3. Studies of Cancer in Animals

3.1 Experimental conventions

3.1.1 *Species studied*

The experimental induction of skin cancers in mice following exposure to a mercury-arc lamp was first reported by Findlay (1928). Initially, haired albino mice were used, but hairless and nude mice are now preferred.

An important development was the use of the hairless mouse as a model (Winkelmann *et al.*, 1960, 1963). In haired animals, the fur provides effective protection of the skin against UVR. This limits investigations to sparsely haired skin regions, mainly the ears, as, in long-term experiments with frequent exposures, the mechanical trauma caused by shaving might influence the process of tumorigenesis. The skin of hairless mice differs, however, from human skin in many respects. It is, for instance, much thinner and has abnormal hair follicles. The hairless mouse does, however, have a thymus and a functioning immune system, in contrast to the nude mouse (Eaton *et al.*, 1978; Hoover *et al.*, 1987). Many recent studies on carcinogenesis induced by UVR used the hairless mouse model (Forbes *et al.*, 1981; de Gruijl *et al.*, 1983; Gallagher *et al.*, 1984b). The changing designations of 'Skh' mice are listed in Table 30. Skin tumorigenicity has been evaluated experimentally in only a relatively small number of species other than the mouse.

Table 30. Alternative designations used for 'Skh' outbred stocks of hairless mice

Phenotype	1970–86	After 1986	Synonyms used in the literature	Inbred strains derived from Skh:hr stock[a]
Albino[b]	Skh: hairless-1	Skh:hr I	Sk-1; Skh-1; Skh/Hr-1; Skh:HR; HRA/Skh-1; Skh-hr1	HRA/Skh (Temple University, Philadelphia, PA, USA)
Pigmented[c] (any colour)	Skh: hairless-2	Skh:hr II	Sk-2; Skh-2; Skh/Hr-2	HRA/Skh-1 (University of Sydney, Sydney, Australia)

[a]From Forbes *et al.* (1990)
[b]Forbes *et al.* (1981); de Gruijl *et al.* (1983)
[c]Davies & Forbes (1988)

3.1.2 *Wavelength ranges*

As noted in section 1.1, for the purposes of this monograph, the UV wavelength range is subdivided according to the convention of the Commission Internationale de l'Eclairage (1987) into: UVA (315–400 nm), UVB (280–315 nm) and UVC (100–280 nm). The UVB

range is generally found to be most effective in inducing skin cancer, i.e., tumorigenesis may be achieved with smaller doses of radiant exposure than with UVA and UVC. A complete discussion of wavelength ranges is given in section 1.1.

3.1.3 *Measured doses*

Many investigators of the carcinogenicity of UVR have reported the type of lamps they used, which are frequently broad-spectrum lamps, sometimes in combination with filters. When estimates of the doses of UVR administered are given, the measuring instrument is usually mentioned and the result is given in terms of irradiance or dose, with no further detail. Such information is of some value, especially for comparing the results of experiments in which the same type of lamps were used.

The action spectrum (see section 1) given in Figure 10 shows that the carcinogenic effectiveness of UVR in hairless mice changes steeply, even by orders of magnitude, over a wavelength range of 10 or 20 nm. This pattern indicates that irradiance must be spectrally specified in order to be meaningful, and not integrated into one value over a broad spectrum. One approach is to give irradiance weighted according to the action spectrum for UV carcinogenesis, but this is available only in provisional form (see Fig. 10 and discussion on pp. 46–47). Another approach is to provide data on erythemally weighted irradiance, since the action spectrum for erythema corresponds approximately to that for carcinogenesis (Forbes *et al.*, 1978). A simple, direct way of calculating this is to relate the doses administered to the minimal erythema dose or to the minimal oedemic dose for the animal being investigated. When investigators supplied such measures of effect, they are mentioned in the summaries below.

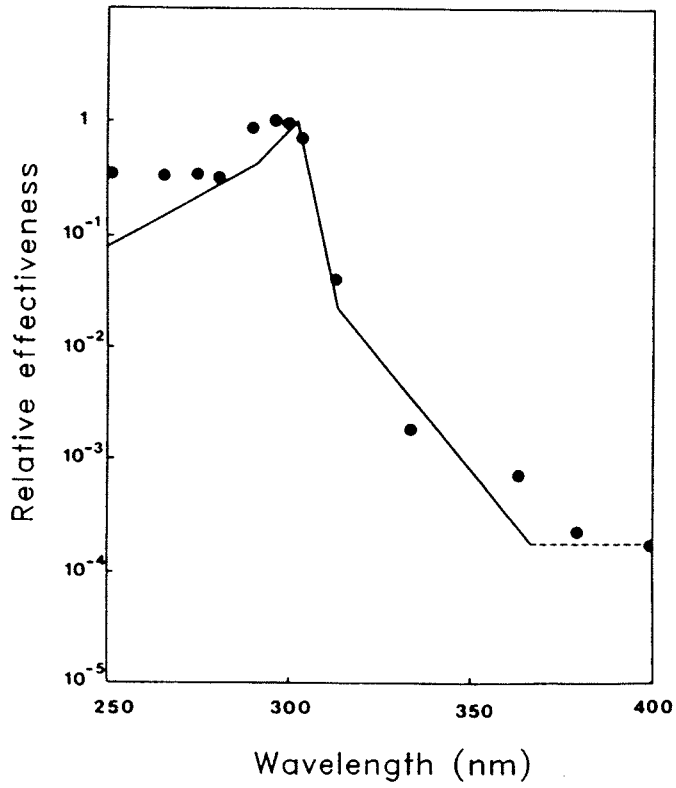
In experimental situations, there is never a perfectly sharp cut-off of wavelengths. The expression 'mainly UVA' is of questionable value, because even if UVB represents only 0.1% of the emission spectrum, it may still dominate the effect (see pp. 144–147, 151 and Fig. 10). Terms such as 'mainly UVB' are used below only when there are good reasons to assume that the effects considered are due mainly to UVB radiation.

3.1.4 *Protocols*

Experimental investigations on the carcinogenicity of UVR, conducted mostly on mice, have been reviewed (Blum, 1959; Urbach *et al.*, 1974; Kripke & Sass, 1978; WHO, 1979; van der Leun, 1984; Epstein, 1985).

Hundreds of studies have been reported. Most were not designed to test whether or not the radiation used was carcinogenic *per se* but to investigate the process of UV carcinogenesis. The methods used in these studies differ in many respects from those in standard lifetime studies to evaluate the carcinogenicity of chemicals. For example, many studies do not give complete details of the UVR emission spectrum used or exposure dose, do not enumerate all tumours, do not provide data on survival or do not provide histological details of tumours. Control groups are not always included; however, spontaneous skin tumours are rare in mice and rats. In many of the studies presented in detail below, appropriate statistical analyses have been done demonstrating clear dose-related trends in numbers of tumour-bearing animals, number of tumours per animal and/or median time to first tumour.

Fig. 10. Sterenborg–Slaper action spectrum for ultraviolet-induced skin carcinogenesis (1.0-mm tumours) in albino hairless mice. Effectiveness is defined as the reciprocal of the daily dose at each wavelength that leads to tumours of 1-mm diameter in 50% of animals in 265 days, relative to the corresponding value at the wavelength of maximal effectiveness. The effectiveness between 340 and 400 nm represents an average value for that wavelength range.



From van der Leun (1987a)

3.2 Broad-spectrum radiation

3.2.1 *Sunlight*

In one study by Roffo (1934), 600 rats [sex and strain unspecified] were exposed to solar radiation (sunlight) at a latitude of 35 °S in Buenos Aires, Argentina. The average exposure was for 5 h per day, with avoidance of the hours around solar noon in the summer. In the first days, 365 rats died from sunstroke. Of the 235 remaining animals, 165 (70%) developed tumours. There were 140 tumours of the ear (58% squamous-cell carcinomas; 36% spindle-cell sarcomas; 6% carcinosarcomas); 58 eye tumours (tumours of the conjunctiva, 100% spindle-cell sarcomas; tumours of the eyelid, 50% squamous-cell carcinomas and 50% spindle-cell sarcomas); and 15 other tumours, mainly squamous-cell carcinomas, at sites including the nose, tail, paw and neck. In complementary experiments reported in the same paper, groups of animals were exposed either to sunlight filtered through various colours of glass, to radiation from various types of lamp (quartz mercury, glass mercury, neon gas and

filament lamps) or to short Hertzian wavelengths. Tumours [types and sites unspecified] were observed in all 150 animals exposed to quartz mercury lamps; no tumour was induced in any other experimental group. On the basis of this evidence, the author concluded that the carcinogenicity of sunlight could be attributed to UVR.

