Chapter 7
Carcinogenicity

Human studies

Cohort and case–control studies relevant to evaluating associations between sunscreen use and the prevention of human cancer are summarized on pp. 69–85. The results with respect to carcinogenicity are summarized below.

Fifteen case–control studies were available to evaluate the association between sunscreen use and cutaneous melanoma. Eight of these studies showed statistically significantly higher risks for cutaneous melanoma among users of sunscreens than among non-users, the relative risks for the higher categories of use ranging up to 2.6. When adjustment was made in five studies for exposure and sensitivity to the sun, the relative risk fell in two studies and changed little in three. However, none of the adjusted relative risks fell much, if at all, below 1.0. Four of the case–control studies provided little evidence of an effect of sunscreen use on the risk for cutaneous melanoma among all subjects, and three showed statistically significantly lower risks in users of sunscreens than in non-users.

Most of these studies could be taken to support a positive association between sunscreen use and risk for cutaneous melanoma (Fig. 42); however, it was not possible exclude positive confounding between sunscreen use and sun exposure, sun sensitivity and a history of sun-related neoplasia, or negative confounding with other sun-protective behaviour such as use of protective clothing, wearing a hat or staying in the shade as an explanation for this finding. In none of the analyses was adjustment made for measures of history of sun-related neoplasia or other sun protective behaviour. When sun exposure and sun sensitivity were measured and controlled in the analysis, the control was judged to be insufficient to permit full control of confounding.

Two of the case–control studies of cutaneous melanoma that showed significantly increased risks among sunscreen users, however, provide evidence that use of sunscreens in relation to recreational sun exposure might be associated with a particularly high risk for cutaneous melanoma in subgroups of subjects. Autier *et al.* (1995) found that, when the risk for cutaneous melanoma was related to sunbathing during the hottest hours of the day, use of sunscreen and hair colour, the relative risk of those with blonde or red hair who sunbathed during the middle of the day and used sunscreens was 6.3 (95% CI, 1.7–23) with reference to those with black or brown hair who neither sunbathed during the hottest hours nor used sunscreens. These results were consistent with an additive interaction between sunbathing in the hottest hours of the day and use of sunscreens. Similarly, Westerdahl *et al.* (2000) reported a relative risk for cutaneous melanoma of 8.7 (95% CI, 1.0–76) for users of sunscreens compared with non-users when the users stated that they used sunscreens to be able to spend more time in the sun. In addition, although Elwood and Gallagher (1999) did not find an overall effect of sunscreens on the risk for cutaneous melanoma (RR, 1.1; 95% CI, 0.75–1.6), the risk was increased for people who used sunscreens 'only in the first few hours' of solar exposure (RR, 1.6; 95% CI, 1.0–2.5). Two of these studies thus suggest that use of sunscreens leads to spending more time in the sun, particularly during sunbathing, which may increase the risk for cutaneous melanoma.

One cohort study and one case–control study showed increased risks for

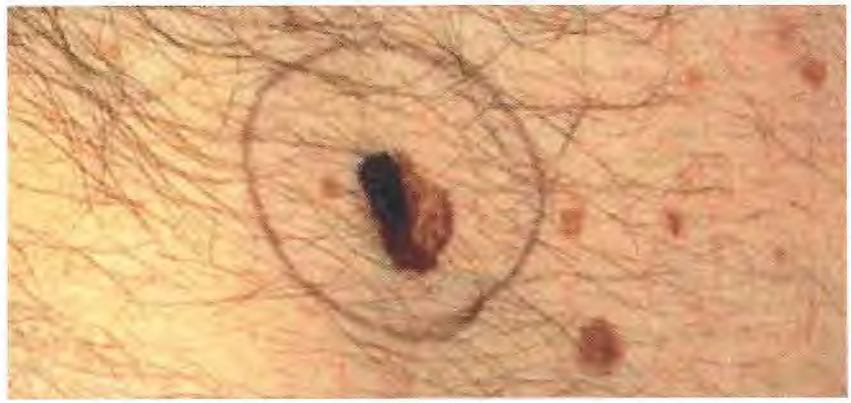


Figure 42 Melanoma of the trunk

basal-cell carcinoma in sunscreen users. No significant association between sunscreen use and cancer risk was observed in one cohort and one case–control study of squamous-cell carcinoma, one of squamous- and basal-cell carcinoma of the skin or one case–control study of squamous-cell carcinoma of the vermilion border of the lip. The same difficulties in control of confounding of sun sensitivity and exposure were present in these studies as in the case-control studies of cutaneous melanoma.

