, sunscreens supplemented with 5-methoxypsoralen were investigated, but only the effects of the sunscreens alone are discussed. The effects of 5-methoxypsoralen are discussed on p. 131. Groups of 20 male and 20 female hairless albino mice (outbred St John's strain [age not specified]) maintained under ambient lighting free from UVR were used to test the capacity of a sunscreen containing two UVB-absorbers, 12.5 μL/mL [1.25%] ethylhexyl methoxycinnamate and 10 mg/mL [1%] 3-benzylidene camphor, in a vehicle of peanut oil, isopropylmyristate and the antioxidants butylated hydroxytoluene and butylated hydroxyanisole, to protect against photocarcinogenesis. [The SPF of the sunscreen was not given.] (Young *et al.*, 1987). Approximately 150 μL of the product or the vehicle were applied to the dorsum of mice 30–60 min before exposure to 17 kJ/m^2 of solar-simulated

Table 22. Effects of sunscreens on carcinogenesis induced by solar-simulated UVR in experimental animals

Strain of mouse	UV absorber	Radiation source	Cumulative dose	Average tumour incidence or multiplicity	Reference
Skh-I hairless	0.75–50% ethylhexyl methoxycinnamate, non- and mutagenic	Solar-simulated UVR, 3 times per week for 8 weeks, TPA x 60 at 10 weeks	14 400 Robertson Berger units	Tumour incidence: 8.3% with 50% sunscreen; 86.4% with 0.75% sunscreen; 100% in controls	Forbes *et al.* (1989)
Skh-I hairless	50% ethylhexyl methoxycinnamate; 35% ethylhexyl PABA	Solar-simulated UVR, 5 times per week for 10 weeks, croton oil 8 times at 200 days	30.8 kJ/m^2 UVB	Tumour incidence: 39% with ethylhexyl methoxycinnamate; 16.5% with ethylhexyl PABA; 100% in controls	Reeve *et al.* (1985)
Hairless St John's strain	1.25% ethylhexyl methoxycinnamate + 1% 3-benzylidene camphor	Solar-simulated UVR, 5 times per week for 45 weeks	90 kJ/m^2 UVB	Tumour multiplicity: 1.09 with sunscreen; 6.0 in controls	Young *et al.* (1987)
Skh-I hairless	0.5% ethylhexyl methoxycinnamate + 0.5% butyl methoxydibenzoylmethane	Solar-simulated UVR, 5 times per week for 73 weeks	146 kJ/m^2 UVB	Tumour incidence: 37% with sunscreen; 67% in controls	Young *et al.* (1990)
Skh-I hairless	5% terephthalylidene dicamphor sulfonic acid, sunburn protection factor, 4; 10% terephthalylidene dicamphor sulfonic acid, sunburn protection factor, 6; 5% ethylhexyl methoxycinnamate, sunburn protection factor, 4	Solar-simulated UVR, 5 times per week for 40 weeks	384 kJ/m^2 UVB	Average tumour latency: 22 weeks with ethylhexyl methoxycinnamate; 26 weeks with 5 or 10% terephthalylidene dicamphor sulfonic acid; 20 weeks in controls	Fourtanier (1996)
Skh-I hairless	9.5% ethylhexyl methoxycinnamate, sunburn protection factor, 6; 7.0% ethylhexyl PABA, sunburn protection factor, 6	Solar-simulated UVR, 5 times per week for 12 weeks	360 MED solar-simulated UVR (sunscreen) or 60 MED solar-simulated UVR (vehicle)	Tumour multiplicity; 1.7 with ethylhexyl PABA; 8.0 with ethylhexyl methoxycinnamate; 17.5 in controls	Domanski *et al.* (1999)
C3H	10% octocrylene + 2% phenylbenzimidazole sulfonic acid, SPF 16; 8% ethylhexyl methoxycinnamate + 2% phenylbenzimidazole sulfonic acid, SPF 15; 9% octocrylene + 0.3% phenylbenzimidazole sulfonic acid + 0.7% terephthalylidene dicamphor sulfonic acid + 3% 3-benzylidene camphor, SPF 15; 10% octocrylene + 0.2% phenylbenzimidazole sulfonic acid + 3.25% terephthalylidene dicamphor sulfonic acid + 1.5% 3-benzylidene camphor, SPF 22	Solar-simulated UVR, 5 times per week for 70 weeks	1589 kJ/m^2 UVB	Tumour incidence: 15% with sunscreens; 100% in controls	Anantha-swamy *et al.* (1999)

Table 22 (contd)

Cumulative	Average tumour incidence or	Reference	Cumulative dose	Average tumour incidence multiplicity	Reference
Skh-I hairless	5% ethylhexyl PABA; 10.8% ethylhexyl methoxycinnamate	DMBA + solar-simulated UVR 5 times per week for 6 weeks	72.9 kJ/m^2 UVB	Tumour incidence: 81.3% with ethylhexyl PABA; 30.8% with ethylhexyl methoxycinnamate; 85.7% in controls	Reeve *et al.* (1990)
C3H haired (shaved)	8% ethylhexyl methoxycinnamate, sunburn protection factor, 4; 7.2% TiO_2, sunburn protection factor, 7	DMBA + solar-simulated UVR 5 times per week for 32 weeks	571 kJ/m^2 UVB	Tumour incidence: 0% with ethylhexyl methoxycinnamate or TiO_2; 87% in controls	Bestak & Halliday (1996)

SPF, sun protection factor (in humans); DMBA, dimethylbenz[*a*]anthracene

UVR (290–400 nm), supplying a daily UVB dose of about 0.4 kJ/m^2. The treatments were continued on 5 days per week for 44–46 weeks, when the rate of survival was approximately 70%, and tumour growth was monitored. Half of the mice were retained for further observation until week 60. All the final tumours were classified histologically, and the data were analysed statistically. Some ventral tumours occurred, a common characteristic in this mouse strain, and were considered to be unrelated to the treatments. Male mice had slightly more tumours by week 25 than female mice, but no difference was evident after this time, and there was no difference in the tumour incidence between the sexes. The sunscreen alone significantly decreased the tumour incidence and reduced the tumour multiplicity progressively, from 6.0 to 1.09 tumours/mouse at 60 weeks. The progressive incidences of malignant tumours (but not benign tumours) were also reduced by the sunscreen, from 12 (no sunscreen) to 3.

In a second study, groups of 30 female Skh-1 hairless albino mice, 8–10 weeks of age, were treated with 100 μL of sunscreen consisting of the UVB absorber ethylhexyl methoxycinnamate at 0.5% and the UVA absorber butyl methoxydibenzoylmethane at 0.5% [SPF not determined] in a vehicle of 20% ethanol in water. The mice were exposed for 20–40 min after the topical applications to 17 kJ/m^2 of solar-simulated UVR from a vertically mounted xenon arc source on 5 days per week for up to 73 weeks, the duration of treatment being determined by the severity of the tumour response. Tumours were monitored up to the end of the study and were then classified histologically. The rate of survival was greater than 80%. The sunscreen alone was significantly protective against photocarcinogenesis, reducing the diameter of tumours, the number of tumours per mouse and the proportion of mice with histologically identified malignancies from 67% to 37% (Young *et al.*, 1990).

In a comparison of the ability of a newly developed broad-spectrum UVA absorber, terephthalylidene dicamphor sulfonic acid, and the predominantly UVB-absorbing ethylhexyl methoxycinnamate (not mutagenic in Ames' test) to protect from photocarcinogenesis, groups of 28 female Skh-1 hairless albino mice were treated with 5% (approximate sunburn protection factor, 4) or 10% terephthalylidene dicamphor sulfonic acid (approximate sunburn protection factor, 6) or 5% ethylhexyl methoxycinnamate (approximate sunburn protection factor, 4) in 0.1 mL of a mineral oil–glycerine–water emulsified vehicle applied to the dorsal skin (approximately 40 cm^2) either 30 min before irradiation with solar-simulated UVR from a filtered xenon arc source on 3 days per week or 30 min after irradiation on 2 alternate days per week. This complex experimental design was used to identify both anti-photocarcinogenic activity and enhancement of photocarcinogenesis by the test compound according to an established protocol. However, because the mice received two or five weekly exposures to UVR without the sunscreen on the skin, the possibility of demonstrating the efficacy of the product was reduced. The weekly dose of UVB was 9.6 kJ/m^2; three of the exposures were through topical sunscreen to 1.6 kJ/m^2 and two were through unprotected skin to 2.4 kJ/m^2. As the MED was determined to be 3.6 kJ/m^2, the daily doses were sub-erythemal. Treatment was continued for 40 weeks, and the animals were observed for a further 10 weeks. The rate of survival was > 90% at 28 weeks, at which time mice with an unacceptably large tumour load were removed. Data shown only graphically in the publication indicate that tumours

began to appear in unprotected mice at 16 weeks, and 100% prevalence was seen at 25 weeks. In mice treated with 5% ethylhexyl methoxycinnamate, 50% prevalence was delayed from 20 weeks in the unprotected mice to 22 weeks, but in those treated with 5% or 10% terephthalylidene dicamphor sulfonic acid, 50% prevalence was delayed until 26 weeks. The difference between the groups was reported to be significant by a log-rank test. Analysis of the average cumulative tumour yields showed no significant protection by 5% ethylhexyl methoxycinnamate but significant protection by 5% and 10% terephthalylidene dicamphor sulfonic acid, with no difference between these two concentrations. The equal sunburn protection factor values (of 4) of 5% ethylhexyl methoxycinnamate and 5% terephthalylidene dicamphor sulfonic acid do not support the observed difference in photocarcinogenic protection (Fourtanier, 1996).

Protection from photocarcinogenesis was studied in groups [size not specified] of inbred female Skh:HR-1 hairless mice, 8–12 weeks old, irradiated with a fluorescent solar-simulated UVR source on 5 days per week for 10 or 12 weeks through 0.2 mL of sunscreen lotions (sunburn protection factor, 6, determined in mice) containing either 9.5% ethylhexyl methoxycinnamate or 7.0% ethylhexyl PABA, spread over the entire dorsal skin 15 min before exposure. The daily dose of solar-simulated UVR was 6 MED [units not given], and control mice received 1 MED of solar-simulated UVR through 0.2 mL of the base lotion after determination of the MED by the increase in mid-dorsal skinfold after 24 h under the experimental conditions. Tumours were monitored for 35 weeks; at 200 days [approximately 30 weeks], the average tumour multiplicity was 17.5 in irradiated mice given base lotion, 8.0 in those given ethylhexyl methoxycinnamate and 1.7 in those given ethylhexyl PABA. The data were not analysed statistically, but it was clear that both sunscreens provided strong protection from photocarcinogenesis when compared with the base lotion and that ethylhexyl PABA offered greater protection than ethylhexyl methoxycinnamate (Domanski *et al.*, 1999).

In a study to assess the relationship between protection against solar-simulated UVR-induced *p53* mutations and protection against skin cancer development, shaved female C3H/HeNCr (MTV^-) mice were treated with complete sunscreen formulations of SPF 15 or 22. Two of these sunscreens contained UVB absorbers only (octocrylene or ethylhexyl methoxycinnamate plus phenylbenzimidazole sulfonic acid), and the other two contained UVA and UVB absorbers (octocrylene plus phenylbenzimidazole sulfonic acid plus terephthalylidene dicamphor sulfonic acid plus 3-benzylidene camphor). All were applied in the same vehicle. Groups of 16 mice, 8–12 weeks of age, were irradiated five times per week with a solar simulator (xenon arc) providing 4.54 kJ/m^2 UVB and 30.2 kJ/m^2 UVA, for 70 weeks. The sunscreens were applied 30 min before exposure at 100 μL/mouse, or approximately 2 mg/cm^2. All mice exposed to UVR only or vehicle plus UVR developed one or more skin tumours after 48 weeks of exposure, whereas only one mouse treated with sunscreen developed a skin tumour at this time. Although additional skin tumours developed in sunscreen-treated mice upon continued irradiation, the frequency was low, only nine of the surviving 60 mice treated with sunscreen having developed skin tumours at week 70, after a total exposure of approximately 1500 kJ/m^2 UVB (Ananthaswamy *et al.*, 1999). Intermediary biomarkers found in this study are described on p. 102.

Co-carcinogenicity with DMBA: The co-carcinogenicity of DMBA and solar-simulated UVR was studied in groups of 15–16 female Skh:HR-1 mice, 20–26 weeks of age. The effect of initiation with a single application of 50 μg of DMBA in 50 μL of acetone followed 1 week later by stepwise increments of solar-simulated UVR administered on 5 days per week from a cellulose acetate-filtered fluorescent tube source, starting from 1 MED daily, for 6 weeks, was compared in mice with unprotected skin or skin to which one of two sunscreens had been applied. The skin of the dorsum received 5% ethylhexyl PABA or 10.8% ethylhexyl methoxycinnamate in ethanolic solutions matched for absorbance at 310 nm in 0.1 mL before irradiation. The cumulative UVB dose was 72.9 kJ/m^2. After the treatments, the mice were observed until week 37. The rate of survival was 93%. The solar-simulated UVR regime resulted in 13.3% tumour incidence and an average multiplicity of 0.2 at 260 days [37 weeks], while DMBA resulted in 26.7% tumour incidence and 0.47 multiplicity. The combination of DMBA plus solar-simulated UVR greatly enhanced the response, so that 85.7% of mice had acquired tumours with an average multiplicity of 2.0. Irradiation through ethylhexyl PABA was not significantly protective and resulted in 81.3% tumour incidence and a multiplicity of 1.44. Irradiation through ethylhexyl methoxycinnamate, however, significantly decreased the tumour incidence to 30.8% and the multiplicity to 0.38, a response that was not significantly different from that to DMBA alone. Neither sunscreen altered the response to DMBA alone. The incidence of squamous-cell carcinoma was 7% after DMBA plus solar-simulated UVR and 13% after DMBA plus solar-simulated UVR plus ethylhexyl PABA, whereas all other treatments resulted only in papillomas during the study period. The papillomas induced by DMBA and all co-carcinogenic treatments regressed, while the papillomas induced by solar-simulated UVR alone did not (Reeve *et al.*, 1990) (Fig. 36).

The co-carcinogenicity of DMBA and solar-simulated UVR was tested in

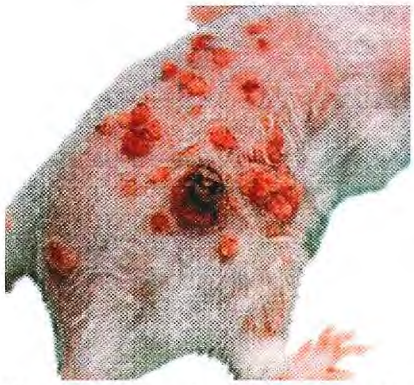

Figure 36 Experimentally induced skin papillomas on a CD1 mouse

groups of 15 shaved female C3H/HeJ mice, 10–12 weeks of age, to test sunscreens containing 8% ethylhexyl methoxycinnamate (sunburn protection factor 4 in the mouse) and 7.2% microfine TiO_2 (sunburn protection factor 7 in the mouse). Mice were initiated with 10 nmol [2.56 μg] of DMBA in 50 μL of acetone 5 days before the beginning of a stepwise incremental regime of solar-simulated UVR from a cellulose acetate-filtered fluorescent tube source (UVB irradiance, 1.7 W/m^2) on 5 days per week for 32 weeks. The initial dose of solar-simulated UVR was 0.4 MED, which was increased by four weekly increments of 30%, but the exposure remained sub-oedemal throughout, giving a cumulative dose of 571 kJ/m^2 UVB and 11.4 MJ/m^2 UVA. Some groups were treated at least 10 min before irradiation with either ethylhexyl methoxycinnamate, microfine TiO_2 or the vehicle, which was an oil-in-water emulsion containing 0.1% butylated hydroxytoluene and 0.5% α-tocopherol, or the vehicle without these antioxidant additives. Tumours ≥ 3 mm in diameter were monitored until week 48. Solar-simulated UVR alone resulted in a 46% tumour incidence at 48 weeks. Whereas DMBA alone did not induce tumours, treatment with DMBA plus solar-simulated UVR resulted in a 87% tumour incidence, with an average onset of 37 weeks. The sunscreen vehicle did not alter this frequency significantly, but both ethylhexyl methoxycinnamate and TiO_2 protected against tumour development at 48 weeks. In the unprotected irradiated groups, the final average tumour multiplicity varied from 1.1 to 1.4, but the differences were not statistically analysed. All the tumours that were examined histologically were squamous-cell carcinomas, and no regressions were recorded. Thus, at low daily doses of solar-simulated UVR, both sunscreens protected from co-carcinogenesis by DMBA plus solar- simulated UVR (Bestak & Halliday, 1996).