In another report by Roffo (1939), 2000 white rats and mice [exact numbers unspecified] were exposed to sunlight for an average of 5 h per day. After three to six months, benign neoplasms and, after seven to nine months, malignant neoplasms of the skin of the ear (88% of all malignant tumours), the forepaw (7.25%), the tail (2%) and nose (one tumour) developed in 600 animals; 25% of the tumours were seen on the eyes. The ear tumours were diagnosed as squamous-cell carcinomas (58%), spindle-cell sarcomas (36%) and carcinosarcomas (6%) by detailed histological examination. Similarly, the paw tumours were diagnosed as squamous-cell carcinomas (42%) and spindle-cell sarcomas (58%); the tumours of the tail were all squamous-cell carcinomas. The distribution of tumours of the eye was similar to that in the study of Roffo (1934). [The Working Group considered that these are exceptional studies which fully document the carcinogenicity of solar radiation in rats and mice, even though quantitative detail is lacking. The resulting neoplasms are described and photographically illustrated in exact detail. The Working Group accepted the weight of evidence contained in these studies as to the carcinogenicity of solar radiation to rats and mice.]

Domestic and other animals of many species (cows, goats, sheep (reviewed by Emmett, 1973), cats (Dorn *et al.*, 1971) and dogs (Madewell *et al.*, 1981; Nikula *et al.*, 1992)) develop skin tumours, and there are good indications that sunlight is involved. The tumours described generally developed in sparsely haired, light-coloured skin. Cancers of the eye occur in many species, including dogs, horses, cats, sheep and swine, but are particularly frequent in cattle (Russell *et al.*, 1956).

3.2.2 *Solar-simulated radiation*

In several investigations on carcinogenesis by UVR, 'solar-simulated radiation' was used (Forbes *et al.*, 1982; Staberg *et al.*, 1983a; Young *et al.*, 1990; Menzies *et al.*, 1991). In one large, particularly informative experiment (Forbes *et al.*, 1982), more than 1000 hairless albino Skh-hr1 mice were exposed to solar-simulated radiation from a xenon arc lamp, with various filters to make the spectral distribution in the UV region similar to that of sunlight under various thicknesses of the ozone layer. The exposures lasted for up to 80 weeks. More than 90% of the mice developed skin tumours, predominantly squamous-cell carcinomas. The time to development of 50% of first tumours was shorter after exposure to the spectra that included higher irradiance in the wavelength range 290–300 nm. The other experiments mentioned were more limited and dealt with more specialized aspects of UV carcinogenesis.

3.2.3 *Sources emitting UVC, UVB and UVA radiation*

Sources emitting radiation in the entire UV wavelength range were used in experiments on UV carcinogenesis mainly between 1930 and 1960.

(*a*) *Mouse*

Grady *et al.* (1943) exposed 605 strain A mice to broad-spectrum UVR at a wide range of doses and irradiances (weekly doses, 3.6–43 $\times$ 10^7 ergs/cm^2 [40–430 kJ/m^2]; Blum &

Lippincott, 1942). The investigation dealt primarily with skin tumours (mainly spindle-cell sarcomas). About 5% of the mice developed tumours of the eye. Histological examination by Lippincott and Blum (1943) showed that the eye tumours arose mostly in the cornea and were spindle-cell sarcomas or fibrosarcomas; haemangioendotheliomas were also found.

A particularly large, informative series of investigations was carried out with unfiltered medium-pressure mercury arc lamps which emitted UVC, UVB and UVA (Blum, 1959). More than 600 strain A mice were irradiated (daily dose, 0.32–8.6 $\times$ 10^7 ergs/cm^2 [3–86 kJ/m^2]) in a series of investigations dealing with various aspects of UV carcinogenesis; the dose–effect relationship was addressed particularly. In most of the experiments, more than 90% of mice developed skin tumours, mainly of the ears, the only site for which quantitative data were given.

(*b*) *Rat*

Findlay (1930) exposed six epilated albino rats to broad-spectrum UVR from a mercury-vapour lamp at a distance of 18 in [46 cm] for 1 min three times a week. Rapidly growing papillomas were reported in one rat. The time required was, however, much longer than in mice exposed similarly, namely, 21 months as compared to eight months for mice.

Putschar and Holtz (1930) exposed 35 rats [strain unspecified] with very low spontaneous tumour incidence to almost continuous irradiation with broad-spectrum UVR from a quartz mercury lamp for 11 months. They reported regular occurrence of skin tumours, including papillomas, squamous-cell carcinomas and, occasionally, basal-cell carcinomas. The tumours were first seen after 27 weeks of exposure.

Huldschinsky (1933) exposed seven white rats to UVR from a solar lamp for 2 h per day, six days per week for one year or more. Another group of five rats was exposed to a quartz lamp emitting a predominantly UVC waveband ($<$ 270 nm). The doses given per session were about 10 times higher than those used in phototherapy. Spindle-cell sarcomas of the eye were found in 2/7 and 5/5 rats in each group, respectively.

Hueper (1942) reported squamous-cell carcinomas and, rarely, spindle-cell carcinomas and sarcomas, round-cell carcinomas and basal-cell carcinomas of the skin in 20 rats [strain unspecified] exposed for up to 10 months to broad-spectrum UVR from a mercury vapour burner (a Hanovia Super S Alpine lamp) at a distance of 75 cm.

In a study by Freeman and Knox (1964), a group of 78 rats (66 pigmented and 12 unpigmented) was exposed to broad-spectrum UVR from mercury lamps at 50 cm from the skin on five days a week for one year; the doses per session corresponded to approximately 1 MED for rat skin. A total of 98 eye tumours developed, with more tumours in pigmented rats. The tumours arose in the corneal stroma; two-thirds were diagnosed as fibrosarcomas and one-third as haemangioendotheliomas.

(*c*) *Hamster*

Hamsters exposed to an irradiation regimen similar to that described above also developed eye tumours (Freeman & Knox, 1964). In 19 animals (9 pigmented, 10 unpigmented) exposed for one year, haemangioendotheliomas and fibrosarcomas developed in 14 eyes.

(d) Guinea-pig

Guinea-pigs were exposed to the same regimen as described above. None of 17 animals developed a tumour of the eye (Freeman & Knox, 1964).

3.3 Sources emitting mainly UVB radiation

Many experiments have been carried out with sources emitting mainly UVB radiation, in which increases in the number of tumour-bearing animals and/or in the number of tumours per animal were seen (Blum, 1959; Winkelmann *et al.*, 1963; Freeman, 1975; Stenbäck, 1975a; Daynes *et al.*, 1977; Kripke, 1977; Spikes *et al.*, 1977; Forbes *et al.*, 1981; de Gruijl *et al.*, 1983; Gallagher *et al.*, 1984b). The most informative studies are described below.

3.3.1 *Mouse*

Freeman (1975) studied carcinogenesis induced by chronic exposure to narrow-band UVB produced by a high-intensity diffraction grating monochromator with a half-power band-width of 5 nm. Exposure was three times per week to one ear of each haired albino mouse. Four wavelengths were used, and the doses were determined as the MED. Of a group of 30 mice exposed to 300 nm (weekly dose, 60 mJ/cm^2), 16 developed squamous-cell carcinomas of the ear. Of a group of 30 mice exposed to 310 nm (weekly dose, 750 mJ/cm^2), 16 survived to 450 days and eight developed five squamous-cell carcinomas, two fibrosarcomas and one angiosarcoma of the ear. No skin tumour was observed among 30 mice irradiated with UVR at 290 nm (weekly dose, 42 mJ/cm^2); of five mice irradiated with 320 nm (weekly dose, 4950 mJ/cm^2), two developed squamous-cell carcinomas of the ear.

Two fibrosarcomas and one unspecified tumour of the eye were reported in 24 C3H/HeN mice bearing 25 skin tumours (mostly fibrosarcomas) after exposure to UVR (168 J/m^2 three times a week) from Westinghouse FS40T12 sunlamps (280–340 nm) (Kripke, 1977).

In the experiment of Forbes *et al.* (1981), groups of 24 male and female hairless albino Skh:HR mice (the changing designations of sources of 'Skh' mice are listed in Table 30), six to eight weeks old, were irradiated on five days per week with Westinghouse FS40T12 sunlamps (see Fig. 9c, p. 64), emitting mainly UVB (with < 1% below 280 nm; two-thirds at 280–320 nm and one-third at > 320 nm). All animals had developed tumours by the end of the experiment (up to 45 weeks), and a dose–response effect was demonstrated, as assessed by time to tumours in 50% of animals (Table 31). Histological examination showed tumours of 4 mm or more in diameter to be squamous-cell carcinomas; those of about 1–4 mm formed a continuum from carcinoma *in situ* to squamous-cell carcinoma, and those less than 1 mm comprised epidermal hyperplasia and squamous metaplasia tending toward carcinoma *in situ*. Less than 1% of tumours were fibrosarcomas.