In seeking to explain the increased incidences of cutaneous melanoma and non-melanocytic skin cancer seen in most white populations, Garland *et al.* (1992, 1993) suggested that the advent of effective UVB sunscreens in the 1960s allowed individuals to remain outdoors in sunny climates for long periods without sunburn. Since these sunscreens did not, however, absorb UVA to any great extent, individuals who used them were probably exposed to substantially more UVA than before. While this is a theoretical possibility, there is no direct support for it in the epidemiological studies.

A further hypothesis was advanced, implicating sunscreens in the increasing incidence of cutaneous melanoma in white populations. It was suggested that sunscreens interfere with the role of UVB in the photosynthesis of vitamin D in human skin (Garland *et al.*, 1990). As 1,25-dihydroxyvitamin D has been shown to inhibit the growth of cutaneous melanoma cells *in vitro* (Frampton *et al.*, 1983), lowered serum levels of this hormone might place sunscreen users at higher risk for this cancer. Weinstock *et al.* (1992), however, found no difference in vitamin D intake from dietary sources among 165 cutaneous melanoma patients and 209 controls, suggesting that vitamin D intake is not associated with the incidence of this cancer. This study does not, however, address the issue of vitamin D synthesis in the skin as a result of exposure to UVR.

Several reviews (Ainsleigh, 1993; Studzinski & Moore, 1995) have suggested that sunlight may protect against a number of cancers, including those of the colon (Garland & Garland, 1980; Garland *et al.*, 1989), prostate (Hanchette & Schwartz, 1992; Gann *et al.*, 1996) and breast (Gorham *et al.*, 1990; Janowsky *et al.*, 1997; John *et al.*, 1999). Most of these studies are descriptive or, if analytical, involved relatively few subjects. In addition, control for confounding in the analytical studies was incomplete.

At present, there is no convincing evidence that broad-spectrum sunscreens increase the risk for cutaneous or internal cancers by decreasing the levels of vitamin D.

Experimental models

Assessment of the carcinogenic risk of sunscreens in experimental animals requires long-term experiments with appropriate models, depending on whether internal organs are targeted or whether the risk is limited to a particular type of skin cancer. At present, the only adequate models for this purpose appear to be for squamous-cell carcinoma.

Carcinogenicity of UVR filters

Because some batches of ethylhexyl methoxycinnamate were found to cause reverse mutation in bacteria (see p. 137), Gallagher *et al.* (1984) assessed the carcinogenicity of mutagenic and non-mutagenic samples. The design of the study and the results with regard to protection are described on p. 93. In the various groups of 20–22 HRA/Skh-1 hairless mice, skin tumours developed in only 4/146 (2.7%) surviving protected mice but in 40–100% of unprotected mice, confirming the protective effect of ethylhexyl methoxycinnamate against UVR-induced skin carcinomas. In order to detect latent tumour initiation, the tumour promoter croton oil was applied to the dorsal skin of sunscreen-treated mice, sunscreen-treated UVR-irradiated mice and untreated mice. While croton oil alone did not promote skin tumours in control mice, ethylhexyl methoxycinnamate may have initiated the skin tumours in 3/16 surviving unirradiated mice. No distinction could be made between the 'mutagenic' and the 'non-mutagenic' samples, since the numbers of mice were too small. Application of croton oil revealed latent skin tumours in 15–46% of mice previously exposed to UVR through ethylhexyl methoxycinnamate, but the two preparations did not differ significantly.