Inorganic sunscreens: ZnO and TiO_2 of small particle size are new developments in inorganic sunscreens, and there are few adequate studies of the efficacy of these products in protecting animals against photocarcinogenesis. Groups of 30 female Skh:HR-1 mice were maintained under ambient lighting free from UVR and received an incremental regime of solar-simulated UVR from a cellulose acetate-filtered fluorescent tube source, starting with a daily dose of 1 MED and increasing in a stepwise manner to 2 MED per day, on 5 days per week for 12 weeks after topical application of 0.3 mL (2 mg/cm^2) of an SPF 15 sunscreen containing aluminium stearate-coated, microfine TiO_2 as the only active ingredient. After the 12 weeks of irradiation, the mice were given topical applications of croton oil on the dorsal skin. Tumours appeared in 100% of the mice treated with solar-simulated UVR plus croton oil [although the figure in the publication indicates a 95% incidence] by 52 weeks, whereas addition of the sunscreen reduced the final incidence to 25%. The study indicates protection against photocarcinogenesis by the TiO_2 sunscreen (Greenoak *et al.*, 1993). [The Working Group noted that the doses of UVR were not stated, and no controls receiving only the vehicle or only croton oil after solar-simulated UVR or after sunscreen alone were included.]

In a second study, a sunscreen containing 3.5% microfine ZnO plus 3.5% ethylhexyl methoxycinnamate, which had a sunburn protection factor of 7 in mice under the study conditions, was tested in the same strain of mice. The protocol was intended to simulate sunscreen use by humans previously exposed without protection to a latent carcinogenic dose of solar-simulated UVR. Thus, the mice were irradiated at selected multiples of the MED through the sunscreen (Greenoak *et al.*, 1998). [The protocol was, however, inconsistent, the UVR was undefined and the control treatments were inadequate.]

Intermediary biomarkers

Evaluation of the preventive effects of sunscreens against UVR-induced skin cancers in animal models is labour-intensive, time-consuming and expensive. In addition, when sunscreens with high SPFs are evaluated, the animals may die before sufficient numbers of tumours are seen to distinguish differences between treatment regimens. One solution is to use priming doses of UVR without protection, but this allows only evaluation of protection against the promotion phase of the tumour process. As experiments for photocarcinogenesis necessarily last more than 1 year, a large number of animals must be included to compensate for accidental deaths. When a large number of animals is exposed, the UVR source must be powerful, and xenon arc lamps, the only source that delivers a spectrum which resembles that of the sun, are particularly expensive. This source emits a considerable amount of infrared radiation, which is difficult to filter out, and the animals must be placed far from the source. As a consequence, the amount of irradiance reaching the skin is reduced and the exposure time must be increased which may result in elimination of the sunscreen by grooming.

For these reasons, earlier, surrogate biomarkers have been used to evaluate the efficacy of sunscreens against UVR-induced skin cancer. Some biomarkers are transient (e.g. DNA damage and sunburn cells), while others are persistent (e.g. *p53* mutations). The markers may be steps in the pathway of photocarcinogenesis and therefore possibly 'causal'; they may only be related to the pathway (e.g. apoptotic cells); or they may simply be associated with exposure to UVR. Only the biomarkers that are in the pathway of photocarcinogenesis are evaluated on p. 144.

Molecular and cellular biomarkers

Studies of molecular and cellular biomarkers are summarized in Table 23.

DNA damage: The inhibition of DNA synthesis and the characteristic lesions, cyclobutane pyrimidine dimers and pyrimidine (6–4) pyrimidone, induced by direct absorption of UVR by DNA have been used to evaluate the protective efficacy of sunscreens in six studies.

In the first study, the ability of seven commercial sunscreens to protect against UVR-induced inhibition of epidermal DNA synthesis was evaluated in the hairless mouse model (HRS/J). Two hours after one exposure to UVB (0.03–1.8 kJ/m^2) delivered by Westinghouse FS20 fluorescent tubes, a significant decrease in tritiated thymidine incorporation was measured in DNA, reflecting a decrease in DNA synthesis. The maximum inhibition (70–80% below that of unexposed ventral skin) occurred at 0.15 kJ/m^2. The SPFs of the sunscreens were between 4 and 15 (claimed on the label of sunscreen product tested) or 4 and 24 (claimed by the manufacturer). A ZnO ointment was also evaluated, but the concentration of the active ingredient and the SPF of this preparation were not given. The sunscreen preparations were applied at 4 μL/cm^2 (about 4 mg/cm^2) and the ZnO ointment at 12 μL/cm^2. Statistically significant differences in the efficacy of the sunscreens were found. The DNA protective factors, defined as the ratio of the UVR dose required to inhibit DNA synthesis by 50% with and without sunscreen, were 4.4 for the SPF 4 sunscreen, 8.4 for the SPF 6 product and 21–27.6 for the SPF 15 products. The ZnO ointment was protective at all UVR doses used. A relatively good correlation was found between the DNA protective factor and the SPF (Walter, 1981).

The ability of 1, 5 or 10% of fine-particle-size TiO_2, 1% PABA or 1% urocanic acid in white petroleum to prevent changes in epidermal DNA synthesis was evaluated in female hairless mice. The animals were exposed to single doses of UVB (4.4, 8.8 or 17.6 kJ/m^2) from unfiltered Toshiba FL20SE-30 fluorescent tubes, and they received approximately 2 mg/cm^2 of the sunscreens. Suppression of DNA synthesis was seen 1 h after exposure of unprotected animals. None of the sunscreens protect against the suppression induced by 8.8 kJ/m^2 UVB, except 5% TiO_2. DNA synthesis was strongly increased (fivefold by 4.4 kJ/m^2 and eightfold by 8.8 kJ/m^2) 48 h after exposure in comparison with the level in unexposed controls. [No data were reported for 17.6 kJ/m^2.] PABA and urocanic acid gave very little protection against this increase, whereas 1% and 5% TiO_2 gave complete protection. The protective effect of TiO_2 increased proportionately with the concentration used, and a dose of 10% TiO_2 was protective against the high dose of UVR (Suzuki, 1987).

The photoprotective effects against the formation of cyclobutane pyrimidine dimers of sunscreen preparations containing 8% ethylhexyl PABA, 7.5% ethylhexyl methoxycinnamate or 6% benzophenone-3 were studied in female C3H/HeNCr (MTV$^-$) mice exposed to a single dose of 5 kJ/m^2 UVB from unfiltered FS40 sunlamps (fluorescent tubes). The SPFs and the absorption spectra of the sunscreen preparations were unknown. The quantities applied were 200–250 μL/mouse. The frequency of cyclobutane pyrimidine dimers in epidermal DNA was determined by an assay sensitive for endonucleases with alkaline agarose gels. The number of cyclobutane pyrimidine dimers was reduced by 91% by ethylhexyl PABA, 86% by ethylhexyl methoxycinnamate and 67% by benzophenone-3. The vehicle had no protective effect (Wolf *et al.*, 1993a).

Pyrimidine dimers were measured by the endonuclease-sensitive assay with alkaline sucrose gradients in epidermal DNA of female Skh:Hr1 mice. Groups of mice were exposed to single dose of solar-simulated UVR (290–400 nm) or UVA (320–400 nm) without topical treatment, or after topical application of 2 mg/cm^2 of the vehicle, a UVB absorber (ethylhexyl methoxycinnamate at 5%) or a broad-spectrum UVA absorber (terephthalylidene dicamphor sulfonic acid at 5%). The sunburn protection factor of the sunscreen preparations determined in the mouse model were similar (4). DNA protection factors were determined for both preparations and were 6.6 for ethylhexyl methoxycinnamate and 11.5 for terephthalylidene dicamphor sulfonic acid with solar-simulated UVR, and 2 and 8, respectively, with UVA. Both UVR filters were effective, but terephthalylidene dicamphor sulfonic acid was significantly more effective than ethylhexyl methoxycinnamate in protecting against the induction of pyrimidine dimers. The vehicle provided a slight but nonsignificant protective effect (Ley & Fourtanier, 1997).

Accumulation of cyclobutane pyrimidine dimers in DNA of female C3H/HeNTac mice exposed to a single irradiance of 2.5 J/m^2 per s of UVB (unfiltered Westinghouse FS20 lamp) for 1 h (about 9 kJ/m^2) was measured after application at approximately 4 mg/cm^2 of various commercial sunscreens (SPF 8, 15 or 30) or preparations containing various concentrations of PABA (5, 10,

Table 23. Molecular and cellular biomarkers of UVR-induced carcinogenesis

Animal species, strain (no. per group)	UVR source	UVR regimen	Sunscreen	Results	Reference
DNA damage					
Mouse, Skh:hr1 (9–15)	Solar-simulated UVR, 2–30 x 10^4 J/m^2, and UVA (xenon), 2–13 x 10^5 J/m^2	Single	5% terephthalylidene dicamphor sulfonic acid (sunburn protective factor, 4); 5% ethylhexylmethoxy-cinnamate (sunburn protective factor, 4)	Significant protection, with DNA protection factor > sunburn protection factor	Ley & Fourtanier (1997)
Sunburn cells (apoptotic cells)					
Mouse, Skh:hr1 (3–4)	Solar-simulated UVR (xenon), 0.11–1.8 kJ/m^2	Single	Commercial sunscreen (SPF 12): ethyl-hexyl methoxycinnamate; benzophenone-3; ZnO; talc. Pure or diluted (12.5, 25 or 50%)	No protection with 12.5% sunscreen, significant protection with 25 and 50%	Sambuco *et al.* (1984)
***p53* mutations**					
Mouse, C3H (20)	Filtered UVB from tubes, 4.5 kJ/m^2 per day	Repeated, 5 days per week, 12 weeks	Phenylbenzimidazole sulfonic acid, 2%; octocrylene, 10% (SPF 15)	Significant protection	Ananthaswamy *et al.* (1997)
			Phenylbenzimidazole sulfonic acid, 0.3%; octocrylene, 9%; terephthalylidene dicamphor sulfonic acid, 0.7%; butyl methoxydibenzoylmethane, 3% (SPF 15)	Significant protection	
Mouse, C3H (16)	Solar-simulated UVB (xenon), 4.5 kJ/m^2 per day	Repeated, 5 days per week, 16 weeks	Phenylbenzimidazole sulfonic acid; 2%; octocrylene, 10% (SPF 15)	Significant protection	Ananthaswamy *et al.* (1999)
			Phenylbenzimidazole sulfonic acid, 0.3%; octocrylene, 9%; terephthalylidene dicamphor sulfonic acid, 0.7%; butyl methoxydibenzoylmethane, 3% (SPF 15)	Significant protection	
			Ethylhexyl methoxycinnamate, 8%; phenylbenzimidazole sulfonic acid, 2% (SPF 15)	Significant protection	
			Octocrylene, 10%; phenylbenzimidole sulfonic acid, 0.2%; butyl methoxy-dibenzoyl methane, 1.5%; terephthalylidene dicamphor sulfonic acid, 3.25% (SPF 22)	Significant protection	

SPF, sun protection factor

15 or 20%) in a neutral cream vehicle. The UVR absorber in the commercial products consisted of combinations of benzophenone-3, ethylhexylmethoxycinnamate and ethylhexyl salicylate or ethylhexyl PABA and glyceryl PABA esters. Their protection factors in the mouse model were not determined. The SPF-8 sunscreen reduced dimer formation to 48% of the control level, but statistically significant protection was seen only with the SPF-15 (approximately 67% of control) and -30 (approximately 88% of control) products. Application of the PABA preparations resulted in a dose-dependent effect. A 10% dispersion was necessary to give significant protection (approximately 45% of controls receiving the vehicle); the 15% dispersion gave about 80% protection, and the 20% dispersion about 90% that in controls (McVean & Liebler, 1997).

The same protocol (female C3H/HeNTac mice, unfiltered FS20 sunlamps, a single dose of 9 kJ/m^2, cyclobutane pyrimidine dimer determination, 4 mg/cm^2 application of sunscreen) was used to evaluate preparations containing a single UVB absorber, 5% ethylhexyl methoxycinnamate or 5% ethylhexyl salicylate, or a single UVA plus UVB absorber, 5% benzophenone-3. The SPFs of the preparations were not measured or specified. Only the ethylhexyl methoxycinnamate preparation significantly inhibited thymine dimer formation, by about 60% compared with controls exposed without sunscreen (McVean & Liebler, 1999).

DNA damage is considered to be a transient, causal early biomarker in the pathway of photocarcinogenesis. All six studies showed protection against DNA damage by sunscreens, but only one study used solar-simulated UVR and provided information on the dose–response relationship.

p53 accumulation and sunburn cells or apoptotic cells: TP53 is expressed after DNA damage, as this protein induces transient cell cycle arrest allowing DNA repair or apoptosis to take place. The apoptotic keratinocytes produced after exposure to UVR have been called'sunburn cells' (Fig. 37). No animal model has been used to measure accumulation of TP53 and protection by sunscreens, but the number of apoptotic cells formed after exposure to UVR has been determined in order to evaluate the efficacy of sunscreens. The cells were counted in histological sections stained with haematoxylin and eosin, except in one study (Okamoto *et al.*, 1999) in which the terminal deoxynucleotidyl transferase-mediated UTP nick and labelling (TUNEL) technique was used.

In the first study, male hairless Skh-1 mice were exposed to increasing doses of solar-simulated UVR (290–400 nm) through a commercial SPF-12 product containing ethylhexyl methoxycinnamate, ZnO, talc and benzophenone-3. This product was applied, diluted in its vehicle to 50, 25 or 12.5% of the original concentration, at a dose of 2 µL/cm^2. The output of the simulator was measured with a Robertson Berger meter. Each animal was exposed for 0, 1, 2, 4, 8 and 16 min (1.7 min corresponds to 0.96 Robertson Berger sunburn unit and is equal to 1 MED or 0.2 kJ/m^2). A progressive increase in the protective effect was seen with increasing relative concentration of the sunscreen, and all treatments were significantly effective, except that with the 12.5% preparation (Sambuco *et al.*, 1984).

In two studies, female C3H/HeN mice were exposed twice a week for 3 weeks to 4.8 kJ/m^2 UVB or to a single dose of UVB at 5 kJ/m^2 delivered by unfiltered FS40 sunlamps. Application at 200–250 µL/mouse of preparations of unknown SPF containing a single UVB absorber (8% ethylhexyl PABA or 7.5% ethylhexyl methoxycinnamate) or a single UVA plus UVB absorber (6% benzophenone-3) almost completely prevented the formation of sunburn cells, whereas the vehicle was ineffective. The three sunscreen preparations did not differ in efficacy (Wolf *et al.*, 1994, 1995).