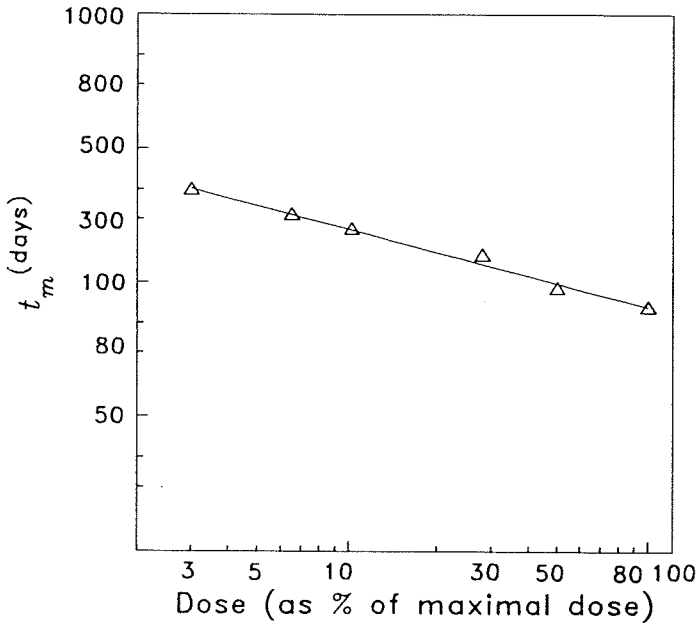
Six groups of 22–44 male and female Skh-hr 1 hairless albino mice (total, 199), six to eight weeks of age, were exposed to daily doses ranging from 57 to 1900 J/m^2 of mainly UVB radiation from Westinghouse FS40TL12 sunlamps; this dose range encompassed a factor of 33. Most of the animals developed skin tumours, although even the highest daily dose was sub-erythemic. A clear-cut relationship was shown between daily dose and time required for 50% of animals to develop skin tumours, which were predominantly squamous-cell carcinomas (Fig. 11). Squamous-cell carcinomas developed in 71% of the mice in the lowest

Table 31. Dose–response to ultraviolet radiation of hairless Skh:HR mice

Daily dose (J/m^2)	Time to 50% tumour incidence (weeks)	Terminated at week
420	38.6	45
587	33.3	45
822	29.2	45
1152	20.0	36
1613	17.6	36
2259	12.9	25

From Forbes *et al.* (1981)

Fig. 11. Dose–effect relationship for the induction of < 1-mm skin tumours in hairless mice by exposure to UVB radiation over a wide range of daily doses; t_m, median induction time



From de Gruijl *et al.* (1983)

dose group, and two skin tumours were reported in a total of 24 nonirradiated control mice (de Gruijl *et al.*, 1983).

In albino hairless Skh:Hr-1 mice irradiated with UVB or UVB plus UVA radiation three times a week for 16 weeks, with a 17-week recovery period, the spectrum for UV tumorigenesis was sharp and had a maximum near 300 nm (Bissett *et al.*, 1989).

3.3.2 *Rat*

Skin tumour induction was studied in a group of 40 shaven female NMR rats, 8–10 weeks old at the start of the experiment. The animals were irradiated chronically at a distance of 37.5 cm for 60 weeks with Westinghouse FS40T12 sunlamps (Fig. 9c), emitting mainly UVB (weekly dose, 5.4–10.8 $\times$ 10^4 J/m^2). A total of 25 skin tumours, most of which were papillomas of the ears, developed in 16/40 animals (Stenbäck, 1975a).

3.3.3 *Hamster*

Stenbäck (1975a) irradiated 40 shaven female Syrian golden hamsters, 8–10 weeks of age, using the same protocol as described above. A total of 30 skin tumours developed in 14/40 animals; 22 were papillomas (14 animals), four were keratoacanthomas (three animals), one was a squamous-cell carcinoma of the skin and three were papillomas of the ear (one animal).

3.3.4 *Guinea-pig*

Stenbäck (1975a) exposed guinea-pigs using the same protocol as above and found skin tumours in 2/25 animals (a fibroma and a trichofolliculoma).

3.3.5 *Fish*

Two hybrid fish strains susceptible to melanocytic neoplasms by UVR were developed by Setlow *et al.* (1989) by crossing platyfish and swordtails. A group of 460 fish were exposed to mainly UVB radiation from Westinghouse FS40 sunlamps, filtered with acetate sheets transmitting > 290 nm or > 304 nm at various doses (150 and 300 J/m^2 per day for > 290 nm; 850 and 1700 J/m^2 per day for > 304 nm) for 1–20 consecutive days. There were 103 controls. Depending on the wavelength, the level, the number of days of exposure and the strain, 19–40% of the irradiated fish developed melanocytic tumours; 13 and 2% of the controls in the two strains, respectively, developed such tumours.

3.3.6 *Opossum*

Monodelphis domestica, a South American opossum, is unusual in showing the phenomenon of photoreactivation (see Glossary) of pyrimidine dimers and erythema (Ley, 1985); it also developed actinic keratoses and skin tumours (mainly fibrosarcomas and squamous-cell carcinomas) on exposure to UVR from an FS-40 sunlamp (280–400 nm) (Ley *et al.*, 1987). Animals were shaved regularly and exposed to mainly UVB radiation from Westinghouse FS40 sunlamps, with relative emissions of 0.04, 0.27, 0.69, 1.0 and 0.09 at a dose of 250 J/m^2 (which is approximately half of an average MED; see Fig. 9c) at 280, 290, 300, 313 and 360 nm, respectively. Eight of 13 animals developed localized melanocytic hyperplasia; 100 weeks after the start of the experiment, melanomas were found in 5/13 surviving animals. *M. domestica* do not develop spontaneous melanomas, as was apparent in a much larger colony not exposed to UVR. Exposure of another group to photoreactivating light after UV irradiation reduced the incidence of melanocytic hyperplasia (3/17); this was considered to be a precursor lesion of the melanomas, although photoreactivation could not be demonstrated in the melanoma (Ley *et al.*, 1989).

[The Working Group noted that the melanocytic lesions induced in fish and the South American opossum differ histologically from human melanoma: they grow to a larger size and do not metastasize readily.]

Ley *et al.* (1991) exposed groups of *M. domestica* to UVR from fluorescent sunlamps (Westinghouse FS40; 280–400 nm with a peak at 313 nm) three times a week for 70 weeks at a dose of 250 J/m^2. Besides skin tumours, tumours of the anterior eye were observed beginning 30 weeks after the start of exposure. At 69 weeks, 50% of the animals had eye tumours, which were classified as fibrosarcomas of the corneal stroma. In animals exposed to UVR followed immediately by photoreactivating light, tumours appeared later and in reduced numbers.

'Cancer eye' in cattle, which includes squamous-cell carcinoma of the eye and the circumocular skin, is thought to be caused by solar UVR. In an attempt to confirm this relationship experimentally (Kopecky *et al.*, 1979), four Hereford cattle (which lack pigment around the eyes) were exposed to UVB radiation from Westinghouse FS40 lamps. Three cows developed grossly observable tumours of the eye, one of which was histopathologically confirmed as a preneoplastic growth.

3.4 Sources emitting mainly UVC radiation

3.4.1 *Mouse*

Carcinogenicity studies have been performed mainly in mice, but no study is available in which animals were exposed solely to UVC radiation. Several studies have been reported in which the source of UVC radiation was low-pressure mercury discharge germicidal lamps, which emit 90–95% of their radiation at wavelength 254 nm and weaker spectral lines in the UVB, UVA and visible light regions (Rusch *et al.*, 1941; Blum & Lippincott, 1942; Forbes & Urbach, 1975; Lill, 1983; Joshi *et al.*, 1984; Sterenborg *et al.*, 1988). In all of these investigations, the exposures induced tumours. Two of the most informative studies are described in more detail below.

A group of 40 female C3H/HeNCr1Br mice were irradiated with these lamps at a weekly dose of 3×10^4 J/m^2. Three animals died without tumours after 9, 43 and 63 weeks of irradiation; all of the other animals had tumours. By 52 weeks, 97% of the animals had developed skin tumours, with a median time to appearance of 43 weeks. The mean number of tumours per tumour-bearing mouse was 2.9. Tumour histology was carried out in 29/37 mice. Of a total of 83 suspected tumours, 66 were squamous-cell carcinomas, 10 were proliferative squamous lesions and 6 were invasive fibrosarcomas; one had the appearance of a cystic dilatation (Lill, 1983). [The Working Group that resulted in *IARC Monographs* volume 40 (IARC, 1986a) noted that the 4% UVB content of the source, representing a weekly dose of 1170 J/m^2, could not be excluded as contributing to the induction of skin tumours.]