In a follow-up study (Forbes *et al.*, 1989; see also p. 94), the protective effect of both the mutagenic and the non-mutagenic ethylhexyl methoxycinnamate was found to be dose-related, but consistently more tumours appeared in mice given the mutagenic preparation. Total protection was obtained with 7.5% non-mutagenic and 50% mutagenic samples. The reason for the reduced protection by the mutagenic sample was not apparent. Some of the skin tumours in unirradiated mice were found to be initiated after promotion with TPA. Whereas the prevalence of skin tumours in control mice treated only with TPA was 8.9%, the mutagenic sample of 7.5% ethylhexyl methoxycinnamate induced four times more tumours (17% prevalence) than the non-mutagenic sample (4.2%). With 50% ethylhexyl methoxycinnamate, however, only 4.2 and 4.3% of the mice responded to each sample, respectively. This experiment therefore provides inconsistent results with regard to the enhancement of carcinogenicity by this agent. [The Working Group noted that the SPF values of the two samples were not measured; a difference might have accounted for the difference in the effective UVR initiating doses.]

A working group convened by IARC (IARC, 1989) evaluated studies of the carcinogenicity of TiO_2 administered to rodents orally, subcutaneously, intraperitoneally, intratracheally or by inhalation. The group concluded that there was *limited evidence* of carcinogenicity in experimental animals exposed to high

doses by inhalation (lung tumours) or to 90 mg given as five weekly intraperitoneal injections (abdominal tumours). Studies of the co-carcinogenicity of TiO_2 in hamsters showed that intratracheal administration of 3 mg weekly for 15 weeks augmented the numbers of benzo[*a*]pyrene-induced benign and malignant tumours of the larynx (papilloma and squamous-cell carcinoma), trachea (papilloma, squamous-cell carcinoma and adenocarcinoma) and lungs (adenoma, adenocarcinoma, squamous-cell carcinoma and anaplastic carcinoma).

Photocarcinogenicity of UVR filters

Relatively little information is available in the open scientific literature on possible enhancement of the carcinogenicity of UVR by sunscreens. Most sunscreen products have been tested primarily to assess their protective effect against the induction of squamous-cell carcinoma when applied before exposure to UVR. All the published studies (see p. 90) showed that UVR-induced skin tumour formation in rodents is inhibited by topical treatment with individual or combinations of sunscreens (Gasparro *et al.*, 1998).

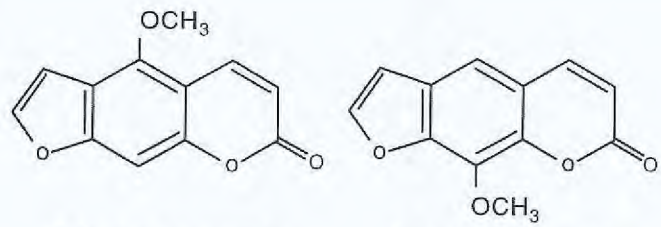
In studies designed to test the long-term safety of these products, the source of UVR used was a solar simulator and the sunscreen was applied after exposure to see whether it had a negative effect on the skin's protective responses to UV irradiation (Sambuco *et al.*, 1991). Terephthalylidene dicamphor sulfonic acid and ethylhexyl methoxycinnamate did not enhance the carcinogenicity of UVR (Fourtanier, 1996). The study was not designed to ascertain protection factors, but a protection factor was calculated as the ratio of the dose required to attain a defined tumour response with the dose that is required to attain the same response under 'protected' conditions. It was calculated that a 5% preparation of ethylhexyl methoxycinnamate offered a protection factor of only 1.3, and the 5 and 10% preparations of terephthalylidene dicamphor sulfonic acid had a protection factor of 2.4. These low factors and the lack of difference between the 5 and 10% preparations may be explained by the design of the study, which limits the ability to detect small differences in protection. To avoid acute or subchronic damage in animals, only small doses of UVR were given through the sunscreens, making it difficult to detect a dose–effect relationship with two concentrations of the same UVR absorber with a difference of no more than 1.5 in the SPF or protection factor.

Carcinogenicity of sunscreens containing photosensitizing tanning agents

The photocarcinogenicity mediated by the furocoumarins has been well documented (IARC, 1987), and there is sufficient evidence that 8- and 5-methoxypsoralen increase the carcinogenic effects of UVA in mouse skin (Fig. 43). Addition of 5-methoxypsoralen to a sunscreen was intended to provide a rapid tan by UVA-driven photosensitization, while the sunscreen offered protection against UVB damage. It was thought that the acquired tan would subsequently protect the skin against solar UVR.

Two studies have addressed the interaction of 5-methoxypsoralen and sunscreens in mice. The protocols and the effects of the sunscreens alone are described on p. 94, whereas the effects of 5-methoxypsoralen are described here.