A further study on the effect of sunscreens on sunburn cell formation was

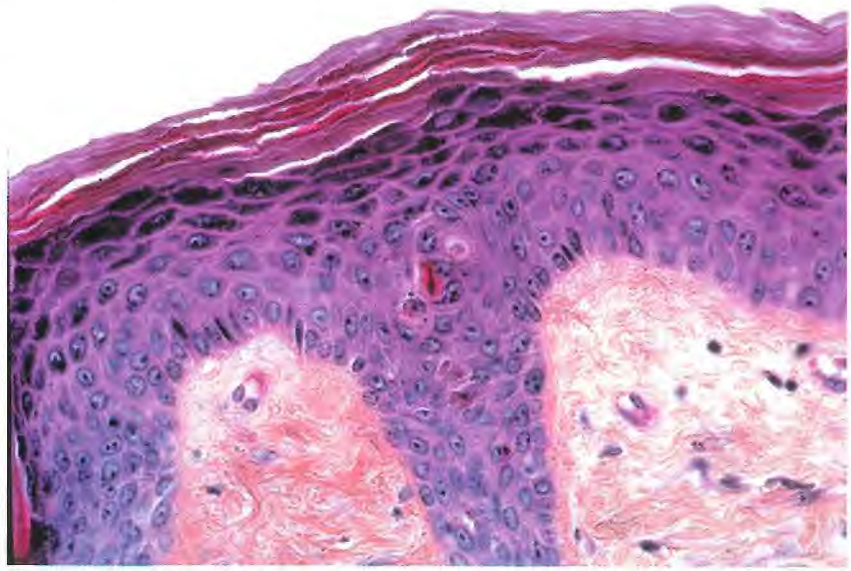

Figure 37 UVR induces keratinocyte apoptosis. The dead cells are called 'sunburn cells' and can be counted on skin sections and used to evaluate the efficacy of sunscreens.

conducted with domestic male Yorkshire pigs. The backs of the animals were treated at 0.1 mL/10 cm^2 twice a day for 3 days and 30 min before irradiation with 3–4 MED of UVB (unfiltered Westinghouse FS40 fluorescent bulbs; 3 kJ/m^2). Biopsy samples were taken after 24 h, and sunburn cells were counted. PABA dissolved at 0.1% in an aqueous solution containing propylene glycol and hydroxypropylcellulose and benzophenone-3 dissolved at 0.25% in ethanol, propylene glycol and water were protective (Darr *et al.*, 1996).

The protection afforded by a SPF-60 commercial sunscreen [composition not given] on the induction of apoptotic cells was measured in female C3H/He mice exposed to a single dose of 0.25 or 0.5 kJ/m^2 of UVB delivered by an unfiltered Toshiba fluorescent sunlamp (FL20SE) with or without application of the sunscreen at 50 µl/mouse 30 min before exposure. Skin biopsy samples were obtained 24 h after exposure, and epidermal sheets were prepared by incubation in EDTA and stained by the TUNEL technique. The cells were enumerated under a fluorescence microscope. The sunscreen was effective in reducing the induction of apoptic cells at both irradiances, with about 90% reduction when compared with unprotected animals (Okamoto *et al.*, 1999).

Sunburn or apoptotic cells are considered to be transient biomarkers of exposure to UVR but are not in the direct pathway of photocarcinogenesis. In five studies, sunscreens protected against the occurrence of this biomarker, but only one study used solar-simulated UVR and provided information on the dose–response relationship in hairless mice.

p53 *mutations:* Cells that acquire mutations at dipyrimidine sites, in particular in the *p53* gene, can expand clonally and may become a precancerous lesion. Two papers from the same authors (Ananthaswamy *et al.*, 1997, 1999) report the results of studies of this marker. Female C3H/HeNCr mice were exposed to repeated doses of UVB delivered by Kodacel filtered FS40 sunlamps (4.5 kJ/m^2 [2.3 MED]) on 5 days per week for 12 weeks. The efficacy of two prototype sunscreen preparations applied at 2 mg/cm^2 was measured by counting the number of *p53* mutations in exposed mouse skin. One sunscreen contained only UVB absorbers, 10% octocrylene and 2% phenylbenzimidazole sulfonic acid, while the other contained two UVA (3% butyl methoxydibenzoylmethane and 0.7% terephthalylidene dicamphor sulfonic acid) and two UVB absorbers (9% ocrocrylene and 0.3% phenylbenzimidazole sulfonic acid). The vehicle and the SPF (15) were similar for the two preparations. After 12 weeks, 9/20 mice exposed to the vehicle plus UVR had CC→TT mutations at codon 148, 154–155 or 175–176. In contrast, only 1 of 20 mice treated with the UVB sunscreen plus UVR and 2 of 20 mice exposed and treated with the UVB plus UVA sunscreen had mutations at these codons.

In the second paper (Ananthaswamy *et al.*, 1999), the same strain of mice and the same techniques were used to analyse *p53* mutations in epidermal DNA, but the animals were exposed for 16 weeks to solar-simulated UVR (4.5 kJ/m^2 UVB and 30.3 kJ/m^2 UVA) on 5 days per week. The dose of UVA plus UVB was 34.8 kJ/m^2 per exposure. Four sunscreens were studied: the same two sunscreens as in the previous study, a SPF-15 product containing 8% ethylhexyl methoxycinnamate and 2% phenylbenzimidazole sulfonic acid (UVB absorbers) and a SPF-22 product containing 10% octocrylene, 0.2% phenylbenzimidazole sulfonic acid, 3.25% terephthalylidene dicamphor sulfonic acid and 1.5% butyl methoxydibenzoyl methane (UVB plus UVA absorbers). All the UVR absorbers were introduced in the same vehicle, and the products were applied at a dose of 2 mg/cm^2. Pooled data for all three codons showed the presence of nine mutations in 16 mice treated with the vehicle (oil-in-water emulsion) plus UVR and in none of 16 mice treated with the SPF-16 (octocrylene plus phenylbenzimidazole sulfonic acid) sunscreen or the SPF-22 (octocrylene plus phenylbenzimidazole sulfonic acid plus terephthalylidene dicamphor sulfonic acid plus butyl methoxydibenzoyl methane) sunscreen. Only 1/16 mice treated with the SPF-15 sunscreen containing ethylhexymethoxycinnamate plus phenylbenzimidazole sulfonic acid and 2/16 of those treated with the SPF-15 sunscreen containing octocrylene plus phenylbenzimidazole sulfonic acid plus terephthalylidene dicamphor sulfonic acid plus butyl methoxydibenzoyl methane had detectable *p53* mutations. Overall, the sunscreens used in this study inhibited the number of UV-induced p53 mutations by 80–100%.

p53 mutations are considered to be a persistent biomarker in the pathway of the development of squamous-cell carcinoma. One study showed that sunscreen products protect against *p53* mutations induced by solar-simulated UVR, but the dose–response relationship was not evaluated.

Immunological end-points

It is now clearly established that UVR induces immune suppression, thus permitting the growth of tumour cells. The immunological impairment caused by UVR can be divided into local and systemic effects. Local immune suppression is defined as a diminished contact hypersensitivity response to haptens, when they are applied at a UVR-irradiated site. Exposure to UVR can also result in a diminished contact hypersensitivity response when haptens are applied at a distant, unirradiated site, and this is referred to as 'systemic immune suppression'. In contrast, delayed-type hypersensitivity reactions occur when antigens are injected. The rejection of melanoma and non-

melanoma skin cancers is also altered by exposure to UVR. UVR-induced immune suppression has also been demonstrated in assays that do not involve tumour cells. In these assays, the immune system is primed to react with a certain simple chemical (the hapten) in a first contact known as sensitization or immunization (the afferent stage), which evokes a (hapten-) specific immune response. Exposure to UVR is then found to suppress the ultimate reaction. In most studies, exposure to UVR occurs before the immunization, although in a few studies it has been done after immunization, before the challenge (efferent stage).

Immunological reactions have been used to evaluate the efficacy of sunscreens. In experimental animals, UVR-induced lack of responsiveness to haptens is associated with the presence of hapten-specific T suppressor cells. The mechanism by which UVR activates the suppressor rather than the effector arm of the immune response is not completely understood; however, alterations in the number and activity of constituents of the dermal immune system (Langerhans cells and dendritic epidermal T cells) and the production of soluble factors (cytokines, neuropeptides, prostaglandins and growth factors) have been implicated. In addition, urocanic acid in the stratum corneum, when isomerized from the *trans* to the *cis* form by UVR, has immune suppressive properties. DNA damage also appears to be involved in immune suppression by UVR. The studies that have been conducted on immunological end-points are summarized in Table 24.

Langerhans cells and dendritic epidermal T cells: The role of sunscreens in preventing alterations in Langerhans cells (Fig. 38) was determined by counting ATPase-positive cells on epidermal sheets. C3Hf/HeN mice were exposed repeatedly to UVR from unfiltered fluorescent FS40 sunlamps, for 30 min/day with 3.22 kJ/m^2 through a commercial sunscreen containing 5% PABA as the UVR absorber. Daily treatment with the sunscreen, 'applied liberally and rubbed in', decreased the number of ATPase-positive cells from the second day of exposure for up to 11 days. In PABA-pretreated animals, a decrease was measured on days 3 and 4, but the number had returned to normal by day 7 or 8 (depending on the experiment). The morphology of the remaining cells in both unprotected and protected animals was altered (Lynch *et al.*, 1981).

HRA:Skh-1 hairless albino and HRA:Skh-2 hairless pigmented mice were exposed for 5 days/week for 4 weeks under six F40BL UVA tubes flanking a single Oliphant FL40SE UVB tube filtered with Kodacel, providing fluorescent solar-simulated UVR. The mice were unrestricted and received increasing doses of UVR, for average cumulated doses of 42 kJ/m^2 UVB and 811 kJ/m^2 UVA. Two SPF-15 sunscreen preparations were evaluated. One contained 6.5% ethylhexyl PABA plus 3% benzophenone-3, and the other 7.5% ethylhexyl methoxycinnamate plus 4.5% benzophenone-3. The vehicles (unspecified base lotions) were different. Simple solutions of 6.5% ethylhexyl PABA or 7.5% ethylhexyl methoxycinnamate in ethanol, dimethyl sulfoxide and acetone were also studied. All the products were applied at 2 mg/cm^2. At the end of the exposure, the mice were killed and epidermal sheets were prepared from excised skin and immunostained to detect Langerhans cells or dendritic epidermal T cells. Langerhans cells were significantly depleted in epidermis exposed for 4 weeks when compared with that from unirradiated mice. The densities of Langerhans cells in animals treated with the ethylhexyl PABA sunscreen, ethylhexyl PABA in solution, the ethylhexyl methoxycinnamate sunscreen or ethylhexyl methoxycinnamate in solution and in unexposed mice did not differ significantly, and the densities in exposed and vehicle-treated mice did not differ from those of the group receiving UVR only. Similar results were found in the two mouse strains. In contrast, ethylhexyl PABA but not ethylhexyl methoxycinnamate protected dendritic

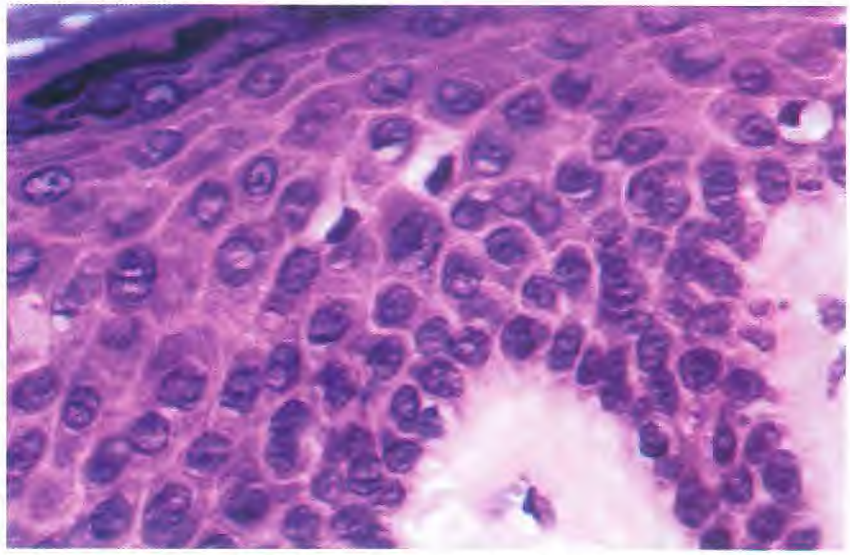

Figure 38 Elongated nuclei surrounded by clear space in mid-squamous layer, the usual site of Langerhans cells

Table 24. Immunological biomarkers of exposure to and effects of UVR

Animal species, strain (no. per group)	UVR source	UVR regimen	Sunscreen	Effect	Results investigated	Reference
Langerhans cells						
Mouse, Skh-1/Skh-2 (6)	Solar-simulated UVR (fluorescent tubes)	Repeated: 5 days per week for 4 weeks (dose increasing by 20% every week)	Commercial sunscreen: ethylhexyl PABA, 6.5%; benzophenone-3, 3% (SPF 15)	Numbers of Langerhans cells (Ia^+) and dendritic epidermal T cells	Significant protection	Ho *et al.* (1992)
		Total dose, 42 kJ/m^2 UVB, 811 kJ/m^2 UVA	Commercial sunscreen: ethylhexyl methoxycinnamate, 7.5%; benzophenone 3, 4.5% (SPF 15)		Significant protection for Langerhans but not dendritic cells	
			Ethylhexyl PABA, 6.5% solution		Significant protection	
			Ethylhexyl methoxycinnamate, 7.5% solution		Significant protection for Langerhans but not dendritic cells	
Mouse, Skh-1 (5–14)	Solar-simulated UVR (fluorescent tubes)	Single dose, 2 MED 1 MED = 5.5 kJ/m^2 UVB, 530 kJ/m^2 UVA	1 or 2 applications	Number of Langerhans cells (Ia^+)	1st application / 2nd application	Walker *et al.* (1994)
			Ethylhexyl methoxycinnamate, 9% in lotion		No protection / Significant protection	
			Ethylhexyl methoxycinnamate, 9% in ethanol		Significant protection / Significant protection	
			Ethylhexyl methoxycinnamate, 9% in lotion	Langerhans cell function (mixed epidermal cell–lymphocyte reaction)	No protection / Significant protection	
			Ethylhexyl methoxycinnamate, 9% in ethanol		No protection / Significant protection	
Mouse, Skh-1 (10)	Solar-simulated UVR (xenon)	Single dose-effect, 2–4 MED 1 MED = 3.7 kJ/m^2 UVB	Terephthalylidene dicamphor sulfonic acid, 5% (sunburn protection factor 4)	Number of Langerhans cells (Ia^+)	Significant protection, less than predicted from sunburn protection factor	Guéniche & Fourtanier (1997)
			Ethylhexyl methoxycinnamate, 5% (sunburn protection factor, 4)		Significant protection, less than predicted from sunburn protection factor	
Mouse, Skh-1 and C3H (5)	Filtered UVB tubes	2 consecutive single doses of 1.8 kJ/m^2 each	5 commercial sunscreens: Ethylhexyl methoxycinnamate, 3.5%; benzophenone-3, 1% (SPF 4)	Number of Langerhans cells (Ia^+) in C3H mice	Significant protection, proportional to SPF	Beasley *et al.* (1998)
			Ethylhexyl methoxycinnamate 7%, benzophenone-3, 2% (SPF 8)		Significant protection, proportional to SPF	

Table 24 (contd)