Sterenborg *et al.* (1988) presented evidence that the tumours they induced in albino hairless mice were indeed due to UVC radiation. Groups of 24 male and female hairless albino mice (Skh-hr1), 6–10 weeks of age, were exposed to UVC radiation from Philips germicidal TUV 40W low-pressure mercury discharge lamps (mainly 254 nm) on seven days a week for 75 min per day at 230, 1460 or 7000 J/m^2 (30 times the MED); this dose was 60% less during the first seven days of the experiment. A total of 65 squamous-cell carcinomas of

the skin were found [number of animals with tumours not specified]. Both the percentage of tumour-bearing animals and the number of tumours per mouse were strongly dose-related. By comparing their results with those of experiments with UVB, the investigators concluded that (i) the UVB emitted by the low-pressure mercury discharge lamps was insufficient to account for the induction of tumours at the rate found, as at least 850 days of exposure to the UVB radiation present would be required to induce skin tumours at the rate observed, as compared to 161 days with the low-pressure mercury discharge lamp used; (ii) there is a qualitative difference between the effects of low-pressure mercury discharge and UVB lamps, in that the tumours induced by the mercury discharge lamps were scattered more widely over the skin of the mice than in the experiments with UVB; and (iii) the dose–effect relationship for tumorigenesis was less steep with the mercury discharge lamps than with UVB sources. [The Working Group noted that the evidence given to exclude UVB as contributing to the induction of skin tumours does not obviate the possibility that some interaction between UVC and UVB radiation led to tumour induction.]

3.4.2 *Rat*

Nine groups of 6 or 12 male CD-1 rats, 28 days of age, were shaved and exposed to varying doses of UVC from Westinghouse G36T6L sterilamps emitting predominantly 254 nm (dose range, 0.08–26.0 $\times$ 10^4 J/m^2). Survival ranged from 75 to 92% for the nine experimental groups. Keratoacanthoma-like skin tumours developed at a yield that was approximately proportional to dose throughout the dose range 0.65–26.0 $\times$ 10^4 J/m^2, although no tumour was observed at 0.32 $\times$ 10^4 J/m^2 or below (Strickland *et al.*, 1979).

3.5 Sources emitting mainly UVA radiation

The carcinogenic properties of UVA radiation received little attention before the introduction of UVA equipment for tanning, which led to the development of powerful sources of UVA. Many experiments have now been performed, using mainly hairless mice, to examine the possible carcinogenicity of UVA radiation (Zigman *et al.*, 1976; Forbes *et al.*, 1982; Berger & Kaase, 1983; Staberg *et al.*, 1983a,b; Kaase *et al.*, 1984; Santamaria *et al.*, 1985; Strickland, 1986; van Weelden *et al.*, 1986; Slaper, 1987; Kligman, 1988 [abstract]; van Weelden *et al.*, 1988; Kligman *et al.*, 1990 [abstract]; Sterenborg & van der Leun, 1990; van Weelden *et al.*, 1990a; Kelfkens *et al.*, 1991a; Kligman *et al.*, 1992). Some have shown no induction of tumours (Staberg *et al.*, 1983a,b; Kaase *et al.*, 1984; Kligman, 1988 [abstract]). [The Working Group noted that the doses may have been too small (daily doses in the range of 160 kJ/m^2) (Staberg *et al.*, 1983b) or the exposure period too short (Berger & Kaase, 1983; Kaase *et al.*, 1984; Kligman, 1988 [abstract]), as noted by the authors in a subsequent report (Kligman *et al.*, 1992).] In the other experiments, tumours were induced. [The Working Group noted that in some of the latter experiments either it is unclear whether UVB radiation was sufficiently excluded from the spectrum (Zigman *et al.*, 1976; Berger & Kaase, 1983; Staberg *et al.*, 1983a; Santamaria *et al.*, 1985) or the exclusion of UVB radiation was not fully convincing (Strickland, 1986).]

Studies in which the exclusion of UVB radiation was documented to be sufficient and which led to the induction of tumours by UVA in hairless mice were reported by van Weelden *et al.* (1986, 1988, 1990a), Slaper (1987), Kligman *et al.* (1990 [abstract], 1992), Sterenborg

and van der Leun (1990) and Kelfkens *et al.* (1991a). A few of the most informative studies are described below.

Groups of 24 male and female albino hairless Skh-hr 1 mice were exposed to UVA radiation from a bank of Philips TL40W/09 fluorescent tubes, filtered through a 10-mm glass plate selected for strong absorption of UVB radiation, for 12 h a day on seven days a week for about one year, at which time the experiment was terminated. The daily dose was 220 kJ/m^2. Most animals developed scratching lesions before they contracted skin tumours, which occurred in all animals; the median time to tumour appearance was 265 days. At the end of the experiment, the larger lesions were examined histologically: 60% were classified as squamous-cell carcinomas, 20% as benign tumours, including papillomas and keratoacanthoma-like lesions, and 20% as mild cellular and nuclear atypia. The histological findings were similar to those observed in a parallel experiment with UVB, but the tumours in the UVA-exposed group appeared over a longer time span. Residual UVB radiation was excluded as the cause of tumours in UVA-exposed mice on quantitative considerations: the authors concluded that more than 100 000 times the UVB present would have been required in order to induce tumorigenesis at the rate observed (van Weelden *et al.*, 1986, 1988).

Groups of 48 male and female hairless albino Skh-hr 1 mice were exposed to 220 kJ/m^2 UVA radiation (> 340 nm) from four high-pressure mercury metal-iodine lamps (Philips HPA 400 W), passed through liquid filters, for 2 h per day on seven days per week for up to 400 days. The spectrum matched that of a lamp used for tanning (the UVASUN 5000); UVB was effectively excluded by the filters. Skin tumours developed in most of the animals, and 31 developed tumours before any scratching was observed. The largest tumours were examined histologically at the end of the experiment: 15/20 tumours examined were squamous-cell carcinomas (Sterenborg & van der Leun, 1990).

The desire to tan safely has raised interest in the possible carcinogenicity of long-wavelength UVA (340–400 nm). In some experiments, UVB was excluded so rigorously that there was also very little UVA in the range 315–340 nm; exposure was therefore mainly to wavelengths in the region of 340–400 nm (van Weelden *et al.*, 1988; Sterenborg & van der Leun, 1990; van Weelden *et al.*, 1990a). These experiments yielded squamous-cell carcinomas in most animals. [The Working Group noted that if these were to be ascribed to the small proportion of shorter-wavelength UVA present in the spectra, a sharp peak in the action spectrum for UV carcinogenesis would have to occur between 330 and 340 nm, which does not appear likely.] In experiments by Kligman *et al.* (1990 [abstract], 1992), wavelengths shorter than 340 nm were filtered out rigorously. Female hairless albino Skh-hr 1 mice were exposed several times per week for 60 weeks to UVA at wavelengths of 340–400 nm at daily doses of 360 and 600 kJ/m^2, as used in artificial suntanning. Eighteen weeks later, 44 surviving mice had 19 skin tumours, mostly papillomas. At week 100, 22 surviving mice had 40 tumours, many of which were considered clinically to be squamous-cell carcinomas.

The carcinogenicity of short-wavelength UVA (315–340 nm) was investigated in one experiment. Groups of 24 male and female albino hairless Skh:hr 1 mice were exposed to average daily doses of 20 or 56 kJ/m^2 radiation from specially developed fluorescent tubes with peak emission near 330 nm (UVB radiation was filtered out efficiently using a glass filter) on seven days a week for 650 days. All mice in the high-dose group developed multiple tumours, first mainly papillomas and later predominantly squamous-cell carcinomas. In the

lower-dose group, three mice developed skin tumours, all of which were papillomas. The lamps also emitted long-wavelength UVA (340–400 nm), but in a proportion considered by the authors to be too small to account for the rate of tumorigenesis observed (Kelfkens *et al.*, 1991a). The investigators estimated the carcinogenic effectiveness of short-wavelength UVA (315–340 nm) to be approximately five times greater than that of long-wavelength UVA (340–400 nm).

3.6 Interaction of wavelengths

In daily life, the skin is exposed frequently to several wavelength ranges (UVA, UVB, UVC) simultaneously, or to different combinations at different times. The simplest explanation of an effect of such combined exposures is 'photoaddition', i.e., each exposure contributes to the effective dose in an additive way. The validity of this hypothesis is one of the assumptions underlying widely used concepts such as 'erythemal effective energy' and the derivation of the action spectrum shown in Figure 10 (p. 141). It implies that any additional exposure to an effective dose, in any wavelength region, increases the carcinogenic effect.

Several studies provide indications, however, that the situation is more complicated. Interactions are seen between the effects of different wavebands that result in deviations from photoaddition (for reviews, see van der Leun, 1987b, 1992). The literature on this topic is controversial and cannot be summarized in detail here. The following two sections form an attempt to give an overview and interpretation.

3.6.1 *Interaction of exposures given on the same day*

Several types of interactions have been reported between different wavelength ranges administered simultaneously or in close temporal proximity. These have led to concepts of processes such as:

- photorecovery: the effect of UVB or UVC is reduced by simultaneous or immediately subsequent exposure to UVA or visible light [The Working Group noted that photoreactivation is a special case of photorecovery but applies only to species that have the 'photoreactivating enzyme', photolyase (see Glossary).];
- photoprotection: the effect of UVB or UVC is reduced by prior administration of UVA or visible light;
- photoaugmentation: the effect of UVB or UVC is enhanced by prior, simultaneous or subsequent administration of UVA or visible light.