Both 5- and 8-methoxypsoralen have phototumorigenic potential in hairless mice (Young *et al.*, 1983). A study was conducted in groups of 20 male and 20 female hairless albino mice (outbred St John's strain) to test the capacity of a sunscreen which contained two UVB absorbers, 12.5 μL/mL (1.25%) ethylhexyl methoxycinnamate and 10 mg/mL (1%) 3-benzylidene camphor, with and without 5-methoxypsoralen at 25 or 50 μg/mL, to protect against photocarcinogenesis. The effect of 5-methoxypsoralen alone was tested in irradiated and unirradiated mice. The treatments were continued on 5 days/week for 44–46 weeks. Tumour growth was monitored, the tumours were classified histologically, and the data were analysed statistically. 5-Methoxypsoralen was clearly carcinogenic at both doses, and the sunscreen gave protection against the tumorigenic effect up to week 40. After that time, however, the mean tumour multiplicity was increased by 25 μg/mL 5-methoxypsoralen in the presence of the sunscreen, from 0.81 to 4.5 tumours per mouse, and the number at 60 weeks (4.5) was similar to that in mice irradiated through the vehicle (6.0). This was significantly greater than the 1.09 tumours per mouse found in sunscreen-protected mice. Thus, solar-simulated UVR-induced photocarcinogenesis was clearly inhibited by the sunscreen alone, and the sunscreen was found to suppress the increased risk incurred by addition of 5-methoxypsoralen to the preparation during the first 40 weeks of treatment. The later increase in tumour incidence due to 5-methoxypsoralen after



5-Methoxypsoralen 8-Methoxypsoralen

Figure 43 Formulae of 5-methoxypsoralen and 8-methoxypsoralen

irradiation was not, however, prevented by the sunscreen (Young *et al.*, 1987).

In a further study, Young *et al.* (1990) treated groups of 30 female Skh-1 hairless albino mice with 100 μL of solutions containing 5-methoxypsoralen at 5, 15 or 50 μg/mL from bergamot oil, with and without added sunscreen consisting of the UVB absorber ethylhexyl methoxycinnamate at 0.5% and the UVA absorber butyl methoxydibenzoylmethane at 0.5% [SPF not determined]. Similar concentrations of 5-methoxypsoralen are found in cosmetics and perfumes. As described on p. 96, the mice were irradiated with solar-simulated UVR 20–40 min after the topical applications on 5 days/week for up to 73 weeks, the duration of treatment being determined by the severity of the tumour response. One group also received solar-simulated UVR 5–6 h after topical application, and a further group was treated and irradiated only every other 4-week period. The protection afforded by the sunscreen alone is described on p. 96. Treatment with 5-methoxy-psoralen and solar-simulated UVR did not induce tumours up to 57 weeks. Subsequently, an increase in the concentration of 5-methoxypsoralen decreased the average time to tumour onset from 62 weeks to 58, 49 and 28 weeks with 5, 15 and 50 μg/mL 5-methoxypsoralen, respectively. Irradiation 5–6 h after topical application of 50 μg/mL 5-methoxy-psoralen alone significantly reduced the time to tumour onset from 65 to 58 weeks. The intermittent treatments also appeared to be protective, but not when analysed in relation to the cumulative dose of solar-simulated UVR, in which case photocarcinogenesis with 50 μg/mL 5-methoxypsoralen alone was enhanced. This indicates that the 4-week 'rest' period did not result in repair of the damage caused by 5-methoxypsoralen and solar-simulated UVR.

As albino mice were used in these two studies, the results are indicative of the cancer risk in the absence of pigmentation. To assess whether psoralen-enhanced pigmentation would provide additional protection against photocarcinogenesis, experiments were conducted with the furocoumarin 6,6,4′-trimethylangelicin instead of 5-methoxypsoralen in pigmented female Skh hairless mice (HRA.HRII-c/+/Skh) (Kipp *et al.*, 1998). 6,4,4′-Trimethylangelicin is more powerful in inducing pigmentation than 5-methoxypsoralen and causes mono-adducts in DNA but considerably fewer cross-links than 5-methoxypsoralen. The authors concluded that epidermal tanning with or without furocoumarin is not effective in preventing skin cancer in this mouse model.