Animal species, strain (no. per group)	UVR source	UVR regimen	Sunscreen	Effect	Results	Reference
Mouse, Skh-1 and C3H (contd)			Ethylhexyl methoxycinnamate 7.5%; benzophenone-3, 4% (SPF 15) Ethylhexyl methoxycinnamate 7.5%; benzophenone-3, 4% octyl salicylate, 5%; homosalate, 5% (SPF 30) Ethyhexyl methoxycinnamate, 7.5%; benzophenone-3, 6%; octyl salicylate 5%; octocrylene, 8% (SPF 45)		Significant protection, proportional to SPF Significant protection (also in Skh-1 mice), proportional to SPF Significant protection, proportional to SPF	
Urocanic acid						
Mouse, Skh (2)	Solar-simulated UVR (fluorescent tubes)	Repeated: 5 x 6 MED or 20 x 6 MED 1 MED = 2 kJ/m^2 UVB plus 24.5 kJ/m2 UVA	Ethylhexyl PABA (sunburn protection factor 6) Ethylhexyl methoxycinnamate (sunburn protection factor, 6)	*trans* to *cis* isomerization	No protection No protection	Reeve (1997)
Cytokines						
Mouse, Skh-1 (10)	Solar-simulated UVR (xenon)	Single dose–effect, 2–4 MED 1 MED = 3.7 kJ/m^2 UVB	Terephthalylidene dicamphor sulfonic acid, 5% (sunburn protection factor, 4) Ethylhexyl methoxycinnamate 5% (sunburn protection factor, 4)	Interleukin-10 in sera	Significant protection, less than predicted from sunburn protection factor Significant protection, less than predicted from sunburn protection factor	Guéniche & Fourtanier (1997)
Contact hypersensitivity, delayed-type hypersensitivity, tumour susceptibility						
Guinea-pig, strain-2 (3)	Sun	Repeated: 5 h/day, 3 days	PABA, 5%	Local contact hypersensitivity to oxazolone	No protection	Morison *et al.* (1985)
Mouse, HRA/Skh (10)	Solar-simulated UVR (xenon)	Repeated: 72 kJ/m^2 per day, 5 days	2 commercial sunscreens: Ethylhexyl PABA, benzophenone-3 (SPF 6) Ethylhexyl PABA, benzophenone-3 (SPF 15)	Systemic contact hypersensitivity to trinitrochlorobenzene	No protection No protection	Fisher *et al.* (1989)
Mouse, HRA/Skh (6)	Solar-simulated UVR (fluorescent tubes)	Repeated: 5 days/week, 4 weeks (increasing dose) Total dose, 42 kJ/m^2 UVB, 811 KJm^2 UVA	2 commercial sunscreens (2 mg/cm^2): Ethylhexyl PABA, 6.5% benzophenone-3, 3% (SPF 15) Ethlhexyl methoxycinnamate, 7.5%, benzophenone-3, 4.5% (SPF 15)	Local contact hypersensitivity to trinitrochlorobenzene	No protection No protection	Ho *et al.* (1992)

Table 24 (contd)

Animal species, strain (no. per group)	UVR source	UVR regimen	Sunscreen	Effect investigated	Results	Reference
Mouse, BALB/c and C3H (6)	Solar-simulated UVR (fluorescent tubes)	Repeated: 5 days/week, 4 weeks, increased by 20% (i) or 30% (ii) Total dose: (i) 70 kJ/m^2 UVB, 1410 kJ/m^2 UVA (ii) 80 kJ/m^2 UVB, 1580 kJ/m^2 UVA for BALB/c; 35 kJ/m^2 UVB, 682 kJ/m^2 UVB for C3H	(i) Ethylhexyl PABA, 8% (sunburn protection factor, 4)	Local contact hypersensitivity to trinitrochloro-benzene	BALB/c mice No protection	Bestak *et al.* (1995)
					Significant protection	
			Ethylhexyl methoxycinnamate, 8% (sunburn protection factor 4)			
			TiO_2, 7.2% (sunburn protection factor, 7)		Significant protection	
			(ii) Ethylhexyl PABA, 8% (sunburn protection factor, 4)		No protection	
			Ethylhexyl methoxycinnamate, 8% (sunburn protection factor, 4)		No protection	
			TIO_2, 7.2% (sun protection factor, 7)		No protection	
			(ii)	Tolerance		
			Ethylhexyl PABA, 8% (sunburn protection factor, 4)		No protection	
			Ethylhexyl methoxycinnamate 8% (sunburn protection factor, 4)		No protection	
			TiO_2, 7.2% (sunburn protection factor, 7)		Significant protection	
			(ii)	Local contact hypersensitivity to trinitrochloro-benzene	C3H mice	
			Ethylhexyl PABA, 8% (sunburn proteciton factor 4)[a]		No protection	
			Ethylhexyl methoxycinnamate (sunburn protection factor, 4)[a]		No protection	
			TiO_2, 7.2% (sunburn protection factor, 7)[a]		Significant protection	
			ZnO (sunburn protection factor, 9)		Significant protection	
			(ii)	Tolerance		
			Ethylhexyl PABA, 8% (sunburn protection factor, 4)[a]		No protection	
			Ethylhexyl methoxycinnamate (sunburn protection factor, 4)[a]		No protection	
			TiO_2, 7.2% (sunburn protection, factor 7)[a]		Significant protection	
			ZnO (sunburn protection factor, 9)		Significant protection	

Table 24 (contd)

Animal species, strain (no. per group)	UVR source	UVR regimen	Sunscreen	Effect investigated	Results	Reference
Mouse, C3H (5)	Filtered UVB tubes	Two single doses: 1.8 kJ/m^2 per day	10 commercial sunscreens: Ethylhexyl methoxycinnamate, 3.5%; benzophenone-3, 1% (SPF, 4) Ethylhexyl methoxycinnamate, 7%; benzophenone-3, 2% (SPF, 8) Ethylhexyl methoxycinnamate, 7.5%; benzophenone-3, 4% (SPF, 15) Ethylhexyl PABA, 8%; benzophenone-3, 4% (SPF, 15) Ethylhexyl methoxycinnamate, benzophenone-3 (SPF, 15) Ethylhexyl PABA, ethylhexyl methoxycinnamate, benzophenone-3 (SPF, 15) Ethylhexyl methoxycinnamate, 7.5% benzophenone-3, 4% (SPF, 15) Ethylhexyl methoxycinnamate, benzophenone-3 (SPF, 15) Ethylhexyl methoxycinnamate, 7.5%; benzophenone-3, 4.5% (SPF, 15) Ethylhexyl methoxycinnamate, 7.5%; octyl salicylate, 5%; homosalate, 5%; benzophenone-3, 4% (SPF, 30)	Local contact hypersensitivity to 2,4-dinitro-1-fluorobenzene	No protection with SPF < 15; significant protection with SPF > 15. Protection increased with quantity applied	Roberts & Beasley (1995)
		Two single doses, 2–15 MISD, dose-effect 1 MISD = 0.9 kJ/m^2	Ethylhexyl methoxycinnamate, 7.5%; benzophenone-3, 4% (SPF, 15)		Significant protection with 2–7.5 MISD; no protection with 15 MISD	
	Solar-simulated UVR (xenon)	Two single doses, 2–60 MISD, dose–effect 1 MISD = 1.35 kJ/m^2	Ethylhexyl methoxycinnamate, 7.5%; benzophenone-3, 4% (SPF, 15)		Significant protection with 2–30 MISD; no protection with 40 MISD	
Mouse, C3H (10–15)	Filtered UVB tubes or solar-simulated UVR (xenon)	Two single doses, dose–effect UVB: 2–15 MISD 1 MISD = 0.9 kJ/m^2 Solar-simulated UVR: 2–60 MISD 1 MISD = 1.35 kJ.m^2	4 commercial sunscreens: Ethylhexyl methoxycinnamate, 3.5%; benzophenone-3, 1% (SPF, 4) Ethylhexyl methoxycinnamate, 7%; benzophenone-3, 2% (SPF, 8)	Local contact hypersensitivity to 2,4-dinitro-1-fluorobenzene	Significant protection against UVB, protection equal to or less than SPF Significant protection against solar-simulated UVR, protection equal to or greater than SPF	Roberts *et al.* (1996)

Table 24 (contd)

Animal species, strain (no. per group)	UVR source	UVR regimen	Sunscreen	Effect	Results investigated	Reference
Mouse, C3H (10–15) (contd)			Ethylhexyl methoxycinnamate, 7.5%; benzophenone-3, 4% (SPF, 15) Ethylhexyl methoxycinnamate, 7.5%; octyl salicylate, 5%; homosalate, 5%; benzophenone-3, 4% (SPF, 30)			
Mouse, C3H (5–10)	Solar-simulated UVR (UVA-340 sunlamps)	Two single doses, 1–60 MISD, dose-effect 1 MISD = 1 kJ/m^2	4 commercial sunscreens: Ethylhexyl methoxycinnamate, 3.5%; benzophenone-3, 1% (SPF, 4) Ethylhexyl methoxycinnamate, 7%; benzophenone-3, 2% (SPF, 8) Ethylhexyl methoxycinnamate, 7.5%; benzophenone-3, 4% (SPF, 15) Ethylhexyl methoxycinnamate, 7.5%; octylsalicylate, 5%; homosalate, 5%, benzophenone-3, 4% (SPF, 30)	Local contact hypersensitivity to 2,4-dinitro-1-fluorobenzene	Significant protection, equal to or greater than SPF	Beasley *et al.* (1996)
Mouse, CH3 (5)	Filtered UVB tubes and solar-simulated UVR (xenon)	Repeated: 1 TISD/day, 5 days/week, 6 weeks 1 TISD = 7.5 kJ/m2 UVB, 25.3 kJ/m2 solar-simulated UVR	4 commercial sunscreens: Ethylhexyl methoxycinnamate, 7%; benzophenone-3, 2% (SPF, 8) Ethylhexylmethoxycinnamate, 7.5%; benzophenone-3, 4% (SPF, 15) Ethylhexyl methoxycinnamate, 7.5% octylsalicylate, 5%; homosalate, 5%; benzophenone-3, 4% 4% (SPF, 30) Ethylhexyl methoxycinnamate, 7.5%; octyl salicylate, 5%; octocrylene, 5%; benzophenone-3, 6% (SPF, 45)	Transplanted tumour incidence and growth	UVB: no protection with SPF 8 or 15; significant protection with SPF 30 and 45 Solar-simulated UVR: significant protection with all sunscreen	Roberts & Beasley (1997a)

Table 24 (contd)

Animal species, strain (no. per group)	UVR source	UVR regimen	Sunscreen	Effect investigated	Results	Reference
Mouse, C3H (5) (contd)			Ethylhexyl methoxycinnamate, 7.5%; octyl salicylate, 5%, homosalate, 5%; benzophenone-3, 4% (SPF, 30) Ethylhexyl methoxycinnamate, 7.5%; octyl salicylate, 5%, octocrylene, 5%; benzophenone-3, 6% (SPF, 45)	Activation of tumour antigen-specific suppressor T cells	Significant protection with both sunscreens	
Mouse, CH3 (5)	Solar-simulated UVR (xenon)	Two single doses, dose effect, 2–15 MISD Local MISD = 1.35 kJ/m^2 Systemic MISD = 6.76 kJ/m^2	2 commercial sunscreens: Ethyhexyl methoxycinnamate, 3.5%; benzophenone-3, 1% (SPF, 4) Ethylhexyl methoxycinnamate, 7%; benzophenone-3, 2% (SPF, 8)	Local and systemic contact hypersensitivity to 2,4-dinitro-1-fluorobenzene	Significant protection, greater than SPF	Roberts & Beasley (1997b)
Mouse, Skh-1 (4–24)	Monochromatic light (TL01, 311 nm)	Single dose–effect, directly on mice or on Transpore tape UVB: 21.8 kJ/m^2 = 2.8 MISD	Ethylhexyl PABA, 4.7% Ethylhexyl methoxycinnamate, 6.3% (SPF, 4)	Systemic contact hypersensitivity to 2,4-dinitro-1-fluorobenzene	Significant protection, equal to sunburn protection factor	Walker & Young (1997)
Mouse, Skh-1 (10)	Solar-simulated UVR (xenon)	Single dose effect, 2–4 MED 1 MED = 3.7 kJ/m^2 UVB	Terephthalylidene dicamsulfonic acid, 5% (SPf, 4) Ethylhexyl methoxycinnamate, 5% (SPF, 4)	Systemic contact hypersensitivity to 2,4-dinitro-1-fluorobenzene	Significant protection, less than sunburn protection factor	Guéniche & Fourtanier (1997)
Mouse, Skh-1 (20)	Solar-simulated UVR (xenon)	Single dose-effect, 0.5–16 MED, 1 MED = 30 kJ/m^2 UVB + 26.2 kJ/m^2 UVA UVA	Octocrylene, 7%; butyl methoxydibenzoylmethane, 3% (SPF, 7; UVA protection factor, 8) Octocrylene, 10%; butyl methoxydibenzoylmethane, 0.3% (SPF, 8; UVA protection factor, 3)	Systemic contact hypersensitivity to 2,4-dinitro-1-fluorobenzene	Significant protection, lower than SPF. The higher the UVA factor, the higher the immunosuppressive protection factor	Fourtanier *et al.* (2000)

Table 24 (contd)

Animal species, strain (no. per group)	UVR source	UVR regimen	Sunscreen	Effect investigated	Results	Reference
Mouse, C3H (5)	Solar-simulated UVR (xenon)	Single dose-effect	Octocrylene, 9%; phenylbenzimidazole sulfonic acid, 0.3% terephthalylidene dicamphor sulfonic acid; 0.7%; butyl methoxydibenzoylmethane, 3% (SPF, 15) Octocrylene, 10%; phenylbenzimidazole sulfonic acid, 0.2%; terephthalylidene dicamphor sulfonic acid, 3.25%; butyl methoxydibenzoylmethane, 1.5% (SPF, 22)	Systemic contact hypersensitivity to 2,4-dinitro-1-fluorobenzene	Significant protection, lower than sun protection factor	Ulrich *et al.* (1999)

SPF, sun protection factor (determined in human skin); MED, maximum erythemal dose; MISD, minimum immunosuppressive dose; TISD, effective tumour immune suppression dose
[a]Containing oxygen radical scavengers

epidermal T cells from the effects of UVR in both mouse strains (Ho *et al.*, 1992).

Male dd-y strain mice received a single irradiance of 0.1 or 1 kJ/m^2 UVB delivered by two unfiltered fluorescent lamp bulbs (Toshiba FL20SE-30). Before exposure, the mice were shaved and 2 mg/cm^2 of two commercial sunscreens were applied. The first contained TiO_2 and had a SPF of 15, and the second contained 7.5% ethylhexyl methoxycinnamate, 5% ethylhexyl salicylate and 8% ethylhexyl PABA. Skin samples were taken at intervals of 2–16 days after exposure to UVB. Adenosine 5'-diphosphate (ADP)-positive cells were counted in epidermal sheets, and the size of Langerhans cells was measured. The population of Langerhans cells was decreased and their size reduced after irradiation with 1 kJ/m^2 UVB, but the population recovered progressively to within normal limits after 16 days and the size within 8 days. Both sunscreens provided protection against the decreased number of Langerhans cells but did not prevent the shrinkage seen after the second day. Exposure to 0.1 kJ/m^2 of UVB induced little change in the Langerhans cells population (Miyagi *et al.*, 1994).

A single UVB absorber, ethylhexyl methoxycinnamate, was evaluated in female Skh-1 hairless albino mouse for its ability to inhibit UVR-induced epidermal Langerhans cells (Ia$^+$) depletion and suppression of the allo-activating capacity of epidermal cells (mixed epidermal cell–lymphocyte reaction). Ethylhexyl methoxycinnamate prepared at 9% in ethanol or a cosmetic lotion was applied before exposure to fluorescent solar-simulated UVR from a combination of two Wolf Helarium UVB/UVA tubes and six Philips TL10 UVA tubes. In experiments with a long exposure, a second application was made halfway through the irradiation. The UVR doses were multiples of 0.3–3 mouse MEDs. A single application of the UVB absorber in both vehicles at 2 mg/cm^2 gave varying degrees of protection from Langerhans cell depletion but afforded no protection against suppression of the mixed epidermal cell–lymphocyte reaction. When the sunscreens were applied twice, there was better protection from Langerhans cell depletion and complete protection from suppression of the mixed epidermal cell–lymphocyte reaction (Walker *et al.*, 1994).

In a study described on page 99, Wolf *et al.* (1995) showed that single UVB absorbers (ethylhexyl PABA or ethylhexyl methoxycinnamate) or a single UVA plus UVB absorber (benzophenone-3) prevented the decrease in the numbers of Langerhans cells and dendritic epidermal T cells induced by a single dose of 5 kJ/m^2 UVB from unfiltered fluorescent tubes in the C3H mouse model.