Photoaugmentation of UVB carcinogenesis by UVA was suggested by several investigators (Urbach *et al.*, 1974; Willis *et al.*, 1981, 1986; Kligman, 1988 [abstract]; Talve *et al.*, 1990) but could not be confirmed by others (Forbes *et al.*, 1978; van Weelden & van der Leun, 1986). The latter investigators found evidence of photorecovery: the effect of UVB plus UVA was smaller than that of the same UVB exposure given alone. The reduction was small; however, UVA reduced the carcinogenic effective dose of UVB by 16%.

Interactions of different wavelength ranges when given simultaneously, prior to or immediately after each other appear to be either nonexistent or unproven, as in the case of photoaugmentation, or small, as in the case of photorecovery. Such interactions currently play a small role in the evaluation of risks (see, for example, Health Council of the

Netherlands, 1986). Other uncertainties in the estimates, such as the dose received, are likely to have a greater influence than interactions. Photoreactivation, is, however, a well-defined process in those species which possess photolyase and may result in reduction of effects.

3.6.2 *Long-term interactions*

A different type of interaction occurs when exposures to one wavelength band are separated temporally from exposures to another. For example, a prolonged course of UVB exposures, by itself sufficient to induce tumours, is compared with an identical UVB course that is preceded or followed by a course of UVA exposures, usually over several weeks.

Forbes *et al.* (1978) exposed hairless mice to tumorigenic UVB or to UVB followed by UVA and visible light for 30 weeks. The longer-wavelength exposures reduced the tumorigenic effect of the UVB. Staberg *et al.* (1983b) gave mice a tumorigenic combination of UVB and UVA and found that subsequent exposures to UVA increased the tumorigenic effect. The UVA was derived from Philips TL40W/09 lamps filtered through 2-mm plain glass to remove the UVB. [The Working Group noted that since the glass transmitted some UVB the increased carcinogenic effect may have been due to added UVB radiation.] Bech-Thomsen *et al.* (1988a) pretreated lightly pigmented hairless female hr/hr C3H/Tif mice with UVA for four weeks before exposure to broad-spectrum UVR. The UVA reduced the carcinogenic effect of the broad-spectrum UVR. This result was not corroborated in a subsequent, similar experiment by the same investigators (Bech-Thomsen *et al.*, 1988b), in which mice were pretreated with radiation from various UVA sources. The purest UVA radiation neither increased nor decreased the carcinogenic effect of UVB.

Slaper (1987) exposed one group of mice daily to UVB and a second group daily to UVA at doses matched for approximately equal carcinogenic effect. In a third group of mice that received the two regimens alternately every week, the carcinogenic effect was less than that in the UVA- or the UVB-exposed group. The effective dose in the alternating regimen was estimated to be 80% that in the UVB regimen. The investigator concluded that both UVA and UVB contributed to the carcinogenic effect of the alternating regimen.

[The Working Group noted that the effect of long-term interactions appears to be similar to that of interactions of exposures given on the same day. Photoaddition gives a reasonable prediction, but the combined effects tend to be slightly less than would be predicted.]

3.7 Additional experimental observations

3.7.1 *Tumour types*

Skin tumours in UV-exposed animals are commonly epidermal, benign papillomas and malignant squamous-cell carcinomas; adnexal neoplasms, mainly basal-cell carcinomas, are less common. Attempts have been made to induce naevi and malignant melanomas. Many tumours are found, since the animals are followed for long periods of time; however, tumours coalesce and regress, and all tumours are not examined histologically.

Squamous-cell carcinoma is the commonest type of tumour found after exposure to UVR. These tumours have been reported in mice exposed to predominantly UVB radiation (Winkelmann *et al.*, 1960, 1963; Epstein & Epstein, 1963; Freeman, 1975; Forbes *et al.*, 1981;

de Gruijl *et al.*, 1983), to predominantly UVA radiation (van Weelden *et al.*, 1988; Sterenborg & van der Leun, 1990) and to predominantly UVC radiation (Lill, 1983; Sterenborg *et al.*, 1988). They have also been found in rats (Putschar & Holtz, 1930; Roffo, 1934, 1939; Hueper, 1942), hamsters (Stenbäck, 1975a) and opossums (Ley *et al.*, 1989) following exposure to broad-spectrum UVR.

Papillomas were reported to be the commonest tumour after exposure of hairless mice to UVR consisting of UVB and UVA (Gallagher *et al.*, 1984b). Papillomas were also reported to precede or accompany squamous-cell carcinomas induced in hairless mice by UVA (van Weelden *et al.*, 1988), UVB (Stenbäck, 1978) or UVC radiation (Sterenborg *et al.*, 1988). Papillomas were also common in rats (Findlay, 1930; Putschar & Holtz, 1930; Stenbäck, 1975a) and hamsters (Stenbäck, 1975a) exposed to broad-spectrum UVR.

The main type of tumour diagnosed after exposure of haired mice to broad-spectrum UVR was *fibrosarcomas* (Grady *et al.*, 1941, 1943). Squamous-cell carcinomas were less common, but the ratio of carcinomas to sarcomas increased with the number of exposures per week (Grady *et al.*, 1943). Spikes *et al.* (1977) reported many squamous-cell carcinomas in clipped C3Hf mice irradiated with UVB, especially at low doses; the high-dose group had a much higher proportion of fibrosarcomas. The investigators suggested that the type of tumour induced might be dose-dependent. Norbury and Kripke (1978) found that the type of tumour might depend on immunological factors. They compared UVB tumorigenesis in normal C3H/HeN (MTV^-) mice, in T cell-depleted mice and in T cell-depleted mice reconstituted with thymus grafts. In the normal mice, fibrosarcomas predominated; in the T-cell depleted, reconstituted mice, squamous-cell carcinomas predominated. Spindle-cell sarcomas were reported in rats irradiated with sunlight (Roffo, 1934), and fibrosarcomas were seen in opossums irradiated with UVB (Ley *et al.*, 1989).

The diagnosis of fibrosarcoma was questioned by Morison *et al.* (1986). After C3H/HeNCr (mammary tumour virus-free) haired pigmented mice were exposed to mainly UVB radiation, the tumours induced were almost all squamous-cell carcinomas. The investigators noted that the same type of tumour had been diagnosed in many previous reports as fibrosarcoma; they diagnosed squamous-cell carcinomas by studying specific markers for cell differentiation in the tumours. In a study by Phelps *et al.* (1989) in which hairless albino Skh/hr-1 mice were exposed to UVA and UVB at 0.3 J/cm^2 [30 kJ/m^2], all mice developed epidermal neoplasia and 25% of animals developed spindle-cell tumours that resembled human atypical fibroxanthoma. [The Working Group noted that earlier studies did not use presently available cellular markers.]

Keratoacanthomas and similar benign epidermal neoplasms have been reported in mice exposed to UVB (Stenbäck, 1978), rats exposed to UVB and UVC (Strickland *et al.*, 1979) and hamsters exposed to UVB (Stenbäck, 1975a).

Actinic keratosis, or solar keratosis, a precursor lesion of squamous-cell carcinomas, has been reported in hairless mice exposed to UVA and UVB (Kligman & Kligman, 1981) and in haired mice exposed to UVB (Stenbäck, 1978).

Basal-cell carcinomas have not been reported in studies in mice. A few studies on UV carcinogenesis in nude mice, which have a deficient immune system, have been reported (Eaton *et al.*, 1978; Anderson & Rice, 1987; Hoover *et al.*, 1987). The skin tumours induced

by mainly UVB radiation in these studies were mostly squamous-cell carcinomas, but in the experiments reported by Anderson and Rice (1987) in nude mice of BALB/c background there were several basal-cell carcinomas. Basal-cell carcinomas were found occasionally in rats exposed to broad-spectrum UVR (Putschar & Holtz, 1930; Hueper, 1942). [The Working Group noted that the classification of these neoplasms and their relation to the corresponding neoplasms in humans is not clear.]

There is no report in which cutaneous *malignant melanoma* was induced in mice by UVR alone (Epstein, 1990; van Weelden *et al.*, 1990b; Husain *et al.*, 1991), in spite of concerted attempts to achieve this.

No study was found in which the primary objective was to examine the susceptibility of the eye to UVR; rather, eye tumours were found incidentally in studies designed to investigate skin carcinogenesis. All of the tumours of the eye identified in these reports involved superficial parts of the eye (cornea and conjunctiva); no tumour of the interior eye was reported.

Studies of the effect of UVR on tumour induction in other organs (lymphoma in mice) are few and were not designed to determine this effect (Ebbesen, 1981; Joshi *et al.*, 1986). [The Working Group considered that the data were inadequate for evaluation and that data on survival among treated and control groups, sample selection and analysis of data were limited.]

3.7.2 *Dose and effect*

Quantitative information is available mainly on the induction of squamous-cell carcinoma in mice. In most of the experiments, exposure was regular, several times per week or every day, until tumours developed. The daily doses of UVR required for skin tumorigenesis are usually well below those present outdoors in the environment, and most experiments have been conducted with UVB doses lower than those required to elicit acute reactions in mouse skin (erythema or oedema). In one experiment in hairless mice, with a UVB dose 33 times lower than that required for acute reactions, 71% of the skin tumours were squamous-cell carcinomas (de Gruijl *et al.*, 1983). The effectiveness of UVB radiation is increased at lower dose rates (Kelfkens *et al.*, 1991b).

The higher the dose given, the less time it takes for tumours to appear. In most experiments, the time required for 50% of mice to develop tumours ranged between a few months and one year. By maximizing the exposure regimen in hairless mice (escalating doses of UVB radiation), the time could be reduced to 18 days (Willis *et al.*, 1981). In a few experiments, in both mice and rats, skin tumours resulted from a single exposure to UVB radiation (Hsu *et al.*, 1975; Strickland *et al.*, 1979); in mice, this required a dose that first caused skin ulceration: hairless mice, 60 kJ/m^2 (Hsu *et al.*, 1975); Sencar mice, 29 kJ/m^2 (Strickland, 1982).

Quantitative dose–effect relationships have been derived for mice exposed regularly (usually daily) to UVR. The median time to first tumour, t_m, has been used as a measure of the effect and is related to dose level. Dose–effect relationships of the form

$$t_m = c\, D^{-r},$$

where c is a constant incorporating the susceptibility of the strain of mice as well as the effectiveness of the radiation spectrum, D is the daily dose of radiation and r is a numerical

exponent giving the steepness of the relationship, have been proposed by several authors. Estimates of r vary from 0.2 (Sterenborg *et al.*, 1988) for small tumours of the skin induced by UVC radiation in hairless mice, to 0.5 (Blum *et al.*, 1959) for large tumours on the ears of haired mice induced by broad-spectrum UVR and to 0.6 (de Gruijl *et al.*, 1983) for small tumours induced by broad-band UVB in hairless mice. Figure 11 (p. 145) illustrates the shape of this dose–response relationship for $r = 0.6$; other forms of the relationship have been proposed (Forbes *et al.*, 1982). All of them provide adequate descriptions of the dose–response within the range of the available data, although extrapolations outside this range differ substantially.

3.7.3 *Dose delivery*

The tumorigenic effect of UVR depends not only on the dose but also on the temporal pattern of exposure. In general, the effectiveness of treatment increases with the number of fractions of the dose per week (Forbes *et al.*, 1981), for both daily and accumulated doses. A daily dose administered over 12 h is more effective than the same daily dose administered in 1 h (Kelfkens *et al.*, 1991b). The same weekly dose is more effective when given over three to five days than if given in one day (Forbes *et al.*, 1981).

3.7.4 *Action spectra*

Ideally, the carcinogenic effectiveness of UVR can be expressed as a continuous function of wavelength. That function, called the action spectrum for UV carcinogenesis, is not yet completely delineated. Freeman (1978) made an early attempt to determine this spectrum and found that it was limited to a few narrow bands around the wavelengths 290, 300, 310 and 320 nm. Narrow-band monochromatic sources are difficult to achieve.

Since that time, various action spectra have been proposed to weight the spectral irradiance of a source. Forbes *et al.* (1982) and Cole *et al.* (1986) determined dose–effect relationships similar to that shown in Figure 11 for many different UV spectra. By weighting these lamp spectra with various existing action spectra for photobiological effects, effective doses were computed for each experiment. In this way, the investigators tried to align the results from the experiments with different UV spectra into one dose–effect relationship. One of the action spectra (MEE48), originally determined for the induction of oedema in mice 48 h after exposure to UVR and which is similar to the human erythema action spectrum, fitted well. The authors concluded that the mouse oedema spectrum was also appropriate for describing skin cancer induction (Cole *et al.*, 1986).

Sterenborg and van der Leun (1987) attempted to determine an action spectrum directly from observations on UV carcinogenesis. They exposed hairless albino mice to seven different lamp spectra under otherwise identical circumstances. The lamp spectra overlapped to some extent, and the action spectrum was derived by mathematical fitting. The analysis yielded an action spectrum for the wavelength range 250–360 nm. Slaper (1987) added observations in the UVA region and extended the action spectrum throughout the UVA range (see Fig. 10, p. 141).

The action spectrum shown in Figure 10 is for albino hairless Skh-hr 1 mice with an end-point of 1.0-mm tumours. Although different end-points may yield different action spectra, this curve shows good agreement in the UVB range with the MEE48 spectrum and

also with the observations of Freeman (1978) for wavelengths 300, 310 and 320 nm. [The Working Group noted that the action spectrum for UV carcinogenesis in the wavelength range 300–320 nm may be considered a good approximation.] The different shapes of Figure 10 and MEE48 in the UVC reflect a scarcity of data in this wavelength range. [The Working Group noted that the action spectrum for carcinogenesis by UVC is still highly uncertain.] The MEE48 left widely different options open for the action spectrum of long-wavelength UVA: the effectiveness in the wavelength range 330–400 nm could be either zero or as high as 0.0002 (Cole *et al.*, 1986). More recent data on the carcinogenesis of UVA, used to construct the curve in Figure 10, indicate a mean effectiveness of 0.00015 in this range (Slaper, 1987). [The Working Group noted that this value for the carcinogenic effectiveness for UVA may be regarded as an estimate of the order of magnitude.]

3.7.5 *Pigmentation*

Pigment was reported to be protective against tumours arising from the conjunctiva in cattle (Anderson, 1963).

Freeman and Knox (1964) also examined the influence of pigmentation in a group of 78 rats composed of 66 pigmented rats of various strains (black, black and white, grey-brown, grey and white) and 12 albinos. Under the same irradiation regimen, the pigmented rats developed tumours in 73% of eyes and the albinos in only 8%. The tumour yield was consistently higher in the pigmented strains than in the albinos. In nine pigmented and 10 albino hamsters exposed for one year, 50% of pigmented animals and 25% of non-pigmented animals developed eye tumours.

Davies and Forbes (1988) exposed closely related albino hairless Skh-hr 1 mice and pigmented hairless Skh-hr 2 mice to broadband UVR from a filtered xenon arc lamp. Especially at high doses, the latent period until 50% of animals had first tumours was longer in Skh-hr 2 mice.

van Weelden *et al.* (1990a) derived mice of different degrees of pigmentation—'browns' and 'blacks'—by selective breeding from Skh-hr 2 stock and exposed 24 albinos (Skh-hr 1) (van Weelden *et al.*, 1988), 16 'browns' and eight 'blacks' to UVA radiation. The brown mice were less susceptible to skin tumours than the albinos, but the more heavily pigmented blacks were as susceptible as the albinos: the median times for tumour induction were 265 days for albinos, 267 days for blacks and 375 days for browns (van Weelden *et al.*, 1990a).

3.8 Administration with known chemical carcinogens

Since UVR alone produces tumours, it is a 'complete' carcinogen and may thus be involved in cocarcinogenicity. Several investigators have attempted to determine whether UVR has tumour 'initiating' and/or tumour 'promoting' activity when tested in a traditional two-stage protocol. For the purposes of this monograph, a 'tumour initiator' is defined as an agent that, at a stated amount and upon administration once, is incapable of causing tumours in the population of animals unless the skin is subsequently treated with a 'tumour promoter'. A 'tumour promoter' is defined as an agent that, under stated conditions is incapable of causing tumours unless the skin was previously treated with a 'tumour initiator'. The test systems used embody a number of variables, not all of which were necessarily considered by

the authors. For example, UVR has also been shown to influence the immune system, and polycyclic aromatic hydrocarbons are photochemically active.

3.8.1 *Administration with polycyclic aromatic hydrocarbons*

Most of the studies summarized below demonstrate that UVR has a cocarcinogenic action with other carcinogens. Other reports provide additional information on cocarcinogenesis, on photolysis of polycyclic aromatic hydrocarbons and on other interference with chemical carcinogenesis (Clark, 1964; Ito, 1966; Santamaria *et al.*, 1966; Davies *et al.*, 1972 [abstract]; Shabad & Litvinova, 1972; Stenbäck & Shubik, 1973; Stenbäck, 1975b; Roberts & Daynes, 1980; Gensler & Welch, 1992).

(*a*) *3,4-Benzo[a]pyrene*

Groups of 18 female SPF (specific pathogen-free) BALB/c mice, six weeks of age, received 30-min exposures on the shaved dorsal skin to UVB from a Westinghouse FS40 sunlamp (280–320 nm) five times a week for 13 weeks (total dose, 7.0×10^5 J/m^2) or no UVB exposure followed one week later by twice weekly applications of 0, 0.1 or 1.0 mg 3,4-benzo[*a*]pyrene in acetone on the shaved ventral skin for 20 (acetone only), 20 or 10 weeks, respectively. Pre-exposure to UVB enhanced tumour growth in the high-dose group: 29 tumours (of 20 examined histologically, 90% were squamous-cell carcinomas and 10% undifferentiated sarcomas) in the UVB-pretreated group compared to two (squamous-cell carcinomas) in the non-irradiated 3,4-benzo[*a*]pyrene-treated animals 18 weeks after the first treatment with 3,4-benzo[*a*]pyrene. No such effect was seen in the low-dose group (Gensler & Bowden, 1987; Gensler, 1988a).