Two single UVR absorbers, 5% terephthalylidene dicamphor sulfonic acid (a broad-spectrum UVA absorber) and 5% ethylhexyl methoxycinnamate (a UVB absorber), in the same vehicle were evaluated in female hairless Skh-1 mice exposed to a xenon solar simulator. The two preparations both have a sunburn protection factor determined in the mouse model of about 4 and were applied at 2 mg/cm^2 30 min before exposure to UVR. Dose–response relationships were obtained at 0, 2, 3 and 4 mouse MEDs. The end-points evaluated were inflammation, inhibition of systemic contact hypersensitivity, reaction to dinitrofluorobenzene, release of IL-10 (an immunomodulatory cytokine) in the sera and the number of Langerhans cells. The last two end-points were examined at the end of the contact hypersensitivity protocol, 13 days after exposure to UVR. Exposure to 2 MED induced a 70% decrease in the number of Langerhans cells. The vehicle had no effect. The ethylhexyl methoxycinnamate preparation protected against the effect of 2 MED but not against higher doses, whereas terephthalylidene dicamphor sulfonic acid protected against doses up to 3 MED (Guéniche & Fourtanier, 1997).

Five commercial sunscreen lotions (SPF 4, 8, 15, 30, 45) were compared for their ability to prevent depletion of epidermal Langerhans cells after application at 2 mg/cm^2. Female C3H or hairless Skh-1 mice were exposed on two conse-cutive days to 1.8 kJ/m^2 delivered by Kodacel filtered Westinghouse FS20 sunlamps. Biopsy samples were taken 24 h after the last exposure, and epidermal sheets were stained for Langerhans cells (Ia$^+$). The number of these cells was depleted by ~ 75% in unprotected Skh1 exposed mice or those receiving placebo lotion, and the SPF 30 sunscreen completely prevented this depletion. In the C3H mice, all the sunscreens provided protection against Langerhans cell depletion, which was proportional to the labelled SPF (Beasley *et al.*, 1998).

Urocanic acid: The effect of single UVB absorbers (5% ethylhexyl PABA, sunburn protection factor, 5; and 5% ethylhexyl methoxycinnamate, sunburn protection factor, 7) in the same vehicle (a cosmetic emulsion) on the photoisomerization of urocanic acid was studied in female Skh: HR-2 pigmented mice irradiated with a single unfiltered FL40SE UVB fluorescent tube three times daily at 1 or 5 MED. Urocanic acid was extracted from dorsal epidermal scrapings obtained from skin excised immediately after irradiation. The sunscreens were applied at 3–5 mg/cm^2. Irradiation of skin that was unprotected or treated only with base lotion resulted in photoisomerization of 25% and 23% of the epidermal urocanic acid to *cis*-urocanic acid, respectively, whereas the percentage after application of ethylhexyl methoxycinnamate was 7% after three times 1 MED and 3% after three times 5 MED; that after application of ethylhexyl PABA was 2% after three times 1 MED and 0% after three times 5 MED. Thus, topical sunscreen application, independently of the nature of the UVB absorber, effectively prevented *cis*-urocanic acid formation in the epidermis in response to exposure to UVB (Reeve *et al.*, 1994).

The efficacy of some UVB absorbers against photoisomerization of urocanic acid was also evaluated in Skh:HR hairless mice exposed to fluorescent solar-simulated UVR. Neither ethylhexyl PABA nor ethylhexyl methoxycinnamate (both sunburn protection factor 6) prevented the *trans* to *cis* isomerization induced by repeated exposure (five times 6 MED or 20 times 6 MED). Between 22 and 29% photoisomerization occurred in all exposed groups (Reeve, 1997).

Cytokines: In the study of Guéniche and Fourtanier (1997) described above, the effects of sunscreens on the release of an immunomodulatory cytokine (IL-10) by UVR was studied in animals. The IL-10 in sera significantly increased in the irradiated untreated and vehicle-treated groups (from 82.7 pg/mL to 147.5 pg/mL). The groups treated with ethylhexyl methoxycinnamate were protected after exposure to 2 MED but not after 3 MED. Those receiving terephthalylidene dicamphor sulfonic acid were protected at doses up to 3 MED.

Local and systemic immune suppression and susceptibility to implanted tumours: The role of sunscreens in preventing UVR-induced immune suppression was studied in C3Hf/HeN mice sensitized with dinitrofluorobenzene 24 and 48 h after the last exposure to UVR on the unexposed or exposed back. The light sources were unfiltered Westinghouse FS40 sunlamps, and the exposure (3.22 kJ/m^2) was repeated on days 0, 1, 2, 3, 4, 6 and 7 for 30 min/day. The challenge was given 4 days after the last exposure on the footpad, and the swelling was measured 24 h later. A commercial sunscreen containing 5% PABA, applied liberally and rubbed in, failed to protect against the inhibition of local contact hypersensitivity induced by repeated doses of UVB at 3.22 kJ/m^2 (Lynch *et al.*, 1981).

PABA, ethylhexyl PABA, glyceryl PABA and benzophenone-3 in single or combined formulations, with labelled SPFs of 5–15, were applied at 0.3–0.5 mL/mouse to female C3Hf/HeN mice exposed under FS40 sunlamps for 3 or 4 weeks, and tumour growth was determined after subcutaneous transplantation of syngeneic UVR-induced tumour fragments (fibrosarcoma cells). PABA had no effect on the induction of the tumour-susceptible state after 3 weeks of exposure and treatment, and the tumour growth in the sunscreen-treated mice was equivalent to that in the unprotected animals. An almost complete lack of protection against the acquisition of tumour susceptibility after 4 weeks of UVB irradiation was also found in animals treated with the mixture of ethylhexyl PABA and glyceryl PABA, benzophenone-3 or all three products together (Gurish *et al.*, 1981).

Application of 5% PABA partially but significantly protected female C3H/HeNCR (MTV$^-$) mice against UVB-induced inhibition of systemic contact hypersensitivity to oxazolone but gave no statistically significant protection to BALB/cAnNCR mice against the induction of susceptibility to transplanted tumours. Histological evaluation of the skin showed that the sunscreen had not offered complete protection. The animals were exposed with or without sunscreen or vehicle to repeated doses of UVB from unfiltered Westinghouse sunlamps at 18 kJ/m^2 per day, for 3 days for the contact hypersensitivity or three times per week for 8 or 12 weeks for tumour susceptibility testing (Morison, 1984).

In a further study, 5% PABA applied liberally before and 2 h after exposure to UVR slightly protected female guinea-pigs exposed to sunlight (5 h per day for 3 days) against inhibition of the systemic contact hypersensitivity reaction to oxazolone and inflammation (Morison *et al.*, 1985).

The efficacy of SPF 6 and 15 commercial sunscreens containing ethylhexyl PABA and benzophenone-3 in preventing systemic suppression of contact hypersensitivity to trinitrochlorobenzene was tested in inbred albino HRA/Skh hairless mice exposed to either UVB from unfiltered fluorescent tubes or solar-simulated UVR. The sunburn protection factor of the product was verified in mice and found to agree well with that on the labels of the products, which were applied at 2 μL/cm^2. Under these conditions, the two sunscreens did not prevent the suppression of contact hypersensitivity induced by either source of UVR (Fisher *et al.*, 1989).

Two commercial SPF-15 sunscreens, one containing 7.5% ethylhexyl-methoxycinnamate and 4.5% benzophenone-3 and the other 6.5% ethylhexyl PABA and 3% benzophenone-3, applied at 2–3 mg/cm^2 in different vehicles were tested in inbred male Skh:HR-1 hairless albino mice exposed at 1.2 kJ/m^2 per day to a single unfiltered Oliphant FL40SE UVB fluorescent tube on 3 consecutive days. The ethylhexyl methoxycinnamate but not the ethylhexyl PABA product inhibited UVR-induced systemic suppression of contact hypersensitivity to dinitrofluorobenzene and susceptibility to transplanted tumours, but the two preparations were equally effective in preventing erythema and oedema. The tumour cells were injected 21 days after the first exposure to UVB, and tumour growth was monitored for up to 30 days (Reeve *et al.*, 1991).

The efficacy of similar commercial preparations was tested in hairless inbred albino HRA:Skh-1 mice in a study described in the subsection on Langerhans cells. The mice were sensitized by local application of trinitrochlorobenzene on irradiated skin. Neither preparation prevented inhibition of local contact hypersensitivity (Ho *et al.*, 1992).

The efficacy of 7.5% ethylhexyl methoxycinnamate was compared with that of 8% ethylhexyl PABA and 6% benzophenone-3 in four studies. The UVR

source was unfiltered FS40 sunlamps. The sunburn protection factors of the products were not determined in the mouse model, and the SPFs given were rated by the manufacturer. The sunscreens were applied at doses of 200–250 μL/mouse. In two studies, UVR-induced local and systemic inhibition of contact hypersensitivity to dinitrofluorobenzene and oedema in female C3H/HeNCr mice were used as end-points. All the sunscreens prevented oedema and suppression of local contact hypersensitivity after exposure to two or five MEDs (Wolf *et al.*, 1993b, 1995). In another study, the sunscreens prevented oedema but only partially protected against systemic suppression of delayed-type hypersensitivity to *Candida albicans* (Wolf *et al.*, 1993a). In the fourth study, UVR was found to enhance the growth of melanoma cells injected into the irradiated ears of mice. Mice were exposed to UVR twice a week at 4.8 kJ/m^2 for 3 weeks before injection of the tumour cells. Application of the sunscreens prevented oedema and histological damage but offered no protection against the UVR-enhanced growth of melanoma cells (Wolf *et al.*, 1994).

In the study of the capacity of sunscreens to prevent isomerization of urocanic acid by exposure to UVR, protection against suppression of the systemic contact hypersensitivity reaction to oxazolone was also measured. Only 15% ethylhexyl PABA protected against suppression induced by three times 1 MED. When 15 MED were given, none of the concentrations of ethylhexyl PABA was protective (Reeve *et al.*, 1994).

The ability of two organic UVB absorbers (8% ethylhexyl methoxycinnamate and 8% ethylhexyl PABA) and two inorganic sunscreens (7.2% microfine TiO_2 and a commercial ZnO cream) to protect the dermal immune system from 4 weeks' exposure to fluorescent solar-simulated UVR was studied in inbred female BALB/c or inbred female C3H/HeJ mice exposed to sub-erythemal doses of UVR, 5 days/week for 4 weeks. Each week, the exposure time was increased by 20% (protocol (i)) or 30% (protocol (ii)). The sunburn protection factors of the formulations were measured with a solar simulator in both strains of mouse and found to vary between 4 and 9. The quantity of sunscreen applied was 2 mg/cm^2. In the experiment with C3H mice and UVR protocol (ii), oxygen radical scavengers were added to the ethylhexyl methoxycinnamate, ethylhexyl PABA and TiO_2 sunscreens. The end-points were local and systemic suppression of contact hypersensitivity to trinitrochlorobenzene. Tolerance, which is the failure to develop a secondary contact hypersensitivity response, was also evaluated. With protocol (i), which induced local but not systemic immune suppression or tolerance in BALB/c mice, ethylhexyl PABA exacerbated the immune suppression, whereas ethylhexy methoxy- cinnamate and TiO_2 protected the immune system. When the cumulative dose was increased by 12.7% (protocol (ii)), causing systemic immune suppression and tolerance, none of the sunscreens protected from immune suppression, but ethylhexyl methoxycinnamate provided partial and TiO_2 complete protection from tolerance. In the C3H/He mice, ethylhexyl methoxycinnamate provided some protection, whereas TiO_2 and ZnO provided complete protection from systemic immune suppression; ethylhexyl PABA did not protect. In this mouse strain, only TiO_2 and ZnO were completely effective against tolerance. Ethylhexyl methoxycinnamate was partially protective. The authors concluded that sunscreens can protect from local and systemic immune suppression, although this protection is limited and is not related to the sunburn protection factor of the sunscreens or the MED of the mouse strain. Instead, protection seemed to be provided by sunscreens with a broad absorption spectrum (Bestak *et al.*, 1995).

Commercial sunscreens containing combinations of UVR absorbers and labelled SPFs of 4–45 were examined in five studies in female C3H/HeNHsd mice. In the first study, three UVR sources were used: unfiltered FS20 sunlamps, Kodacel filtered FS20 sunlamps and a solar simulator. Mice were exposed on 2 consecutive days. The ability of the sunscreens to prevent local suppression of contact hypersensitivity to dinitrofluorobenzene was studied. Mice protected by SPF-4 and -8 sunscreens and exposed to filtered sunlamps (1.8 kJ/m^2 per exposure) showed contact hypersensitivity responses that were significantly greater than those of the unprotected (placebo treated) control groups, whereas animals protected with SPF-15 and -30 sunscreens mounted responses similar to those of the unirradiated controls. The effects of the amount of a SPF-15 sunscreen containing 7.5% ethylhexyl methoxycinnamate and 4% benzophenone-3 on different UVR spectra were tested by comparing application of 4, 2 or 1 mg/cm^2 on one side and the three UVR sources on the other side. The two higher concentrations of sunscreen provided protection, whereas 1 mg/cm^2 did not, and the level of immune protection was related to the UVR source used, with solar simulator > filtered FS20 sunlamps > unfiltered FS20 sunlamps. The immune protection factor of the SPF-15 sunscreen was 30 for the solar simulator, 7.5 for the filtered sunlamps and 2 for the unfiltered sunlamps (Roberts & Beasley, 1995).

In the second study, the effects of four commercial sunscreen lotions of SPF 4, 8, 15 and 30 applied at 2 mg/cm^2 on the immune protection factor as measured by local suppression of contact hypersensitivity to dinitrofluorobenzene was evaluated with the same three UVR sources. The immune protection factors of the four sunscreens exceeded the labelled SPF in tests conducted with the solar simulator, but the values were significantly lower than

the labelled SPF in tests with unfiltered and filtered FS20 sunlamps. The immune protection factors for the SPF-4, -8, -15 and -30 sunscreens were 15, 15, 30 and 60, respectively, in tests conducted with the solar simulator, 1, 2, 4, and 4 with the unfiltered FS20 sunlamps and 4, 8, 8 and 8 withthe filtered sunlamps (Roberts *et al.*, 1996).

The third study evaluated the UVA-340 sunlamp, which emits a near solar UVR spectrum. The same sunscreens as used in the previous study (SPF 4–30) were evaluated in the same test for local contact hypersensitivity at the same applied dose (2 mg/cm^2). The immune protection factors obtained were 8, 15, 15 and 30, equal to or greater the level of protection predicted by the labelled SPF (Beasley *et al.*, 1996).

In the fourth study, the influence of UVR spectrum on the tumour immune protective capacity of four commercial sunscreens (SPF 8–45; 2 mg/cm^2 applied) was evaluated. Tumour immune suppression was evaluated by the incidence and growth rate of transplanted tumours. The UVR sources were unfiltered FS20 sunlamps, Kodacel filtered FS20 sunlamps and a solar simulator. Tumours were transplanted after 6 weeks of exposure on 5 days per week to doses of 5, 7.5 or 25.3 kJ/m^2, depending on the UVR source. The tumour immune protection levels matched those predicted by the labelled SPF when sunscreen-protected mice were exposed to the solar simulator, and the SPF-30 and -45 sunscreens also blocked activation of tumour antigen-specific suppressor T lymphocytes. In comparison, when Kodacel filtered FS20 sunlamps were used, sunscreens with SPFs > 15 provided partial to complete protection with regard to tumour incidence, and all the sunscreens reduced the tumour growth rates. None of the sunscreens provided measurable tumour immune protection for mice exposed to unfiltered FS20 sunlamps (Roberts & Beasley, 1997a).

In the final study in this series, two commercial sunscreens (SPF-4 and SPF-8) were evaluated with respect to local and systemic contact hypersensitivity to dinitrofluorobenzene after exposure to a solar simulator. Dose–effect relationships were established for these two end-points, and immune protection factors were determined. These factors exceeded the SPFs, with values of 15 for local protection and 8 for systemic protection with the SPF-4 sunscreen and 15 for local protection and 15 for systemic protection with the SPF-8 product (Roberts & Beasley, 1997b).