(*b*) *7,12-Dimethylbenz[a]anthracene*

In an attempt to assess the promoting effects of UVR, groups of 15–31 male and 16–22 female Swiss albino mice, 11–18 weeks of age, received a single application of two drops (0.1 ml) of 0 or 0.5% 7,12-dimethylbenz[*a*]anthracene (DMBA) in acetone on the posterior half of the dorsal skin, followed 14 days later by exposures to UVB (280–320 nm; high-pressure Hanovia hot quartz contact lamp) twice a week for 67 weeks (total dose, 13.33×10^7 ergs/cm^2 [133 kJ/m^2]) or no exposure. At the end of the UVB treatment, 16/31 mice treated with DMBA and UVB had developed 19 skin tumours, compared to 4/41 and 0/47, respectively, among mice treated with DMBA alone and UVB alone. Exposure to UVB also enhanced the multiplicity and degree of malignancy of DMBA-induced tumours (Epstein & Epstein, 1962).

Groups of 26–42 male and female outbred hairless mice, 7–12 weeks old, received a single application of two drops (0.1 ml) of 0 or 0.5% DMBA in acetone, followed six weeks later by exposures to UVB (280–320 nm; high-pressure Hanovia hot quartz contact lamp) three times a week for 29 weeks (total dose, 15.34×10^7 ergs/cm^2 [153 kJ/m^2]) or no exposure. All animals were observed for 63 weeks. UVB exposure produced skin tumours in 22/26 animals, and DMBA treatment alone in 3/41; acetone alone produce no skin tumour. Exposure to UVB following DMBA treatment enhanced carcinogenicity with regard to appearance time (first tumour observed at 14 weeks compared to 30 in the group treated with DMBA alone and 20 in that given UVB alone), multiplicity at 58 weeks after DMBA

treatment (40 in 24 animals compared to 22 in 26 animals treated with UVB alone and 3 in 41 animals treated with DMBA alone) and degree of malignancy. Two 'melanomas' appeared in the group receiving the combined treatment (Epstein, 1965).

Groups of 18–46 outbred hairless pigmented mice [sex unspecified], 8–11 weeks old, received a single application of 0.05 ml of 0.4% DMBA (0.2 mg) in acetone or no DMBA. After 13 months, mice treated with DMBA had developed pigmented lesions ('blue naevi') in the treated areas. For the following seven months, mice received UVB (280–320 nm; high-pressure Hanovia hot quartz contact lamp) three times a week or no UVB treatment. Exposure to UVB following DMBA treatment enhanced the growth of naevi into malignant-appearing pigmented tumours ('melanomas'): 5/18 *versus* 0/41 in the group treated with DMBA alone and 0/39 in the group treated with UVB alone (Epstein *et al.*, 1967). [The Working Group noted the limited reporting on metastases.]

A group of 56 B6D2F$_1$/J mice [sex unspecified], six weeks of age, was irradiated with UVB (280–340 nm; Westinghouse FS40 sunlamp) dorsally for 30 min per day on five days per week (Roberts & Daynes, 1980) for 11.5 weeks (total dose, 6.2×10^5 J/m^2). A control group of 41 mice received no irradiation. Both groups subsequently received a single application of 100 μg DMBA in 0.1 ml acetone on the shaved ventral skin, followed four days later by applications of 5 μg 12-*O*-tetradecanoylphorbol 13-acetate (TPA) three times a week for 32 weeks. Tumour yield was significantly decreased at 32 weeks (2.2 *versus* 4.8 tumours/mouse) in the pre-irradiated mice (Gensler, 1988b).

Groups of 20–24 female hairless Skh-hr 2 mice, six to eight weeks old, received a single application of 0 or 0.5% DMBA in acetone on the dorsal skin. Two weeks later, the animals were irradiated with UVB (290–320 nm; Westinghouse FS40-T12 sunlamp), UVA (320–400 nm; GTE-Sylvania fluorescent black light tubes) or a combination of UVA plus UVB three times a week for 30 weeks or were not irradiated, and were observed for 12 months. All mice receiving DMBA treatment developed multiple 'blue naevi'; virtually none of the untreated mice or mice that received UVR treatment only showed this effect. Irradiation of DMBA-treated animals induced a higher incidence of papillomas (70–100%), squamous-cell carcinomas (30–80%), melanomas (25–33%) and lymphomas (21–50%), than exposure to UVA alone (0–32% papillomas, 0–47% squamous-cell carcinomas, no melanoma and no lymphoma) or to DMBA alone (90, 25, 0 and 5% of these tumours, respectively). The authors also examined selected lesions induced by DMBA alone or by DMBA with UVR for the presence of H- or N-*ras* mutations. Mutations at codon 61 in N-*ras* were present in three (two induced by DMBA plus UVR, one by DMBA alone) out of eight of the early pigmented lesions examined and in one out of three of the malignant melanomas examined (induced by DMBA plus UVR); no H-*ras* mutation was observed (Husain *et al.*, 1991). [The Working Group noted that lesions were not induced by UVR alone.]

3.8.2 *Administration with other agents with promoting activity*

These studies were designed to evaluate the action of UVR as a tumour initiator.

(*a*) *Croton oil*

Groups of 15–53 male and 9–30 female random-bred hairless mice, 9–12 weeks old, received a single exposure to UVB (280–320 nm; high-pressure Hanovia hot quartz contact

lamp) for 30 s (1.3×10^7 ergs/cm^3 [13 kJ/m^2]) or no exposure, followed two weeks later by applications to the dorsal skin of 0 or 0.1 ml croton oil in acetone twice a week for 18 months. Neither UVB exposure nor croton oil alone produced any skin tumour over the course of the study. The group of 79 mice that received both UVB exposure and croton oil had eight persistent skin tumours (one per mouse) (Epstein & Roth, 1968).

Groups of 30 female Swiss mice, eight weeks old, received UVB once (5.5×10^7 ergs/cm^2 [55 kJ/m^2]) from Westinghouse FS40T12 lamps or croton oil (0.02 ml of a 2.5% solution, twice a week for 30 weeks); a group of 60 mice received UVB followed after 10 days by croton oil for life. UVB alone produced no tumour; croton oil alone produced regressing tumours, and the combination produced 11 tumours (four papillomas, four fibromas and three regressing tumours) in seven mice (Stenbäck, 1975c).

Groups of 40 male haired mice (random-bred 'Hall' strain), 18 weeks of age, were clipped and exposed once to UVC (medium-pressure mercury discharge lamp). One group received no further treatment; the other received one application of croton oil one day before irradiation and, beginning two weeks later, received applications of 0.25 ml croton oil (0.5% solution) once a week for 30 weeks. By 35 weeks, the groups had 20 and 23 survivors, with 0 and 12 skin tumours, respectively (Pound, 1970).

(*b*) *12-O-Tetradecanoylphorbol 13-acetate*

Six groups of 25 eight-week-old female C3H/HeNCr(MTV$^-$) mice were irradiated with UVB (Westinghouse FS40 sunlamps) on the shaved dorsum for 30 min, five times a week for two weeks (total dose, 1.44×10^5 J/m^2), followed two weeks later by 'promotion' with applications of 0 or 5 μg TPA in acetone twice a week. Ventral irradiation for 30 min, three times a week for 12 weeks (total dose, 4.54×10^5 J/m^2) (to produce a 'systemic' effect) was begun two weeks after completion of dorsal initiation. At 70 weeks, UVB exposure of the dorsum alone had produced no tumour, and dorsal applications of TPA alone had produced a 5% incidence of tumours. The combination of these treatments produced a 41% tumour incidence. Ventral irradiation of animals that had received TPA only produced a 33% incidence, and ventral irradiation of mice that had received both UVB and TPA produced a 100% incidence. The authors suggested that these findings reflect a systemic effect—possibly suppression of immune surveillance or a biochemical influence on the epidermal growth regulatory system (Strickland *et al.*, 1985).

(*c*) *Benzoyl peroxide*

Benzoyl peroxide is considered to be a prototype promoter of two-stage chemical carcinogenesis in the skin (Slaga *et al.*, 1981). The studies summarized below were motivated, however, by concerns about the safety of using this compound for treating acne vulgaris.

Groups of Uscd (Hr) stock hairless albino mice (total, 148) [sex unspecified], three to four months old, were exposed on the posterior half of the back to UVR (Hanovia hot quartz contact lamp emitting primarily UVB; 270 mJ/cm^2 [2.7 kJ/m^2]) three times a week for eight weeks. Four weeks later, the mice were divided into four groups. The final skin tumour incidences at the irradiated sites were: 38% in the group that received applications of 0.1 ml of a 0.1% solution of croton oil in acetone on the back skin five times a week for the duration of the experiment (62 weeks); 5% in the group that received applications of acetone alone;

8% in mice that received applications of the benzoyl peroxide base; and 8% in those that received applications of a 5% lotion of benzoyl peroxide in water five times a week for the duration of the study (Epstein, 1988).

Five groups of Oslo hairless mice (16 males and 16 females) were irradiated under Philips HP3114 sunlamps (mostly UVB) twice a week for 52 weeks (total dose, 26.5 J/cm^2 [265 kJ/m^2]). The mice were treated before or after each exposure with 5% benzoyl peroxide in gel, with the gel alone or with no chemical. Throughout the study, the groups were indistinguishable in terms of the proportion with one of more tumours (median latent period, approximately 40 weeks) and of the total number of tumours per survivor (approximately 1.5 at 40 weeks and approximately 4 at 48 weeks). Thus, benzoyl peroxide did not enhance photocarcinogenesis. The study also included several groups of SENCAR mice treated topically with DMBA once (51.2 μg) or with vehicle followed by benzoyl peroxide twice a week. Benzoyl peroxide reduced the number of DMBA-induced tumours (Iversen, 1988). Two unresolved concerns were raised by the author: Firstly, the fact that benzoyl peroxide reduced the tumorigenicity of DMBA was contrary to the author's previous experience (Iversen, 1986) and to that of several others; secondly, the UVR dose used in this study was lower (total dose, 265 kJ/m^2) than that used in the 1986 study (total dose, 480 kJ/m^2), but the tumour response was significantly greater.

(d) Methyl ethyl ketone peroxide

A postulated mechanism for tumour promotion involves the generation of free radicals, possibly with reactive oxygen species, leading to enhanced lipid peroxidation and DNA damage and/or cell phenotype. A study was therefore designed to test whether methyl ethyl ketone peroxide (MEKP), which is known to produce lipid-peroxidizing activity *in vivo*, acts as a tumour promotor in skin 'initiated' by UVR. Furthermore, since glutathione has been shown to be a major endogenous reducing agent which protects against lipid peroxidation, the study also tested diethyl maleate (DEM), which is known to deplete the intracellular level of glutathione in mouse skin.

Groups of 24 male and female hairless albino mice (14–16 weeks old) were irradiated with UVB (280–320 nm; Westinghouse FS40 fluorescent sunlamps; 2054 J/m^2 daily) for 18 weeks. Three weeks later, topical application of MEKP (20 μl containing 0 or 10 μg MEKP) was begun and continued twice a week for 25 weeks. Other groups received DEM (0 or 1 μg in dibutyl phthalate) 1 h before each MEKP application. Otherwise identical control groups received either the chemical treatments or UVB alone. At 46 weeks, the groups that did not receive UVB irradiation had at most two tumours on two mice (among 21 survivors in mice exposed to MEKP plus DEM). Exposure to UVB produced five tumours in four mice exposed to the solvent, out of 19 survivors; 11 tumours in eight mice exposed to MEKP, out of 21 survivors; and 18 tumours in nine mice exposed to MEKP plus DEM, out of 16 survivors. Using tumour onset rate analysis (Peto *et al.*, 1980), the overall effect of MEKP was statistically significant. Tumour enhancement by MEKP was greater in the presence of DEM (Logani *et al.*, 1984).

3.9 Interaction with immunosuppressive agents

Investigations have been reported on agents known to influence immunological responses in humans and on agents chosen to test some aspect of immunological response in mice. [The Working Group noted that in most cases the effect on the immune system of the animals was not evaluated directly; these agents have effects other than immunosuppression, which may explain their interaction with photocarcinogenesis.]

Three groups of 12 male Skh-Hr1 hairless mice, eight weeks of age, were irradiated with 280–320 nm UVB (Westinghouse FS40T12 sunlamps) on five days per week for 30 weeks at daily doses of 470 J/m^2. Two weeks after the first UVB exposure, one group received subcutaneous injections of 0.1 ml *anti-mouse lymphocytic serum* twice a week for 20 weeks; a second received intraperitoneal injections of 12 mg/kg bw *6-mercaptopurine* (Purinethol) five times a week for 20 weeks; and a third received intraperitoneal injections of 0.1 ml isotonic saline five times a week for 20 weeks. Treatment with anti-mouse lymphocytic serum resulted in an earlier appearance and a greater numbers of tumours than did treatment with saline; in contrast, 6-mercaptopurine appeared to delay the appearance of tumours (Nathanson *et al.*, 1976).

Groups of 24–28 female albino HRA/Skh-1 hairless mice, 21–35 weeks of age, were irradiated with UVR (UVB from an Oliphant FL40SE tube and UVA from six Sylvania 40BL tubes) to simulate the UVR portion of terrestrial sunlight on five days per week for 10 weeks to achieve a MED. At the same time, the animals received intraperitoneal injections of 15 mg/kg bw *azathioprine* in 0.1 ml glycine buffer, 10.6 mg/kg bw *cyclophosphamide* in 0.1 ml glycine buffer or 0.1 ml vehicle alone. At day 200, mice receiving UV irradiation alone had a tumour incidence of 77%; those also receiving azathioprine had an incidence of 96% (marginally significant enhancement of tumour growth); and those receiving cyclophosphamide had an incidence of 85% (nonsignificant increase) (Reeve *et al.*, 1985).

Groups of 15 female albino HRS/J hairless hr/hr mice, eight weeks old, were irradiated with UVB (280–320 nm; Westinghouse FS40 sunlamps) on five days a week for 24 weeks; further groups also received injections of 4 or 8 mg/kg bw *azathioprine* or 10 or 25 mg/kg bw *cyclosporine* three times a week. The mean latent period for tumour development was 16 weeks in the group receiving UV irradiation only and 12–13 weeks in the groups also receiving azathioprine or cyclosporine, indicating enhancement of photocarcinogenesis by both drugs (Nelson *et al.*, 1987).

Groups of female C3H/HeN(MTV$^-$) mice [initial numbers unspecified], four to six weeks of age, received grafts of fragments of an antigenic ('regressor') tumour (fibrosarcoma) previously induced in a host animal by UVB. Some animals received no further treatment; other groups received UVB irradiation (Westinghouse FS40; 5 kJ/m^2 per day on five days a week for four to six weeks), subcutaneous injections of 25 or 75 mg/kg bw *cyclosporine* once a day on eight consecutive days, or injections of 20 mg/kg bw *cyclophosphamide* 1, 3, 6, 9 and 13 days after tumour challenge. Tumours grew progressively in the groups treated with UVB or cyclosporine, but not in the groups receiving no further treatment or cyclophosphamide (Servilla *et al.*, 1987).

Groups of six female albino HRA/Skh-1 hairless mice, 10–12 weeks of age, were irradiated with UVA plus UVB (one Oliphant FL40SE tube and three Sylvania F4/350 BL tubes)

on five days a week until death (about 35 weeks). During that time, they were also injected intraperitoneally with 15 mg/kg bw *azathioprine*, 20 mg/kg bw *prednisolone* or 15 mg/kg bw *cyclophosphamide* in 0.1 ml saline or given 60 mg/kg bw *cyclosporine* in 0.1 ml peanut oil by gavage or 0.1 ml vehicle alone. Azathioprine, cyclophosphamide and cyclosporine all significantly enhanced photocarcinogenesis with regard to median latent periods and tumour multiplicity. Prednisolone did not enhance this effect, nor did it interfere with the enhancement by other drugs when given in combination with them (Kelly *et al.*, 1987).

Groups of 15–32 female albino Skh-hr 1 hairless mice, 10–12 weeks of age, were irradiated with UVA plus UVB (250–700 nm; one Oliphant FL40SE tube, three Sylvania F40/350 BL tubes and two True-Lite [Duro-Test Corp] tubes) on five days per week for 12 weeks. Two weeks after the first irradiation, mice received intraperitoneal injections on five days a week of 15 mg/kg bw *azathioprine* or *6-mercaptopurine* in 0.1 ml saline or 0.1 ml vehicle alone. Both compounds significantly enhanced skin photocarcinogenesis with regard to median latent period, proportion of malignant:benign growths and tumour multiplicity (Kelly *et al.*, 1989).

3.10 Molecular genetics of animal skin tumours induced by ultraviolet radiation

Three skin papillomas and three skin carcinomas produced in female SENCAR mice after a single exposure to UVB (280–315 nm; Westinghouse FS20; 70 kJ/m^2) were examined for *ras* gene alterations. A five- to 10-fold increase in cHa-*ras* RNA gene expression associated with the gene amplification was found in papillomas and carcinomas, while DNA from carcinomas, but not from papillomas, induced foci in the NIH-3T3 cell transfection assay (Husain *et al.*, 1990).