The relationship between photoprotection against inflammation and immune suppression offered by two UVB filters (4.7% ethylhexyl PABA and 6.3% ethylhexyl methoxycinnamate) was studied in female HRA.HRII-c/+/Skh mice exposed to single doses of monochromatic UVB (Philips TL01 tubes, λ_{max} = 311 nm). A UVR dose–response curve without sunscreen was established for the two end-points. The dose of UVB for 50% immune suppression was lower than that for 50% maximal inflammation (oedema). Ethylhexyl PABA and ethylhexyl methoxycinnamate in the same vehicle totally prevented the oedema and partially prevented the systemic suppression of contact hypersensitivity to dinitrofluorobenzene induced by a single dose of UVB (21.8 kJ/m^2 or 2.8 minimum immune suppression doses). Similar responses were obtained when the sunscreens were applied topically or on a tape placed above the cages. In studies of UVB dose–response relationships for inflammation and immune suppression in mice treated topically with ethylhexyl methoxycinnamate, this sunscreen had a protection factor of 4 for both inflammation and immune suppression (Walker & Young, 1997).

In a study reported in the subsection on Langerhans cells and cytokines, Guéniche and Fourtanier (1997) studied the protection afforded by two UVR absorbers (terephthalylidene dicamphor sulfonic acid and ethylhexyl methoxycinnamate) against inhibition of systemic contact hypersensitivity to dinitrofluorobenzene induced in Skh-1 mice by various doses of solar-simulated UVR. With a UVR dose equivalent to 2 MED, the contact hypersensitivity response was inhibited by 60–70% in untreated or vehicle-treated exposed mice compared with control mice. Application of either sunscreen protected against doses up to 2 MED; at higher doses, ethylhexyl methoxycinnamate did not protect whereas terephthalylidene dicamphor sulfonic acid significantly protected the animals against doses up to 4 MED. The immune protection factors, calculated as the ratio of the minimum immune suppressive dose with and without sunscreen, were 1.6 for 5% ethylhexyl methoxycinnamate and 2.5 for 5% terephthalylidene dicamphor sulfonic acid. Thus, under these experimental conditions, the immune protection capacity of these two sunscreens is lower than their capacity to protect against UVR-induced inflammation.

The level of immune protection afforded by two broad-spectrum sunscreens with SPF 7–8 (determined for both humans and mice) but with different UVA protection levels (determined in humans by the persistent pigment darkening method as 8 or 3) was tested in female Skh-1 hairless albino mice. The two products contained the same filters against UVB (octocrylene) and UVA (butyl methoxydibenzoylmethane) in the same vehicle but at different concentrations; they were applied at 2 mg/cm^2. Solar-simulated UVR dose–response curves for inflammation and systemic suppression of contact hypersensitivity to dinitrofluorobenzene were generated and used to derive protection factors. Both sunscreens protected against suppression of contact hypersensitivity, but the product with the higher UVA protection factor gave significantly greater protection. The techniques used to

determine immune protection factors gave similar results for a given sunscreen, but the immune protection factors were always lower than the SPFs (Fourtanier *et al.*, 2000).

In a study of UVR-induced suppression of the systemic contact hypersensitivity response to dinitrofluorobenzene and protection by sunscreens, dose–response curves for UVR-induced immune suppression were generated with a xenon solar simulator in C3H/HeN mice with and without application of sunscreens. Two broad-spectrum products (SPF-15 and SPF-22) containing UVA and UBV absorbers in the same vehicle were applied 30 min before a single exposure. Both products protected, but the immune protection factors, obtained as the ratios of UVR doses inducing 50% suppression, were lower than the SPFs. The immune protection factor for the SPF-15 sunscreen was 2.3 and that for the SPF-22 product was 4.2 (Ullrich *et al.*, 1999).

Immune suppression is considered to be in the causal pathway of photocarcinogenesis; however, an appropriate biomarker of carcinogenic risk has not been established. Most of the studies showed that sunscreens provide some protection against various end-points in UVR-induced immune suppression.

Photoageing

Chronic exposure to UVR profoundly damages the dermis and the epidermis of human and animal skin. These alterations are called 'photoageing' (Fig. 39). As they occur, like carcinogenesis, in response to cumulative exposure, evaluation of the prevention of photoageing can be used as a biomarker of protection by sunscreens against UVR-induced damage. These studies are summarized in Table 25. This biomarker is also described on p. 85.

Numerous studies have been conducted by Kligman and colleagues in Skh hairless mice of the ability of sunscreens to protect against connective tissue damage induced by repeated exposure to UVR. In the first study, two sunscreens containing 2% ethylhexyl PABA (SPF 2) or 7% ethylhexyl PABA plus 3% benzophenone-3 (SPF 15) were applied to Skh-1 and Skh-2 mice exposed to repeated doses of UVB (unfiltered Westinghouse FS20) at six human MEDs per exposure, three times a week for 30 weeks, followed by 15 weeks of observation. Skin samples were taken at 10-week intervals and were stained to reveal changes in the dermis. The unprotected, irradiated animals showed considerable dermal damage. The SPF-15 sunscreen completely prevented these changes, but the SPF-2 sunscreen was less effective. A surprising histological finding was the extent of the repair of the dermis after irradiation ceased (Kligman *et al.*, 1982).

The second study focused on whether repair would occur if animals were protected by sunscreens after dermal damage was induced and irradiation was continued. Female albino hairless Skh-1 mice were exposed to a daily dose of UVR of 1.7 kJ/m^2 from unfiltered Westinghouse FS20 sunlamps three times weekly for 30 weeks. Commercial sunscreens of SPF 6 (5% ethylhexyl PABA) and SPF 15 (7% ethylhexyl PABA and 3% benzophenone-3) were applied after 10 and 20 weeks of irradiation. Both sunscreens, but especially the SPF 15, allowed repair of previously damaged dermis during continued irradiation (Kligman *et al.*, 1983).

In the third study, the contributions of UVA and UVB to connective tissue damage in female albino Skh-1 hairless mice and the protection afforded by a commercial SPF-15 broad-spectrum sunscreen (7% ethylhexyl PABA and 3% benzophenone-3) was evaluated. Substantial protection against these effects was found (Kligman *et al.*, 1985).

In the fourth study, three groups of female albino Skh-1 hairless mice received a cumulative dose of solar-simulated UVR from a xenon arc that was 10 and 16 times a previously determined minimal photoageing dose over periods of 18 and 30 weeks. Each twice-weekly exposure was designed to equal the SPF value of the first sunscreen, an SPF-7 product containing the UVB absorber ethylhexyl methoxycinnamate. The second sunscreen contained ethylhexyl methoxycinnamate and a UVA absorber (benzophenone-3) and had an SPF of 16. The third, with an SPF of 18, contained ethylhexyl methoxycinnamate, benzophenone-3 and butyl methoxydibenzoyl methane. Considerable damage to the dermal matrix was seen in the group given the SPF 7 product, with greater damage at 30 weeks than at 18. The SPF-16 sunscreen was highly protective at 18 weeks, but the damage at 30 weeks was still significant. The SPF-18 sunscreen, with the broadest spectral absorption, provided the greatest protection at both times. Thus, prevention of sunburn does not guarantee that photoageing will not occur during chronic exposure (Kligman *et al.*, 1996).

A study was conducted in female albino Skh-1 hairless mice to determine the substantivity of sunscreen products with various SPFs and their ability to protect against chronic photodamage. Waterproof commercial sunscreens containing only ethylhexyl PABA (SPF 2) as the UVR absorber or containing ethylhexyl PABA plus benzophenone-3 (SPF 4 and 8) were evaluated. The mice

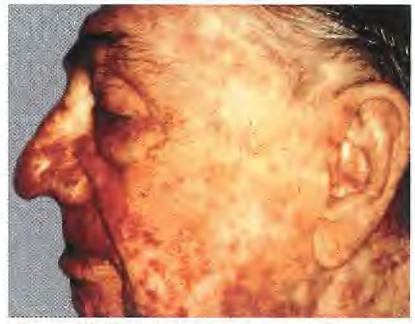

Figure 39 Elderly Australian man with extensive sun damage

Table 25. Photoageing as a biomarker of UVR-induced carcinogenesis

Animal species, strain (no. per group)	UVR source	UVR regimen	Sunscreen	Effect investigated	Results	Reference
Mouse, Skh-1 (12)	Solar-simulated UVR (xenon) and UVA (xenon or tubes)	Repeated: 3 days/week, 34 weeks with UVA at 300–1350 kJ/m^2 per day or solar-simulated UVR at 70 kJ/m^2 per day	Ethylhexyl PABA, 7%; benzophenone-3, 3% (SPF 15)	Connective tissue damage	Significant protection	Kligman *et al.* (1985)
Mouse, Skh-1 (18)	Solar-simulated UVR (xenon)	Repeated: 7 MED/day (increasing doses during weeks 1 and 2), 2 days/week, 18 or 30 weeks 2.8 kJ/m^2 per day	Ethylhexyl methoxycinnamate (SPF 7) Ethylhexyl methoxycinnamate, benzophenone-3 (SPF 16) Ethylhexyl methoxycinnamate, benzophenone-3, butyl methoxydibenzoylmethane (SPF 18)	Epidermal and dermal damage	No protection with SPF 7 Significant protection with SPF 16 and 18, but lower than predicted by SPF	Kligman *et al.* (1996)
Mouse, Skh-1 (12)	Solar-simulated UVR and UVA (fluorescent tubes)	Repeated: 5 days/week, 16 or 32 weeks Solar-simulated UVR: 213.5 kJ/m^2 per day UVA: 199–222 kJ/m^2 per day	Ethylhexyl methoxycinnamate, 2% Ethylhexyl methoxycinnamate, 2%; butyl methoxydibenzoylmethane, 0.75% Ethylhexyl methoxycinnamate, 2%; butyl methoxydibenzoylmethane, 2% Butyl methoxydibenzoylmethane, 0.75%	Clinical, histological and biochemical alterations	Significant protection against clinical and histological alterations; no protection against biochemical alterations, except for third product (significant protection)	Harrison *et al.* (1991)
Mouse, MF1/hr (20)	UVA (xenon)	Repeated: 350 kJ/m^2 per day, 3 days/week, 52 weeks	Terephthalylidene dicamphor sulfonic acid 5% (SPF 5)	Clinical, histological and biochemical alterations	Significant protection	Fourtanier *et al.* (1992)

SPF, sun protection factor; MED, maximum erythemal dose

were exposed under unfiltered FS40 sunlamps at 0.3 kJ/m^2 (about 0.5 MED) three times weekly for 28, 17 and 22 weeks for the SPF-2, SPF-4 and SPF-8 products, respectively. When the sunscreens were applied 15 min or less before each UVB exposure, they protected against skin wrinkling, and an increased delay in the onset of wrinkles was seen with increasing SPF. As the time between treatment and irradiation increased (from 0 to 8 h), however, the protection afforded by all the sunscreens diminished rapidly (Bissett *et al.*, 1991).

The protection against chronic photodamage afforded by two single UVR absorbers, one UVB (2% ethylhexyl methoxycinnamate) and one UVA (0.75 or 2% butyl methoxydibenzoylmethane), or a combination of the two was examined in female albino Skh-1 hairless mice irradiated on the back for 8 h/day, 5 days/week for 16 weeks with fluorescent solar-simulated UVR or UVA or for 32 weeks with UVA alone. All of the UVR-exposed mice showed histological and biochemical damage, recorded as an increased proportion of type III collagen. Application of the UVB sunscreen resulted in marked protection against all non-biochemical end-points. Addition of 0.75% of the UVA absorber gave no clear advantage, but addition of 2% of the UVA absorber reduced the biochemical changes and connective tissue damage. UVA irradiation for 16 weeks caused no histological or biochemical changes, but the mice irradiated with UVA for 32 weeks showed slight dermal damage. These changes were not modified by the 0.75% UVA sunscreen (Harrison *et al.*, 1991).

In a study in female albino MF1/hr hairless mice, sub-erythemal doses of pure UVA enhanced the numerous changes observed during chronological ageing. The photoprotective properties of a broad-spectrum UVA absorber, terephthalylidene dicamphor sulfonic acid, against UVA-induced damage were assessed in 3-month-old albino hairless mice exposed for 1 year to 350 kJ/m^2 (about 0.5 MED) of UVA from a xenon source filtered through a WG 345/2 mm (Schott) filter. One group of animals received a formulation containing 5% of the sunscreen before irradiation, while another was untreated. All the changes induced by chronic exposure to UVA were reduced or abolished by the sunscreen (Fourtanier *et al.*, 1992).

Photoageing is a persistent biomarker of chronic exposure to UVR and is not in the pathway of photocarcinogenesis. Seven studies showed that sunscreens can reduce photoageing caused by either solar-simulated UVR or UVA. There was no clear dose–effect relationship.

In-vitro models

Measurement of optical transmission

Testing of sunscreen products to determine the SPF *in vivo* is time-consuming and expensive in terms of volunteer time. Several attempts have therefore been made to develop a reliable *in-vitro* method for assessing new sunscreen formulations. A reliable *in-vitro* method would also permit systematic studies of the performance of sunscreens under various conditions, such as in water (e.g. water temperature, salinity and turbulence) or normal use (e.g. sand abrasion). Assays exist to measure the transmission of UVR through a substrate before and after application of a sunscreen. The ratio of the transmission without and with the sunscreen gives a measure of photoprotection.

A wide range of substrates has been used to measure transmission through sunscreens. These include wool (Wurst *et al.*, 1978; Greiter *et al.*, 1979), pig skin (Greiter *et al.*, 1979), lyophilized pig epidermis (Stamper, 1990), hairless mouse epidermis (Sayre *et al.*, 1979, 1980; Cole & van Fossen, 1988, 1990), human stratum corneum (Kammeyer *et al.*, 1987; Pearse & Edwards, 1993), synthetic skin casts (Stockdale, 1987; Ferguson *et al.*, 1988), surgical tape (Diffey & Robson, 1989; Sellers & Carpenter, 1992; Keeley *et al.*, 1993), a combination of a biomembrane barrier with a biomacromolecular matrix (Gordon, 1992), roughened quartz plates (Diffey *et al.*, 1997; DeFlandre & Lang, 1988), glass plates (Berset *et al.*, 1996), reconstructed human epidermis (Marginean Lazar *et al.*, 1997) and excised human epidermis (Brown & Diffey, 1986; Marginean Lazar *et al.*, 1997; Stokes & Diffey, 1997a,b, 1999a,b, 2000).

Systems used for detection of the transmitted UVR include either a broad-band radiometer or a scanning spectroradiometer. The most reliable results are obtained when protection factors are measurement spectroradiometrically, the method being as follows. The spectral transmission of UVR through the substrate is measured on a wavelength-by-wavelength basis both with and without the sunscreen. The source of UVR must have a continuous spectrum over the wavelength range of interest (normally 290–400 nm), but the shape of the spectrum is unimportant, and there is no need to simulate the solar spectrum. Indeed, this is undesirable, since the intensity of radiation in the UVB region (290–315 nm) will be so low that the signal-to-noise ratio can become compromised. The transmittance of the sunscreen at wavelength λ nm ($T(\lambda)$) is equal to the ratio of the photocurrent measured through the substrate with the sunscreen applied to that before the sunscreen is applied. $T(\lambda)$ is usually measured in 5-nm steps from 290 to 400 nm. The SPF is then calculated as:

$$\sum_{290}^{400} E(\lambda)\varepsilon(\lambda)\Delta\lambda \Big/ \sum_{290}^{400} E(\lambda)\varepsilon(\lambda)T(\lambda)\Delta\lambda$$

where $E(\lambda)$ is the spectral irradiance of terrestrial UVR under defined conditions, $\varepsilon(\lambda)$ is the erythema action spectrum (McKinlay & Diffey, 1987) and $\Delta\lambda$ is the wavelength step (e.g. 5 nm). The exact numerical values of the derived SPF depend on the choice of $E(\lambda)$. For example, if $E(\lambda)$ is chosen to represent midday winter sunlight at latitude 60° N, a different SPF will be obtained from that when $E(\lambda)$ is selected to represent midday summer sunlight at latitude 20° N.

The most reliable substrate is without doubt excised human epidermis, because, unlike with other substrates, interactions between sunscreen and skin are taken into account. Comprehensive studies with this *ex-vivo* substrate, yielding excellent agreement with SPFs obtained by phototesting in human volunteers *in vivo* (Stokes *et al.*, 1998), have been reported (Stokes & Diffey, 1997a,b, 1999a,b, 2000). In this method, skin is taken from the underside of the female breast during an operation for breast reduction. It is obtained by a process known as de-epidermalization, the principle of which is to remove the epidermis and epidermal appendages while leaving the deepest layers of the dermis *in situ*. The samples of skin are placed in a water bath at 60 °C for 45 s. When the epidermis is removed from the water bath, it is gently separated from the dermis by peeling. Sheets of epidermis can be stored at 4 °C for several weeks without loss of barrier function (Schaefer & Redelmeier, 1996).

DNA damage

In an *in-vitro* test system, PABA strongly protected against damage induced by UVA and UVB irradiation in calf thymus DNA in the presence of Fenton reagents. This result was attributed to the free-radical scavenging properties of PABA (Shih & Hu, 1996).

Ethylhexyl dimethyl PABA protected against broad-spectrum UVR-induced endonuclease-sensitive sites (pyrimidine dimers) in cultured human keratinocytes if the product was not applied directly to the cells. When the sunscreen was in contact with the cells, however, a large increase in photo-induced strand breakage was seen (see also p. 137) (Gulston & Knowland, 1999).

Inhibition of semi-conservative DNA synthesis or repair synthesis

PABA and amyl dimethyl PABA spread on glass protect against semi-conservative DNA synthesis or UVB-induced repair synthesis in cultured human fibroblasts (Arase & Jung, 1986). This system is not widely used, and the results are difficult to reproduce among laboratories.

Biomarkers in cells and skin equivalents

Living cells in culture have been used to test sunscreen products and active ingredients (e.g. Marrot *et al.*, 1999). In order to mimic the three-dimensional structure of the skin, keratinocytes and fibroblasts are grown in collagen-containing matrices (Nelson & Gay, 1993; Augustin *et al.*, 1997a). The responses measured after exposure to UVR include morphological changes, cytotoxicity and the release of pro-inflammatory mediators, such as IL-1-α, tumour necrosis factor-α and prostaglandin E2. The most commonly observed result in a number of studies with various sunscreens was a relative increase in survival in the presence of sunscreen as compared with controls with no sunscreen (Nelson & Gay, 1993; Augustin *et al.*, 1997b; Sun *et al.*, 1999).

In an exploratory study with terephthalylidene dicamphor sulfonic acid, a wide range of markers, including nuclear *p53* induction in keratinocytes, melanogenesis in melanocytes, plasmid DNA damage, mutation in *Saccharomyces cerevisiae* and DNA damage as measured in the Comet assay in keratinocytes, was used to demonstrate protection against UVR emitted by a solar simulator (Marrot *et al.*, 1999).

Antimutagenicity in short-term assays

In a comprehensive study of protection against mutagenesis, Mondon and Shahin (1992) used three *in-vitro* systems — haploid and diploid cells of the yeast *Saccharomyces pombe* and Chinese hamster V79 cells — to investigate a range of mutational end-points, including specific base changes and frameshift mutations in the yeast and the broad range of mutations detected with the thioguanine-resistance marker in the rodent cell line. PABA and 4-(2-oxo-3-bornylidene)methylphenyl trimethylammonium methyl sulfate (a benzylidene camphor derivative synthesized in the laboratory) were tested for their capacity to protect against mutation induced by UVB from a Westinghouse FS20 sunlamp. Both sunscreens protected against mutation in a concentration-dependent fashion, the second being consistently more effective than PABA at equal concentrations, although absorption at equal concentrations was not determined. The authors concluded that PABA was less protective because it counteracted photosensitization. The assays were clearly effective for determining photomutagenicity and the photoprotective effects of sunscreens and indicated that the greater the sensitivity of the mutant allele to alteration, the greater the protection factor observed (Hodges *et al.*, 1977; Sutherland & Griffin, 1984).

Mechanisms of cancer prevention by sunscreens

Sunscreens absorb solar UVR and may reduce the exposure of the skin to this carcinogen. This phenomenon and its relationship to skin cancer have been investigated intensively by reference to a range of cellular and molecular changes induced by UVR and other relevant events.

Solar radiation is a complete carcinogen (IARC, 1992), and UVR is the part of the spectrum that has been implicated in

skin carcinogenesis. An early step in skin carcinogenesis involves the induction of DNA damage, which then leads to a cascade of events, including cell cycle arrest, DNA repair, apoptosis, mutation and neoplastic transformation. Efficient removal of DNA lesions by cellular repair processes appears to be a critical step in the prevention of tumour formation. Errors during the repair of these lesions lead to the incorporation of wrong bases into the genetic material. The unrepaired lesions may also disrupt cellular processes by obstructing the DNA and RNA synthesizing machinery. These mistakes often result in mutation, leading to loss or inappropriate expression of the affected genes. Genetic alterations in *p53*, *patched (ptc), smoothened (smo) or sonic hedgehog (shh)* genes appear to play an important role in the development of squamous- and basal-cell carcinomas (Brash *et al.*, 1991; Ziegler *et al.*, 1993; Gailani *et al.*, 1996; Fan *et al.*, 1997; Oro *et al.*, 1997; Xie *et al.*, 1998).

The role of solar radiation in the pathogenesis of human cutaneous melanoma is more complex than that in squamous-cell carcinomas. Genetic alterations in *p16* and N-*ras* genes have been implicated in melanoma development (Kamb *et al.*, 1994; Hussussian *et al.*, 1994), and the C→T and CC→TT mutations in the *p16* gene have been detected in human melanoma cell lines (Liu *et al.*, 1995; Pollock *et al.*, 1995). The presence of these UVR-induced 'signature' mutations suggests that UVR present in sunlight plays a role in the induction of cutaneous melanomas in humans. In primary melanomas, however, only one CC→TT mutation has been described among some 25 *p16* mutations analysed (Kumar *et al.*, 1998, 1999). Mutations in the N-*ras* oncogene are also reported to play a role in melanoma development (Padua *et al.*, 1985; Keijzer *et al.*, 1989; van't Veer *et al.*, 1989).

Although commercial sunscreens were originally developed to protect against sunburn, laboratory studies have shown that some sunscreens are also efficient in protecting against UVR-induced DNA damage, skin ageing, sunburn cell formation, immune suppression and development of skin cancer in animal models (see p. 98). Sunscreens can also prevent the emergence of actinic keratoses in humans (Naylor *et al.*, 1995) and help reduce the incidence of skin cancer in patients with xeroderma pigmentosum (Kondoh *et al.*, 1994).

Because UVR-induced DNA damage, *p53* mutation, proliferation and immune suppression are key events in skin cancer development, inhibition of one or more of these events may protect against it. Sunscreens would be expected to afford protection against all these events because they should interfere with the primary event, induction of DNA damage. Of the various biological end-points used in studies of sunscreens, some represent acute effects of UVR (e.g. erythema, DNA damage and expression of *p53* and *p21*), while others represent chronic effects (e.g. *p53* mutation and skin cancer). The protective efficacy of sunscreens may vary depending upon the end-point under study.

Inhibition of UVR-induced DNA damage

UVR, particularly wavelengths in the UVC and UVB spectra, induces predominantly two types of DNA photoproducts, cyclobutane-type pyrimidine dimers (Setlow & Carrier, 1966) and pyrimidine (6–4) pyrimidone or (6–4) photoproducts (Mitchell & Nairn, 1989). Both lesions are formed exclusively in runs of tandemly located pyrimidine residues which are often 'hot spots' for UVR-induced DNA damage and mutation. Tornaletti and Pfeifer (1994) demonstrated that mutation 'hot spots' for *p53* in skin cancers are also 'slow spots' for DNA repair. In addition to pyrimidine dimers and (6–4) photoproducts, UVR induces other types of DNA lesions, such as cytosine photohydrates, purine photoproducts and single-strand breaks (Weiss & Duker, 1987; Doetsch *et al.*, 1988; Gallagher & Duker, 1989). UVA radiation and visible light (> 400–500 nm) are known to cause DNA damage indirectly by producing reactive oxygen species such as superoxide anion, singlet oxygen and hydrogen peroxide via unknown endogenous photosensitizers (Peak *et al.*, 1987). These highly-reactive, short-lived molecules produce single-strand breaks, DNA–protein cross-links and altered bases in DNA. There is some evidence that altered bases, particularly 7,8-dihydro-8-oxo-guanine (8-hydroxyguanine) are produced more frequently than single-strand breaks or DNA–protein cross-links by UVA and visible light (Tchou *et al.*, 1991, 1992; Boiteux, 1993; Pflaum *et al.*, 1994).

Because UVR-induced DNA damage is a prerequisite for initiating the process of photocarcinogenesis, inhibition of this event can protect against a wide array of events associated with skin cancer development. Several sunscreen ingredients have been tested for their efficacy to protect against UVR-induced pyrimidine dimers in human and mouse skin.

An SPF-15 sunscreen formulation containing 7.5% ethylhexyl methoxycinnamate and 4.5% benzophenone-3 reduced the number of pyrimidine dimers in human skin induced by solar-simulated UVR (Freeman *et al.*, 1988). Similarly, an SPF-10 sunscreen gave protection against UVB-induced pyrimidine dimers in human skin (van Praag *et al.*, 1993). In two later studies, the degree of photoprotection was correlated with the erythemal response (Bykov *et al.*, 1998b; Young *et al.*, 2000). One of them found an association between DNA damage and the erythemal response, whereas no association was found in the other.

Experiments in mice analogous to those in human skin have also shown

that several organic sunscreen products, including ethylhexyl methoxycinnamate, benzophenone-3 and PABA and its derivatives, reduce UVR-induced DNA damage (Walter, 1981; Walter & DeQuoy, 1980; Wolf *et al.*, 1993a). Topical administration of a broad-spectrum UVA absorber containing 5% tetraphthalylidene dicamphor sulfonic acid was significantly more effective than a UVB absorber (5% ethylhexyl methoxycinnamate) in preventing the induction of pyrimidine dimers in hairless mouse skin irradiated with solar-simulated UVR (Ley & Fourtanier, 1997). In contrast, Suzuki (1987) found that 1% PABA and 1% urocanic acid afforded little or no protection against DNA damage induced by irradiation of hairless mice with a broad-spectrum UVR source (peak emission, 305 nm). [The Working Group noted that this study is hard to interpret because no attempt was made to relate the SPF to a DNA protection factor.]

Topical administration of ZnO or TiO_2 provided protection against DNA damage induced by unfiltered UVB in hairless mice (Walter, 1981; Suzuki, 1987).

Inhibition of UVR-induced p53 *and* p21$^{Waf1/Cip1}$ *expression*

UVR induces high levels of *p53* expression (Maltzman & Czyzyk, 1984; Campbell *et al.*, 1993; Hall *et al.*, 1993; Lu & Lane, 1993; Zhan *et al.*, 1993), which in turn activates the transcription of downstream genes responsible for cell-cycle arrest at the G_1–S transition (Kastan *et al.*, 1991, 1995). The G_1–S arrest results, at least partly, from *p53* transactivation of *p21*$^{Waf1/Cip1}$, which binds to and inactivates the cyclin-dependent kinases required for cell-cycle progression (El-Deiry *et al.*, 1993; Harper *et al.*, 1993; Xiong *et al.*, 1993). Growth arrest may allow the cells to repair the DNA damage. However, *p53* can also cause apoptosis of cells with excessive unrepaired DNA damage (Reed, 1994; Ziegler *et al.*, 1994) by activation of *bax* and/or down-regulation of *bcl-2* expression (Gillardon *et al.*, 1994; Miyashita *et al.*, 1994; Miyashita & Reed, 1995). Recent studies showed that Fas–Fas–ligand interaction is essential for the elimination of cells containing UVR-induced DNA damage (Hill *et al.*, 1999).

Because UV irradiation results in over-expression of TP53 and TP21$^{Waf1/Cip1}$ proteins in human and mouse cells *in vitro* and *in vivo*, these two proteins have been used as indicators of DNA damage in studies of photoprotection by sunscreens. Application of 0.2 ml/cm^2 of a sunscreen containing ethylhexyl methoxycinnamate and benzophenone-3 to human skin 15 min before irradiation with Supavasun 3000, a broad-band UVR source, nearly eliminated UVR-induced expression of TP53 and TP21$^{Waf1/Cip1}$ proteins (Pontén *et al.*, 1995; see also section 4.1.5 (b)). Similarly, application of an SPF-25 sunscreen containing 18% TiO_2 to human skin before irradiation with UVB (300 nm) from a monochromatic light source prevented induction of TP21$^{Waf1/Cip1}$ protein (El-Deiry *et al.*, 1995). Repeated application of a sunscreen containing benzophenone-3, butyl methoxydibenzoylmethane and ethylhexyl methoxycinnamate to human skin before each exposure to natural sunlight for 5–9 weeks caused a 33% reduction in the number of TP53-positive cells. Surprisingly, however, a near total barrier, blue denim fabric with an SPF of 1700, resulted in only a 66% reduction in the number of TP53-positive cells in chronically sun-exposed human skin (Berne *et al.*, 1998). The protection afforded by two sunscreens with identical SPF but different UVA protection factors was compared by measuring nuclear accumulation of TP53 protein in human skin biopsy samples after chronic UV irradiation with a solar simulator. The two sunscreens only partially decreased the number of TP53-positive cells. The two sunscreens with different UVA protection factors, as determined by the persistent pigment darkening method, provided different levels of photoprotection against nuclear TP53 accumulation. Because TP53 and TP21$^{Waf1/Cip1}$ proteins are induced after DNA damage, it is reasonable to conclude that sunscreens prevent DNA damage and thereby prevent induction of TP53 and TP21$^{Waf1/Cip1}$ (Seité *et al.*, 2000a).

A study was conducted to investigate whether sunscreens with different SPFs can protect against UVR-induced *p53* expression. Application of sunscreens with SPFs of 8, 30 or 40 to the buttock skin of 12 volunteers 15 min before irradiation with 3 MED of UVR from a solar simulator caused a decrease in the number of TP53-positive cells and a decrease in the intensity of immunostaining for TP53 when compared with that seen in the buttock skin of volunteers irradiated with 1 MED of UVR (Krekels, *et al.*, 2000).

Reduction of UVR-induced p53 mutations

Analyses of human skin cancers and UVR-induced mouse skin cancers for *p53* mutations have provided new insights into the molecular mechanisms by which UVR induces skin cancer. Both human and mouse UVR-induced skin cancers harbour unique mutations (C→T and CC→TT transitions) in *p53* at a high frequency (50–100%) (Brash *et al.*, 1991; Kress *et al.*, 1992; Kanjilal *et al.*, 1993; Ziegler *et al.*, 1993; Dumaz *et al.*, 1997; Ananthaswamy *et al.*, 1998). Mutations in the *p53* gene have been shown to precede the appearance of skin cancer. For example, *p53* mutations have been detected in sun-exposed skin from healthy volunteers and from skin cancer patients, and can serve as an indicator of prior solar exposure (Nakazawa *et al.*, 1994; Kanjilal *et al.*, 1995; Urano *et al.*, 1995; Ouhtit *et al.*, 1997). Furthermore, UVR-specific *p53* mutations were found in actinic keratoses (Nelson *et al.*, 1994; Ziegler *et al.*, 1994; Ren *et al.* (1996). Jonason *et*

al. (1996) demonstrated that whole, mounted preparations of human skin contained clonal patches of keratinocytes with mutated TP53.

Experiments to determine the timing of *p53* mutation in relation to skin cancer development have been performed in the mouse model of photocarcinogenesis. The presence of mutant *p53*-positive clusters was reported in UVB-irradiated mouse skin well before the appearance of skin tumours (Berg *et al.*, 1996). Similarly, *p53* mutations in UVB-irradiated C3H mouse skin were detected as early as week 4 of chronic UVR irradiation, and the frequency of *p53* mutations increased progressively, reaching 50% at week 12 (Ananthaswamy *et al.*, 1997).

The finding that *p53* mutations arise early during UVR-induced skin carcinogenesis suggests that they might be used as an early biological marker of the efficacy of sunscreens in photoprotection. Several organic SPF-15 sunscreens containing either UVB absorbers (10% octocrylene and 2% phenylbenzimidazole sulfonic acid) or UVB plus UVA absorbers (9% octocrylene, 0.3% phenyl-benzimidazole sulfonic acid, 3% butyl methoxydibenzoylmethane and 0.7% terephthalylidene dicamphor sulfonic acid) have been shown to protect C3H mouse skin against *p53* mutations induced by UVB plus UVA from Kodacel-filtered FS40 sunlamps or a solar simulator (Ananthaswamy *et al.*, 1997, 1999). By using a highly sensitive technique of allele-specific polymerase chain reaction, these investigators demonstrated that application of UVB or UVB plus UVA sunscreens onto the shaved dorsal skin of C3H mice 30 min before each exposure to radiation for 12–16 weeks resulted in a 80–100% reduction in CC→TT *p53* mutations when compared with the frequency in unprotected mouse skin.

The reduction of *p53* mutations and skin cancer induction by sunscreens can be attributed to their ability to protect the skin against UVR-induced DNA damage. The UVB sunscreens used in studies of Ananthaswamy *et al.* (1997, 1999) appeared to be as effective as the UVB plus UVA sunscreens in inhibiting *p53* mutation. This finding is not unexpected because only UVB-induced mutations (CC→TT) were assayed; UVA-induced mutations, which are predominantly G→T transversions, were not assessed (Drobetsky *et al.*, 1995; Sage *et al.*, 1996). However, it is unlikely that UVA-type mutations play a role in UVR-induced skin carcinogenesis because they are seldom present in human or mouse UVR-induced skin cancers. In support of this contention, it was shown that even the mouse skin tumours induced by massive doses of UVA did not contain G→T transversions in the *p53* gene (van Kranen *et al.*, 1997). UVA therefore probably plays a minor role in the initiation of squamous-cell carcinoma.

Protection against p53 mutations in skin cancers arising in routine users of sunscreens

Although numerous epidemiological studies have been performed to assess the protective effects of sunscreens against melanoma and other skin cancers, none have addressed the mechanistic aspects. However, a recent study addressed the important question of whether basal-cell carcinomas arising in routine users of sunscreens are similar to or different from those arising in non-users (Rosenstein *et al.*, 1999). The findings suggest that the commercial sunscreens tested were quite effective in preventing UVB-induced mutations in basal-cell carcinomas.

Inhibition of UVR-induced non-melanoma skin cancer in mice

In several studies in mice, almost all the sunscreens tested protected against UVR-induced skin cancer (Kligman *et al.*, 1980; Wulf *et al.*, 1982; Forbes *et al.*, 1989; Flindt-Hansen *et al.* 1990a,b; Fourtanier, 1996). It was also shown *in vitro* that UVR-irradiated PABA solution was still effective in protecting mice against UVR-induced skin cancer (Flindt-Hansen *et al.*, 1989). This suggests that, although irradiated PABA solution containing degradative photoproducts can enhance pyrimidine dimer formation and is potentially mutagenic, it can still protect mice against UVR-induced skin cancer. A second implication of this study is that the activity of a sunscreen *in vitro* has little relevance *in vivo*, and caution should be exercised in extrapolating data obtained *in vitro* to the situation *in vivo*.

Nonetheless, sunscreen formulations containing UVB absorbers or UVB plus UVA absorbers with SPFs of 15–22 were effective in protecting mice against skin cancers induced by a solar simulator. In this study, 100% of mice that received a cumulative dose of 1000 kJ/m^2 of UVB only or vehicle plus UVB developed skin tumours, whereas the probability of tumour development was 2% in mice treated with the sunscreens and 1000 kJ/m^2 of UVB and 15% in mice treated with sunscreens plus 1500 kJ/m^2 of UVB. The sunscreen formulations containing only UVB absorbers were as effective as those containing both UVB and UVA absorbers in inhibiting UVR-induced *p53* mutations and skin cancer. This suggests that, under the experimental conditions used, attenuating UVA with sunscreens containing UVA absorbers does not provide a detectable increase in photoprotection against *p53* mutations or skin cancer above that provided by sunscreen formulations containing only UVB absorbers. The added protective effect of UVA sunscreens is difficult to estimate, however, because of the small numbers of animals affected. These results suggest that the mutagenic and carcinogenic effects of solar-simulated UVR are due mainly to UVB and not UVA wavelengths. The sunscreens used in this study protected the mice against not only UVR-induced *p53* mutations but also skin cancers. The authors therefore

concluded that inhibition of *p53* mutations is a useful early marker of photoprotection against an important initiating event in UVR-induced carcinogenesis (Ananthaswamy *et al.*, 1999). Additional markers of promotional events in the multistep process of photocarcinogenesis are needed to assess the role of sunscreens in protecting against skin cancer induction.

Protection against UVR-induced immune suppression

UVR is known to suppress various types of immune response (see section 4.2.2 (b)). In particular, irradiation before immunization suppresses the induction of contact hypersensitivity (Noonan *et al.*, 1981) and delayed-type hypersensitivity responses (Ullrich, 1986). UVR-induced DNA damage has been shown to play a role in some types of immune suppression (Applegate *et al.*, 1989; Kripke *et al.*, 1992; Vink *et al.*, 1996, 1997). In studies of the effects of enhanced repair of UVR-induced pyrimidine dimers on UVR-induced immune suppression, it was found that topical application of liposomes containing T4N5 endonuclease or DNA photolyase to mouse skin following UV irradiation abrogated UVR-induced suppression of contact hypersensitivity (Kripke *et al.*, 1992; Vink *et al.*, 1996, 1997). In addition to DNA damage, UVR can also isomerize urocanic acid present in the stratum corneum from the native *trans* to the *cis* form, which in turn causes immune suppression in mice (De Fabo & Noonan, 1983; Noonan & De Fabo, 1992). However, although sunscreens can block UV-induced isomerization of *trans-* to *the cis*-urocanic acid, there was no apparent correlation between the formation of *cis*-urocanic acid and suppression of the induction of contact hypersensitivity (Reeve *et al.*, 1994).

Most published results suggest that sunscreens do afford protection against UVR-induced immune suppression. Some of the early failures to demonstrate such protection can be attributed to use of non-solar UVR sources (Gurish *et al.*, 1981; Lynch *et al.*, 1981) or use of a sunscreen designed to absorb one wavelength of light (e.g. UVB) when immune suppression was induced by a different wavelength (e.g. UVA; Hersey *et al.*, 1988). Roberts *et al.* (1996) found that considerable immune suppressive energy was contained in wavelengths below 295 nm emitted from an FS40 sunlamp. Transmission of 'non-solar' UVR (UVC and short-wave UVB) through a sunscreen was 15 times greater when the sunlamp was used than when a solar-simulated source was employed. These findings illustrate the limitations of using non-solar UVR sources to determine the efficacy of a sunscreen.

A second issue is the immunological end-point chosen and the wavelengths of UVR responsible for suppressing that end-point. Roberts *et al.* (1996) found that UVB-absorbing sunscreens were effective in preventing contact hypersensitivity induced by solar-simulated UVR. Sunscreens were reported to be ineffective in protecting against UVR-induced inhibition of NK cell function in humans (Hersey *et al.*, 1987). This may have been due to the design of the study (see p. 90), or the wavelengths involved in suppressing NK function may lie within the UVA region of the solar spectrum (Hersey *et al.*, 1988). Since the formulation used in this study absorbs primarily UVB and would not have provided substantial protection against UVA, it is not surprising that it did not block immune suppression. The absorptive qualities of the sunscreen being tested, the UVR wavelengths responsible for the biological effect measured and the spectral output of the artificial UVR source used in any particular study are important in determining the efficacy of a sunscreen.

As exposure to UVR before immunization suppresses the induction of contact hypersensitivity, many investigators have examined the protective effect of sunscreens against these reactions. Although some failed to find any protection (Fisher *et al.*, 1989; Ho *et al.*, 1992), in most studies sunscreens protected against immune suppression. The degree of protection, however, varied greatly. While some authors reported total protection (Reeve *et al.*, 1991; Wolf *et al.*, 1993b; Beasley *et al.*, 1996; Roberts *et al.*, 1996; Roberts & Beasley, 1997b), others found that the degree of immune protection was less than the degree of protection against erythema and/or oedema (Wolf *et al.*, 1993a; Bestak *et al.*, 1995; Whitmore & Morison, 1995; Hayag *et al.*, 1997; see pp. 87 and 102). The reasons for these discrepancies are not entirely clear. As solar-simulated light was not used in all the studies mentioned above, the complication of irrelevant wavebands and incomplete absorption of UVC and short-wave UVB may be a contributing factor. Walker and Young (1997) compared protection from oedema and suppression of contact hypersensitivity induced by monochromatic UVB, in order to eliminate confounding due to differences in action spectra for these end-points. Under these conditions, the protection factors for ethylhexyl methoxycinnamate were the same but the mice were more sensitive to immune suppression than to the induction of oedema. Similar results were obtained when sunscreens were applied to the mice and on Transpore tape above the mice. These data suggest that reports of lack of immune protection are not due to interactions between sunscreens and the skin.

Bestak *et al.* (1995) found better protection with broad-spectrum sunscreens (UVA plus UVB absorbers). Roberts and Beasley (1997b) reported that the SPF was equal to or exceeded the immune protection factor when a minimal immune suppressive dose (i.e. the amount of UVR required to induce 50% immune suppression) calculated from UVR dose–response curves (with solar-simulated light) was used in studies of protection by sunscreens. Fourtanier *et al.* (2000) conducted a study in mice to

compare two sunscreens with the same SPF but different levels of UVA protection. The sunscreen with the higher UVA protection factor provided better protection against suppression of induction of contact hypersensitivity, but the immune protection factor was lower than the SPF. Three methods for the determination of the immune protection factor gave comparable results. Despite the reported discrepancies in the degree of immune protection, sunscreens do appear to afford protection against immune suppression of contact hypersensitivity and delayed-type hypersensitivity to varying degrees.

Effect on the immune response to recall antigens or contact hypersensitivity in humans

In the studies summarized above, normal mice or unsensitized volunteers were exposed to UVR and then immunized with an antigen or a contact allergen. Measurement of the immune response to recall antigens such as diphtheria, tetanus toxoid and tuberculin—antigens that most people encounter during childhood immunizations—offers a unique advantage in that no active immunization is required. Rather, the effect of exposure to UVR on the elicitation of the delayed-type hypersensitivity reaction is measured. Using a UVB-absorbing sunscreen, Hersey *et al.* (1987) found no protection against UVR-induced immune suppression of delayed-type hypersensitivity to recall antigens. Immune protection was observed, however, in two studies with broad-spectrum sunscreens (Moyal *et al.*, 1997; Moyal, 1998; see p. 87). The degree of immune protection to recall antigens may depend on the total UVR-absorbing properties of the sunscreen. Volunteers exposed chronically to natural sunlight were treated with two sunscreen preparations, one with a SPF of 15 and a UVA protection factor of 6 and the second with a SPF of 30 and a UVA protection factor of 12. While both blocked sunlight-induced erythema, only the SPF-30 sunscreen blocked immune suppression (Moyal, 1998).

The effect of sunscreen application on UVR-induced suppression of the elicitation of contact hypersensitivity to dinitrochlorobenzene or nickel has been examined. High-SPF sunscreens protected against UVB-induced suppression of contact hypersensitivity (Whitmore & Morison, 1995; Hayag *et al.*, 1997). Damian *et al.* (1997) found that application of a broad-spectrum sunscreen protected against immune suppression; no protection was seen with a UVB-absorbing sunscreen alone, but addition of a UVA absorber to the sunscreen preparation made it effective. A similar result was obtained by Serre *et al.* (1997), who examined UVR-induced (solar-simulated light) suppression of induction of contact hypersensitivity in humans. Complete protection was achieved only when the sunscreen formulation absorbed both UVB and UVA. Nonetheless, Damian *et al.* (1999) generated linear dose–response curves for suppression of nickel contact hypersensitivity and demonstrated that the immune protection factor of a sunscreen can be determined in humans *in vivo*.

Effect on UVR-induced suppression of tumour rejection

UVR-induced murine skin tumours are highly antigenic and are immunologically rejected when transplanted into normal mice (Kripke, 1974). Exposure of mice to a sub-carcinogenic dose of UVR suppresses tumour rejection and allows the antigenic tumours to grow progressively (Kripke & Fisher, 1976; Fisher & Kripke, 1977). A series of studies was performed in mice to determine whether sunscreens can protect mice against the UVR-induced suppression of skin cancer rejection. Most of the studies showed sunscreens to be effective (Morison & Kelley, 1985; Reeve *et al.*, 1991; Roberts & Beasley, 1997a), although two exceptions were noted (Gurish *et al.*, 1981; Morison, 1984).

In studies of the effect of sunscreens on the growth of transplanted melanoma cells in UVR-irradiated mouse skin, Wolf *et al.* (1994) took advantage of the fact that irradiation of the skin promotes the growth of melanoma cells transplanted into the irradiated site and is an immunologically mediated phenomenon (Donawho & Kripke, 1991). Although sunscreen preparations containing UVB only or both UVB and UVA absorbers blocked UVR-induced inflammation and the accumulation of sunburn cells in the skin, they failed to prevent the UVR-induced enhancement of melanoma growth. These results may have a number of explanations. The design of the study may not have been suitable for detecting a protective effect, as the dose–response relationship was not analysed (see p. 112). As unfiltered FS40 lamps were used as a source of UVR, the failure of the sunscreens to absorb UVC and incomplete absorption of short-wave UVB may play a role. The mechanism by which UVR enhances melanoma growth is not entirely clear, but it has been suggested (Donawho *et al.*, 1998) that UVR-induced photoisomerization of urocanic acid is involved. Because wavelengths in the UVA region of the solar spectrum efficiently convert *trans*-urocanic acid into the *cis* isoform (Gibbs *et al.*, 1993), use of a sunscreen with a higher absorbancy of UVA could block UVR-enhanced melanoma growth, as in the situation described above (Moyal, 1998).

Other immunological end- points

Other immunological end-points have been used to determine the efficacy of sunscreens. Studies have addressed the ability of sunscreens to block the depletion of epidermal Langerhans cells (Miyagi *et al.*, 1994; Walker *et al.*, 1994; Hayag *et al.*, 1997; Neale *et al.*, 1997; Beasley *et al.*, 1998; Hochberg & Enk, 1999), UVR-induced suppression of

allostimulatory activity in the mixed epidermal cell–lymphocyte reaction (van Praag *et al.*, 1991; Walker *et al.*, 1994; Davenport *et al.*, 1997; Hurks *et al.*, 1997), depression of antigen-presenting cells function in a conventional mixed lymphocyte reaction *in vitro* (Mommaas *et al.*, 1990), induction of IL-10 in mouse serum (Guéniche & Fourtanier, 1997) and in human skin (Hochberg & Enk, 1999) or suppression of NK function (Hersey *et al.*, 1987, 1988). For the most part, sunscreen application has been shown to afford some degree of protection. The most notable exception is the inability of sunscreens to block UVR-induced suppression of NK function, but, as mentioned above, this phenomenon is UVA-dependent and the sunscreens available when this study was performed were UVB absorbers.

Possible reasons for differences between the immune protection factor and SPF

The studies summarized above show a general lack of agreement between the immune protection factor and SPF. The possible reasons for this lack of agreement are:

- Use of non-solar sources, especially those containing UVC;
- Lack of assessment of the SPF (or erythemal protection factor) with the experimental source and in the same experimental model, and therefore reliance on the labelled SPF;
- Lack of dose–response relationships for immune suppression and inflammation (in humans and mice), and drawing of conclusions for various end-points from 'arbitrary' doses of UVR without reference to dose–response curves;
- Possible differences in action spectra for erythema and immune suppression;
- Comparison of an acute end-point (SPF) with the results of studies of repeated exposure;
- Lack of standard protocols for determining the immune protection factor.